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The protective potential of Tiron in models of direct and physiologically induced reactive oxygen species

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The Protective Potential of Tiron in Models of Direct and Physiologically Induced Reactive Oxygen Species

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The Protective Potential of Tiron in Models of Direct and Physiologically Induced Reactive Oxygen Species

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Certificate of Ethical Approval

Applicant:

Danielle Meyer

Project Title:

Tiron is protective against ROS-induced damage in vitro

This is to certify that the above named applicant has completed the Coventry University Ethical Approval process and their project has been confirmed and approved as Medium Risk

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The above shows the most recent certificate of ethical approval, for historic ethical approval related to this thesis see Appendix 1.

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This thesis is dedicated to my brother Jonathan Williams and my much-missed uncle, Jon Roberts. Forever in my thoughts as I strive to be the best that I can be x

What a journey this has been!

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Abstract

Reactive oxygen species (ROS) derived damage and accompanying oxidative stress have been identified in a number of pathological conditions. As a result, antioxidant supplementation has been increasingly investigated to reduce ROS and inhibit the accumulation of oxidative damage. Tiron, 4,5-Dihydroxy-1,3-benzenedisulfonic acid disodium salt monohydrate, is a synthetic vitamin E analogue first characterised in 1964. Described chemically as a spin trap, Tiron has also been considered a superoxide scavenger and previously shown to alleviate oxidative stress to a high degree in human skin cells.

This thesis aimed to evaluate the use of Tiron comparatively to protect against ROS-induced damage in three different human immortalised cell lines. Renal proximal epithelial tubular (HKC-8), bronchial epithelial cells (BEAS-2B) and astroglial (SVGp12) cells were pre-treated with Tiron for 24 hours prior to the induction of ROS with H_2O_2 or an optimised physiological method through exposure to high glucose or hypoxia. Protection against ROS-induced damage in all models was assessed through the measurement of ROS, mitochondrial DNA (mtDNA) strand breaks and malondialdehyde (MDA) concentration. The effects of prolonged exposure to Tiron, co-treatment at the time of ROS induction and Tiron's influence on Nrf2 protein expression were also investigated.

Tiron pre-treatment was significantly protective against ROS-induced mtDNA strand breaks, significantly reduced ROS and MDA levels and was independent of the Nrf2 pathway in all cell lines. Co-treatment with Tiron was also significantly protective in HKC-8 and BEAS-2B cells. However, prolonged supplementation with Tiron led to a significant increase in Nrf2 protein expression across all cell lines, providing some insight into its mechanism.

Overall, this thesis demonstrates a promising use for Tiron supplementation in the protection against ROS damage, building upon previously published literature of Tiron's use as a ROS reducing agent. The finding that Tiron consistently offered significantly high degrees of protection independent of cell type and pre- or co-treatment methods in models of both direct and physiologically-induced ROS indicates the therapeutic potential of this ROS reducing agent. Further studies are now required to support the use of Tiron in disease-specific models before clinical translation.

Publications from this Thesis

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'The Use of Antioxidant Supplementation to Prevent ROS-Induced Mitochondrial Damage' **Poster and abstract**. *HLS Symposium*, Coventry, U.K, March 2015.

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List of Abbreviations

8OHdG	8-hydrocyo-2'-deoxyguanosine
AD	Alzheimer's disease
ADP	Adenosine diphosphate
AGE	Advanced glycation end-products
AMP	Adenosine monophosphate
APP	Amyloid peptide precursor
ARE	Antioxidant response element
ATP	Adenonsine triphosphate
BAL	Bronchoalveolar fluid
BBB	Blood brain barrier
BEAS-2B	Human bronchial epithelial cells
BEGM	Bronchial epithelial cell growth medium
BER	Base excision repair pathway
Bp	Base pairs
COPD	Chronic obstructive pulmonary disorder
CREB	Cyclic adenosine monophosphate-response element binding
CBP	Cyclic adenosine monophosphate-response element binding binding protein
CT	Cycle threshold
CuSOD	Copper superoxide dismutase
DAG	Diacylglycerol
DAMPs	Damage-associated patterns
DCF	2',7'-dichlorofluorescein
DCFDA	2'7'-dichlorofluorescein diacetate
DKD	Diabetic kidney disease / diabetic nephropathy
DM	Diabetes mellitus
DMEM	Dulbecco's modified eagle's media
DMEM/F12	DMEM with Ham's F12 nutrient mixture
DMSO	Dimethyl sulfoxide
DOPA	Dihydroxyphenylalanine
DSB	Double strand breaks
EDTA	Ethylenediaminetetraacetic acid
EDTA-Na_2.2H_2O	EDTA-disodium dihydrate
EMEM	Eagle's minimum essential medium
eNOS	Endothelial nitric oxide synthase

ETC	Electron transport chain
$FAD^+/FADH_2$	Flavin adenine dinucleotide
FBS	Foetal bovine serum
Fc	Fragment crystallisable
Fe^{2+}	Ferrous iron
Fe^{3+}	Ferric Cation
FIH	Factor inhibiting hypoxia inducible factor α
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GFAT	Glutamine:fructose-6-phosphate amidotransferase
GLUT	Glucose transporter
GPx	Glutathione peroxidase
GSH	Glutathione (reduced)
GSSG	Glutathione (oxidised)
H^+	Hydrogen ion
H_2O_2	Hydrogen peroxide
HDFn	Human dermal fibroblasts
HIF	Hypoxia inducible factors
HIV	Human Immunodeficiency Virus
HKC-8	Human renal proximal tubular epithelial cells
HNE	4-hydroxynoneal
HO-1	Hemeoxygenase-1
HR	Homologous repair
HRE	Hypoxia response element
HRP	Horseradish peroxidase
I/R	Ischaemia/reperfusion
ICAM-1	Intracellular adhesion molecule-1
IgE	Immunoglobulin E
IL-	Interleukin
iPSC	Induced pluripotent stem cells
IRS	Insulin receptor substrates
ITS	Insulin-transferrin-selenium
JNK	c-Jun N-terminal kinases
KCl	Potassium chloride
Keap1	Kelch-like ECH-associated protein 1
KH_2PO_4	Monopotassium phosphate
kb	Kilobase

LD50	Lethal dose reducing cell viability to 50%
LDH	Lactate dehydrogenase
LOO·	Peroxyl radical
MAA	Malondialdehyde acetaldehyde
Maf	Musculoaponeurotic fibrosarcoma
MAPK	Mitogen-activated protein kinase
MCI	Mild cognitive impairment
MDA	Malondialdehyde
MMR	Mismatch repair
MnSOD	Manganese superoxide dismutase
MnTBAP	Manganese (III) tetrakis (4-benzoic acid) porphyrin chloride
mtDNA	Mitochondrial DNA
$Na_2HPO_4.12H_2O$	Sodium dihydrogen phosphate dodecahydrate
NaCl	Sodium chloride
NAC	N-acetylcysteine
NAD+/NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
nDNA	Nuclear DNA
NER	Nucleotide excision repair pathway
NF-κB	Nuclear factor kappa (κ)-light-chain-enhancer of activated B cells
NHEJ	Non-homologous end joining
NLRP3	NACHT, LRR and PYD domains-containing protein 3
NO	Nitric oxide
NOX	Nicotinamide adenine dinucleotide phosphate oxidase
NQO1	Nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase 1
Nrf2	Nuclear factor (erythroid-derived 2)-like 2
$O_2\cdot^-$	Superoxide anion
$O_2\cdot^{2-}$	Peroxide anion
OH·	Hydroxyl anion
OXPHOS	Oxidative phosphorylation
PAMPs	Pathogen-associated molecular patterns
PARP-1	Poly(ADP-ribose) polymerase 1
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction

PD	Parkinson's disease
PEDF	Pigment epithelium-derived factor
PHD	Proline hydroxylase
PI3K	Phosphoinositide 3-kinase
PKC	Protein kinase C
PRR	Pattern recognition receptor
PUFA	Polyunsaturated fatty acids
pVHL	Von Hippel-Lindau tumour suppressor protein
qPCR	Quantitative polymerase chain reaction
RAGE	Receptor of advanced glycation end products (AGE)
RISP	Rieske iron-sulphur protein
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SBT	Seabuckthorn leaf extract
SDS	Sodium dodecyl sulphate
SEM	Standard error of mean
SGLT1/2	Sodium-glucose cotransporters 1/2
SNc	Sustantia nigra pars compacta
SOD	Superoxide dismutase
SRB	Sulforhodamine B
SSB	Single-strand break
SVGp12	Human foetal astrocyte cells
T1D	Type 1 diabetes mellitus
T25	$25cm^2$ cell culture flask
T2D	Type 2 diabetes mellitus
T75	$75cm^2$ cell culture flask
TBA	Thiobarbituric acid
TBARS	TBA reactive substances
TBE	Tris borate EDTA
tBHP	Tert-butyl hydroperoxide
TBS	Tris buffered saline
TCA	Tricarboxylic acid cycle
TGF-β1	Transforming growth factor β1
TNF-α	Tumour necrosis factor alpha
UDP-GlcNAc	Uridine diphosphate N-acetylglucosamine
UVR	Ultraviolet radiation

VEGF	Vascular endothelial growth factor
ZnSOD	Zinc superoxide dismutase

Chapter 1 – Introduction

1.1 General Introduction

Reactive oxygen species (ROS) are volatile free radicals containing one or more unpaired electrons in their outer electron shell, rendering them reactive (Li et al. 2013, Halliwell 2013). Under normal physiological conditions, ROS are involved in a variety of homeostatic actions including signal transduction, regulation of redox, cell growth and phagocytic activity. However, exposure to high concentrations of ROS can be causative of oxidative stress, defined as the imbalance between ROS concentration and the antioxidant defence system (Bullón et al. 2015).

Oxidative stress can have a significant effect on biological systems that are integral to signalling and homeostasis and can be identified by numerous biomarkers. These include oxidation products of DNA, lipid peroxidation by-products, or a reduction of antioxidant enzyme activity (Bickers and Athar 2006); resultant damage has been attributed to the aetiopathogenesis of numerous diseases and has also been identified as a carcinogenic catalyst (Pisoschi and Pop 2015, Waypa et al. 2016).

There is a comprehensive evolutionary defence system in operation to protect the body against oxidative stress. This system utilises endogenous antioxidants to reduce and quench ROS (Plotnikov et al. 2013). Without exogenous inducers, 2-5% of O_2 is converted into ROS, which is well managed by host antioxidants (Umeno et al. 2017). However, exposure to ultraviolet radiation (UVR), environmental toxins and ROS-inducing pharmaceuticals, just to name a few, can lead to ROS generation beyond manageable limits (Birch-Machin et al. 2013). This can result in a cascade of detrimental effects including those detailed above as well as biological signal disruption and stimulation of the inflammatory response (Birch-Machin et al. 2013, Bullón et al. 2015). Therefore, there is increasing interest in the prevention of ROS-induced damage and disease, with ROS being identified within the pathology of diseases including chronic pulmonary obstructive disorder (COPD) and diabetes mellitus (DM). As such, there is interest in the exploitation of exogenous antioxidant supplementation to combat this, such as with the water-soluble ROS reducing agent Tiron.

1.2 The Formation of Reactive Oxygen Species (ROS)

1.2.1 Formation of ROS within the Mitochondria

Incomplete or partial reduction of molecular O_2 within the mitochondria leads to the production of O_2 metabolites such as the superoxide anion ($O_2^{\cdot -}$), hydroxyl ($OH^{\cdot}$), peroxide ($O_2^{\cdot 2-}$) and hydrogen peroxide (H_2O_2). The aforementioned metabolites are collectively termed ROS (Camello Almaraz et al. 2006). $O_2^{\cdot -}$ is the most commonly described ROS in this field and is a precursor for other ROS including $OH^{\cdot}$, H_2O_2 and peroxyl radicals ($LOO^{\cdot}$) (Carocho and Ferreira 2013). The incomplete reduction of O_2 largely occurs within the mitochondria as a result of insufficient oxidative phosphorylation (OXPHOS) and electron 'leakage' from the electron transport chain (ETC) during aerobic respiration (Chen et al. 2007).

The mitochondrion is an organelle responsible for the majority of cellular O_2 consumption and are functionally associated with the generation of adenosine triphosphate (ATP) through OXPHOS, first identified in 1961 (Mitchell 1961, Mitchell 2011, Li et al. 2013). The transfer of carbon through the tricarboxylic acid (TCA) cycle oxidises reduced nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) to NAD^+ and FAD^+ and facilitates the passage of electrons through the ETC via four complexes (I-IV) (Vendelbo and Nair 2011). The passage of electrons prompts proton release into the intermembrane space of the mitochondrion by complexes I, III and IV, creating a proton gradient to permit ATP synthase to produce ATP at complex V through a series of reduction and oxidation reactions, defined as chemiosmotic theory (Vendelbo and Nair 2011).

Electron passage through the ETC (Figure 1.1) is facilitated by the transporter coenzyme ubiquinone, sourced at complex I. Alongside NADH oxidation, coenzyme Q, also known as ubiquinone, is reduced to ubiquinol through the binding of two electrons at the quinone binding site (Pryde and Hirst 2011, Waypa et al. 2016). The newly formed ubiquinol then migrates to deposit two electrons at a time at the quinol binding site in complex III. Here, one electron is transferred to the Rieske iron-sulphur protein (RISP) of complex III before passage to complex IV. The second electron is typically removed by B cytochromes, capable of relegating ubiquinone back into the membrane (Waypa et al. 2016).

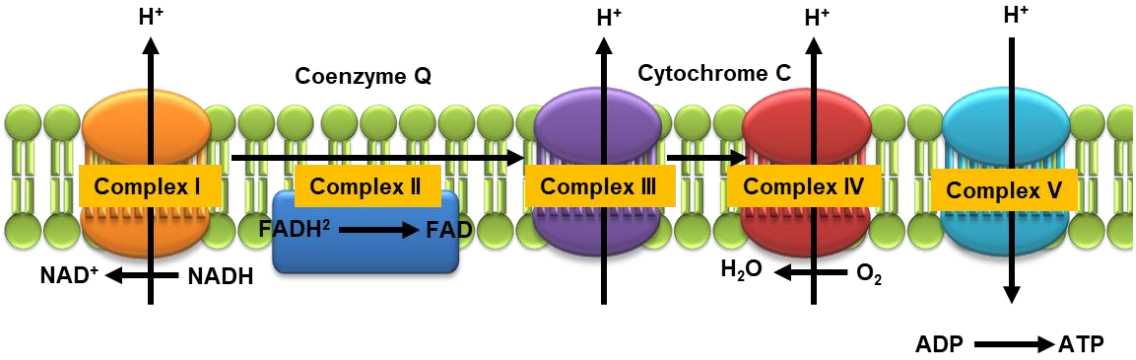

***Figure 1.1**. **The electron transport chain (ETC) during oxidative phosphorylation (OXPHOS)**. Taking place in the mitochondrial inner membrane, electrons are transported along a proton gradient, resulting in the release of hydrogen ions (H^+), through complexes I to IV and the oxidation of nicotinamide adenine dinucleotide (NADH to NAD^+) and flavin adenine dinucleotide ($FADH_2$ to FAD). The energy generated through this proton gradient results in the production of adenosine triphosphate (ATP) at complex V by phosphorylation of adenosine diphosphate (ADP). Original figure, adapted from* (Chen et al. 2007, Vendelbo and Nair 2011).

The production of $O_2^{\cdot-}$ by complex I has been postulated as the predominant source of ROS in the ETC. However, the activities of all complexes have been shown to contribute to the generation of ROS (Kussmaul and Hirst 2006, Chong et al. 2014). Complex III has also been identified as a key contributor to the formation of ROS and the effects on cell signalling (Chong et al. 2014, Forkink et al. 2015, Thummasorn et al. 2018).

H_2O_2 is formed following the protonation, or proton transfer of $O_2^{\cdot-}$ as a result of the addition of an electron to $O_2^{\cdot-}$ (Murphy 2009). $O_2^{\cdot-}$ itself is dismutated, a process of simultaneous reduction and oxidation to H_2O_2 by the antioxidant enzyme superoxide dismutase (SOD), which is freely available in the mitochondrial membrane and intermembrane space. This has led to the characterisation of H_2O_2 as a mitochondria-formed ROS. $O_2^{\cdot-}$ has a short half-life and cannot successfully reach the mitochondrial outer membrane without conversion to peroxynitrite, limiting its influence as a signalling molecule and has therefore been superseded by the more stable and available H_2O_2 in the study and induction of ROS and associated effects across published research (Clément and Pervaiz 2001, Gough and Cotter 2011, Li et al. 2013). However, the mitochondria are not the only source of ROS physiologically.

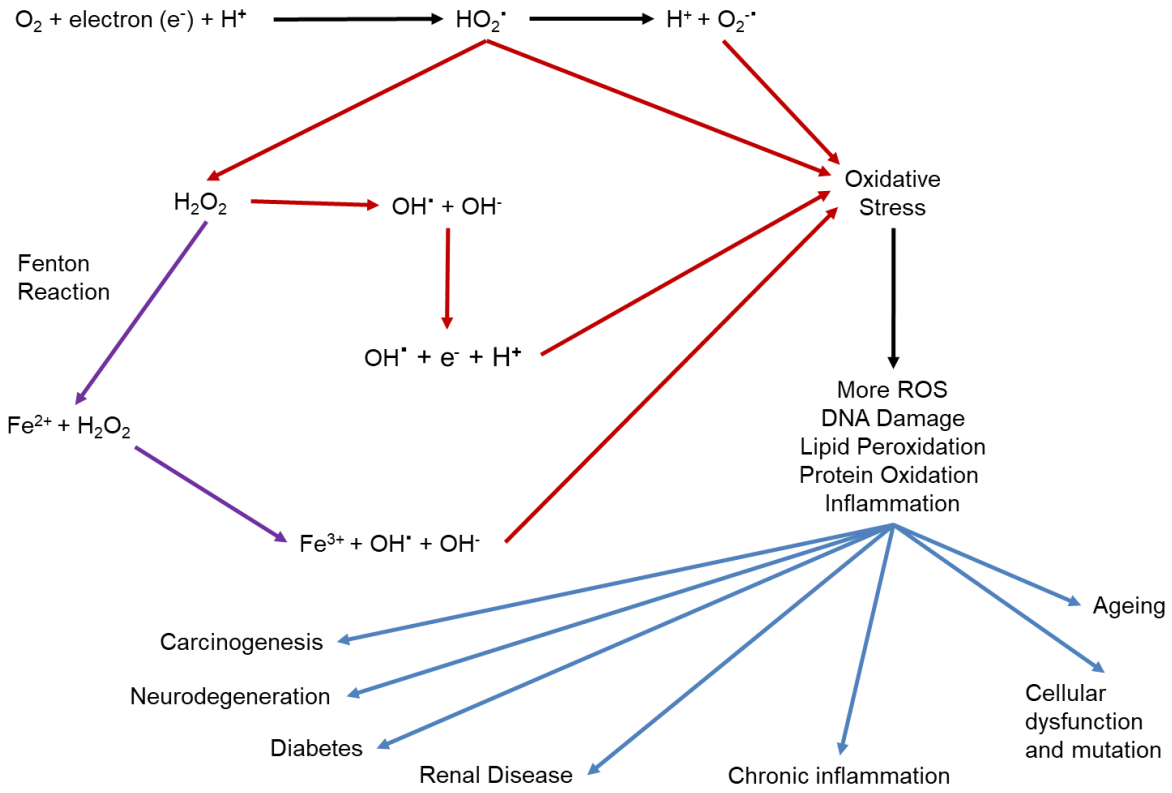

Figure 1.2. Overview of the formation of reactive oxygen species (ROS) and potential consequences. *The leakage of electrons (e^-) and generation of hydrogen ions (H^+) from the electron transport chain (ETC) result in the production of a hydroperoxyl radical ($HO_2\cdot$) which can further induce the formation of ROS such as superoxide ($O_2\cdot^-$) and hydrogen peroxide (H_2O_2). H_2O_2 can lead to the production of hydroxyl radicals ($OH\cdot$) directly or through interaction with ferrous (Fe^{2+}) ions, through the Fenton reaction and resulting in the production of ferric (Fe^3) cations and $OH\cdot$. Together, these contribute to an environment of oxidative stress, which can form more ROS and also lead to DNA damage, lipid peroxidation, protein oxidation and inflammation, all of which have been attributed to cellular dysfunction, mutation and within disease pathologies. Original figure, adapted from (Murphy 2009, Vendelbo and Nair 2011, Pryde and Hirst 2011).*

1.2.2 Formation of ROS Outside of the Mitochondria

1.2.2.1 The Fenton Reaction

With the majority of endogenous ROS sourced from the mitochondria, additional ROS produced outside of this are capable of augmenting physiological concentrations. Free radical production catalysed by transition metals was first reported by Dr H.J.H Fenton in 1876 (Kanti Das et al. 2015). The Fenton reaction, defined as the reaction between transition metals and

H_2O_2 with a ferrous iron (Fe^{2+}) catalyst, is favoured thermodynamically due to H_2O_2 having a higher reduction potential compared with other aqueous iron molecules (Figure 1.2) (Li et al. 2014, Zhou et al. 2016). Reactions also occur with ligands containing a ferric cation (Fe^{3+}) however these are unfavourable in comparison. A biological example of the Fenton reaction involves the oxidation of haem; haem within haemoglobin contains Fe^{2+} which in the presence of O_2 can produce $O_2^{\cdot-}$ and haem-Fe^{3+} (Angelelli et al. 2005).

To facilitate the Fenton reaction, H_2O_2 crosses cellular membranes to react with transition metals; leading to ROS formation, particularly $OH^{\cdot}$, which can lead to the re-reduction of $O_2^{\cdot-}$ and H_2O_2, creating a cycle of ROS regeneration (Thomas et al. 2009). The $OH^{\cdot}$ radical is a highly aggressive molecule, responsible for widespread oxidative damage within the body. The generation of ROS via the Fenton reaction has been implicated in the development of neurodegenerative diseases such as Alzheimer's disease (AD) (Smith et al. 1997, Lim et al. 2007). However, due to their high reactivity, these radicals have a short life span and are utilised further in hydrogen abstraction and transfer of electrons (Bossmann et al. 2004). Other metals including copper, zinc and aluminium are also capable of facilitating the Fenton reaction to generate $OH^{\cdot}$. However, whether the Fenton reaction is capable of forming anything other than $OH^{\cdot}$ is uncertain and it is also frequently referred to as iron catalysed H_2O_2 decomposition (Mantzavinos 2003, Barreiro et al. 2007). Additional to this, ROS can be sourced pathologically in stress conditions including hyperglycaemia and hypoxia.

1.2.2.2 Glucose

Hyperglycaemia, defined as high concentrations of glucose, is symptomatic of the metabolic disorder diabetes mellitus (DM) and has been found to cause oxidative stress both directly and through its production of ROS. (Han et al. 2005, Shokrzadeh et al. 2016, Zhai and Wang 2017). The induction of oxidative stress and ROS as a result of hyperglycaemia has been hypothesised to be due to increased activity across multiple mechanisms including the polyol pathway, protein kinase C (PKC), hexosamine pathway and augmented formation of advanced glycation end-products (AGEs) (Figure 1.3) (Evans et al. 2003, Ceriello and Motz 2004). These four mechanisms can induce oxidative stress and the associated damage, and are commonly associated with the development of secondary diabetic complications such as diabetic kidney disease and augment the risk of cardiovascular disease (Kachhawa et al. 2017).

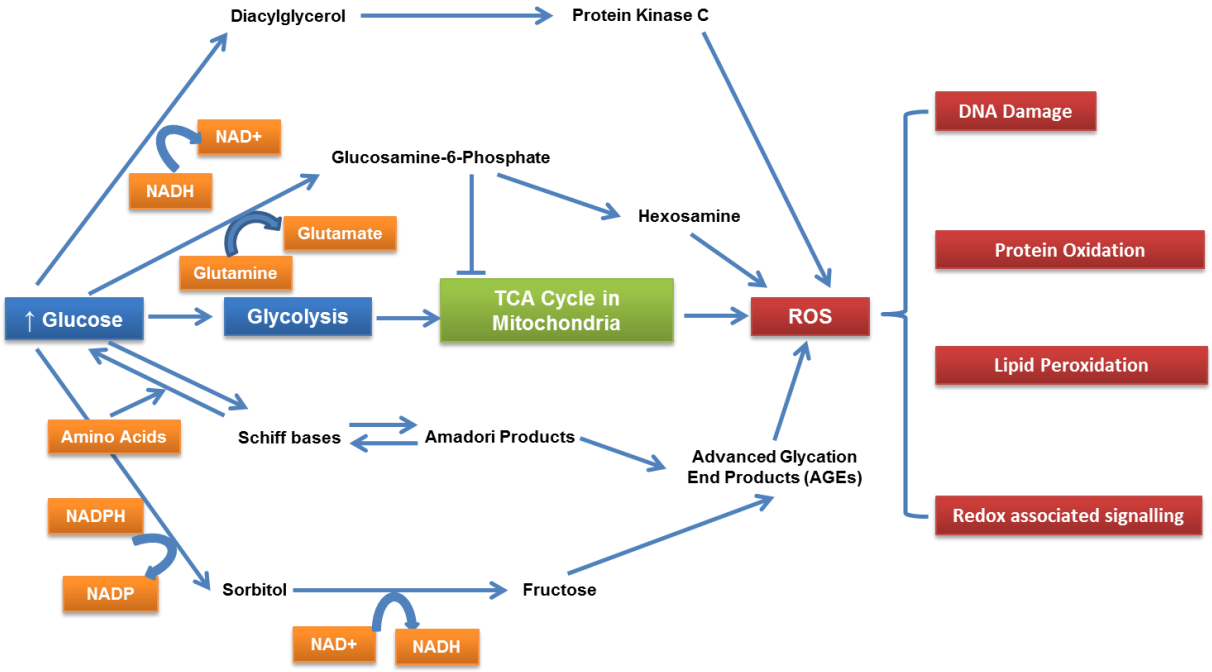

Figure 1.3 A schematic summarising the role of hyperglycaemia in the induction of reactive oxygen species (ROS) and resultant damage. *Elevated concentrations of glucose result in the generation of ROS through increases in sorbitol, resulting in the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH to NADP) and reduction of nicotinamide adenine dinucleotide (NAD^+ to NADH), advanced glycation end products, hexosamine from glucosamine-6-phosphate, protein kinase C through formation of Diacylglycerol and NADH reduction as well as the citric acid (TCA) cycle. This production of ROS contributes to oxidative stress and the induction of related damage. Original figure, adapted from (Ceriello and Motz 2004, Buse 2006, Giacco and Brownlee 2010, Williamson and Ido 2012, Prasad and Dhar 2014).*

The polyol pathway facilitates the formation of sorbitol following glucose reduction in the presence of NADPH (nicotinamide adenine dinucleotide phosphate) and aldose reductase, found specifically in the kidney as well as the heart (Williamson and Ido 2012). Under normal physiological conditions, this pathway utilises little glucose in the production of sorbitol, with glycolysis being the dominant metabolic pathway (Mathebula 2015). Any sorbitol formed in this pathway is oxidised to fructose by sorbitol dehydrogenase, instigating the reduction of NAD^+ to NADH, which can be readily oxidised within ETC at complex I, facilitating the formation of ATP but also contributing to ROS formation after NADH has been translocated from the cytoplasm to the mitochondria (Turrens 2003, Williamson and Ido 2012, Luo et al. 2015). During hyperglycaemia, hexokinase over saturates the glycolytic pathway, leading to enhanced activity of the polyol pathway and resulting in elevated sorbitol and NADH production, increasing the production of $O_2^{\bullet-}$ and other ROS within the ETC through NADH

oxidisation to NAD^+ (Berrone et al. 2006, Giacco and Brownlee 2010, Williamson and Ido 2012). This has been shown in isolated bovine heart mitochondria where NADH oxidation induced $O_2^{\cdot-}$ (Pryde and Hirst 2011) and supported in a type 1 diabetes (T1D) rat model where complex I activity was increased correlated with ROS formation (Raza et al. 2011). In addition to this, increases in the sorbitol pathway also lead to a decrease in NADPH which is utilised in the regeneration of glutathione, a ROS scavenger. Thus any decrease in NADPH reduces glutathione levels, resulting in increased ROS levels and oxidative stress as a result of polyol pathway activation (Chung et al. 2003, Obrosova 2005, Giacco and Brownlee 2010). This has been shown in cardiomyocytes treatment with high glucose concentrations, where NADPH oxidase (NOX) catalytic activity increased the oxidation of $NADP^+$ from NADPH, producing a $O_2^{\cdot-}$ by-product and depleting NADPH and glutathione levels (Balteau et al. 2011).

The production of AGEs involves the non-enzymatic glycation of protein amino acids resulting in Schiff bases through the Maillard reaction, a reaction between amino acids and carbonyl containing sugars such as glucose (Prasad and Dhar 2014). These Schiff bases can be rearranged spontaneously to form Amadori products as well as early glycation products, which can undergo auto-oxidation to form AGEs or reactive carbonyls capable of altering protein structure, increasing lipid oxidation and modifying the extracellular matrix (Du et al. 2003, Han et al. 2005). AGEs are irreversible and their accumulation can increase occupation of macrophage AGE-cellular receptors, increasing inflammation (Sinha et al. 2013, Jin et al. 2015). ROS are generated as by-products of AGE formation as a result of increased NOX activity following binding to receptor of AGE (RAGE), alongside cytokines and other mediators of inflammation themselves generating ROS, further compromising health (Hakim and Pflueger 2010, Guimaraes et al. 2010). AGEs are also capable of impairing mitochondrial function through ETC inhibition, promoting electron leakage and $O_2^{\cdot-}$ formation (Li et al. 2016). The diverse array of damage that can occur as a result of AGE production makes targeting these and associated intermediates, in addition to treatment of hyperglycaemia, an attractive prospect (Palsamy and Subramanian 2011).

An increase in the hexosamine pathway can also augment ROS during hyperglycaemia through $O_2^{\cdot-}$ production. This occurs from the conversion of fructose-6-phosphate to N-acetylglucosamine-6-phosphate, facilitated by glutamine:fructose-6-phosphate amidotransferase (GFAT), and leads to the eventual formation of uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) (Kaneto et al. 2001). This can inhibit GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and enzymes integral to the ETC, as well as reduce NADPH synthesis reducing endogenous antioxidant availability (Sinha et al. 2013). UDP-GlcNAc is an intracellular signalling molecule which initiates the formation of

glycoprotein, glycolipids and proteoglycans. Some glycosylation products formed in this pathway contribute to post-translational protein modification including within insulin receptor substrates, adding to the theory that flux within the hexosamine pathway can increase insulin resistance (Kaneto et al. 2001, Buse 2006). Formation of glucosamine-6-phosphate leads to inhibition of the pentose phosphate pathway, vital in the regeneration of glutathione, and thus reduces the endogenous ROS defence (Buse 2006). It has been shown that hyperglycaemia-induced mitochondrial $O_2^{\cdot-}$ augmentation can inhibit GAPDH and upregulate the hexosamine pathway (Du et al. 2000). Like the polyol pathway, hyperglycaemia induction of the hexosamine pathway does not directly produce ROS but contributes to the accumulation of ROS-induced damage through the reduction of NADPH (Kaneto et al. 2001, Evans et al. 2003, Buse 2006).

Finally, induction of PKC has also been associated with hyperglycaemia. This is an extensively evidenced pathway, with activation apparent across all tissues during hyperglycaemia (Geraldes and King 2010). Activation of PKC can occur as a result of increased glucose concentrations as well as increased ROS and AGE availability, both directly and indirectly as a result of hyperglycaemia. However, enhanced PKC activation during hyperglycaemia has been attributed to elevated diacylglycerol (DAG) levels, found to increase ROS production through NOX activity (Inoguchi et al. 2000, Giacco and Brownlee 2010). ROS production has been previously linked to glucose stimulated PKC activation with inhibition of PKC and DAG shown to reduce ROS *in vitro* (Lee et al. 2004, Ramana et al. 2005). As well as hyperglycaemia, other cellular stressors such as hypoxia are also found to elevate ROS production physiologically.

1.2.2.3 Hypoxia

Hypoxia is a state of abnormally low concentrations of O_2 and is accommodated for in the first instance by altering metabolism to enhance the breakdown of glycogen into glucose through the hypoxia inducible factor (HIF)-1α induced upregulation of glycolytic enzymes (Callapina et al. 2005, Ten and Starkov 2012). This adaptability maintains glycolysis whilst also decreasing the rate of RNA and protein synthesis to allow ATP to meet energy demands until O_2 supply is restored (Ten and Starkov 2012, Terraneo et al. 2017). However, a consequence of hypoxia is increased acidosis, therefore function may only be sustained for a short period before damage occurs.

Under normal conditions, HIF-1α is rapidly targeted for degradation by proline hydroxylase (PHD), which hydroxylates the proline residues of HIF-1α to hydroxyproline. The new hydroxyproline residues permit binding to the von Hippel-Lindau tumour suppressor protein (pVHL) and facilitates degradation of HIF-1α by ubiquitination (Van Welden et al. 2017, Zhou

et al. 2017). Hydroxylation of HIF-α asparaginyl residues by factor inhibiting HIF-1 (FIH) further inhibits binding of the cyclic adenosine monophosphate(AMP)-response element binding (CREB) binding protein (CBP) and histone acetyltransferase p300 (CBP/p300) transcription co-activators, which facilitates HIF-1α binding to DNA at the hypoxia response element (HRE) (De Saedeleer et al. 2012, Van Welden et al. 2017, Zhou et al. 2017).

During hypoxia, proline hydroxylase does not oxidise the proline residues, thus inhibiting cytosolic ubiquitination of HIF-1α. As a result, HIF-1α accumulates, translocates to the nucleus and forms a heterodimer with the constitutively expressed and metabolically stable HIF-1β and CBP/p300 (Figure 1.4) (Dewitz et al. 2017, Van Welden et al. 2017). This stabilises HIF-1α, promotes binding at the HRE and initiates transcription of genes integral to promoting cell survival including vascular endothelial growth factor (VEGF) and erythropoietin, involved in angiogenesis and erythropoiesis respectively (Keitzmann and Görlach 2005, Ren et al. 2008, Jiang et al. 2012, Zhou et al. 2017). Glucose transportation, facilitated by glucose transporters (GLUT), is rate limiting in glucose metabolism. Therefore, to maintain adequate energy provision GLUT and glycolytic enzymes are also upregulated in response to hypoxia. The upregulation of GLUT1 specifically by HIF-1α in response to hypoxia has been demonstrated in mouse chondrocytes (Ren et al. 2008), human placental choriocarcinoma cells (Baumann et al. 2007) and human colon carcinoma (HCT116) cells (Lu et al. 2016). HIF-1α stabilisation also upregulates glycolytic enzymes, including hexokinase and pyruvate kinase, the latter of which is capable of facilitating HIF-1α transcriptional activity by metabolising pyruvate to lactate, inhibiting PHD activity (De Saedeleer et al. 2012) and supported by HIF-1α knockdown models demonstrating reduced glycolysis and increased apoptosis in human synovial fibroblasts (Del Rey et al. 2017).

Due to the decrease in O_2 during hypoxia, it would be anticipated that ROS formation would be hindered by decreased mitochondrial activity. However, paradoxically, oxidative stress can be augmented in conditions of just 1.5% O_2 (Waypa et al. 2016). ROS generation as a result of hypoxia has been well documented, with heavy involvement of complex III and the ROS produced sufficient to stabilise HIF-1α (Chandel et al. 2000, Enomoto et al. 2002, Kietzmann and Görlach 2005). ROS have also been found to stabilised HIF-1α through inhibition of PHD activity shown with exogeneous H_2O_2 (10 μM) supplementation *in vitro* in human hepatocellular carcinoma (Hep3B) cells (Pan et al. 2007). The production of ROS within hypoxia has also been suggested to be dependent on the availability of nitric oxide (NO). NO has been found to nitrosylate HIF-1α cysteine 533 residues within the oxygen-dependent degradation domain, which also hosts its proline residues, preventing its pVHL binding and subsequent degradation within a murine breast tumour model (Li et al. 2007). Similarly, inhibition of NO synthases and ROS generation with ascorbic acid has been found to reduce

HIF-1α accumulation in normoxia, the term used to describe normal levels of O_2 experienced within tissue or blood but also atmospheric O_2 (Quintero et al. 2006).

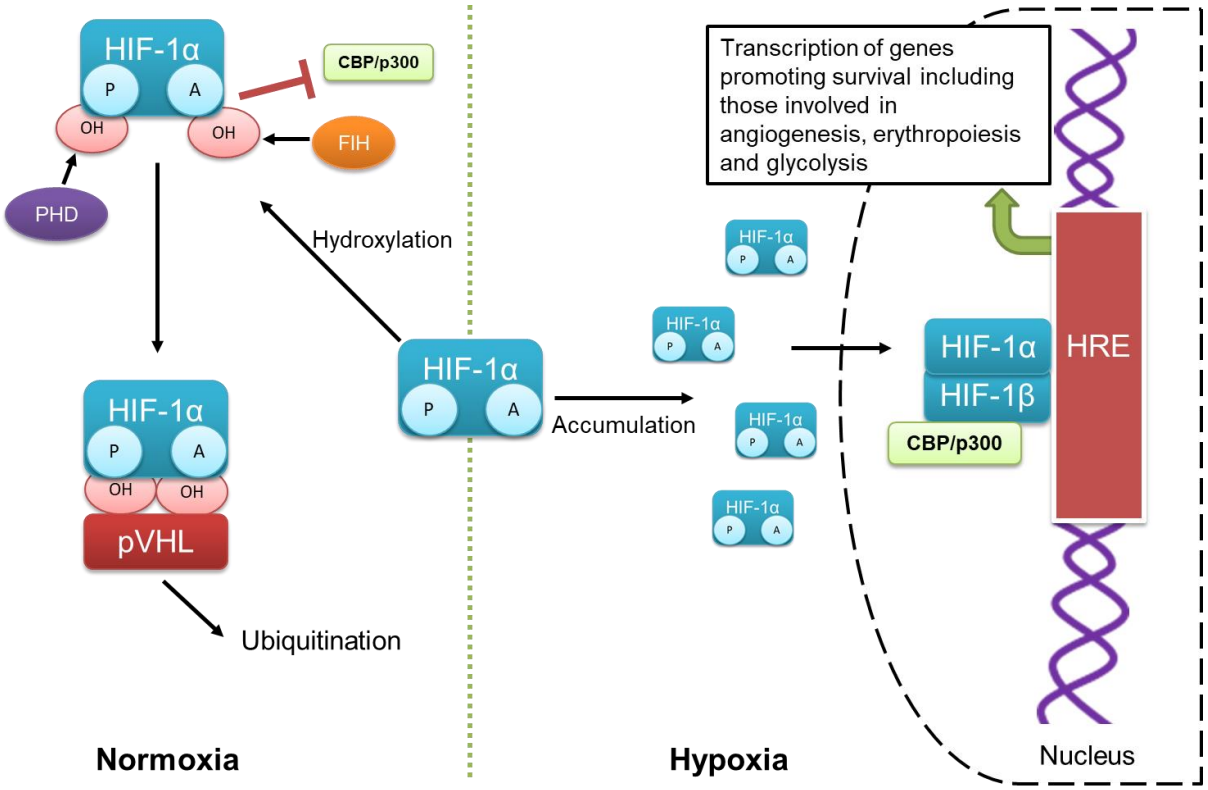

Figure 1.4 A summary of hypoxia-inducible factor (HIF)-1α action in both normoxia and hypoxia. *During normoxia, HIF-1α's proline (P) and asparaginyl (A) residues are hydroxylated by proline hydroxylase (PHD) and factor inhibiting HIF-1 (FIH) respectively, with the latter inhibiting HIF-1α binding to cyclic adenosine monophosphate-response element binding binding protein and histone acetyltransferase p300 (CRB/p300) transcription co-activators. Following hydroxylation, von Hippel-Lindau tumour suppressor protein (pVHL) forms a complex with HIF-1α and targets it for ubiquitination. During hypoxia, PHD and FIH are inhibited, permitting HIF-1α to accumulate and translocate to the nucleus where it forms a heterodimer with HIF-1β and CRP/p300, binding to the hypoxia response element (HRE) and facilitating transcription of survival promoting genes. Original figure adapted from (Ren et al. 2008, De Saedeleer et al. 2012, Van Weldon et al. 2017).*

Failure to restore O_2 results in a loss of ATP, increased calcium and AMP and disruption of the cellular ion gradient leading to the production of endothelial nitric oxide synthase (eNOS) and ROS (Farrukh et al. 2014). Deprivation of O_2 is particularly problematic in two organs, the lungs and the brain. Within the brain, hypoxia causes damage to the blood brain barrier (BBB) and initiates an inflammatory response, leading to the production of more BBB damaging ROS (Lochhead et al. 2010). Furthermore, hypoxia in the lungs is characteristic of a number of

pulmonary pathologies, including COPD, and in acute situations induces inflammation, oedema and increased pressure in the pulmonary artery (Araneda and Tuesta 2012).

Hypoxia also has an interdependent relationship with inflammation which underlies the majority of oxidative stress induced damage. An example of this is the use of inflammatory cytokines to stabilise HIF (Jiang et al. 2010). Activation of HIF also leads to apoptosis inhibition within neutrophils, increasing ROS production rather than damaged cell elimination (Bartels et al. 2013). Equally, inflamed tissues or lesions can become hypoxic themselves leading to an increase in inflammatory mediator recruitment and ROS production (Eltzschig and Carmeliet 2011). With the resultant generation of ROS during hypoxia, this too could be targeted alongside restoring O_2 concentrations. Reduction of ROS could therefore limit hypoxic complications and potentially improve prognosis.

1.2.2.4 Inflammation

The process of inflammation involves a cycle of continuous ROS production, with the first example of ROS being produced by macrophages and neutrophils, which are the first line of defence in response to tissue injury or pathogen invasion. Resting neutrophils, monocytes and macrophages consume varied amounts of O_2, with macrophages consuming the most due to a higher number of mitochondria (Ogawa et al. 2008, Castaneda et al. 2017). When recruited, inflammatory mediators such as macrophages and neutrophils increase their O_2 uptake markedly and experience a 'respiratory burst' of up to 20 times their resting consumption. This results in more OXPHOS, increasing $O_2^{\cdot-}$ and consequential ROS production (Castaneda et al. 2017).

Inflammation is regulated to cease following pathogen or insult removal and successful tissue repair. However, if the inflammation process is prolonged, often through the loss of redox homeostasis and moderate concentrations of ROS, this can lead to chronic inflammation and accretion of ROS production (Oberg et al. 2004, Khansari et al. 2009, Richter et al. 2015). This can lead to redox imbalance and exacerbate oxidative stress, capable of overwhelming host antioxidant defence mechanisms (Reuter et al. 2010). As a result, more molecular damage is accumulated, jeopardising genomic stability and triggering cell apoptosis to restore homeostasis and prevent tumorigenesis (Pálmai-Pallag and Bachrati 2014). Not only does chronic inflammation increase ROS, it also reduces DNA repair efficiency and diminishes mitochondrial respiration and functionality, leading to the accumulation of DNA damage (Pálmai-Pallag and Bachrati 2014). When cells enter apoptosis or necrosis, DNA fragments are released into circulation and are recognised by the immune response to be cleared. Failure to remove this debris and further accumulation of cell-free DNA perpetuates the immune response (Pálmai-Pallag and Bachrati 2014, Ozaki et al. 2015). Chronic inflammation can also

increase the susceptibility of the host to autoimmune, cardiovascular, pulmonary and neurodegenerative diseases as well as some cancers (Reuter et al. 2010). As a result, the involvement of ROS within inflammation could be a therapeutic target, particularly when combined with existing interventions. ROS reducing agent supplementation could be protective in cases of prolonged or unnecessary inflammatory response; this has been demonstrated in both an *in vitro* and *in vivo* model of sepsis, characterised by inflammation and resulting in organ dysfunction (Kong et al. 2011, Lowes et al. 2013).

1.3 Consequences of ROS and Oxidative Stress

1.3.1 DNA Damage

1.3.1.1 Types of DNA Damage

It has been estimated that a cell can encounter up to 100,000 ROS attacks daily (Godic et al. 2014). These attacks can be induced by endogenous and exogenous ROS, creating an environment of oxidative stress. This can be due to either or both decreased antioxidant concentration and activity through mutation or absence from dietary intake, or increased production of ROS through inflammation, xenobiotic and environmental exposure (Shokolenko et al. 2009, Andreyev et al. 2015). The extent of damage is dependent on cell age, cell cycle phase, state of differentiation, mechanisms of DNA repair and its antioxidant capacity (Ramos-Espinosa et al. 2012).

ROS-induced DNA damage ranges from oxidative modification of nucleotides, to generation of abasic sites forming isolated and clustered DNA lesions with both single strand (SSB) and double strand breaks (DSB) within DNA (Chang et al. 2007, Ricci et al. 2008, Tatsch et al. 2015). These events can be catalysed through elevated ROS, due to inflammation or exogenous influence and are summarised in Figure 1.5 (Shokolenko et al. 2009, da Silva 2016).

In the first instance, damage to DNA halts the process of replication until the damage can be repaired. Isolated DNA lesions and abasic sites are repaired by the base excision repair pathway (BER) (Bohgaki et al. 2010, Kim and He 2014). The nucleotide excision repair pathway (NER) is responsible for the removal of strand crosslinks and clustered lesions through employment of the global genome repair sub pathway. This pathway repairs genomic lesions pre-transcription as well as using transcription-coupled repair to eliminate transcribed lesions, whilst the mismatch repair (MMR) fixes mismatched bases as well as minor inserted or deleted base regions (Bohgaki et al. 2010, Kim and He 2014).

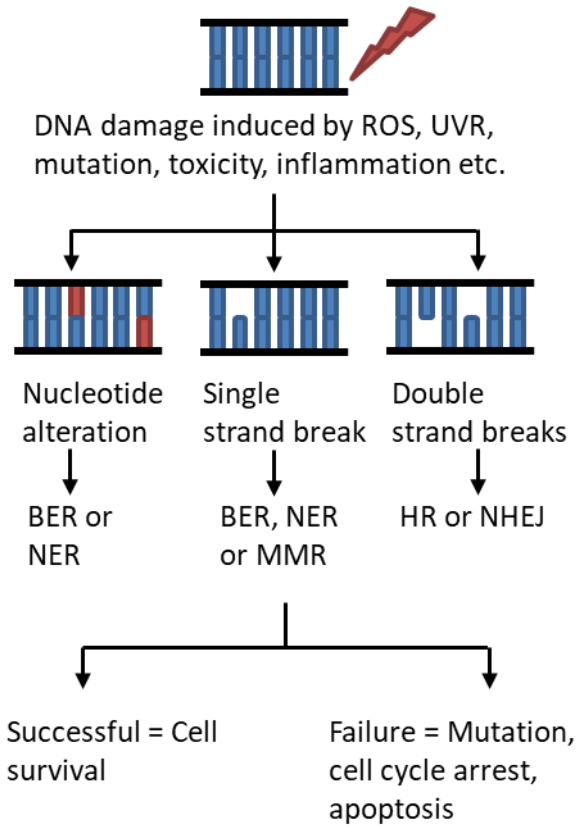

Figure 1.5 A summary of DNA repair pathways induction*. Upon the detection of nucleotide alterations, the base excision (BER) or nucleotide excision (NER) repair pathways are executed. If single strand breaks are identified, the mismatch repair (MMR) pathway, BER or NER are initiated. However, double strand breaks require homologous recombination (HR) or non-homologous end joining (NHEJ). If DNA damage can be successfully repaired, cellular integrity is maintained. Failure to repair damage effectively compromises the cell and can lead to mutation, which can be further replicated, or induction of apoptosis. Original figure, adapted from (Bohgaki et al. 2010, Ramos-Espinosa et al. 2012, Pálmai-Pallag and Bachrati 2014).*

DNA strand breaks occur as a result of multiple ROS attacks at a single site, this is commonly found as a OH· cluster but can also be as a result of incomplete nucleotide repair (Tatsch et al. 2012). Further DNA damage can arise from incomplete BER and NER as well as secondary mutagenic radicals that arise from oxidation of macromolecules, such as lipid hydroperoxides. (Pálmai-Pallag and Bachrati 2014). In order to repair DSB, the stage of the cell cycle determines whether homologous recombination (HR) or non-homologous end joining (NHEJ) is preferable. DSBs identified by HR following binding to the MRN complex involve the endonuclease and Rad51 facilitated formation of a new and undamaged DNA strand (Li and

Heyer 2008). However, DSB repair by NHEJ is the most commonly employed mechanism. This involves the binding of the nuclease enzyme Artemis to facilitate the ligation of two blunt ends following excision of the damaged portion of the strand (Lamarche et al. 2010). However, this process does not generate a restored DNA strand and increases the susceptibility to mutagenesis (Jena 2012). Therefore, it would be beneficial to reduce or inhibit the development of strand breaks within DNA to diminish the risk of disease pathogenesis associated with ROS. One way to model the prevention of DNA damage is through the isolation and evaluation of mitochondrial DNA. This can be achieved following induction of damage such as through exposure to H_2O_2 or physiologically induced ROS as produced by hypoxia or high glucose concentrations.

1.3.1.2 MtDNA Damage as a Biomarker

The mitochondria are pivotal to the theory of aging, a theory first conceptualised in 1956 by Denham Harman. Aging is believed to be potentiated by ROS and oxidative stress and increased acquisition of damage related to age (Harman 1956, Viña 2004). It is suggested that the production of ROS in aging leads to a self-propagating cycle of ROS. As a result, research into oxidative stress is fundamental to understanding the progression of aging as well as the implication it has on the development of so called 'age-related' diseases including neurodegenerative disease (Van Houten et al. 2006, Gupta et al. 2014, Sanders et al. 2014). Whilst this is not a new field of study, there has been inconsistent evidence, particularly in human studies, demonstrating the interaction between disease and oxidative damage, which highlights a gap in understanding this correlation as well as the future role of antioxidant supplementation.

Mitochondrial number and structure are dependent on the energy requirements of individual tissue (Youle and van der Bliek 2012). They are comprised of two phospholipid bilayers, arranged into four compartments: the outer membrane, intermembrane space, inner membrane and matrix (Bolisetty and Jaimes 2013). The mitochondrial matrix contains mitochondrial DNA (mtDNA) as well as being a site for NADH and $FADH_2$ generation, both of which are substrates for the ETC and ATP generation (Youle and van der Bliek 2012, Bolisetty and Jaimes 2013). The mitochondrial genome contains 16,569 base pairs (bp) of circular unmethylated DNA (Gilkerson and Schon 2008), which codes for 37 genes with 13 corresponding to ETC complexes and the rest coding for transfer and ribosomal RNAs needed for protein synthesis within the mitochondria (Malik and Czajka 2013).

MtDNA does not possess protective histones or introns, unlike nuclear DNA (nDNA) (Lagouge and Larsson 2013). Instead there is dependence on the nucleoid protein-DNA complexes for protection within the mitochondrial matrix (Gilkerson et al. 2013). This makes it more susceptible to damage coupled with its close proximity to the ETC and the sites of ROS formation. Similarly, mtDNA is readily exposed to damage by secondary ROS production such as through lipid peroxidation due to the high lipid content within the mitochondria (Gebert et al. 2009, Horvath and Daum 2013, Tatsuta et al. 2013). Consequently, and considering that mtDNA is hypothesised to have up to a 10-fold increased risk of sustaining damage compared to nDNA (Alexeyev et al. 2013), mtDNA is more likely to sustain ROS associated damage and as such, is preferential for the study of oxidative DNA damage over nDNA. Five main types of mtDNA damage have been identified including, damage through alkylation which can occur with chemotherapy as well as endogenous methylation, abasic site formation due to hydrolytic base deamination or glycosidic bonds hydrolysis, adduct formation, base mismatch acquired through replication following nucleotide modification and finally, single and double strand breaks (De Bont and van Larebeke 2004).

Further to this, MtDNA, of which hundreds of thousands of copies are contained within an individual cell, contains a mix of mutant or damaged DNA alongside wild-type DNA, which can be distributed at random amongst daughter cells (Elliott et al. 2008, Wallace and Chalkia 2013, Li et al. 2015). Termed heteroplasmy, it has been estimated that 90% of individuals host at least one mitochondrial heteroplasmy, with 20% of those carrying heteroplasmy associated with mitochondrial diseases, such as Leber's hereditary optic neuropathy (LHON) (Schaefer et al. 2007, Ye et al. 2014). For example, three different point mutations have been identified in mtDNA, G11778A, T14484C and G3460A, resulting in loss of vision from deterioration of the retinal ganglion in LHON. These point mutations have been found to alter the functionality of complex I within the ETC, causing a deficit in ATP synthesis. Resultant increases in ROS and apoptosis have also been identified in LHON and treatment with idebenone, structurally related to coenzyme Q10, has been found to reduce this, resulting in recovery of vision (Qi et al. 2007, Klopstock et al. 2013).

Over time and with replication, the proportion of mutant mtDNA may accumulate and induce a shift towards a cellular mutant phenotype rather than predominantly wild-type (Figure 1.6). This shift impacts cellular function, including decreases in OXPHOS and ATP synthesis, and has been correlated with the onset of disease, such as LHON. Studies within elderly populations have indicated that there is also an age-related elevation in heteroplasmic frequency in skeletal muscle (Figueiredo et al. 2009, Grady et al. 2018). Similarly, the single site mutation A3243G has been attributed to neurological, metabolic and cardiopulmonary deficits, evidenced in a study of 789 elderly persons (Tranah et al. 2018). However, the

threshold at which mutant DNA content influences phenotype varies between tissues and disease. For example, individuals with less than 70% T8993G mutations are asymptomatic for neurogenic muscle weakness, ataxia and retinitis pigmentosa (NARP), a mitochondrially inherited neurodegenerative disorder (Gorman et al. 2016).

Heteroplasmy has also been evidenced in diseases other than those that are mitochondrially inherited. A study found that those with type 2 diabetes (T2D) or coronary artery disease measured a significantly higher frequency of the T16189C substitution within mtDNA compared to healthy controls (Mueller et al. 2011). The A3243G mutation has also been found in 4-fold greater frequency in T2D patients compared to healthy counterparts (Nomiyama et al. 2006). The 4977bp 'common' deletion within mtDNA has been found to cause ETC disruption and has been identified in AD and colorectal cancer as well as mitochondrially inherited diseases (Chen et al. 2011, Krishnan et al. 2012, Zhang et al. 2015). As a result of its interaction with the ETC, this deletion has also been found to augment mitochondrial ROS production, which itself can increase mutant mtDNA. Therefore, there is scope to evaluate ROS reducing strategies to decrease the ROS produced by the 4977bp deletion, or other mutations, and limit the perpetuated risk of further mutant mtDNA accumulation and subsequent heteroplasmy. However, the occurrence of mitochondrial heteroplasmy gives researchers an opportunity to investigate mtDNA damage or mutation within a population of cells without significant loss, such as would be the case with nDNA damage. As a result, use of the mitochondria as biomarkers for oxidative damage, for reasons outlined in this section, is an elementary component to gaining understanding of potential therapeutic opportunities and is preferential over the use of nDNA.

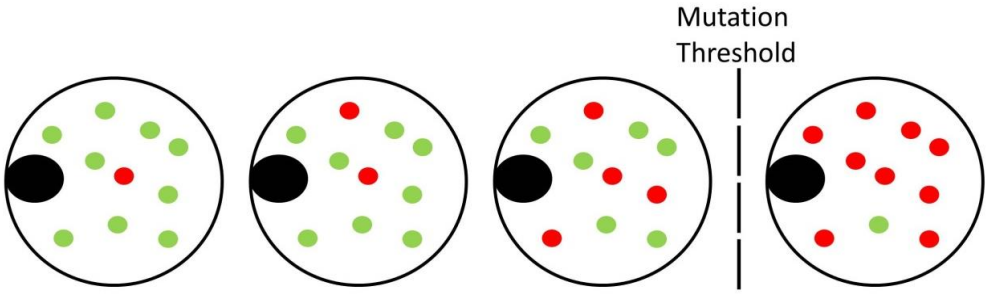

Figure 1.6 Illustration of heteroplasmy in the mitochondria. *Heteroplasmy describes the presence of both mutant and wild-type mitochondrial DNA (mtDNA), where the proportion determines the penetrance of disease. When the mutation threshold is exceeded, the levels of mutant mtDNA, shown in red, are greater than the wild-type (green), compromising cellular function. Original figure created based on (Gilkerson and Schon 2008, Wallace and Chalkia 2013).*

1.3.1.3 Consequences of DNA Damage

As detailed above, DNA damage, of nuclear as well as mitochondrial origin, halts the cell cycle to enable DNA repair pathways to target damaged DNA and prevent the erroneous replication of mutations which can lead to cell compromise and the development of disease, including cancer (Li and Heyer 2008). However, when DNA damage is in excess, apoptosis is mediated by p53, as a tumour suppression response, or through parylation. Parylation occurs when the poly(ADP-ribose) polymerase 1 (PARP-1) binds to damaged DNA and catalyses the transfer of ADP-ribose moieties from donor NAD^+ substrates to synthesise poly(ADP-ribose) (PAR) chains. PAR chains recruit DNA-repairing enzymes such as DNA ligases or polymerases to initiate DNA repair such as through BER (Reinhardt and Schumacher 2012, Yao et al. 2013, Wei and Yu 2016). When DNA damage is at a low or moderate level, ADP-ribosylation facilitates DNA damage repair in a survival promoting function (Wei and Yu 2016). However, with high levels of damage and subsequent activation of PARP-1 and PAR chain hypersynthesis, the cellular pool of NAD+ becomes depleted, which can decrease the efficiency of PARP-1 mediated DNA repair. The reduced availability of NAD^+ also impacts the synthesis of ATP, with decreased concentrations unable to be reduced to NADH, hindering the passage of electrons through the ETC, which can result in cellular compromise leading to apoptosis and necrosis (Alano et al. 2004, Alano et al. 2010, Wei and Yu 2016). Supporting this, NAD^+ depletion has been correlated with both astroglial and neuronal cell death with increased apoptosis-inducing factor translocation, which was reduced with exogenous NAD^+ supplementation, demonstrating the importance of NAD^+ in cell survival (Alano et al. 2004, Alano et al. 2010). Similarly, PARP-1 inhibition has been shown to increase cigarette smoke induced DNA strand break damage within a murine model and reduce associated cellular senescence (Yao et al. 2013). However, PARP-1 inhibition has also been targeted by chemotherapy agents such as Olaparib®, reducing DNA repair pathway efficacy and thus directing DNA damaged cells, such as encountered within cancer, towards apoptosis (Liu et al. 2018).

Apoptosis, comprised of an intrinsic and extrinsic pathway responsible for the activation of caspases, is the process of cell programmed death to remove senescent, damaged or mutated cells (Sinha et al. 2013). This occurs through the shrinkage of cells, condensation of chromatin and fragmentation of DNA. Oxidative stress and damage have been well documented as a cause of premature apoptosis and has also been attributed to physiological aging (Sinha et al. 2013, Antognelli et al. 2014, Bucchieri et al. 2015, Guo et al. 2015). Excessive apoptosis has been implicated within a number of disease pathologies including; cardiomyopathy, contributing to vascular remodelling; Human immunodeficiency virus (HIV), which can result in T cell depletion; and chronic kidney disease (Cummins and Badley 2010, Favaloro et al.

2012, Nemmar et al. 2017). Within DM, high levels of oxidative damage have been correlated with a PARP-1 decrease and increased apoptotic activity leading to testicular dysfunction within a rat model demonstrating that excessive DNA damage accumulation and corresponding apoptotic induction can result in cellular or organ dysfunction (Kilarkaje et al. 2014). Similarly, DNA damage induced through hyperglycaemia has led to diabetic nephropathy following renal cell apoptosis and indicating that targeting the reduction of DNA damage, such as through ROS inhibition, could reduce the incidence of cellular dysfunction or secondary complications within disease (Verzola et al. 2004, Verzola et al. 2007).

Another consequence of DNA damage is the induction of autophagy, a self-sacrificing cellular mechanism stimulated to restore homeostasis. When stimulated, autophagy occurs in three forms to promote cell survival; macroautophagy, the main pathway which is used to eradicated damaged organelles encapsulated in an autophagosome for degradation by lysosomal hydrolase; microautophagy, the direct engulfing of material within the cytoplasm into the lysosome where degradative enzymes are available; and chaperone-mediated autophagy, a significantly more complex pathway requiring chaperone-substrate protein complex formation for translocation to the lysosome before degradation (Shintani and Klionskey 2004, Fader et al. 2012, Nixon and Yang 2012). However, whilst an efficient DNA damage response mechanism targeting proteins and organelles, autophagy can become cytotoxic with both apoptosis and autophagy considered instrument in neuronal cell death within neurodegenerative disease (Fader et al. 2012, Nixon and Yang 2012). Therefore, reducing the accumulation of DNA damage and subsequent apoptotic and autophagic activation may reduce detrimental cell loss within some disease pathologies.

1.3.2 Lipid Peroxidation

1.3.2.1 Induction of Lipid Peroxidation by ROS

Lipids are characterised into apolar triglycerides and polar lipids such as those comprising the lipid bilayer. These polyunsaturated fatty acids (PUFA) can be oxidised following ROS targeting of the carbon-carbon double bonds within the lipid, resulting in a LOO· and H_2O (Esterbauer 1993, Ayala et al. 2014). Like ROS, lipid peroxidation can have both detrimental and beneficial effects with low levels promoting cell survival and high levels causing apoptosis and necrosis (Ayala et al. 2014).

Lipid peroxidation occurs in three steps: initiation, propagation and termination. Initiation involves the addition of ROS, such as OH· from the Fenton reaction, to a lipid, forming H_2O as a by-product. The more double-bonds within PUFA, the more susceptible it is to oxidative damage (Yoshida et al. 2013). Propagation involves the stabilisation of the fatty acid radical

with O_2 to create peroxy-fatty acid radicals through hydrogen removal from a local lipid. However, this product is still unstable and readily reacts with a free circulating lipid. This leads to the generation of another radical, such as lipid hydroperoxide, in a continuous cycle and demonstrates that a lone initiation is capable of stimulating the formation of multiple radicals (Spickett et al. 2010, Ayala et al. 2014, Mihalas et al. 2017). Hydroperoxides are able to induce more lipid radicals, feeding the propagation process, and can also be converted into peroxyl or alkoxyl radicals, also furthering propagation (Ayala et al. 2014). Termination of this chain reaction occurs when a non-radical product is formed instead of a radical; occurring when the concentration of radicals reaches a point where the reaction of two radical products is inescapable. This results in the formation of malondialdehyde (MDA) or 4-hydroxynoneal (HNE) (Gupta et al. 2009). However, the time in which it takes to get from propagation to termination is dependent on the antioxidant response and results in multiple peroxyl radicals being formed in the meantime, contributing to oxidative stress (Agmon and Stockwell 2017).

MDA is a prominent and highly reactive biomarker of lipid peroxidation, produced as a result of the thromboxane A_2 arachidonic acid precursor or through cleavage of the endoperoxide peroxidation intermediate (Ayala et al. 2014). MDA induced adducts formed with free amino acids are known as advanced lipid peroxidation end-products, whilst adducts known as MDA acetaldehyde are formed with MDA's own intermediate acetaldehyde (Ayala et al. 2014). Furthermore, MDA is capable of reacting with cellular proteins and DNA, causing molecular damage (Monzo-Beltran et al. 2017, Singh et al. 2017).

1.3.2.2 Consequences of Lipid Peroxidation

Additional to the cytotoxicity of by-products such as MDA and HNE, Lipid peroxidation impairs membrane fluidity, allowing phospholipids to easily move between the lipid bilayers and increase H_2O leakage. This causes not only membrane damage but also the inactivation of enzymes and transport proteins (Mihalas et al. 2017, Singh et al. 2017). It has been reported that lipid membrane fluidity within AD patients results in thinner cortex membrane layers compared to age-matched controls (Mason et al. 1992). Furthermore, fluidity within the inner mitochondrial membrane of AD patient platelets has also been identified, demonstrating the consequence of lipid peroxidation within disease (Mason et al. 1992, Mecocci et al 1996, Ortiz et al. 2008). Additionally, peroxidation of lipids can act as a proinflammatory mediator through the mimicking of platelet activating factors. Through this, platelet aggregation and phagocyte recruitment are initiated and have been implicated within asthma pathology (Fitzpatrick et al. 2014).

Lipid peroxidation has also been found to initiate apoptosis and autophagy in the beneficial removal of dysfunctional cells, as described in section 1.3.1.3 (Adibhatia and Hatcher 2009,

Catalá 2009). However, apoptosis induced by lipid peroxidation has also been correlated with the development of atherosclerotic plaques as well as cell loss within DM, demonstrating the need to target ROS to minimise the exacerbation of oxidative stress and disease prior to the induction of apoptosis (Allen et al. 2003, Hartung et al. 2005, Van Vré et al. 2012). In support of this, Nicotinamide supplementation has been shown to reduce lipid peroxidation as well as apoptosis and PARP-1 activity within Sprague-Dawley rats treated with amyloid β peptide to simulate AD (Bayrakdar et al. 2014). Therefore, this demonstrates the role that interventional supplementation can have in reducing oxidative stress and damage as well as maintaining cell viability, particularly in disease.

1.4 Pathophysiological Role of ROS

1.4.1 Diabetes Mellitus

In 2017, an estimated 451 million people aged 18 to 99 years were suffering from DM (Cho et al. 2018). Between 2015 and 2017, the number of people diagnosed with DM increased from 3,590,501 to 3,689,509 in the UK alone (Diabetes UK 2017) and the global incidence has been projected to rise to 693 million by 2045 (Cho et al. 2018).

Detailed in 1.2.2.2, the clinical measurement of hyperglycaemia is symptomatic of DM and results from impaired insulin production or resistance. DM is subcategorised into T1D as insulin dependent and type 2 (T2D) as insulin independent, with ROS involvement identified in both (Fakhruddin et al. 2017). T1D results from a T-cell mediated autoimmune destruction of the β-cells in pancreatic islets of Langerhans, which leads to a loss of insulin synthesis and early onset of DM (Buse 2006, Padgett et al. 2013). Whilst the T1D initiating event remains unclear, oxidative stress has been implicated in the associated inflammation and generation of ROS as a result of β cell apoptosis (Matough et al. 2012), as evidenced using SOD mimetics (Padgett et al. 2013).

T2D accounts for approximately 90% of DM diagnoses and is caused by a resistance to insulin, strongly influenced by diet (Fakhruddin et al. 2017). The initiation of insulin resistance has been clinically identified as a predisposing factor for T2D (Hurrle and Hsu 2017). During physiological activation of the insulin signal transduction pathway, responsible for the maintenance of glucose homeostasis, H_2O_2 is produced by NOX to promote the phosphorylation of insulin receptor substrates (IRS) at their serine residue. This leads to phosphoionositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathway signalling, facilitating lipogenesis, glycogen synthesis (Rains and Jain 2011, Dong et al. 2016) and GLUT4 translocation, a glucose transporting protein found in adipose tissue (de Alvaro et al. 2004). Production of H_2O_2 within this cascade demonstrates a physiologically beneficial

role for ROS. However, persistent activation of the insulin cascade leads to insulin resistance, which can culminate in β-cell fatigue and reduced insulin production. This persistent activation leads to an increase in ROS which is no longer physiologically beneficial and results in glucose increases to hyperglycaemic levels, a shared characteristic of both T1D and T2D (Nathan et al. 2005).

The generation of ROS as a result of hyperglycaemia is not only capable of distorting glucose metabolism and cellular function but is also self-propagating. The production of ROS in this manner has been correlated with oxidative damage and the development of micro and macro-vascular diabetic complications. As a result, the ROS produced during hyperglycaemia could be a therapeutic target. A number of studies have used high concentrations of glucose to provide an *in vitro* model of diabetes with ROS production, cell viability reduction and other ROS-associated damage identified (Czajka et al. 2015, Araoye and Ckless 2016, Yang et al. 2016). A study in murine macrophages demonstrated elevation of ROS coupled with an increase in antioxidant concentrations, including catalase and SOD, with 24-hour glucose treatment. Whilst demonstrating a correlation between high glucose and oxidative stress, the levels of ROS were generated at 25 and 50 mM did not differ, whilst 100mM showed a nearly 2-fold increase. (Araoye and Ckless 2016). This demonstrates that modelling with glucose requires optimisation to induce significant levels of ROS and associated damage, even at higher concentrations that would not be encountered physiologically. *In vitro* modelling of this nature is not only preferential economically but also alleviates the reliance on *in vivo* models, such as the induction of T1D and T2D in Streptozotocin treated rats particularly in early investigative studies of potential therapeutics (Schmatz et al. 2012, Yazgan et al. 2015). Furthermore, the use of *in vitro* modelling facilitates the production of comparable results which are not influenced by genetic variation or uncontrolled external stimuli, providing robust and reproducible results in preliminary studies.

1.4.2 Pulmonary Disease

The prevalence of chronic pulmonary conditions such as asthma and COPD have become a major health problem (Allen et al. 2009, Tsiligianni and van der Molen 2010). It has been estimated that asthma alone affects 300 million people worldwide and costs the NHS in excess of £1 billion per annum across England and Wales (Reddy 2011, Mukherjee et al. 2014). The incidence of COPD increased from 227 to 384 million between 1990-2010, indicating that COPD too is a rising health problem and was considered the 3rd most common cause of death globally in 2016 (Adeloye et al. 2015, World Health Organisation 2018) .

Asthma is a condition whereby the symptoms of airway hyperresponsiveness, constriction and inflammation, can be reversed with timely intervention. The condition involves the influx of

inflammatory mediators including eosinophils, macrophages and neutrophils, in response to an allergen (Louhelainen et al. 2009, Erle and Sheppard 2014). This inflammatory response is the driving force behind oxidative stress in asthma (Dworski 2000). ROS generation as a result of inflammatory cell action can result in oxidative stress and damage, which can also further perpetuate inflammation and oxidative stress. For example, ROS generation facilitated by eosinophils has been correlated with increased concentrations of IL-5 whilst TNF-α (tumour necrosis factor α) has been found to upregulate ROS as well as intracellular adhesion molecule-1 (ICAM-1) within the airway, influencing airway remodelling (Kim et al. 2008, Qu et al. 2017). ROS has also been correlated with the release of histamine through the upregulation of immunoglobulin E (IgE), which binds to mast cells to facilitate histamine release during allergen exposure (Kim et al. 2013, Qu et al. 2017). Further supporting the involvement of ROS in asthma, patient samples have demonstrated an increase in lipid peroxidation and lower serum activity of endogenous antioxidants, including SOD (Wood et al. 2003, Comhair et al. 2005, Wedes et al. 2009)

Considered a global but preventable health issue, COPD is characterised as a condition with persistent and progressive restriction of airflow associated with chronic inflammation of the bronchioles (Vestbo et al. 2013). The most common factor in the development of COPD is smoking, with history of smoking correlated with the development of the condition in up to 90% of patients (Kirkham and Rahman 2006). Tobacco smoke contains toxic and mutagenic substances as well as ROS, capable of causing widespread oxidative damage. Additional to ROS-induced damage with $O_2^{\cdot-}$ and Fenton reaction generation of $OH^{\cdot}$, the tar found in cigarettes is capable of causing DNA adducts and formation of 8-hydroxy-2'-deoxyguanosine (8OHdG) (Valavanidis et al. 2013). ROS have been found to stimulate the release of inflammatory mediators in COPD, leading to the formation of more ROS (Louhelainen et al. 2008). Oxidative stress in COPD has been directly evidenced by increased xanthine oxidase, a facilitator of $O_2^{\cdot-}$ and lipid peroxidation, within free bronchoalveolar fluid (BALF) and plasma in COPD patients (Louhelainen et al. 2008, Wiegman et al. 2015).

Hypoxia is a prominent problem within the pulmonary system with pathological and physiological implications. Hypoxia has also been specifically identified in airway hyperresponsiveness and constriction, common to both asthma and COPD (Kirkham and Rahman 2006). As detailed in section 1.2.2.3, hypoxia contributes to cellular dysfunction as well as initiates and exacerbates the inflammatory response resulting in the generation of ROS through the increased expression of inflammatory cytokines such as IL-8 and TNFα (Araneda and Tuesta 2012, Baek et al. 2013, Dewitz et al. 2017). Hypoxia within pulmonary conditions has also been suggested to exacerbate the inflammatory response to allergens and cigarette

smoke, leading to increased ROS production and have also been found to increase pulmonary hypertension (Huerta-Yepez et al. 2008, Olea et al. 2011).

With the ROS generation in COPD and asthma, there is an opportunity to investigate strategies to reduce or protect against oxidative damage within pulmonary models, potentially with the use of antioxidant supplementation. This could be investigated in multiple ways including protection against direct ROS damage or through amelioration of physiologically derived ROS such as hypoxia-induced.

1.4.3 Neurodegenerative Disease

AD is a chronic neurodegenerative disease which is characterised by cognitive decline and memory loss. It was estimated that 850,000 people were living with AD in the UK in 2014 (Donegan et al. 2017). Whilst the cause of AD is poorly understood, oxidative stress has been identified within disease pathology (Di Matteo and Esposito 2003, Schrag et al. 2013). Oxidative stress has also been identified in the pathology of Parkinson's Disease (PD). PD is also a neurodegenerative condition, like AD, but of both genetic and idiopathic origin, the latter of which accounts for up to 95% of diagnoses (Hwang 2013). In 2005, it was suggested that between 4.1 and 4.6 million people over the age of 50 were diagnosed in western Europe, with incidence predicted to double by 2030 (Dorsey et al. 2007).

Hypoxia is a documented inducer of oxidative stress and ROS within neurodegenerative diseases, identified within AD and PD as well as correlated with increases in lipid peroxidation and cognitive decline (Carbonell and Rama 2009, Popa-Wagner et al. 2013, Liu et al. 2016, Kandikattu et al. 2017). Hypoxia has been specifically found to exacerbate clinical manifestations such as augmented β-secretase synthesis, responsible partly for the cleavage of the amyloid peptide precursor (APP), as well as direct induction of ROS (Smith et al. 2003, Sun et al. 2006, Liu et al. 2016). The presence of neurofibrillary tangles of hyperphosphorylated tau proteins and amyloid plaques, a deposit of both amyloid-β peptides and metal ions in AD pathophysiology have been attributed to the generation of ROS (de Vries et al. 2008, Azizi et al. 2015). As AD progresses, the amyloid-β peptides accumulate and impact on patient's memory and learning. This can lead to neuronal cell toxicity and cell compromise, causing brain atrophy (Hullinger and Puglielli 2016). These events are synergistic of causing ROS, with metal ions specifically binding to histidine residues and resulting in toxicity, reduced energy metabolism and compromising redox state. This culminates in production of H_2O_2 and further ROS generation (Viña et al. 2004). Research originating from 1996 has implicated free radicals in the development of AD and evidenced this in *ex vivo* samples (Good et al. 1996, Torres et al. 2011, Mecocci and Polidori 2012). Similarly, oxidative stress and reduced antioxidant capacity has been evidenced *in vitro*, *in*

vivo and in AD patients (Schipper et al. 2006, Askarova et al. 2011, Gubandru et al. 2013, Sanders et al. 2014).

Physiological hallmarks of PD are a loss of dopaminergic neurons, accompanied by astrocyte compromise and the presence of Lewy body deposits in the substantia nigra pars compacta (SNc) within the midbrain (Uttara et al. 2009, Flynn and Melov 2013). The SNc, instrumental in inducing movement, contains the most evidence of oxidative damage including oxidised DNA and lipids (Alam et al. 2002, Sasaki et al. 2006, Hwang 2013). Increased oxidative damage within the mitochondrial of the SNc have been identified in PD alongside augmented formation of the dopamine oxidative by-product cysteinyl-DOPA (Dihydroxyphenylalanine) (Alam et al. 2002, Dias et al. 2013). There is a rich abundance of neuromelanin within this area of the brain, which gives the dopamine neurons a black pigment. Neuromelanin is believed to provide antioxidant-like protection against oxidative stress within the brain (Sasaki et al. 2006). However, in a PD diseased brain, the loss of dopaminergic neurons reduces this pigmentation and neuromelanin and leading to the development of PD symptoms. This demonstrates the presence of oxidative stress as well as the need for antioxidant supplementation to preserve dopaminergic neurons and neuromelanin (Sidoryk-Wegrzynowicz et al. 2011).

The characteristic Lewy bodies found within the SNc are eosinophilic clusters, dense in electrons and comprised mostly of α-synuclein. Whilst the function of Lewy bodies is unknown, these biomarkers of dopaminergic neuronal compromise can be readily oxidised, phosphorylated or ubiquinated which contribute to ROS production and oxidative damage (Gu et al. 2010). Abnormal concentrations of α-synuclein within the mitochondria have also been correlated with dysfunction of the ETC and oxidative stress in PD and could therefore provide a therapeutic target (Devi et al. 2008). Specifically, dysfunction at ETC complex I has been identified in PD patients and has fuelled the hypothesis of ROS involvement in PD. Impaired ETC promotes incomplete OXPHOS and electron leakage, generating ROS (Drechsel and Patel 2008, Grunewald et al. 2016). Increased iron levels, susceptible to the Fenton reaction, have also been identified in SNc alongside lipid peroxidation and DNA oxidation in PD patients post-mortem (Drechsel and Patel 2008).

The presence of oxidative stress within AD and PD pathology has therefore led to an increase in research to understand the involvement of ROS in neurodegenerative disease progression. Additionally, there is increasing investigation into potential ameliorative strategies such as with antioxidants, primarily to delay progression or relieve symptoms.

Astrocytes are the most abundant glial cell within the central nervous system and are integral in the maintenance of brain homeostasis (Kuchibhotla et al. 2010). They are involved in

neurotransmitter regulation (Yang et al. 2009), inflammatory response through reactive astrogliosis (Olsen et al. 2018) and synapse activity modulation (Yang et al. 2009, Reynolds et al. 2019). Elevated levels of calcium (Ca^{2+}) have been identified within AD and correlated with astrocyte hyperactivity, resulting in greater release of glutamate, and amyloid-β plaque formation (Lovell et al. 2001, Pierrot et al. 2006, Hoey et al. 2009, Kuchibhotla et al. 2010, Ujita et al. 2017). This has been correlated with increases in ROS and lipid peroxidation in murine models and patient samples and found to induce neurotoxicity alongside Ca^{2+} dependent reduction of glutathione (Lovell et al. 2001, Abramov et al. 2003, Brustovetskey et al. 2005, Kuchibhotla et al. 2009, Gozalez-Reyes et al. 2017). Altered uptake of the neurotransmitter glutamate by astrocytes, whereby inefficient uptake leads to glutamate receptor overstimulation and neuronal excitotoxicity, has been considered a hallmark of early astrocyte dysfunction and has been associated with cognitive decline and identified in AD (Mookherjee et al. 2011). Therefore, in both incidences there is opportunity to explore the use of antioxidants to mitigate the cytotoxicity that is encountered during AD or PD (Herrera et al. 2001, Frade et al. 2008). With such a variety of interactions, any alteration in function or loss of astrocytes could significantly compromise neuronal cell integrity as well as brain function, influencing disease severity. The latter of which has been shown previously, where increased astrocyte apoptosis was correlated with dementia severity in patient samples as well as neuronal cell loss (Broe et al. 2004).

The protective role of astrocytes has been identified in both AD and PD, but is more clearly defined in AD. For example, reactive astrogliosis, the process by which astrocytes transform into an inflammatory state triggered by local environment changes, has been reported within AD (Wyss-Coray et al. 2003, Carter et al. 2012, Olsen et al. 2018). In AD, astrogliosis centralises around the formation of amyloid-β plaques in a perceived protective capacity, with astrocytes shown to internalise amyloid-β, mediated by apoplipoprotein E, a protein that is inefficient alone at degrading amyloid-β in AD (Koistinaho et al. 2004, Nielsen et al. 2010, Kobayashi et al. 2018). However, the progression of AD symptoms indicates that the process of astrogliosis is not sustained, whether this is as a result of uncontrollable accumulating amyloid-β or a loss of astrocytes is unclear. However, amyloid-β depletes glutathione and leaves astrocytes vulnerable to oxidative stress, which could explain the loss of astrocytes associated with AD chronicity (Abramov et al. 2003). Therefore, the use of antioxidants may reduce the extent of oxidative damage and prevent cell loss (Abramov et al. 2003). Furthermore, it has been shown in AD post-mortem samples that the scavenger receptor B1 is defective compared to healthy controls, a receptor that mediates the internalisation of amyloid-β. This receptor class are stimulated by oxidized lipids and decreases have been associated with oxidative damage (Van Eck et al. 2007). Therefore, as disease progresses

astrocytes may lose this receptor functionality which then corresponds to increases in amyloid-β deposits and oxidative damage (Mulder et al. 2012); another opportunity for amelioration with antioxidant supplementation. Astrogliosis has also been reported in PD, with evidence suggesting both neuroprotective and toxic roles (Chen et al. 2009, di Domenico et al. 2019). Conversely in PD, astrocytes internalise α-synuclein which has been correlated with neuronal cell compromise rather than protection (Song et al. 2009, Halliday and Stevens 2011). Similarly, the role of astrocytes and astrogliosis have been found to aid disease progression, particularly in the SNc (Tong et al. 2015). Therefore, there is an alternative role for antioxidants within PD whereby they could be used to reduce the inflammatory response associated with astrogliosis, such as suggested with guggulipid, a myrrh extract, in rat astrocytoma cells (Niranjan et al. 2012, Venkateshappa et al. 2011, Reichenbach et al. 2019).

Whilst their precise involvement in neurodegenerative disease progression has yet to be fully elucidated but considering the diverse role they play in neuronal cell preservation as well as maintaining brain function, minimising astrocyte cell loss or activity through ROS reducing strategies could have future implications in delaying neurodegeneration disease.

1.5 Antioxidants

1.5.1 The Role of Antioxidants

Antioxidants are generated *in vivo* as well as sourced exogenously through the diet, nutritional supplements and, more recently, cosmetics. The term ‘antioxidant’ is complex in its definition and dependent on the industry and application. As a biological agent, antioxidants can be defined as substrates that delay, remove or prevent oxidative stress to a target molecule even when at low concentrations (Pisoschi and Pop 2015). This correlates with their action as reducing agents capable of donating electrons and aiding free radical reduction (Saeidnia and Abdollahi 2013). Within the food industry antioxidants can be used as preservatives to inhibit the peroxidation of lipids within food products or used as polymerisation agents in forming rubber or plastics within the chemical industry (Ajiboye and Scott 1982, Szeto et al. 2002, Komethi et al. 2012, Clissold et al. 2018).

Whilst there are active biological mechanisms used to repair ROS-induced damage, endogenous antioxidants are the first line defence with the objective to maintain the homeostasis of pro-oxidants with antioxidants, thus prevent oxidative stress (Birben et al. 2012). The mechanism by which antioxidants maintain oxidative balance is dependent on the antioxidant type. The main mechanisms utilised include the neutralising of ROS through

catalytic reactions, inhibition of metal ion reactivity in the Haber-Weiss reaction, the breakage of side chains targeting them for degradation, or by quenching unpaired electrons to form a stable molecule (Rajendran et al. 2014).

1.5.2 The Antioxidant Response Pathway

Antioxidants were originally conceived to only act in a radical neutralising capacity. However, there is now evidence to suggest that they also act as mediators of redox signalling (Holmström and Finkel 2014). The most compelling evidence for this action is the activation of the antioxidant response pathway, which maintains ROS production to low concentrations. This is initiated by the regulation of nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and its complex formation with the antioxidant response element (ARE) exposed on genes that are responsive to stress (Bolisetty and Jaimes 2013). It has been suggested that as well as inhibiting ROS and oxidative stress, antioxidants are capable of inducing oxidative damage repair mechanisms through PI3K and Nrf2 signalling pathways. This involves specific targeting for removal or repair of oxidised proteins by enzymes such as those of the peroxidase family (Pisoschi and Pop 2015).

Nrf2 is a p45 dimer and member of the musculaoaponeurotic fibrosarcoma (Maf) protein family, which is responsible for modulating both anti-inflammatory and antioxidant signalling (Popa-Wagner et al. 2013). Nrf2 is expressed in all tissues but sequestered in the cytosol by kelch-like ECH-associated protein 1 (Keap1). Keap1 promotes Nrf2 ubiquitination and degradation, facilitated by Cullin 3 and associated ubiquitin E3 ligase synthesis, to maintain its half-life at less than 20 minutes and mediate only essential Nrf2-associated gene translation (Figure 1.7) (Cheng et al. 2011, Suzuki et al. 2013).

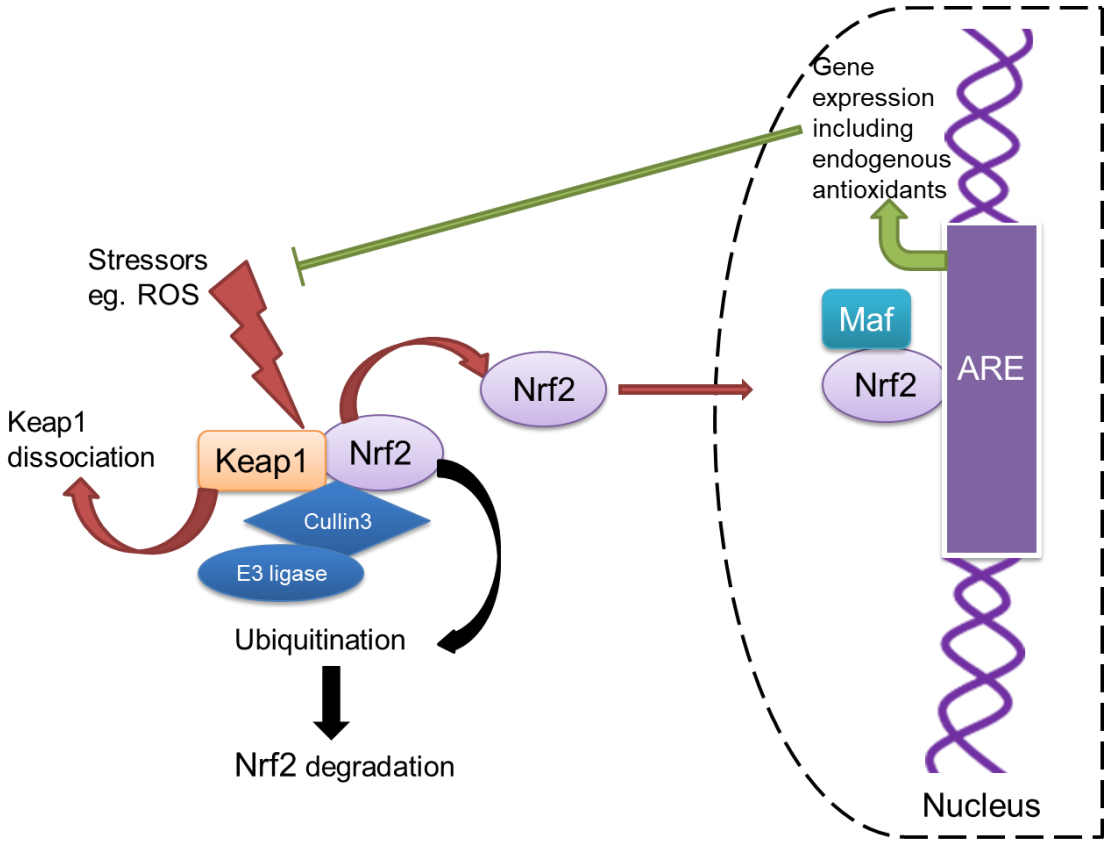

Figure 1.7 *Summary diagram of nuclear factor erythroid 2-related factor 2 (Nrf2) activation following exposure to stressors including reactive oxygen species (ROS). Nrf2 is predominantly sequestered in the cytoplasm by Kelch like-ECH-associated protein 1 (Keap1) and ubiquitinated by Cullin 3, facilitated by E3 ligase. Upon exposure to ROS, Nrf2 dissociates from Keap1 and is translocated to the nucleus where it forms a heterodimer with a musculoaponeurotic fibrosarcoma (Maf) protein and binds to the antioxidant response element (ARE). Original figure summarised from* (Cheng et al. 2011, Scapagnini et al. 2011, Suzuki et al. 2013).

Increased oxidative stress stimulates Nrf2 phosphorylation by PKC activation, permitting Nrf2 to disassociate from Keap1 and translocate to the nucleus for complementary binding to the ARE (Ben-Yehuda Greenwald et al. 2016). Complementary binding facilitates gene transcription of endogenous antioxidants and xenobiotic metabolising enzymes such as thioredoxin and NADPH (Richardson et al. 2015). The cysteine residues of Keap1, specifically Cys273 and 288 within its intervening region domain, are also readily oxidised by ROS leading to Nrf2 dissociation from the Keap1 complex (Li et al. 2004). This oxidative modification compromises the Keap1-Cullin 3 and E3 ligase complex, decreasing ubiquitination frequency

and leading to an overall Nrf2 accumulation and antioxidant response intensification (Taguchi et al. 2011).

1.5.3 Endogenous Antioxidants

Endogenous antioxidants, as induced by Nrf2 pathway activation, are located in the cell membrane and are categorised as either enzymatic or non-enzymatic (Halliwell 2013). As these enzymes are integral in antioxidant defence and also detoxification systems, their expression can be deemed pivotal in the prevention of mutagenesis and disease (Rajendran et al. 2014).

SOD, an enzymatic endogenous antioxidant, is a highly efficient mitochondrial antioxidant capable of eliminating $O_2^{\cdot -}$ in all compartments of the mitochondria. It exists in three isomers; SOD1, also known as CuSOD or ZnSOD; SOD2, also known as MnSOD; and SOD3, similar to SOD1 but located in the extracellular mitochondrial matrix rather than the cytosol (Turrens 2003, Fukai and Ushio-Fukai 2011, Lönn et al. 2012). A reduction in SOD has been identified in a number of disease pathologies including AD and asthma, with clinical trials suggesting that SOD supplementation could partially alleviate oxidative stress (Rosenfeld et al. 1996, Ozcankaya and Delibas 2002, Davis et al. 2003, Dumont et al. 2009). Catalase, another endogenous enzymatic antioxidant, has a high affinity for H_2O_2, catalysing its decomposition into O_2 and H_2O. Similar to SOD, a number of studies have aimed to increase catalase to alleviate oxidative stress using both *in vitro* and *in vivo* models with positive effects on biomarkers of oxidative stress (Noeman et al. 2011, de Farias et al. 2016, Singh et al. 2017). A transgenic murine model of catalase overexpression demonstrated the benefit of catalase action in reducing H_2O_2 production, oxidative damage as well as adverse cardiac pathology (Schriner et al. 2005).

Non-enzymatic, also considered metabolic, endogenous antioxidants are those that are a metabolic by-product and include glutathione and ubiquinone (Pham-Huy et al. 2008). Glutathione exists in both a reduced (GSH) and oxidised state (GSSG), with the ratio between the two being identified as an important biomarker of health and subsequent decline in this ratio indicative of oxidative stress. This has been particularly attributed to the development of neurodegenerative disease (Johnson et al. 2012, Sung et al. 2013). Ubiquinone, also known as coenzyme Q10, was first isolated in 1957 and is sourced both endogenously from the mitochondrial inner membrane and lipoproteins present in plasma (Prakash et al. 2010). Involved directly in the ETC to facilitate ATP formation, it is considered a free radical scavenger and is ideally situated within cellular membranes to inhibit lipid peroxidation (Prakash et al. 2010, Sourris et al. 2012).

1.5.4 Exogenous Antioxidants

The predominant source of exogenous antioxidants is through ingestion of fruits and vegetables in which at least 3,100 antioxidants have been identified. Some postulate that without dietary intake, the endogenous antioxidant system would be inefficient, which highlights the opportunity to investigate exogenous supplementation to benefit health and reduce disease incidence (Bouayed and Bohn 2010, Rajendran et al. 2014). Examples of dietary antioxidants include vitamins A, C, and E as well as lycopene, selenium and polyphenols (Pham-Huy et al. 2008). Dietary antioxidant deficiency has long been correlated with the development of chronic conditions and disease, with individual nutrients being specifically linked to certain conditions including xerophthalmia, a progressive loss of vision (Sommer 2008). The New Zealand CHALICE study identified a correlation between high levels of vitamin C and improved metabolic health, including lowered risk of developing DM, in participants over 50 years old, indicating the importance of good nutritional health when compared to participants with lower levels of vitamin C (Pearson et al. 2017).

Another subset of exogenous antioxidants are phenol-based compounds. These are secondary metabolites of plant origin which are diverse in source and structure with the group subdivided into flavonoids and non-flavonoids. Together they are found in the majority of the 350 foods consumed by the human population that are derived from plants (Tomé-Carneiro et al. 2013). The flavonoid polyphenols include quercetin and catechin and are components found in green tea (Tomé-Carneiro et al. 2013). Recently flavonoids have been the subject of many clinical trials as well as scrutinised *in vitro*, with results indicating these compounds to be anti-inflammatory as well as protective against DNA damage (Min and Ebeler 2009, Bisht et al. 2010, Kapoor and Kakkar 2012, Panat et al. 2016). Non-flavonoid compounds include caffeix acid, pentagalloyl glucose and resveratrol (Bisht et al. 2010). Resveratrol is a widely researched antioxidant supplement evaluated *in vitro*, *in vivo* and in clinical trials to reduce oxidative damage as well as investigated in models of COPD, asthma, AD and cancer (Rahman 2008, González et al. 2011, Ma et al. 2014, Berman et al. 2017).

1.5.5 Tiron

Tiron, 4,5-Dihydroxy-1,3-benzenedisulfonic acid disodium salt monohydrate, is a synthetic vitamin E analogue first characterised in 1964 (Figure 1.8) (McBryde 1964). In 1970, Tiron was identified as having beneficial ROS reducing properties and the interest in its therapeutic use has grown (Hatefi and Hanstein 1970). Tiron has been classified as a $O_2^{\cdot-}$ detector molecule and a diphenol, which reacts readily with $O_2^{\cdot-}$ to produce a reactive semiquinone by-product (Yang et al. 2007, Taiwo 2008). Tiron has also been described chemically as a spin

trap, capable of metal chelation additional to its cell-permeable ROS reducing ability (Taiwo 2008, Vorobjeva and Pinegin 2016).

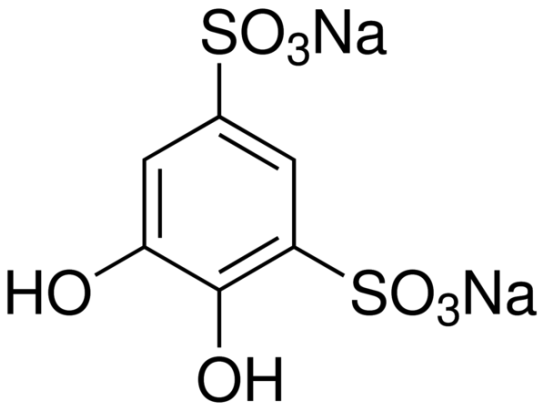

Figure 1.8 Chemical formulation of Tiron *(Sigma-Aldrich, UK)*

Tiron's precise mechanism of action has yet to be comprehensively ascertained despite Tiron being widely used *in vitro*. Studies have used Tiron to partially alleviate metal toxicity, due to its metal chelating ability, and to confirm the mechanism of action of other compounds including Bortezomib, an anti-cancer agent (Ling et al. 2003, Sharma et al. 2007). As a result of its ROS reducing ability, Tiron demonstrated that Bortezomib's mechanism of action involved the induction of ROS evidenced by the decreased production of ROS and increased cell survival (Ling et al. 2003).

Tiron has also been used for a proof-of-concept control, indicating a role for ROS and oxidative stress within disease pathology (Gao et al. 2007, Won et al. 2012, Fan et al. 2014). For example, Tiron has highlighted the role of ROS within DM in bovine endothelial aortic cells by reducing AGE-induced ROS production (He et al. 2011). Similarly, Tiron demonstrated HIF-1α stabilisation during normoxia and its accumulation as a result of hypoxia in an investigation of the role of nitric oxide during hypoxia (Callapina et al. 2005).

Tiron has also been used in the reduction of oxidative stress as a control antioxidant compared to other exogenous supplementation, frequently mirroring or exceeding the results obtained with the antioxidant of interest (Vaquero et al. 2004, Yang et al. 2007, Kim et al. 2011, Alleva et al. 2016, 2018). For example, Tiron demonstrated amelioration of phorbol-12-myristate-13-acetate (PMA) induced ROS at increasing concentrations (0.2-2 mM) and exceeding results obtained with Trolox and Tempol (Vorobjeva and Pinegin 2016). Tiron has also demonstrated significant ROS protection comparable to, but in some studies exceeding, Resveratrol and vitamin C against the effects of the chemotherapeutic temozolomide (Lin et al. 2012). However, when administered with temozolomide, Tiron demonstrated an increase in apoptosis, compromising human glioblastoma (U87MG) cells and thereby enhancing the

chemotherapeutic effect of temozolomide (Lin et al. 2012). This study demonstrated not only the antioxidant effect of Tiron comparable to other exogenous antioxidants, but also indicates a wider use to therapeutically intensify the efficacy of some xenobiotics.

Despite evidence of good tolerance *in vitro* and *in vivo*, there is an absence of data relating to the clinical use of Tiron. This could be as a result of lack of clarity as to Tiron's precise mechanism of action and the range of protection it could elicit, such as against mtDNA damage, in 'normal' cells rather than cancerous and of different origins. Therefore, there is an investigative opportunity to explore the protective use of Tiron to establish its clinical potential.

1.5.6 Current Status of Antioxidant Research

Since the publication of the Denham Harman's free radical theory of aging in the 1950s, there have been a number of studies investigating the use of antioxidant supplements to improve longevity as well as disease prevention (Sackesen et al. 2008, Mecocci and Polidori 2012, Gupta et al. 2012, Halliwell 2013). For example, Hydroxytyrosol, an extract from olive oil has demonstrated significant protective effects across multiple clinical trials. It has demonstrated protectivity against mercury toxicity in human erythrocytes (Officioso et al. 2018) and ameliorated oxidative stress through Nrf2 activation and ROS scavenging in rat pheochromocytoma cells (Peng et al. 2015). In healthy volunteers, it has also demonstrated reductions in MDA but increases in antioxidant status and SOD expression (Colica et al. 2017). Recently, hydroxytyrosol has shown to improve fetal antioxidant status, improve glucose metabolism and reduce DNA hypomethylation correlated with oxidative stress and alleviate intrauterine growth restriction in expectant mothers (Garcia-Contreras et al. 2019). Currently, hydroxytyrosol has not demonstrated adverse effects following supplementation and could have beneficial effects in diseases associated with aging including neurodegenerative disease (de Pablos et al. 2019, Smeriglio et al. 2019).

Resveratrol is a popular antioxidant examined in the prevention or treatment of disease or toxicity. *In vitro* evidence has demonstrated Resveratrol's oxidative damage amelioration in human erthryocytes and lymphocytes (Suwalsky et al. 2015, Albuquerque et al. 2015) and demonstrated efficacy within the treatment of neurodegenerative disease through activation of autophagy to reduce oxidative damage (Wang et al. 2018). *In vivo*, Resveratrol has demonstrated protection against copper oxide toxicity (Khalid et al. 2018), inhibit the reproductive toxicity associated with deoxynivalenol, an ingestible mycotoxin found in food grains (Kolesarova et al. 2012) and shown to improve left ventricular relaxation associated with T2D through oxidative stress inhibition (Zhang et al. 2010). Clinically, Resveratrol has been shown to be well tolerated in healthy individuals (Sergides et al. 2016) and to have beneficial results in improving glucose control and insulin sensitivity in T2D patients (Zhu et

al. 2017, Seyyedebrahimi et al. 2018) as well as an anti-inflammatory effect in AD patients (Moussa et al. 2017). However, it has also been reported that Resveratrol has pro-oxidative and cytotoxic effects depending on the length treatment and potential conjugate formation such as with α-dicarbonyls, which are increased during hyperglycaemia (Martins et al. 2014, Arcanjo et al. 2018). Furthermore, interactions with vital metabolising enzymes including CYP1A2 and CYP3A4 have been found with Resveratrol supplementation (Chow et al. 2010, Hyrsova et al. 2019) including pre-treatment and reducing the clearance of the anticonvulsant carbamazepine through inhibition of the CYP3A4 enzyme in a healthy population, which could lead to toxicity through increased accumulation (Bedada and Nearati 2015). These findings indicate that supplementation with Resveratrol, whilst potentially beneficial in reducing oxidative stress, may have adverse drug interactions which may inadvertently lead to reduced treatment efficacy or toxicity and demonstrates the need for considerable research into the pharmacokinetics and pharmacodynamics of antioxidant compounds being studied in clinical trials.

As with Resveratrol, Vitamin E has been widely researched for its antioxidant benefits. *In vitro* and *in vivo* evidence has demonstrated reduction of oxidative stress in models of preeclampsia (Fiore and Capasso 2008), asthma (Cui et al. 2019) and osteoarthritis (Bhatti et al. 2017). Vitamin E deficiency *in vivo* has also been correlated with cognitive decline and oxidative stress (Fukui et al. 2015) as well as motor hypofunction (Yoshida et al. 2010), with elevation in vitamin E levels found to decrease the risk of developing AD (Mangialasche et al. 2010, Dong et al. 2018). Supporting this, it has been shown that vitamin E supplementation slowed cognitive decline in patients with mild to moderate AD (Dysken et al. 2014). On the other hand there have also been mixed results in the treatment of AD with vitamin E, where supplementation has been found to have no significant effect, contrary to other publications, and indicating more mechanisms are involved (Fraina et al. 2017, Kryscio et al. 2017). Adverse effects with vitamin E have also been reported with reproductive toxicity observed in rats leading to decreased synapse density and plasticity within the hippocampus and causing deficits maintained into adulthood (Betti et al. 2011, Salucci et al. 2014). Additionally, vitamin E supplementation has been correlated with an increased risk of prostate cancer within the healthy male population (Klein et al. 2011). Therefore, the incidence of deficit or disease needs consideration in the use of vitamin E as a supplement and warrants further scrutiny, highlighting the need to expand antioxidant supplementation research.

The inconsistency in translation of *in vitro* data to human trials with antioxidant supplementation or the more recent discovery of adverse effects can potentially be attributed to an absence of understanding of the pharmacology, particularly in the case of Resveratrol. This is the caveat with the use of poorly understood vitamin supplementation and therefore

advocates the study of compounds more thoroughly at cellular level before escalation to *in vivo* or clinic. To address this, there has been an increase in fundamental research into exogenous antioxidant supplementation which re-focus draws upon both *in vitro* and *in vivo* evidence, leading to a better understanding of the antioxidant mechanism and prediction of positive responses in clinic (Öztürk et al. 2017).

Consideration must also be given to the role of antioxidant supplementation in the essential physiological interaction of low concentrations of ROS. Thus, significant work is required to ascertain the thresholds at which supplementation can be rendered beneficial as well as before toxicity is encountered. These elementary uncertainties demonstrate the infancy of this field of understanding, regardless of the extensive use of antioxidant supplementation within clinical trials. As a result, it is paramount that functional and pharmacological studies are undertaken across a multitude of tissues *in vitro* before optimisation for dosing and eventual admission into human trials.

1.6 Aims of this Research

The aim of this research was to develop evidence to support or counter the use of Tiron as a protective antioxidant against ROS induced cellular damage. This was evaluated in three human cell lines including renal proximal tubular, bronchial epithelial and astroglial cells. These cell lines were selected due to their clinical relevance, in which each cell line has been identified as ROS-damaged within diabetes, COPD and Alzheimer's disease. The following was evaluated in each cell line:

a) Assessment of the effect of Tiron on cell viability at different concentrations, identifying a suitable test concentration.

b) Evaluation of Tiron's use to inhibit the formation of DNA strand breaks caused by H_2O_2 treatment or physiological inducers of ROS using a quantitative polymerase chain reaction (qPCR) method.

c) Examination of Tiron's effect on lipid peroxidation through MDA detection after treatment with both ROS-inducing methods.

d) Exploration of the effect of Tiron on the Nrf2 pathway in the presence of H_2O_2 through ELISA.

Chapter 2 – Methods and Materials

To achieve the research aims detailed in the previous chapter, *in vitro* methodology was used and is detailed below.

2.1 Reagents and Buffers

Name	Formula/Recipe/Dilution	Use
H_2O_2	H_2O_2 was diluted from 9.8 M (Sigma-Aldrich, UK) to a stock concentration of 10 mM by adding 5 µL of 9.8 M H_2O_2 to 10 ml serum-free culture medium, protected from light and diluted as appropriate.	Direct application of ROS
Phosphate buffered saline (PBS)	8.0 g/L sodium chloride (NaCl), 0.2g/L potassium chloride (KCl), 2.9 g/L sodium dihydrogen phosphate dodecahydrate ($Na_2HPO_4.12H_2O$) and 0.2 g/L monopotassium phosphate (KH_2PO_4) in 1 L of deionised H_2O. All salts were supplied by Fisher Scientific (UK).	Extensively used as a wash buffer across all protocols
Tiron	The following stock solutions were made, sterile filtered using a 0.22 µm syringe filter (Fisher, UK) and diluted as appropriate; 25.78 mg in 20 ml PBS to give 4 M stock; 161mg in 2ml PBS to give 250 mM stock.	ROS reducing agent
Tris borate EDTA (TBE)	X10 stock formulated by dissolving 108 g/L tris base, 55 g/L boric acid, 9.5 g/L; EDTA-disodium dihydrate (EDTA-$Na_2.2H_2O$) and adjusted to pH 8.0 in 1 L distilled water. Stock was diluted as appropriate.	Used during gel electrophoresis

***Table 2.1**. Formulation of reagent and buffer stock solutions.*

2.2 Cell Culture

2.2.1 Cell Culture Maintenance

Human renal epithelial tubular (HKC-8), human bronchial epithelial (BEAS-2B) and human astroglial (SVGp12) immortalised cell lines were used in this thesis. Each cell line was routinely cultured in 75 cm^2 (T75) cell culture flasks (Fisher, UK) and 20 ml culture medium, with passaging taking place at 80% confluency to prevent morphological changes and cell differentiation. Similarly, cells were not passaged beyond 25. All cells were incubated at 37 ^oC in 5% CO_2 in a NU-5500 IR Autoflow direct heat CO_2 incubator (NUAIRE, UK) unless otherwise stated.

In general, cells for passage were trypsinised for 10 minutes at 37 ^oC with 2 ml Trypsin-EDTA (x2.5) (Labtech, UK). Trypsin, a digestive enzyme derived from the precursor trypsinogen, elicits its action at 37 ^oC and degrades cellular bonds with the culture flask, causing cells to detach. An equal volume of culture medium was added to the trypsinised cells, as the foetal bovine serum (FBS) present within culture medium contains neutralising anti-trypsinogen, and cells were pelleted by centrifugation (5 minutes, 350 x g) (Brunner et al. 2010). The cell pellet was resuspended in culture medium and passaged appropriately depending on requirements. When required for assay, cells were counted using a haemocytometer to ensure a correct and consistent seeding density was plated.

The cell culture methods specific to each cell line, including the culture medium, are found in the chapters relevant to each cell line.

2.2.2 Cell Storage and Revival

Cells for prolonged storage were suspended in solution containing 10% dimethyl sulfoxide (DMSO) with 90% FBS at a density of $1x10^6$ cells/ml. This was then transferred into a cryovial for slow freeze before storing in liquid nitrogen.

When required, stored cells were thawed in a 37 ^oC water bath. The contents of the cryovial were transferred into a 25 cm^2 culture flask (T25) (Fisher, UK). The culture medium (10 ml) was changed after 24 hours to remove the DMSO from the culture, eliminating any potential growth inhibition. These cells were transferred to a T75 after 3-5 days and were not used experimentally for at least a week (3 passages), allowing adequate growth to re-establish the culture.

2.2.3 Mycoplasma Testing

2.2.3.1 Sample Preparation

Each cell line was routinely tested for mycoplasma contamination using the LookOut® Mycoplasma PCR Detection Kit and recommended Jumpstart™ Taq DNA polymerase (Sigma-Aldrich, UK). Mycoplasma is a virulent contaminant within cell culture, which is inconspicuous compared to the presence of yeast or other bacterial infection. Hallmarks of mycoplasma contamination include impaired cell growth and morphological changes, the latter of which can render the affected cell line a different subtype (Burnett and Penn 2013). This procedure was carried out according to manufacturer's guidelines. In brief; cell culture supernatant was aliquoted and incubated at 95 ^{o}C for 5 minutes and centrifuged for 5 seconds to remove extra cellular debris prior to adding it to polymerase chain reaction (PCR) mixture. 2 µl of culture supernatant was added to 23 µl kit supplied rehydration buffer in duplicate, which was comprised of 0.5 µl of DNA polymerase per test. Along with a kit supplied positive and negative control the thermal cycler profile was conducted using a Mastercycler® nexus (Eppendorf, UK), the conditions can be found in Table 2.2. Following this, samples were cooled to 4^{o}C and separated via agarose gel electrophoresis.

Stage	Time (seconds)	Temperature (^{o}C)
Heating	120	94
Denaturation	30	94
Annealing	30	55
Extension	40	72

Table 2.2*. Conditions for mycoplasma PCR analysis conducted for 40 cycles.*

2.2.3.2 Gel Electrophoresis

PCR amplicons were separated using gel electrophoresis as described above. Each agarose gel was made to 1.2% with TBE and heated until dissolved. 2 µl of GelRed nucleic acid stain (Biotium, UK) was added to the agarose once cooled and before pouring into the gel tank (Fisher, UK). The gel was left to set, with the comb and endplates in place, before filling the apparatus with TBE. 5 µl of loading dye was added to each sample and mixed by continuous pipetting, before loading 10 µl of the sample into each well of the gel additional to a 10 µl DNA ladder (New England Biolabs, UK). Samples were separated at 50 volts for 45-60 minutes and imaged using a GelDoc™EZ System (Bio-Rad, UK) and Image Lab™ software (Bio-Rad, UK). Mycoplasma negative samples had a band of 481 base pairs (bp), whilst positive samples had

a band at 259bp. Samples were considered to be mycoplasma contaminated at 260±8bp compared to kit-supplied controls. The visualisation of both bands was indicative of light to moderate contamination in the sample, whilst a single band around 259bp was considered heavily contamination as directed by the manufacturer.

2.3 ROS Induction

2.3.1 Direct Application of ROS with H_2O_2

ROS was directly applied to cell culture using H_2O_2 and has been used across literature to model oxidative stress and associated damage (Baek et al. 2003, Makino et al. 2003, Singh et al. 2007, Du et al. 2017). H_2O_2 was purchased at a stock concentration of 9.8 M (30% w/w solution) from Sigma-Aldrich (UK), stored in aliquots at 4 ^oC in the dark and changed monthly to prevent using denatured stock. All concentrations of H_2O_2 were diluted to a 10 mM stock (Table 2.1) in the relevant serum-free cell culture medium before treatment of cells for 1 hour at room temperature and protected from light. A singular concentration of H_2O_2 to sufficiently induce damage was determined within a range (0.25-10 mM) on an individual cell line basis using the mtDNA strand break assay (sections 3.2.2.1, 4.2.2.1 and 5.2.2.1).

2.3.2 Physiological ROS Induction

2.3.2.1 Glucose

D-Glucose solution (Gibco, UK) supplemented into cell culture medium was used to mimic hyperglycaemic conditions in HKC-8 cells. The relationship between oxidative stress and high glucose concentrations are detailed in chapters 1 and the rationale for use in chapter 3. Extensive optimisation of glucose concentration, over a range of 25-150 mM, as well as incubation time were required and is documented in chapter 3.

2.3.2.2 Hypoxia

Hypoxia was used to induce ROS in both BEAS-2B and SVGp12 cells (chapter 4 and 5 respectively). Hypoxia has been associated with dysfunction of the mitochondria additional to its induction of oxidative stress as detailed in chapter 1. For this research, optimisation was required to identify a suitable length of time (1-120 hours) to induce significant ROS and mtDNA damage ascertained prior to experimentation using a New Brunswick Galaxy 48 R (Eppendorf, UK) incubator with 1% O_2 and 5% CO_2. Cells treated as the normoxic control were maintained under normal culture conditions at atmospheric O_2 (21%).

2.3.3 Confirmation of ROS formation

2.3.3.1 Principle of ROS Detection Assay

A 2',7'-dichlorofluorescin diacetate (DCFDA) detection kit was purchased from Abcam (UK) and used to confirm the generation of non-specific ROS within live cell culture. DCFDA is a cell permeable compound deacetylated to a non-fluorescent molecule by cellular esterases. Detectable ROS are then capable of oxidising the molecule to 2',7'-dichlorofluorescein (DCF), which emits a measurable fluorescence deemed proportional to the ROS within cells and is measurable fluorometrically and with flow cytometry (Figure 2.1) (Eruslanov and Kusmartsev 2010).

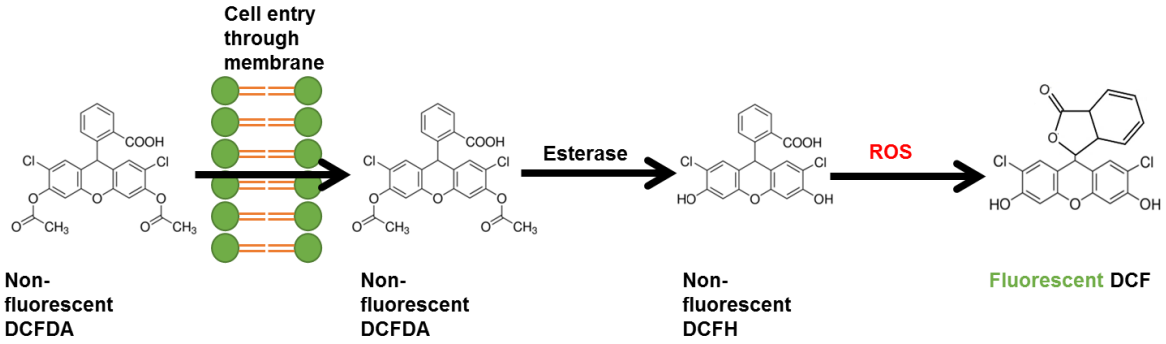

Figure 2.1 *Schematic of the DCFDA reaction to form the fluorescent product for quantification DCF as a result of esterase activity converting DCFDA to DCFH before oxidisation to DCF through ROS interaction, original figure created based on DCFDA assay principle (Eruslanov and Kusmartsev 2010).*

2.3.3.2 Fluorometric Measurement

The assay staining was performed according to manufacturer's instructions and utilised in the optimisation of ROS production by H_2O_2, glucose and hypoxia. In brief; cells were seeded at 25,000 cells per well in a black sided, clear bottomed 96 well micro-titre plate (Fisher, UK) and incubated for 24 hours before application of treatment. Following completion of treatment, DCFDA dye was applied to the culture at 25 µM per well and protected from light during a 45-minute incubation at 37 ^{o}C. The cells were washed with 1x kit buffer before ROS-inducing compounds were applied. Tert-Butyl hydroperoxide (tBHP), an oxidiser of DCFDA, at a concentration of 50 µM for 4 hours was used to validate this assay as a positive control instructed by the manufacturer. Finally, the fluorescence was measured with buffer and vehicle control 'blank' readings subtracted from all data points prior to analysis. The fluorescence emitted by oxidised DCF was measured using a FLUOstar OPTIMA (BMG Labtech, UK) with an excitation and emission wavelength of 485 nm and 520 nm respectively. This method was

set up in parallel with the cell viability assays and provided good reproducibility between replicates. Fluorometric measurement in this assay was chosen for its high through-put capabilities during optimisation and used to indicate the production of ROS by various treatments, including H_2O_2, glucose and hypoxia, over multiple concentrations and time courses. This data was used alongside the measurement of mtDNA strand breaks through qPCR (section 2.5.4) and demonstrated concentration or time related increases in ROS production.

2.3.3.3 Flow Cytometry

DCF oxidation was also quantified with flow cytometry using a BD Accuri™ C6 Plus flow cytometer (BD Biosciences, UK). For this protocol, cells were grown to 70% confluency in T25 flasks and treated with optimised H_2O_2, high glucose or hypoxia following treatment with Tiron if applicable (described in section 2.4). In preparation for staining, cells were trypsinised and stained with 20 µM DCFDA in cell culture medium and incubated for 30 minutes at 37 ^{o}C either before or after ROS treatment, detailed in the relevant chapter (3.2.2.3, 4.2.2.3, 5.2.2.3). For analysis, the DCF was excited at 488 nm and detected at 535 nm. The flow cytometry method provided a more detailed evaluation of intracellular ROS production and the effects of treatment, through discrimination between stained and unstained cells within larger populations, singlet population identification and debris exclusion through applying appropriate gating strategies, compared to the fluorometric read-out that indicated the average emission detected per well. The results obtained by flow cytometry supported the assays modelling oxidative damage, indicating Tiron's potential use in reducing such damage.

2.3.3.4 Data Analysis

Both methods were presented within this thesis to demonstrate the optimisation assays as well as the effects of Tiron treatment. DCF oxidisation measured fluorometrically was determined following subtraction of blank readings from all measurements and calculating the fold change compared to the untreated control. For flow cytometry analysis, DCF fluorescence intensity was obtained using BD Accuri™ C6 Plus software (BD Biosciences, UK) and averaged per treatment. A one-way ANOVA with a Dunnett's post-hoc test applied to data sets evaluating a concentration or incubation range for a single treatment, such as H_2O_2 or hypoxia. A Tukey post-hoc test was applied to identify differences between treatment groups with flow cytometry. Furthermore, a two-way ANOVA with Bonferroni correction post-hoc test was applied where two variables were measured such as concentration or incubation alongside ROS induction. Data was presented graphically as mean ± SEM (GraphPad Prism, USA).

2.4 Treatment with Tiron

2.4.1 Formulation of Tiron

Tiron (Sigma-Aldrich, UK) was dissolved in PBS to a concentration of 250 mM stock, or 4 M in the assessment of the dose at which Tiron reduced cell viability to 50% (LD50), as detailed in Table 2.1. The stock was then diluted to required and optimised concentrations in culture medium (1-10 mM). Unless stated, cell culture medium containing Tiron was removed prior to the induction or application of ROS in each cell line, described as 'pre-treatment' throughout this thesis.

2.4.2 Cell Viability

2.4.2.1 Purpose of Cell Viability Assessment

Cell viability was assessed to establish an LD50 for Tiron in each cell line using a concentration range of 0 to 2 M. Cell viability was then assessed at lower concentrations (1-10 mM) in order to determine a suitable test concentration that did not significantly affect cell viability. The effects of prolonged (48-120 hours) treatment with Tiron was also assessed at the test concentration, as directed in each chapter. As Tiron was solubilised in PBS before dilution with appropriate culture medium, a vehicle control with appropriate PBS volumes was included in each viability assay detailed below.

2.4.2.2 Mitochondria Dependent Viability Assessment

A resazurin based colourimetric toxicology assay, known as the TOX-8 assay, was purchased from Sigma-Aldrich (UK) and used to assess the effect of compounds on cell viability. The TOX-8 assay indicates the integrity of the mitochondria through the catalytic ability of mitochondrial oxidoreductases facilitating the reduction of resazurin to resorufin and dihydroresorufin. This reduction induces a colourimetric change where the rate of dye reduction, from blue to pink, is proportional to cellular viability measured spectrophotometrically (Figure 2.2) (Anoopkumar-Dukie et al. 2005, Riss et al. 2013).

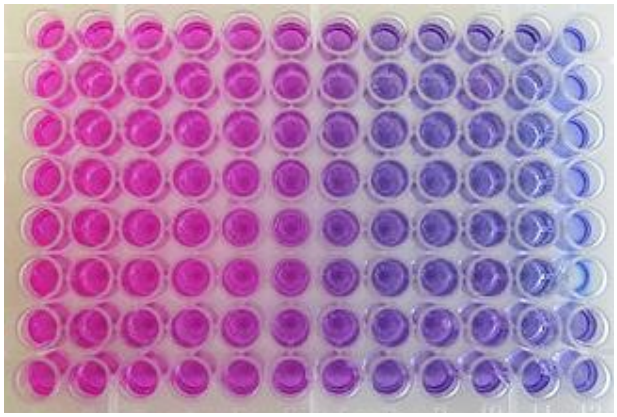

Figure 2.2 *Colourimetric reduction of resazurin (blue) to resorufin (pink) proportional to cell viability. This image demonstrates a cell viability reduction gradient from viable to 50% viability (left to right) and is an original figure.*

The TOX-8 assay was carried out in a 96 well micro-titre plate (Fisher, UK) with cells seeded at 10,000 cells per well and incubated overnight. After 24 hours, with a confluency of at least 60%, the culture medium was replaced with test concentrations of Tiron, H_2O_2 and glucose diluted in culture medium. Once the relevant treatments were applied to each well, they were incubated for a further 24-120 hours depending on the cell line and assay aim. In the assessment of hypoxic effect on cell viability, separate plates were set up for those cultured in hypoxic as well as normal conditions. Prior to analysis, 10 µl of TOX-8 reagent was added to each well, where the cells were at least 80% confluent, and incubated for 2 hours. The results were then read spectrophotometrically at 592 nm with a 690 nm reference using a Sunrise plate-reader and associated XFluor4^{TM} software (Tecan, USA).

2.4.2.3 Mitochondria Independent Viability Assessment

A second assay independent of the mitochondria and metabolic activity was also used to assess cell viability, to support the results of the TOX-8 kit. A sulforhodamine B (SRB) based assay kit (Sigma-Aldrich, UK), also known as TOX-6, was used to indicate cell number, something that is not correlated accurately with metabolic-based stains such as resazurin. This assay relies on SRB's ability to bind with cellular proteins that have been fixed with trichloroacetic acid (TCA) and therefore culminates as a measurement of cellular density following treatment (Vichai and Kirtikara 2006). The SRB pink aminoxanthene dye contains sulfonic groups which, under acidic conditions, bind to cellular amino-acid residues (Figure 2.3). This dye is solubilised for detection when conditions are alkaline through the addition of Tris base (Voigt 2005, Vichai and Kirtikara 2006).

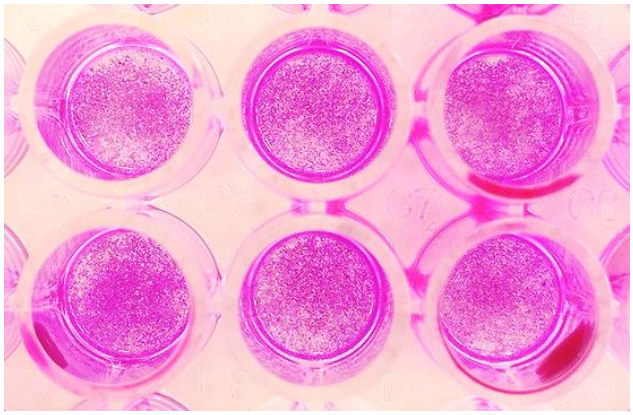

Figure 2.3 *An original figure depicting air dried wells containing cells stained with SRB solution before solubilisation with tris base solution.*

For this assay, cells were seeded and treated as described in section 2.4.2.2. Following the completion of treatment (Tiron or ROS as required), 25 µl of 50% (w/v) trichloroacetic acid solution, a fixative, was added on top of culture medium and incubated at 4 ^{o}C for 1 hour. Plates were then washed with PBS and air dried. Following this, 50 µl of 0.4% SRB solution was added to each well and stained for 30 minutes. The stain was removed and rinsed rapidly with 1% acetic acid solution. Once air dried, SRB was solubilised with 100 µl of 10 mM tris base solution with 5 minutes of gyration before reading spectrophotometrically at 565nm absorbance with a reference of 690nm.

2.4.2.4 Data Analysis

All data was normalised against the untreated control using Excel (Microsoft, USA) and was analysed according to manufacturer's guidance as follows; for TOX-8 assay the mean absorbance (n≥6) for the untreated control was divided by the mean absorbance at the test concentration and given as a percentage; for the SRB assay, the calculation was the inverse following subtraction of the blank control. The LD50 was calculated using the dose-response vs. normalised response function using statistical software (GraphPad Prism, USA). A one-way ANOVA with Dunnett's post-hoc test was conducted in data sets which measured an increase in concentration or treatment duration only. A two-way ANOVA was applied with Bonferroni correction post-hoc test where two variables were quantified, the effect of concentration or treatment duration, and the effect of ROS. Data was presented graphically as mean ± the standard error of mean (SEM) (GraphPad Prism, USA).

2.5 Measurement of ROS Damage

2.5.1 Method Principle

Quantitative polymerase chain reaction (qPCR) assays are frequently used to detect DNA damage within different biological samples. The assay used in this thesis was conceptualised by *Birch-Machin et al* at Newcastle University and utilised primers specific to the mitochondrial genome to identify mtDNA strand breaks (Oyewole et al. 2014, Hanna et al. 2018). As detailed in chapter 1, the mitochondrion is an excellent biomarker for DNA damage due to its absence of protective histones and limited repair mechanisms (Malik and Czajka 2013, Bowman et al. 2013, Stewart and Chinnery 2015). Through qPCR, a new formed mtDNA product is detectable as a PCR amplicon following successful binding of the SYBR green dye, emitting a measurable fluorescence. The generated CT (cycle threshold) value represents the number of cycles required before the product was sufficiently amplified to a threshold (Hunter et al. 2010).

2.5.2 DNA Extraction

Prior to the assessment of mtDNA strand breaks by qPCR, cells were seeded at a density of $5x10^5$ cells per 60 mm dish (Fisher,UK) and with cell-specific treatment conditions, as highlighted in the relevant chapters (sections 3.2, 4.2 and 5.2) .

Following treatment completion, cells were trypsinised and DNA extractred using the QIAmp DNA mini kit following the manufacturer's protocol (Qiagen, UK). The concentration of extracted nucleic acid was estimated using a NanoDrop™One spectrophotometer (Thermo Fisher Scientific, UK) at 260 nm wavelength against kit-supplied buffer AE as the blank reagent. This step was performed to evaluate the quality of extracted DNA, with samples demonstrating undistorted curves of greater than 20 ng/µl analysed further by qPCR. DNA was stored at 4 ^oC until required for analysis and dilution to 10 ng/µl with PCR-grade H_2O (Gibco, UK).

2.5.3 Determination of Mitochondrial Copy Number with an 83bp Amplicon

Following DNA extraction, a small (83bp) region of the mitochondria unlikely to contain damage, was used to align mitochondrial copy before assessment of mtDNA strand breaks (Passos et al. 2007, Gilkerson and Schon 2008, Malik et al. 2011, Gonzalez-Hunt et al. 2016). The method of DNA extraction used extracted all DNA, rather than mtDNA in isolation, therefore the nucleic acid content was determined as detailed in 2.5.1. A region of 83bp, located at position 16042 to 16124 of the mitochondrial genome, was then amplified by qPCR (Bio-Rad CFX Connect™ (Bio-Rad, UK)) to determine the relative copy number of

mitochondria per extracted DNA sample. The presence of damaged mtDNA within this region has been hypothesised to be unlikely due to its short sequence length, indicating the suitability of its use to align mitochondrial copy number (Passos et al. 2007, Hunter et al. 2010, Rothfuss et al. 2010). Approximation of the relative mitochondrial copy number meant that equal concentrations of mtDNA could be measured for their mtDNA damage, providing comparative results.

For this qPCR reaction, the reagent mastermix, primers and qPCR amplification settings for are outlined in Tables 2.3-2.5. For mitochondrial copy number alignment, DNA samples were diluted with PCR-grade H_2O (Gibco, UK) and reanalysed for confirmation.

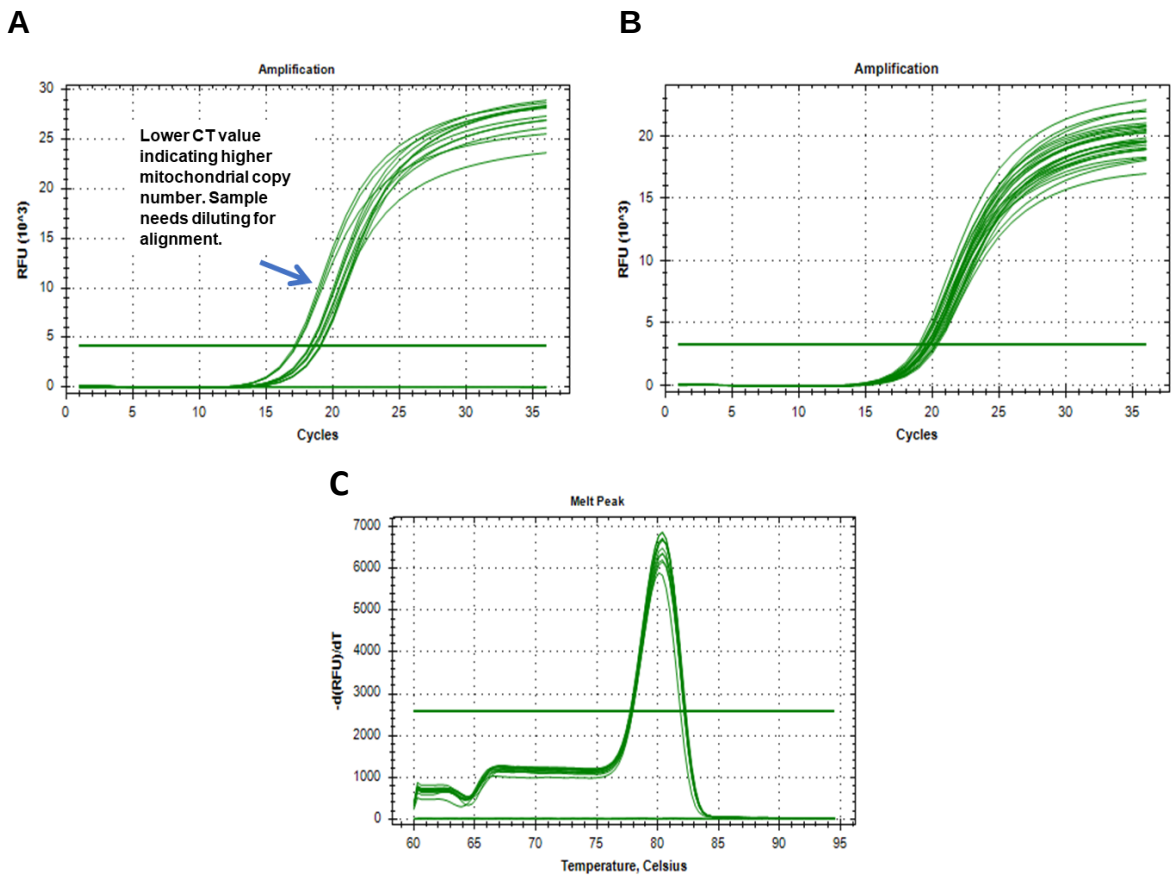

***Figure 2.4**. An example of 83bp qPCR assay determining mitochondrial copy number. (A) Identification of samples that require additional dilutions, (B) copy number alignment across all samples and (C) confirmation of product formation with melt curve generation.*

Reagent (Concentration)	Volume per sample (µl)	Final Concentration
Forward primer (10 µM) (Life Technologies, UK)	1	0.46 µM
Reverse primer (10 µM) (Life Technologies, UK)	1	0.46 µM
SYBR® Green JumpStart™ *Taq* Readymix™ (x2) (Sigma-Aldrich, UK)	12.5	x1
PCR-grade H_2O (Gibco, UK)	7.5	-
DNA	3	-

***Table 2.3**. Reagents for the mastermix used in 83bp qPCR.*

Stage	83bp Assay		1kb Assay	
	Time (seconds)	Temperature (ºC)	Time (seconds)	Temperature (ºC)
Heating	15	94	600	95
Denaturation	45	60	15	95
Annealing	45	72	15	60
Extension	120	72	55	72
Additional Extension	120	72	420	72
Melt Curve (Denaturation)	15	95	15	95
Melt Curve (Annealing)	5	60	60	55
Melt Curve (Extension)	X	X	15	95

***Table 2.4**. Conditions for copy number (83bp) and strand break (1kb) assessment by qPCR where denaturation, annealing and extension stages were repeated for 35 and 40 cycles respectively before additional extension and melt curve generation.*

Assay	Primer Direction	Oligonucleotide Sequence (5' to 3')
83bp	Forward	GAT TTG GGT ACC ACC CAA GTA TTG
	Reverse	AAT ATT CAT GGT GGC TGG CAG TA
1kb	Forward	CTG TTC TTT CAT GGG GAA GC
	Reverse	AAA GTG CAT ACC GCC AAA AG

***Table 2.5**. Primers used for 83bp and 1kb qPCR assay.*

2.5.4 Assessment of MtDNA Strand Breaks with a 1kb Amplicon

MtDNA strand break damage was identified through the amplification of a 1kb region (Figure 2.4), spanning positions 16040 to 404 and encompassing the mitochondrial control region. The control region is responsible for the replication of mtDNA therefore, damage present in this region could become erroneously replicated leading to mutation, apoptosis or disease as well as interruption of replication (Rothfuss et al. 2010). The identification of strand breaks through this qPCR method exploits the inefficiency of DNA polymerase to progress upon identification of strand breaks, lesions or significant mutation within mtDNA (Santos et al. 2006, Hanna et al. 2018). As a result of this inefficiency, strand break presence is determined by an increase in cycle threshold (CT) value. This increase in CT value indicates that additional qPCR cycles are required to generate the amplicon compared to the untreated control (Santos et al. 2006, Passos et al. 2007). The identification of mtDNA damage through the use of mtDNA specific primers negates the need to isolate the mitochondria. This assay quantifies mtDNA strand break damage amongst a determined concentration, or copy number, within a sample. This provides a more robust mechanism of damage visualisation, through reduction of variability, and accounts for the varying degrees of accumulated damage due to mitochondrial heteroplasmicity.

Each DNA extracted sample was diluted as determined in the 83bp assay (section 2.5.3). Displayed in Figure 2.5, a high CT value, such as 22, signalled an increased number of cycles were required to reach the amplification threshold. This indicated the presence of mtDNA damage hindering the efficiency of DNA polymerase. The untreated samples were used comparatively to identify the presence of damage, with measured CT values significantly lower in these samples, such as 18, and determined to have lower levels of mtDNA damage. The mastermix reagents, amplification settings and primers for this assay are detailed in Tables 2.5 and 2.6.

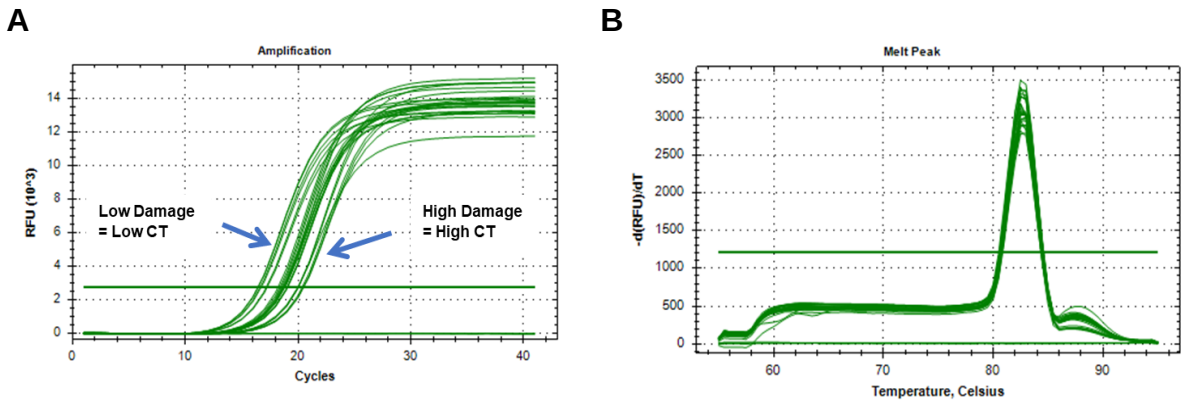

***Figure 2.5**. An example of 1kb qPCR assay identifying the presence of mtDNA stand breaks. (A) Amplification of 1kb production and (B) confirmation of product formation with melt curve generation.*

Reagent (Concentration)	**Volume per sample (µl)**	**Final Concentration**
Forward primer (10 µM) (Life Technologies, UK)	0.5	0.30 µM
Reverse primer (10 µM) (Life Technologies, UK)	0.5	0.30 µM
SensiMix™ SYBR® No-ROX (x2) (Bioline, UK)	10	x1
PCR-grade H_2O (Gibco, UK)	6	-
DNA	3	-

***Table 2.6**. Reagents for the mastermix used in 1kb qPCR.*

2.5.5 qPCR Instrumentation

All qPCR analysis of extracted DNA samples was conducted using a Bio-Rad CFX Connect™ with results reviewed in CFX Manager™ software (Bio-Rad, UK). A melt curve was generated at the end of each qPCR assay as confirmation of product formation (Figures 2.4 and 2.5).

2.5.6 Data Analysis

CT values obtained from qPCR analysis were normalised to the untreated control and expressed as a fold change in CT value. This was calculated by subtracting the sample CT value from the mean of the untreated control to obtain the change in CT value using Excel (Microsoft, USA). As a single CT was indicative of a 2-fold change in damage, the change in CT value was raised to the power of 2 to express a fold change in CT value (Figure 2.6). A one-way ANOVA with Dunnett's post-hoc test was performed to determine statistical significance between concentrations or treatment durations whilst a Tukey post-hoc was applied to determine the differences between treatment groups. A two-way ANOVA with

Bonferroni correction post-hoc determined the significance in data sets containing two variables, the induction of ROS and the effects of treatment concentration or duration. Data was presented as mean ± SEM (GraphPad Prism, USA).

$$Fold\ change\ in\ CT = 2^{(mean\ control\ CT\ value - sample\ CT\ value)}$$

$$Fold\ change\ in\ CT = 2^{(18.43-20.51)} = 0.24$$

***Figure 2.6**. An example calculation of fold change in CT value used in the mtDNA strand break assay.*

2.6 Quantification of Lipid Peroxidation

2.6.1 Measurement of Malondialdehyde

The availability of ROS has been correlated with increases in lipid peroxidation. Furthermore, the increased lipid peroxidation can contribute to the accumulation of ROS as a result of its own reactivity as detailed in section 1.3.2 (Niki 2014, Pérez-Rodríguez et al. 2015). As a biomarker of oxidative stress, lipid peroxidation was incorporated in this study to better understand if Tiron could elicit similar protective effects against ROS-induced lipid peroxidation.

To evaluate the effect on lipid peroxidation, the by-product MDA was measured colourimetrically using the lipid peroxidation (MDA) assay kit (Sigma-Aldrich, UK). MDA is an end product of hydroperoxide decomposition following ROS reaction with PUFA's and can exist in many configurations within aqueous solution (Tangvarasittichai et al. 2009, Cooley and Lunte 2011). Measurement of MDA and associated aldehyde by-products by thiobarbituric acid (TBA) has long been used as a simple and cost-effective assay to determine lipid peroxidation (Spickett et al. 2010). Two TBA molecules can react with a single MDA molecule to form a stable product coloured pink, suitable for detection (Tangvarasittichai et al. 2009).

For this assay, cells were seeded at a density of $5x10^5$ cells per 60 mm dish (Fisher,UK) and incubated. After 24 hours, the culture medium was replaced with test concentrations of Tiron for a further 24 hours followed by H_2O_2 or glucose diluted in culture medium or exposed to hypoxia as appropriate. Cells were then trypinised and treated according to the manufacturer's protocol. Finally, 200 µl of each sample was added to a 96 well plate for analysis at 532 nm absorbance using an Epoch 2 spectrophotometer (Biotek, USA). Comparative MDA standards supplied by the kit were prepared per assay and were processed alongside the cell samples after homogenisation (Figure 2.7).

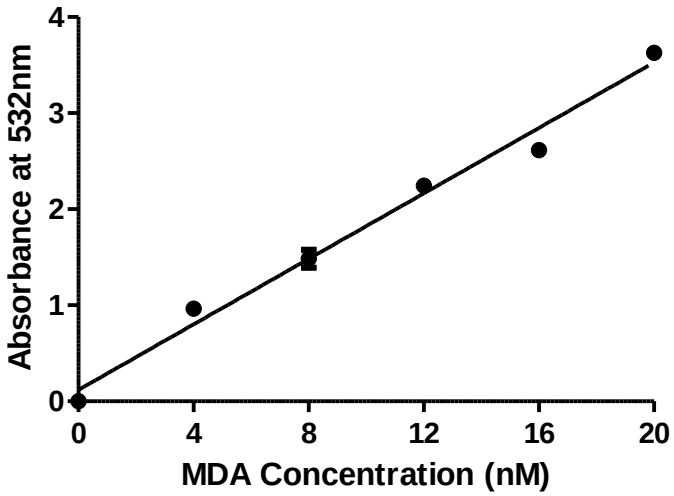

Figure 2.7*. Results generated with kit-supplied standards demonstrating increasing MDA concentration (Sigma-Aldrich, UK).*

2.6.2 Data Analysis

The blank (0) MDA standard solution was subtracted from all readings to correct the background. The MDA standards were plotted to generate a standard curve (Figure 2.7) and the amount of MDA in each sample was then extrapolated from this (GraphPad Prism, USA). The relevant concentration of MDA per sample was then calculated by dividing the amount of MDA in sample (nM) obtained from the standard curve by the sample volume and multiplying by the dilution factor. Significance was determined by one-way ANOVA with Tukey post-hoc test or two-way ANOVA with Bonferroni correction post-hoc test where two variables were measured, such as ROS induction and the effects of concentration or duration of treatment and presented graphically as mean ± SEM (GraphPad Prism, USA).

2.7 Nrf2 Protein Expression

2.7.1 Qualitative Measurement by ELISA

Nrf2, as detailed in chapter 1, is integral to the activation of the antioxidant response pathway and coordinating the translation of endogenous antioxidants including SOD and catalase. A colourimetric ELISA assay (KA3262, Abnova, UK) was used to evaluate the Nrf2 protein expression within human cells following Tiron treatment and ROS-induction, this was to determine if Tiron's protective mechanism was independent of the Nrf2 antioxidant pathway across all cell lines. H_2O_2 was used as known acute inducer of Nrf2 across all cell lines rather than physiological ROS methods to directly evidence Tiron's evidence on Nrf2. Furthermore, the effect of prolonged treatment with Tiron on Nrf2 expression was also evaluated to enquire as to its potential use as a supplement.

This assay used a primary rabbit antibody targeted to Nrf2 and binding of the HRP-conjugate secondary antibody to the fragment crystallisable (Fc) region of the primary antibody to catalyse a colourimetric change with substrate addition and quantify the live protein expression as a result of treatment with Tiron and H_2O_2. A mouse GAPDH antibody was used as the housekeeping control in this assay.

Cells were seeded at 10,000 cells per well in a 96 well micro-titre plate (Fisher, UK) and treated as previously described in section 2.3 and 2.4. Following treatment, cell culture medium was removed, and cells were washed with tris buffered saline (TBS) twice and prepared as directed by the manufacturer (Abnova, UK). Following addition of the kit-supplied stop solution, absorbance was measured immediately at 450nm using an Epoch 2 spectrophotometer (Biotek, USA) (Figure 2.8B). An additional crystal violet stain was also carried out as recommended by the manufacturer, this stained the cell nuclei and gave absorbance measurements proportional to the cell density (Figure 2.8C) with the absorbance read at 595nm using the same spectrophotometer.

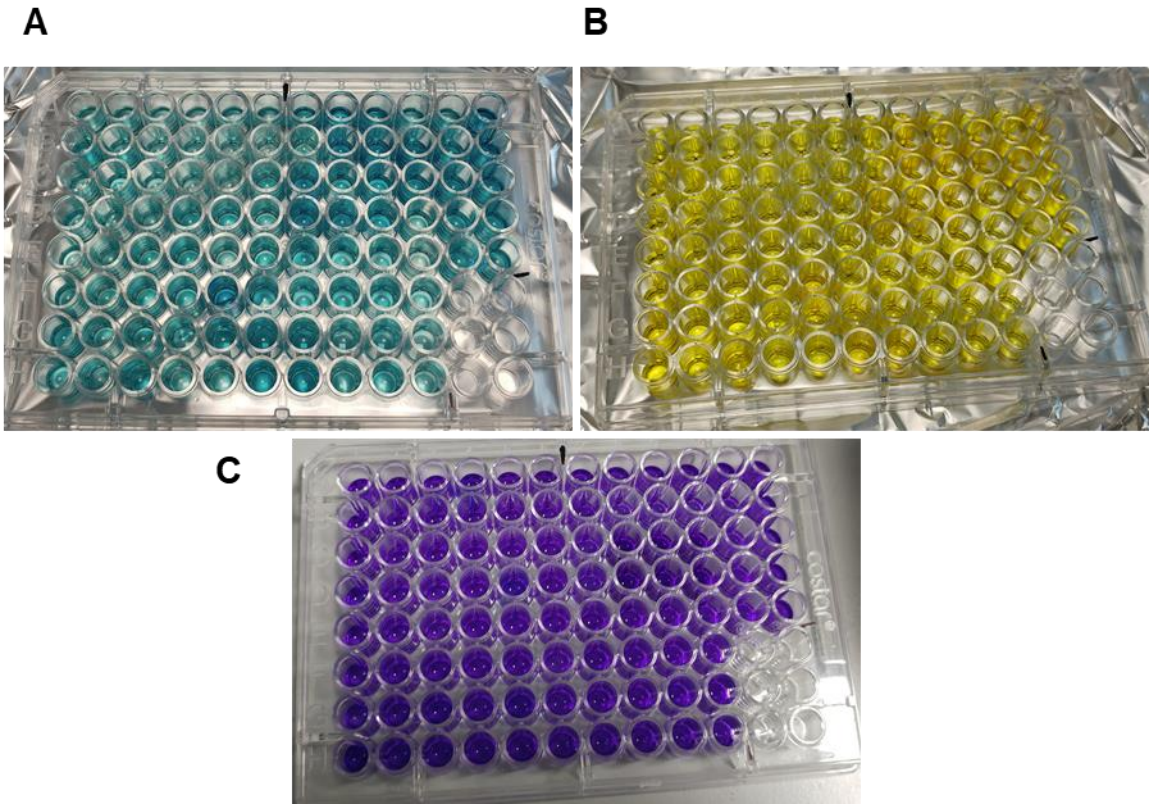

***Figure 2.8**. Original figure representing the stages of colour change within the Nrf2 assay where blue (A) corresponds to the addition of substrate to cells, yellow (B) signifies addition of the stop solution before absorbance reading at 450nm and purple (C) is the final crystal violet stain before reading at 595nm.*

2.7.2 Data Analysis

All results were normalised to the GAPDH control and crystal violet data was normalised by dividing the readings at 450nm by those obtained at 595nm (Figure 2.9) in Excel and expressed as a percentage of the untreated control (Microsoft, USA). Significance was determined by one-way ANOVA with Tukey post-hoc test to determine the difference between treatment groups. A two-way ANOVA with Bonferroni correction post-hoc evaluated the effect of ROS induction alongside concentration or duration of Tiron treatment and presented graphically as mean ± SEM (GraphPad Prism, USA).

$$Normalised\ to\ GAPDH$$

$$= measured\ value\ \div measured\ GAPDH\ value$$

$$Normalised\ to\ crystal\ violet$$

$$= normalised\ GAPDH\ value\ \div measured\ crystal\ violet\ value$$

***Figure 2.9**. An example calculation of normalisation to GADPH and crystal violet controls as directed by manufacturer (Abnova, UK).*

Chapter 3 – The Effect of Tiron in Human Renal Proximal Tubular Epithelial Cells

3.1 Introduction

3.1.1 Oxidative Stress within Diabetes Mellitus

Research has highlighted a role of ROS in the development and progression of DM and its associated microvascular complication, diabetic kidney disease/diabetic nephropathy (DKD). The development of DKD has been found to be causative of end stage renal failure, with diabetic patients having an increased risk of developing DKD during their lifetime (Hakim and Pflueger 2010, Fowler 2011, Gheith et al. 2016, Fakhruddin et al. 2017).

Although involvement of ROS has been identified in T1D, its involvement in T2D has been studied more extensively (Tiganis 2011, Calderon-Salinas et al. 2011, Tatsch et al. 2015). However, regardless of subcategory, DM leads to increased blood glucose concentrations which is pivotal to the progression of disease. Increases in oxidative stress, ROS production and lipid peroxidation have been identified with increasing glucose concentrations *in vitro* and in clinic (Han et al. 2005, Shokrzadeh et al. 2016, Monzo-Beltran et al. 2017, Zhai and Wang 2017). Hyperglycaemia-induced ROS originate from 4 pathways: polyol pathway, protein kinase C, hexosamine pathway and AGE formation (Kachhawa et al. 2017). These are outlined in chapter 1 and the role played in oxidative stress has been well modelled *in vitro* (Kaneto et al. 2001, Lee et al. 2004, Berrone et al. 2006, Lin et al. 2012).

The development of microvascular complications such as DKD have also been identified as dependent on the duration and severity of hyperglycaemia (Forbes et al. 2008). This warrants investigation into interventions to either reduce hyperglycaemia or its associated damage (Nathan et al. 2005). It has been shown that the incidence of DKD can be reduced by more than 50% when efficient glucose control is employed, demonstrating the importance of hyperglycaemic control and potential intervention (Nathan et al. 2005). Correlating with the prolonged exposure to hyperglycaemia, structural changes within primary human proximal tubular epithelial cells isolated from patients with T2D have been identified (Rahmoune et al. 2005). Proximal tubular epithelial cells do not have an adaptive response to high glucose environments, in that they fail to downregulate glucose transportation, leading to glucose hyperabsorption (Vallon 2011). Glucose entry into proximal tubular cells is mediated by sodium-glucose cotransporters 1 and 2 (SGLT1 and SGLT2), with SGLT2 being responsible for overall glucose reabsorption. As such, SGLT2 inhibitors can be incorporated into DM treatment regimens with the aim to control glucose uptake (Vallon et al. 2011, Umanath and

Lewis 2018). Nevertheless, due to the aberrant and continuous uptake of glucose, structural changes within proximal tubular cells have been identified in early DM and contribute to the development of DKD (Vallon 2011, Sun et al. 2016). The development of glomerulosclerosis as well as mesangial expansion, known as Kimmelstiel-Wilson nodules, within DKD has been hypothesised to correlate with proximal tubular damage (Vallon 2011). This leads to increased inflammation and endothelial cell dysfunction causing an elevation in ROS production and oxidative stress; a vicious cycle of ROS generation and one requiring intervention to halt progression or initial development (Gross et al. 2005, Rahmoune et al. 2005).

Between 1990 and 2010, the number of deaths as a result of DKD increased by 94% (Lozano et al. 2012). The prevalence of DKD parallels with the incidence of DM and with the estimation that one in ten adults will be diagnosed with DM by 2030, study into potential interventions is highly topical, whether this be preventative of diabetic complications or incorporated into a therapeutic regime (Bandeira et al. 2012, Umanath and Lewis 2018). As ROS generation in DM is self-perpetuating, the use of antioxidants could be beneficial in reducing oxidative stress and the worsening of symptoms.

Tiron, a ROS reducing agent, has been shown to equal or exceed the effects of Resveratrol and N-acetylcysteine (NAC) *in vitro* in primary and immortalised skin fibroblasts (Gallego-Villar et al. 2014, Oyewole et al. 2014). These antioxidants are potent against glucose-induced oxidative stress *in vitro* in human retinal vascular endothelial (Vaquero et al. 2004), cardiomyocytes (Guo et al. 2015) as well as in an *in vivo* rabbit model of obesity (Jimoh et al. 2018) and in clinical trials (Liao et al. 2017). Several studies have demonstrated tolerance of Tiron *in vivo* as well as its use to recover from oxidative stress following exposure to increased ROS (Kumar Nirala et al. 2009, Morgan et al. 2017). However, to date there has been an absence of research into the use of Tiron to alleviate glucose-mediated oxidative damage.

3.1.2 Aim

The aim of this study was to determine the potential protective capacity of Tiron against cellular ROS in an *in vitro* model of kidney injury. This was explored within this chapter through treatment of human renal proximal tubular epithelial cells (HKC-8) with Tiron, in models of both direct and physiological ROS induction.

3.2 Methods

3.2.1 Cell Culture

The HKC-8 cells used in this chapter were kindly provided by Newcastle University. HKC-8 cells are a SV40 transformed cell line derived from the renal cortex. HKC-8 cells have been found to be comparable to primary proximal tubular cells with the added benefit of longevity through immortalisation (Racusen et al. 1997).

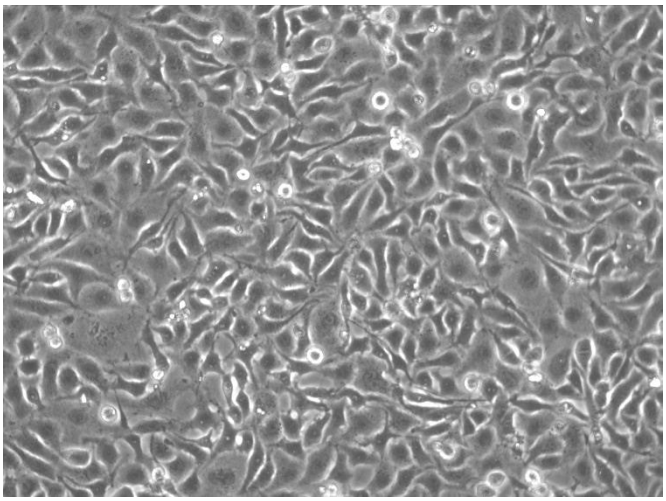

Figure 3.1. *The morphology of HKC-8 cells at 90% confluency, passage 10. Original figure obtained prior to cell passage using a TS100 eclipse microscope (Nikon, UK) and coolLED camera (Microtec, UK).*

HKC-8 cells were cultured in Dulbecco's modified Eagle's media (DMEM) with Ham's F12 nutrient mixture (DMEM/F12) containing GlutaMAX (Gibco, UK) containing 1% insulin-transferrin-selenium supplement (ITS) (Gibco, UK). FBS and penicillin:streptomycin (5U/ml) were supplemented at 5 and 1% respectively. All cell culture consumables were coated with 1% gelatin (Sigma-Aldrich, UK) prior to cell culture to support cell adherence. The cell culture routine was performed as detailed in section 2.2.

3.2.2 Cell Treatment Protocol

3.2.2.1 The Induction of ROS

HKC-8 cells were treated with H_2O_2 according to section 2.3.1. A concentration range of 1-10 mM was optimised in both the cell viability and mtDNA strand break assays. This was performed to obtain a concentration of H_2O_2 at which significant mtDNA damage occurred.

3.2.2.2 The Induction of Hyperglycaemia

This protocol was used to mimic consistently elevated glucose concentrations similar to that physiologically observed in poorly managed diabetes. Cells were treated with culture medium containing D-Glucose (25-150 mM) for 24 hours under standard culture conditions (detailed in section 2.2). The HKC-8 culture medium contained 17.5 mM of glucose ordinarily, which was considered when formulating final concentrations. Total glucose concentration was optimised to induce sufficient ROS production and mtDNA strand break damage, assessed by the DCFDA assay (section 2.3.3) and qPCR respectively (section 2.5). Low and high concentrations of glucose have been used previously to observe the degree of protection capable with test compounds, such as the Chinese medicine Tangluoning (Yang et al. 2017), as well as induce levels comparable to the ROS generating control such as Antimycin A (Araoye and Ckless 2016) or the extent of gene expression such as Neuritin, a neuroptrophic growth factor found to be diminished with after 300mM glucose compared to 150mM (Min et al 2012). As such a high concentration of glucose (150 mM) alongside a lower concentration was used to try to obtain ROS production within HKC-8 cells, which were already accustomed to DMEM containing high glucose (17.5 mM), comparable to observed with H_2O_2 and in turn, observe if Tiron could elicit a similar response or any, even at the lower concentration.

To investigate Tiron's potential incorporation into a treatment regime, Tiron was supplemented concomitantly with glucose. HKC-8 cells were cultured with 25 mM glucose containing Tiron over 72 hours. Both Tiron and glucose were replenished in culture daily to maintain consistent concentrations for the duration of the experiment.

3.2.2.3 Confirmation of Intracellular ROS Production

Intracellular ROS concentrations were assessed using the DCFDA assay (Abcam, UK) as detailed in section 2.3.3. During the H_2O_2 treatment protocol, HKC-8 cells were incubated with DCFDA reagent prior to induction of ROS. For glucose-induced ROS, DCFDA was added following completion of 24-hour incubation with glucose due to the stability of the stain. Both methods of DCFDA addition were according to manufacturer's recommendations.

3.2.2.4 Treatment with Tiron

Tiron stock solutions were prepared as directed in section 2.1 and 2.4 and diluted in culture medium as appropriate. The effect of prolonged Tiron exposure was also observed in HKC-8 cells. For this, cells were cultured with Tiron for up to 120 hours before removal from media and replacement with relevant ROS-induction where applicable.

As detailed in section 2.4.2, the effect of Tiron on cell viability was assessed using the TOX-8 and SRB assays (Sigma-Aldrich, UK).

3.2.3 Assessment of ROS-Induced Damage and Data Analysis

All investigations into the effects of Tiron on ROS-induced damage were conducted and analysed in accordance with chapter 2.

3.3 Results

3.3.1 The Effects of Increasing Tiron Concentration on Cell Viability

HKC-8 cells cultured with Tiron for 24 hours were used to estimate Tiron's LD50 using the TOX-8 and SRB assays. A significant decrease ($p \leq 0.001$) in cell viability was recorded between 50 mM (80±0.66%) and 2 M (8±0.18%) in the TOX-8 assay. These were compared to the untreated control (100±0.68%) and presented in Figure 3.2A. The LD50 value was calculated at 322 mM. These results were confirmed with the SRB assay (Tiron 50 mM: 88±1.23%; Tiron 2M: 7±0.51% vs. untreated control 100±0.59%) with a calculated LD50 of 334 mM (Figure 3.2B).

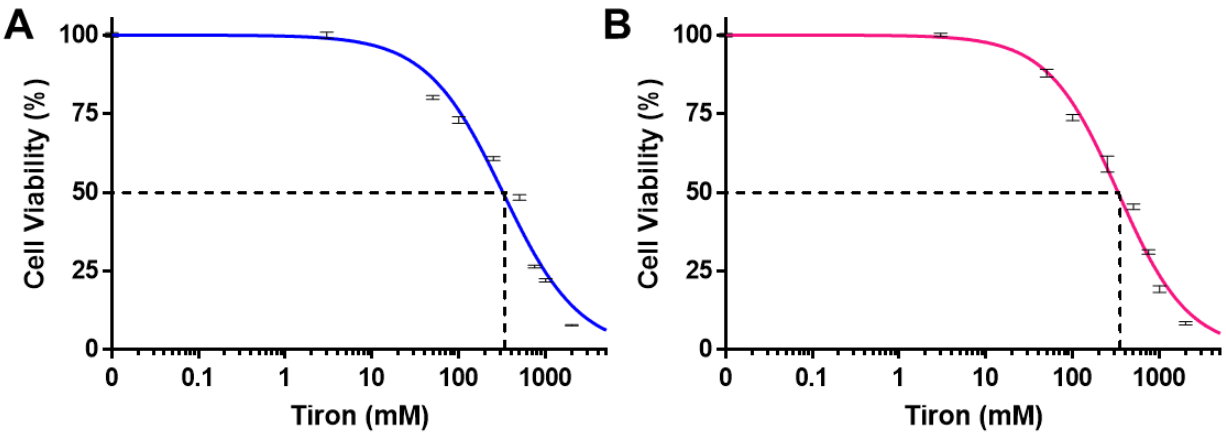

Figure 3.2. *The establishment of Tiron's LD50 in HKC-8 cells following 24-hour treatment (0-2M). The LD50 was calculated as 322 mM and 334 mM, using the TOX-8 (A) and SRB (B) assays. N=9 from 3 independent experiments; mean±SEM.*

A smaller concentration range of Tiron (1-10 mM) was assessed for its effect on HKC-8 cell viability in the TOX-8 and SRB assays, to identify a suitable test concentration following determination of Tiron's LD50. Tiron did not have a significant effect on cell viability when HKC-8 cells were treated with 0-3 mM ($p \geq 0.05$) in the TOX-8 assay (Figure 3.3A), however treatment with 5-10 mM observed a significant decline ($p \leq 0.001$) in cell viability (94±0.77%

and 91±1.46%) compared to the untreated control (100±0.43%) and was confirmed with the SRB assay (Tiron 5 mM: 96±0.98%; Tiron 89±1.8%; vs. untreated control: 100±0.64%) (Figure 3.3B).

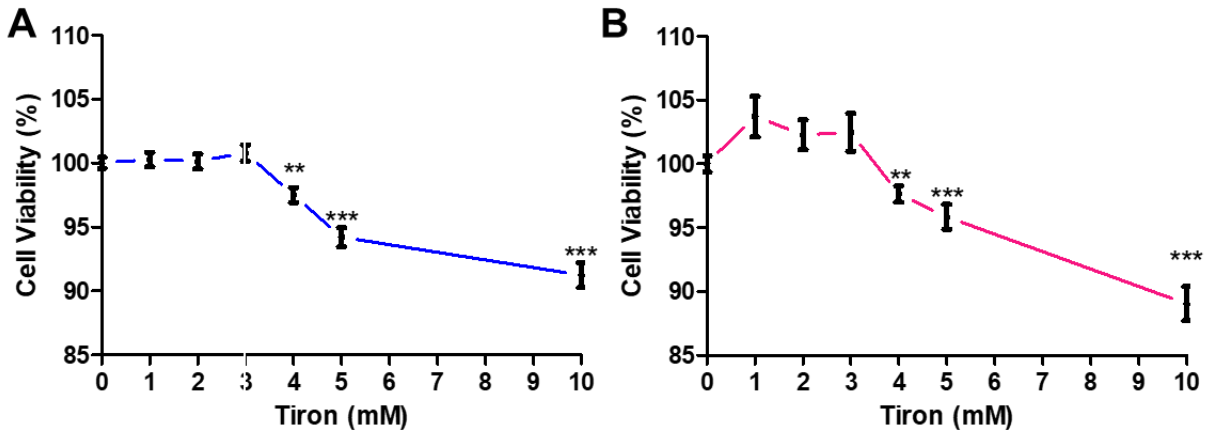

***Figure 3.3**. The effect of 24-hour treatment with Tiron (1-10 mM) on cell viability in HKC-8 cells. Tiron reduced cell viability at 5 mM and above in both the TOX-8 (A) and SRB assays (B). *** p≤0.001 vs. untreated control; n=9 from 3 independent experiments; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

3.3.2 The Optimisation of H_2O_2-Induced ROS Damage Through Titration

A concentration range of H_2O_2 sufficient to decrease cell viability in HKC-8 cells after 1-hour treatment was observed using the TOX-8 and SRB assays (Figure 3.4). Treatment of cells with 1-10 mM H_2O_2 resulted in a significant (p≤0.001) reduction in cell viability compared to the untreated control (H_2O_2 1 mM: 60±1.46%; 5 mM: 51±1.97%; 10 mM: 51±1.72%; vs. 100±0.54%). Similarly, this was also observed in the SRB assay (H_2O_2 1 mM: 63±1.32%; vs. 100±0.88%) (Figure 3.4B).

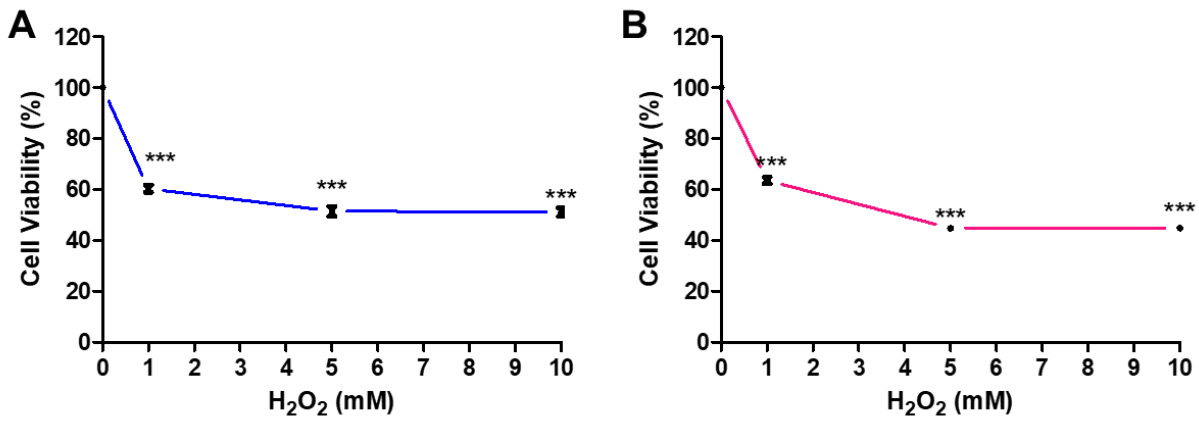

Figure 3.4*. The effect of H_2O_2 (1-10 mM) treatment for 1-hour on HKC-8 cell viability. H_2O_2 significantly reduced cell viability at all concentrations in both the TOX-8 (A) and SRB (B) assays. *** p≤0.001 vs. untreated control; n=9 from 3 independent experiments; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

The same concentration range of H_2O_2, 1-10 mM, was assessed for the induction of mtDNA strand breaks in HKC-8 cells as measured by qPCR. All concentrations of H_2O_2 induced a significant (p≤0.001) fold increase in CT value, indicative of reduced qPCR efficiency (Figure 3.5). This fold increase ranged from 3.24±0.09 to 4.26±0.06, with the highest fold increase observed with 5 mM treatment. As a result, 5 mM was selected as the test H_2O_2 concentration in HKC-8 cells.

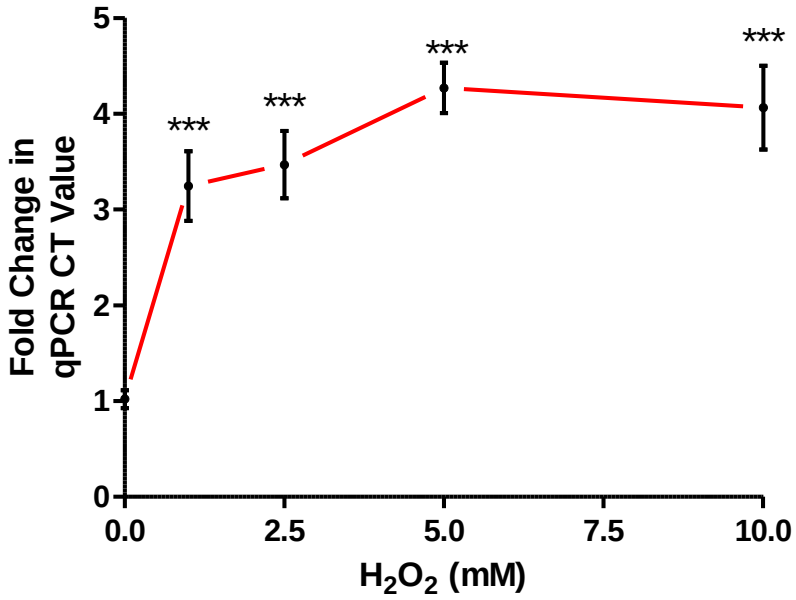

Figure 3.5. *The assessment of significant mtDNA strand break formation with H_2O_2 (1-10 mM) treatment for in HKC-8 cells. 5 mM H_2O_2 induced the most significant mtDNA strand break damage, through analysis of fold change in CT value using qPCR. *** p≤0.001 vs. untreated control; n=6; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

3.3.3 The Effect of Tiron on H_2O_2-Induced ROS Damage

3.3.3.1 The Preservation of Cell Viability with Tiron

HKC-8 cells were treated with Tiron (1-5 mM) for 24 hours before treatment with 5 mM H_2O_2 for 1-hour, the concentration identified in Figure 3.5, to observe the effect of cell viability with the TOX-8 and SRB assays. Tiron treatment and concentration had a significant ($p \leq 0.001$) effect on preserving cell viability. A significant ($p \leq 0.001$) difference was also observed between H_2O_2 and no H_2O_2 treated Tiron samples at each concentration. Treatment with 3 mM Tiron preserved cell viability greater than compared to 5 mM treatment and the H_2O_2 control (Tiron 3 mM: 80±0.86%; 5 mM: 61±1.57%; vs. H_2O_2: 54±1.16%) (Figure 3.6). The results obtained in the SRB assay confirmed this (Tiron 3 mM: 82±1.15%; 5 mM: 58±3.34%; vs. H_2O_2: 52±1.01%).

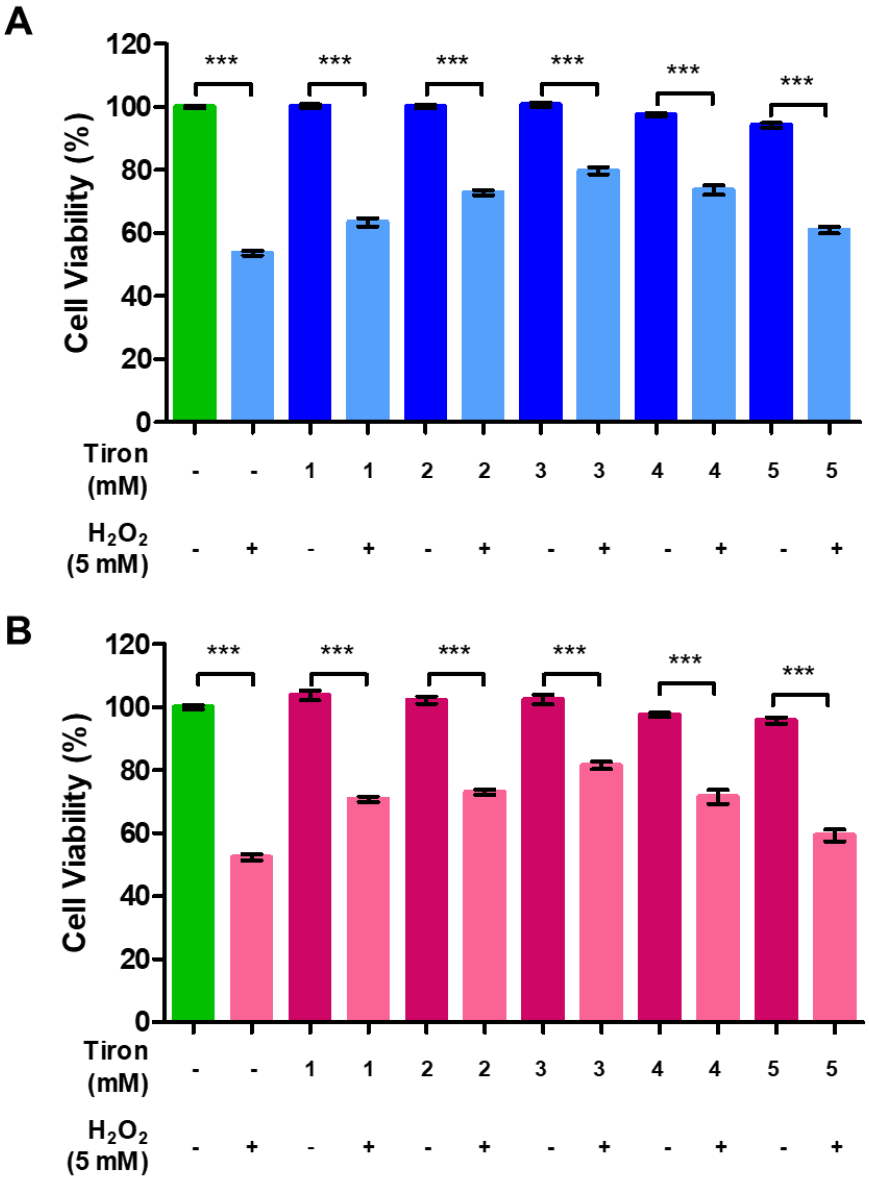

Figure 3.6. *The preservation of cell viability with Tiron (1-5 mM) pre-treatment followed by H_2O_2 (5 mM) exposure. Tiron significantly improved cell viability at all concentrations in the presence of H_2O_2 in HKC-8 cells in both the TOX-8 (A) and SRB (B) assays.* *** $p \leq 0.001$ significant difference *with vs. without H_2O_2 at each Tiron concentration; n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

3.3.3.2 The Prevention of H_2O_2-Induced MtDNA Strand Breaks with Tiron

Against significant (p≤0.001) induction of mtDNA strand break damage with 5 mM H_2O_2, pre-treatment with Tiron at all concentrations was found to be significantly (p≤0.001) protective (Tiron 2 mM: 1.60±0.02; 3 mM: 0.99±0.01; 4 mM: 1.36±0.03; 5 mM: 0.98±0.02; vs. H_2O_2 4.89±0.11) (Figure 3.7). As seen in Figure 3.5 and previous studies, a concentration of 3 mM was selected for further experimentation (Won et al. 2012, Oyewole et al. 2014).

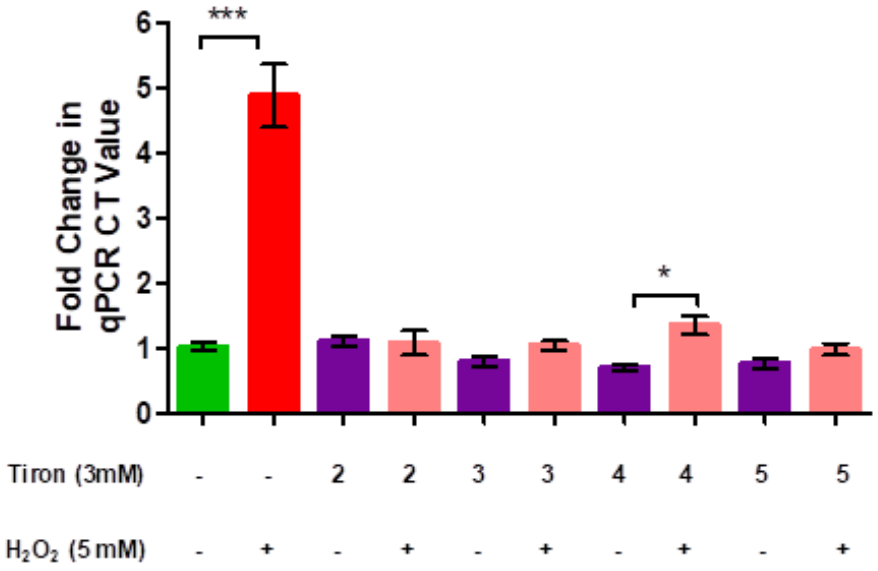

Figure 3.7. *The inhibition of mtDNA strand break damage with Tiron (2-5 mM) assessed by qPCR. Tiron pre-treatment offers significant protection against H_2O_2–induced mtDNA strand breaks in HKC-8 cells at all concentrations. *, *** p≤0.05, 0.001 significant difference with vs. without H_2O_2 at each Tiron concentration; n≥6; mean±SEM, two-way ANOVA with Bonferroni correction.*

3.3.3.3 The Effect of Tiron (3 mM) on H_2O_2-Mediated Intracellular ROS

Shown in Figure 3.8, treatment with H_2O_2 increased intracellular ROS production significantly (p≤0.01) compared to the untreated control (139±11.88% vs. 100±4.77%) as determined by flow cytometry with the DCFDA assay. Pre-treatment with Tiron significantly (p≤0.001) reduced fluorescence intensity by 49±2.53% compared to 5 mM H_2O_2. In the absence of H_2O_2, the Tiron control significantly reduced (p≤0.05) intracellular ROS (66±5.17% vs. 100±4.77%).

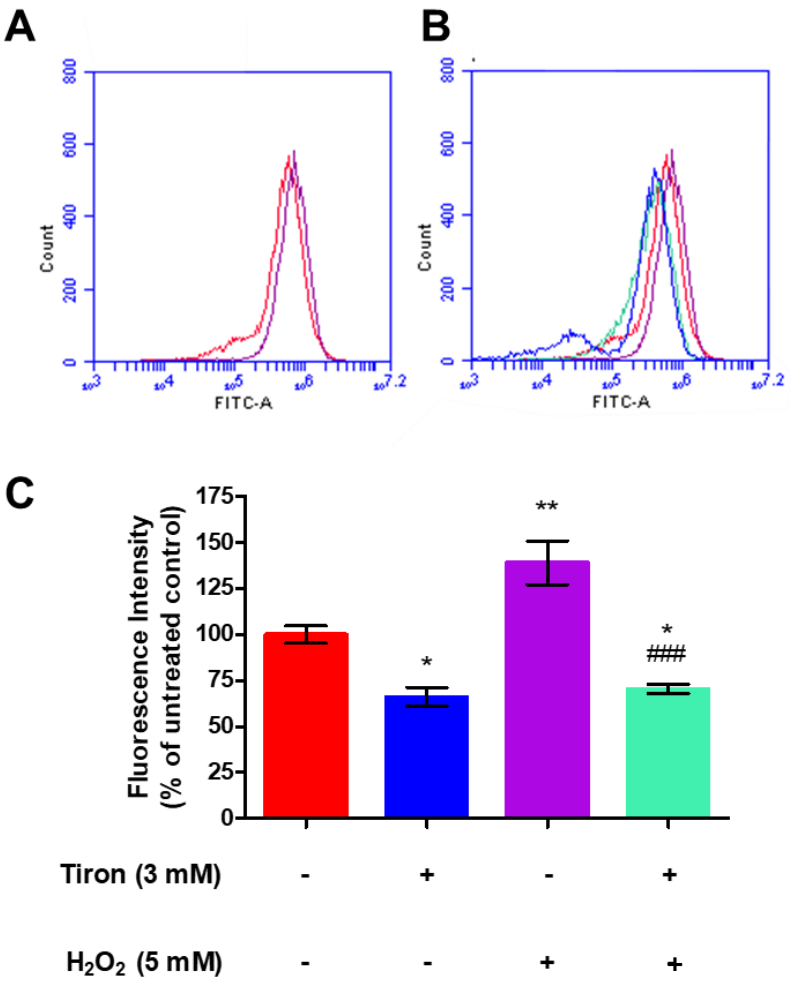

Figure 3.8. *The effects of Tiron (3 mM) on intracellular ROS with and without H_2O_2 (5 mM) with DCFDA by flow cytometry. (A) 5 mM H_2O_2 (purple) treatment significantly augmented ROS compared to the untreated control (red). (B) Tiron pre-treatment (aqua) significantly reduced fluorescence intensity following H_2O_2 treatment comparable to the Tiron only control (blue). (C) Pre-treatment with Tiron significantly reduced fluorescence intensity following H_2O_2 treatment in HKC-8 cells. The colours shown directly relate to Figures 3.8A and 3.8B. *, ** p≤0.05, 0.01 vs. untreated control; ### p≤0.001 vs. H_2O_2; n=6; mean±SEM; one-way ANOVA with Tukey post-hoc.*

3.3.3.4 The Effects of Tiron (3 mM) on Lipid Peroxidation

H_2O_2 (5 mM) significantly ($p \leq 0.001$) increased the production of MDA compared to the untreated control following 1-hour treatment (149±9.36% vs. 100±0.63%) (Figure 3.9). With 24-hour Tiron pre-treatment, MDA production was decreased significantly ($p \leq 0.01$) following H_2O_2 treatment (117±5.50% vs. 149±9.36%). Unlike the effect on mtDNA strand breaks (Figure 3.7) and intracellular ROS production (Figure 3.8), Tiron was unable to restore MDA concentration to baseline.

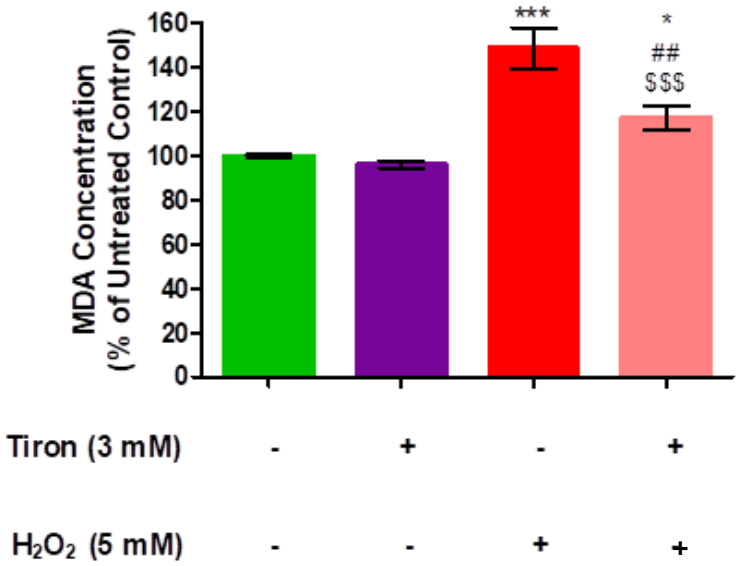

Figure 3.9. *H_2O_2-induced MDA decreases with Tiron (3 mM) pre-treatment. Tiron offered protection against H_2O_2-induced lipid peroxidation in HKC-8 cells through the measurement of the MDA biomarker. *, *** $p \leq 0.05$, 0.001 vs. untreated control; ## $p \leq 0.01$ vs. H_2O_2; $$$ $p \leq 0.001$ vs. Tiron; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

3.3.3.5 The Effect of 24-Hour Treatment with Tiron (3 mM) on Nrf2 Expression

Treatment of HKC-8 cells with 5 mM H_2O_2 induced a significant ($p \leq 0.001$) increase in Nrf2 expression compared to the untreated control (269±7.37% vs. 100±2.84%). Pre-treatment with Tiron did not significantly increase Nrf2 protein expression following H_2O_2 treatment compared to the untreated control (106±4.01% vs. 100±2.84%). Nrf2 expression showed a significant decrease with Tiron pre-treatment ($p \leq 0.001$) compared to the H_2O_2 control (112±7.05% vs. 269±7.37%). Tiron treatment for 24-hours without H_2O_2 also did not increase Nrf2 protein expression (112±7.05% vs. 100±2.84%, $p > 0.05$) (Figure 3.10).

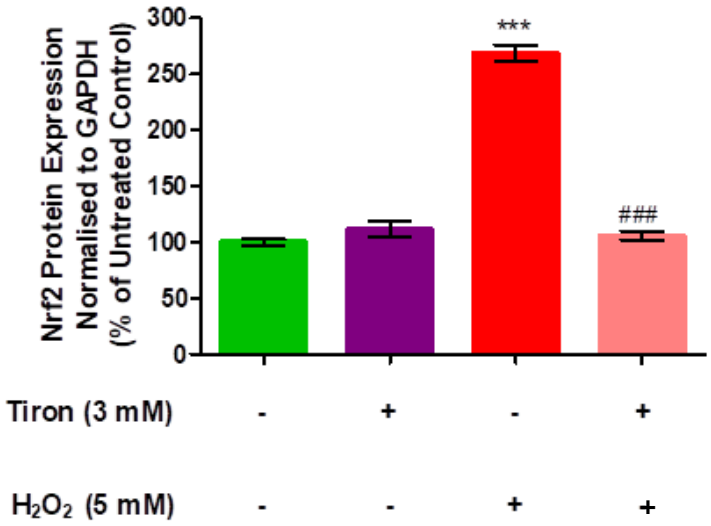

Figure 3.10. *The qualitative evaluation of Nrf2 expression following Tiron (3 mM) treatment for 24 hours with and without H_2O_2. Tiron did not increase Nrf2 expression in HKC-8 cells following ROS induction by H_2O_2. *** $p \leq 0.001$ vs. untreated control; ### $p \leq 0.001$ vs. H_2O_2; n=6; mean±SEM; one-way ANOVA with Tukey post-hoc.*

3.3.4 The Effects of Prolonged Treatment with Tiron (3 mM)

3.3.4.1 The Effect of Prolonged Tiron Treatment on Cell Viability

Treatment with Tiron (3 mM) for 48-72 hours led to a significant ($p \leq 0.001$) reduction in cell viability compared to the untreated control in the TOX-8 assay (48 hours: 70±1.61%; 72 hours: 70±2.40%; vs. 100±1.91%) (Figure 3.11A). Pre-treatment with Tiron for these durations before treatment with 5 mM H_2O_2 significantly ($p \leq 0.001$) preserved cell viability (48 hours: 63±3.14%; 72 hours: 62±1.77%; vs. H_2O_2: 49±2.12%). These observations were consistent with those obtained in the SRB assay (Figure 3.11B) (Tiron 48 hours + H_2O_2: 57±0.722%; Tiron 72 hours + H_2O_2: 57±0.89%; vs. H_2O_2: 45±0.58%; vs. untreated control: 100±0.93%).

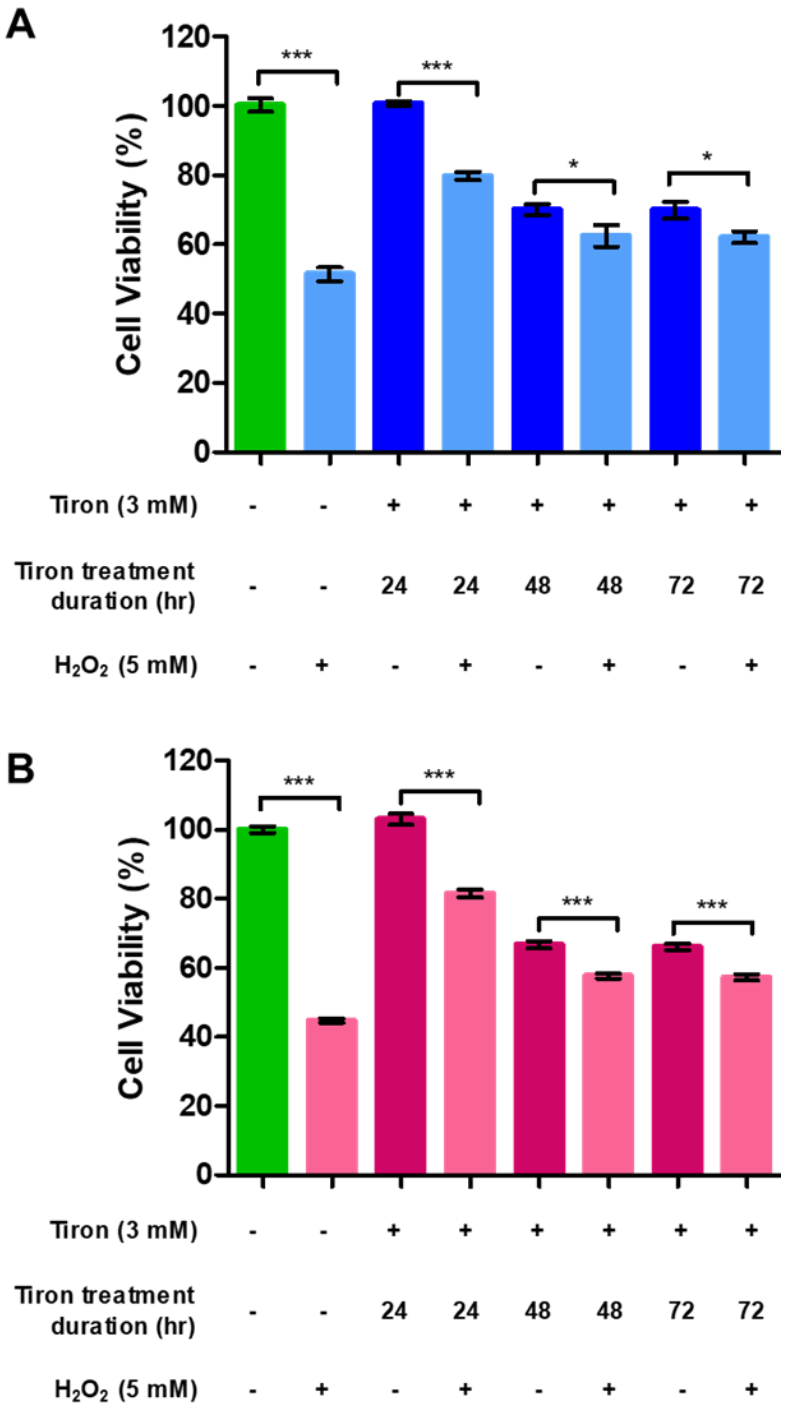

Figure 3.11. *The effect of prolonged treatment (48-72 hours) with Tiron (3 mM) on cell viability. Tiron treatment longer than 24 hours significantly reduced cell viability in both the TOX-8 (A) and SRB (B) assays. *, *** p≤0.05, 0.001 significant difference with vs. without H_2O_2 at each treatment duration; n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

3.3.4.2 The Effects of Prolonged Treatment with Tiron on Intracellular ROS Production

Prolonged treatment with Tiron for 120 hours resulted in a significant ($p \leq 0.001$) fold increase in fluorescence intensity compared to the untreated control measured by the DCFDA assay fluorometrically (Tiron 120 hours: 160±4.91%; vs. 100±1.89%). Shown in Figure 3.12, treatment with H_2O_2 significantly ($p \leq 0.001$) increased fluorescence intensity, indicative of ROS production (278±10.12% vs. 100±1.89%). HKC-8 cells treated with 5 mM H_2O_2 after prolonged Tiron treatment showed no significant difference ($p \geq 0.05$) compared to Tiron only but did have a significant ($p \leq 0.001$) effect on fluorescence intensity compared to the H_2O_2 control, indicating a reduction in intracellular ROS. However, this reduction was not restorative of baseline levels (Tiron 48 hours: 137±6.35%; 120 hours: 166±6.23%; vs. 278±10.12%).

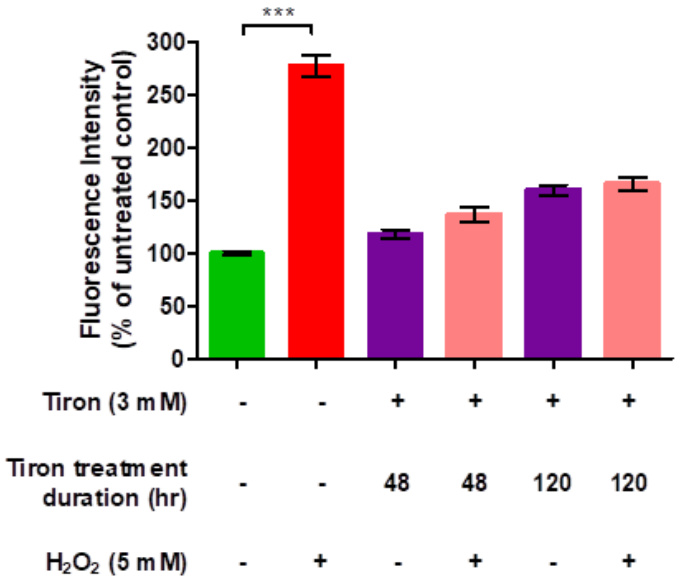

Figure 3.12. *The effects of prolonged treatment (48-120 hours) with Tiron (3 mM) on intracellular ROS. Prolonged treatment with Tiron significantly reduced H_2O_2 increases in fluorescence intensity measured fluorometrically with the DCFDA. *** $p \leq 0.001$ significant difference with vs. without H_2O_2; n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

3.3.4.3 The Expression of Nrf2 Following Prolonged Treatment with Tiron

Treatment with Tiron for 48-120 hours significantly ($p \leq 0.001$) increased the expression of Nrf2, a regulatory protein in the ARE pathway, determined by qualitative ELISA (48 hours: 157±6.10%; 120 hours: 212±14.24%; vs. untreated control: 100±2.84%) (Figure 3.13). Shown in Figure 3.10, treatment with H_2O_2 following prolonged Tiron supplementation led to an increase in Nrf2 protein expression also. This increase in Nrf2 was significantly reduced compared to H_2O_2 (5 mM) treatment alone but not significantly different from Tiron only treatments (48 hours + H_2O_2: 164±9.58%; 120 hours + H_2O_2: 223±16.01%; vs. H_2O_2: 269±7.37%).

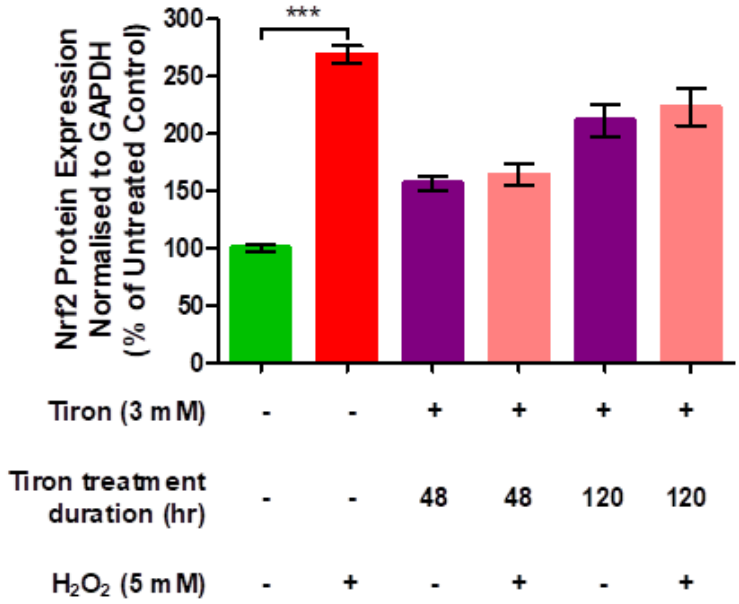

Figure 3.13. *The expression of Nrf2 following prolonged treatment (48-120 hours) with Tiron (3 mM) by ELISA. Tiron augmented Nrf2 expression in HKC-8 cells as due to prolonged incubation with and without ROS induction by H_2O_2. *** $p \leq 0.001$ significant difference with vs. without H_2O_2; n=6; mean±SEM, two-way ANOVA with Bonferroni correction.*

3.3.5 The Optimisation of Glucose-Induced Damage Through Titration

3.3.5.1 The Induction of ROS and mtDNA Damage with Increasing Glucose Concentrations

A significant increase in fluorescence intensity, measured fluorometrically, was observed with glucose (25-150 mM) treatment for 24 hours compared to the control (Glucose 17.5 mM).(Glucose 25 mM: 181±5.12%; 50 mM: 228±8.70%; 75 mM: 260±5.31%; 100 mM: 284±6.82%; 150 mM: 341±11.72%; vs. 100±2.41%) (Figure 3.14). The significant measured increase in fluorescence intensity was indicative of intracellular ROS production in this model.

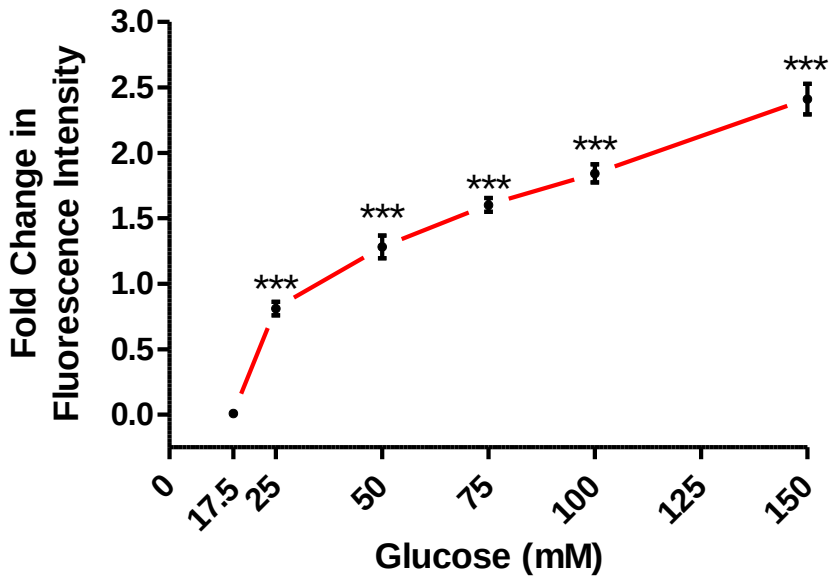

Figure 3.14. *The assessment of increasing concentrations glucose (25-150 mM) on intracellular ROS production. Significant fold change in fluorescence intensity at increasing glucose concentrations were quantified by measuring fluorescent DCFDA in HKC-8 cells *** p≤0.001 vs. control containing 17.5 mM glucose; n≥8 from 3 independent experiments; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

Contrary to the results of Figure 3.14, 25 mM glucose did not induce significant (p≥0.05) mtDNA strand breaks following 24-hour treatment (1.31±0.03 vs. control (17.5 mM) 1.06±0.02) (Figure 3.15). There was a significant (p≤0.001) fold increase in CT value following treatment with 50-150 mM glucose (50 mM: 2.14±0.04; 75 mM: 2.24±0.04; 100 mM: 2.90±0.06; 150 mM: 4.29±0.09; vs. 1.06±0.02). The decrease in CT value was indicative of mtDNA strand break formation due to glucose treatment. Both 50 and 150 mM treatments were selected for further experimentation to assess Tiron's protective capability against a hyperglycaemia mimicking model.

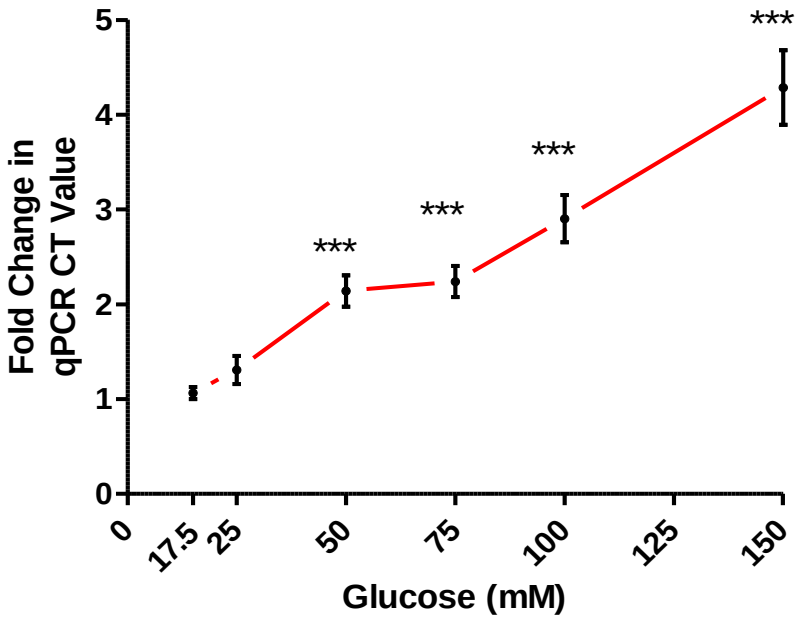

Figure 3.15. *The induction of mtDNA strand breaks with increasing concentrations of glucose (25-150 mM). Glucose at final concentrations 50 to 150 mM produced significant mtDNA strand break damage in HKC-8 cells, through analysis of fold change in CT value using qPCR. *** p≤0.001 vs. control containing 17.5 mM glucose; n=6; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

3.3.6 The Effects of Tiron on Glucose-Induced ROS Damage

3.3.6.1 The Preservation of Cell Viability with Tiron Following Glucose Treatment

Treatment of HKC-8 cells with glucose (25-150 mM) for 24 hours led to significant ($p \leq 0.001$) reduction in cell viability at all concentrations as measured in the TOX-8 assay (Figure 3.16A) (Glucose 25 mM: 82±0.83%; 50 mM: 72±0.50%; 75 mM: 63±0.45%; 100 mM: 57±0.58%; 150 mM: 52±0.49%; vs. control (17.5 mM glucose) 100±0.35%). When glucose was supplemented (final concentration 25-150 mM) following pre-treatment with Tiron (3 mM), there was a significant ($p \leq 0.001$) preservation of cell viability (Glucose 25 mM: 93±0.70%; 50 mM: 82±0.38%; 75 mM: 73±0.31%; 100 mM: 73±0.41%; 150 mM: 71±0.36%; vs. untreated control 100±0.35%). These results were confirmed with the SRB assay (Tiron + Glucose 25 mM: 92±0.72%; + 50 mM: 83±0.28%; + 75 mM: 74±0.72%; + 100 mM: 71±0.44%; + 150 mM: 68±0.37%; vs. control (17.5 mM) 100±0.58%). However, pre-treatment with Tiron across both assays did not inhibit glucose-induced cell viability loss as had been seen with H_2O_2 in Figure 3.6.

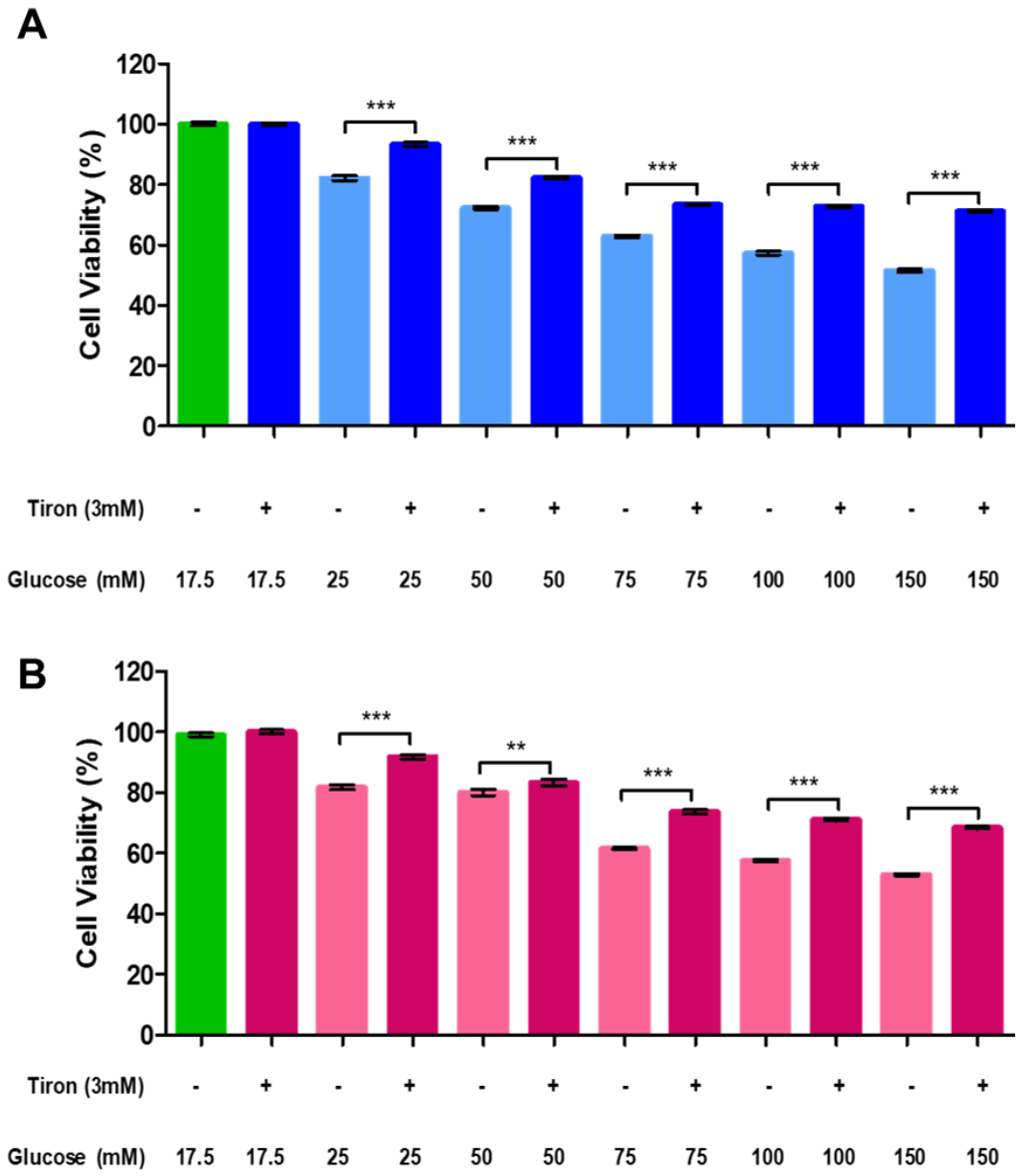

Figure 3.16. *The effect of Tiron (3 mM) pre-treatment on cell viability following glucose exposure (25-150 mM). Tiron preserves cell viability following glucose treatment at all concentrations in HKC-8 cells in both the TOX-8 (A) and SRB (B) assays in the presence of 3 mM Tiron compared to the control, which contained 17.5 mM glucose. *** p≤0.001 significant difference with vs. without tiron treatment at each concentration of glucose; n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni post-hoc.*

3.3.6.2 The Inhibition of Glucose-Induced MtDNA Strand Break Formation with Tiron

In agreement with Figure 3.7, Tiron pre-treatment (3 mM) was shown to be significantly ($p \leq 0.01$) protective against 50 mM glucose-induced mtDNA strand breaks compared to the 50 mM treated control (Tiron + 50 mM: 0.89±0.03; vs. 50 mM: 1.83±0.09) (Figure 3.17). This treatment was not significantly different from the 17.5 mM or Tiron controls (0.89±0.03; vs. 17.5 mM control: 1.06±0.01; vs. 3 mM Tiron: 1.05±0.01). Against supplementation to 150 mM, Tiron offered 97% protection (Tiron + 150 mM; 1.43±0.01; vs. 17.5 mM control 1.06±0.01). This was a significant ($p \leq 0.001$) increase in qPCR efficiency compared to the 150 mM control (Tiron + Glucose 150 mM: 1.43±0.01; vs. 2.07±0.04).

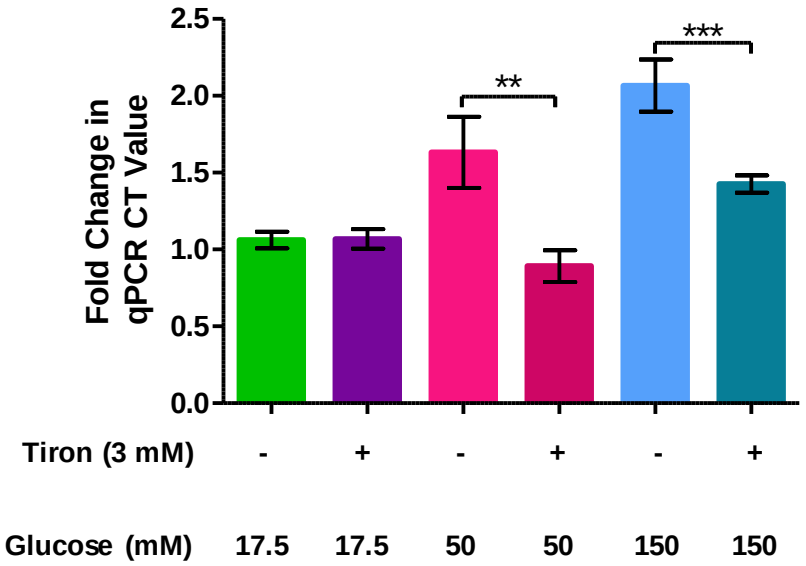

Figure 3.17. *The inhibition of glucose-induced mtDNA strand break formation with Tiron (3 mM) pre-treatment. Tiron offered significant protection against 50 and 150 mM glucose–induced mtDNA strand breaks in HKC-8 cells. **, *** p≤0.01, 0.001 significant difference with vs. without tiron supplementation at each glucose concentration; n≥6; mean±SEM, two-way ANOVA with Bonferroni correction.*

3.3.6.3 The Effect of Tiron on Glucose-Induced ROS Production

In agreement with Figure 3.14, supplementation of glucose to 50 and 150 mM significantly increased fluorescence intensity and intracellular ROS measured by the DCFDA assay (Glucose 50 mM: 140±1.79%; 150 mM: 227±19.03%; vs. control (17.5 mM glucose): 100±4.77%) (Figure 3.18). Tiron pre-treatment significantly ($p \leq 0.001$) reduced this compared to the respective glucose control (Tiron + Glucose 50 mM: 81±1.95% vs. 140±1.79%; + 150 mM: 116±2.34% vs. 227±19.03%).

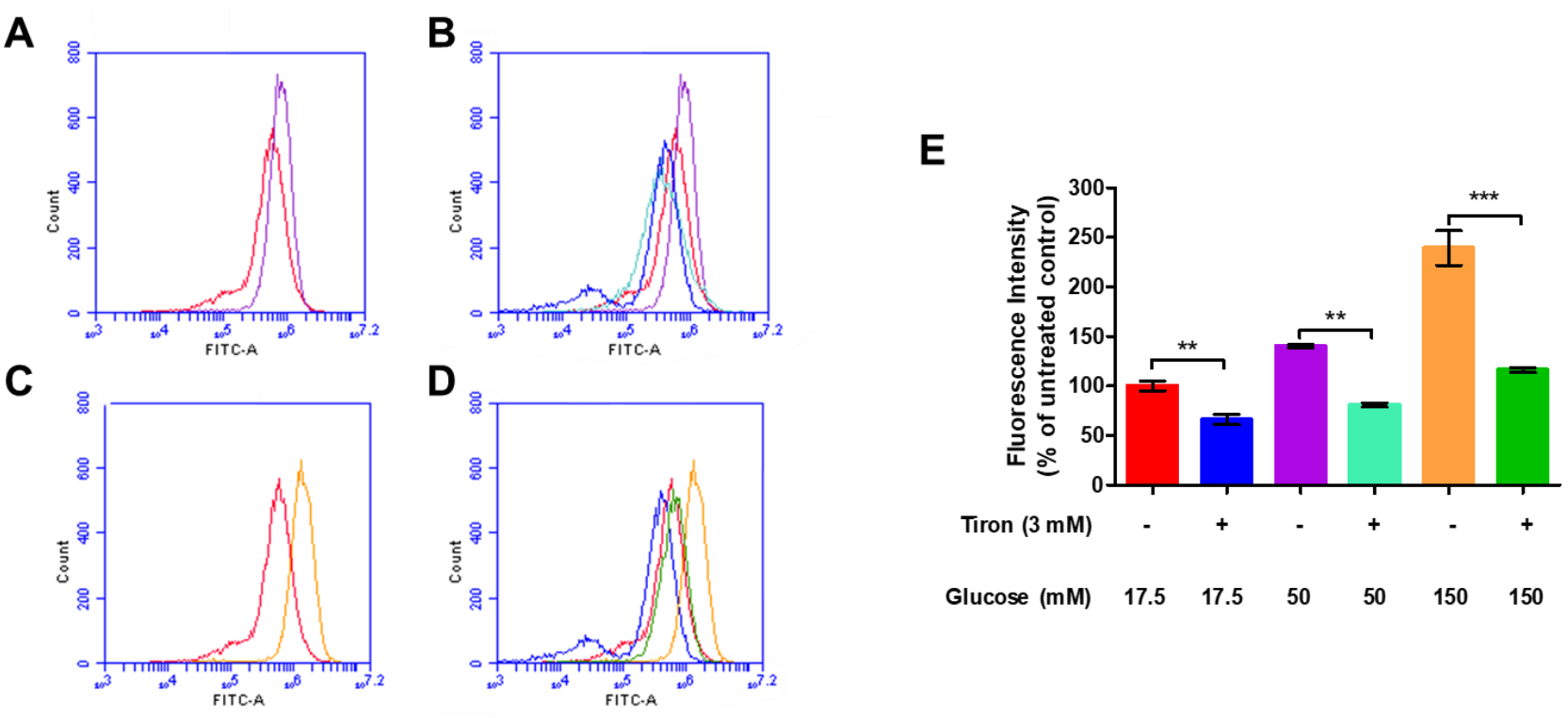

Figure 3.18. *The effects of Tiron (3 mM) on glucose-induced intracellular ROS production (50 and 150 mM) measured with DCFDA by flow cytometry. (A) 50 mM Glucose (purple) significantly increases ROS compared to the control (17.5 mM glucose - red). (B) Pre-treatment with Tiron (aqua) significantly reduced fluorescence intensity with glucose supplementation (50mM final concentration) vs. Tiron only control (blue). (C) 150 mM total glucose (orange) induced significant ROS compared to the 17.5 mM control (red). (D) Tiron pre-treatment (green) significantly reduced 150 mM glucose-induced ROS. (E) Pre-treatment with Tiron significantly reduced glucose-induced DCFDA fluorescence in HKC-8 cells. Colours displayed relate to Figures 3.18A-D. **, *** p≤0.01, 0.001 significant difference with vs. without tiron treatment at each glucose concentration; n=6; mean±SEM, two-way ANOVA with Bonferroni correction*.

3.3.6.4 Glucose-Induced MDA Increase and Tiron Pre-treatment

Following both 50 mM and 150 mM glucose treatment for 24 hours, MDA concentration was significantly ($p \leq 0.001$) increased (Glucose 50 mM: 331±9.21%; 150 mM: 443±10.09%; vs. control (17.5 mM): 100±0.63%) (Figure 3.19). Pre-treatment with Tiron significantly ($p \leq 0.001$) inhibited MDA elevation compared to the relevant glucose control. As seen in Figure 3.9, Tiron had a significant ($p \leq 0.001$) effect but was unable to return MDA concentrations to baseline levels (Tiron + Glucose 50 mM: 164±10.20%;+ 150 mM: 247±5.37%; vs. 100±0.63%).

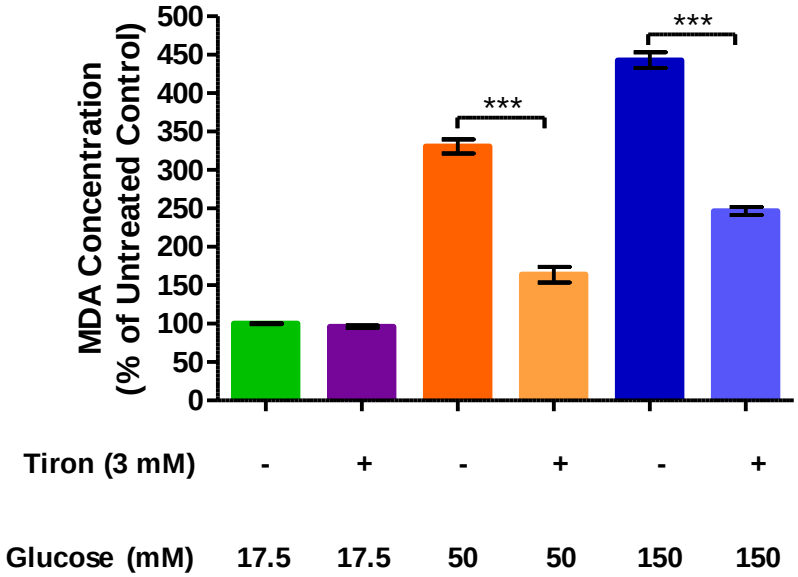

Figure 3.19. *The reduction of glucose-augmented MDA concentrations with Tiron. Tiron offered protection against glucose-induced lipid peroxidation in HKC-8 cells through the measurement of the MDA biomarker. *** $p \leq 0.001$ significant difference with vs. without Tiron supplementation at each glucose concentration; n=6; mean±SEM, two-way ANOVA with Bonferroni correction.*

3.3.7 The Effect of Co-Treatment with Tiron on Glucose-Induced ROS Damage

HKC-8 cells supplemented to a concentration of 25 mM glucose for 72 hours demonstrated a significant reduction in cell viability compared to the control (17.5 mM glucose), measured with the TOX-8 and SRB assays (63±1.83% vs. 100±1.91%) (Figure 3.20). As was observed in Figure 3.11, treatment with 3 mM Tiron for 72 hours significantly reduced cell viability alone (70±2.40% vs. 100±1.91%). Concomittant treatment of glucose and Tiron did not significantly preserve cell viability compared to glucose treatment alone (64±1.83% vs. 63±1.83%). These results were confirmed with the SRB assay (Glucose 25 mM: 61±0.69%; 25 mM + Tiron: 61±0.76%; 3 mM Tiron: 66±0.90%; vs. control: 100±0.93%).

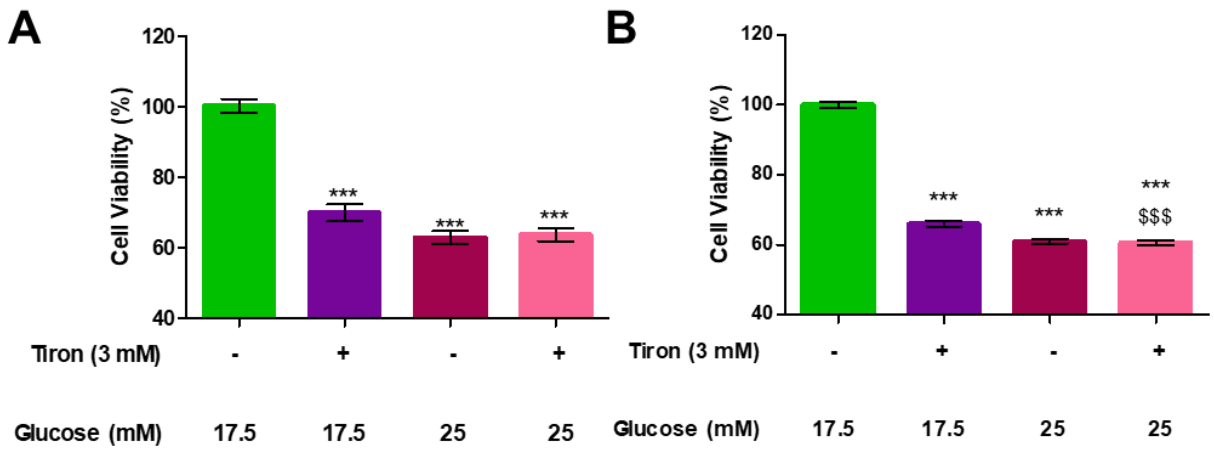

Figure 3.20. *The effect on cell viability of prolonged culture for 72 hours with glucose (25 mM) and Tiron (3 mM). Treatment of both Tiron and glucose significantly reduced cell viability in both the TOX-8 (A) and SRB (B) assays. *** p≤0.001 vs. 17.5mM glucose control; $$$ p≤0.001 vs. Tiron; n=9 from 3 independent experiments; mean±SEM, one-way ANOVA with Tukey post-hoc.*

To explore the possibility of Tiron being incorporated into a therapeutic treatment regime, the conditions outlined above were assessed through qPCR with the mtDNA strand break assay. Presented in Figure 3.21, 72-hour treatment with 25 mM glucose significantly ($p \leq 0.001$) increased the CT value compared to the control (17.5 mM) (2.54±0.08 vs. 1.03±0.03). Concomitant treatment with Tiron led to a significant increase in qPCR efficiency compared to 25 mM glucose treatment (0.53±0.01 vs. 2.54±0.08). Daily Tiron replenishment for 72 hours led to a significant fold change in qPCR CT value compared to the control (0.53±0.01 vs. 1.03±0.03).

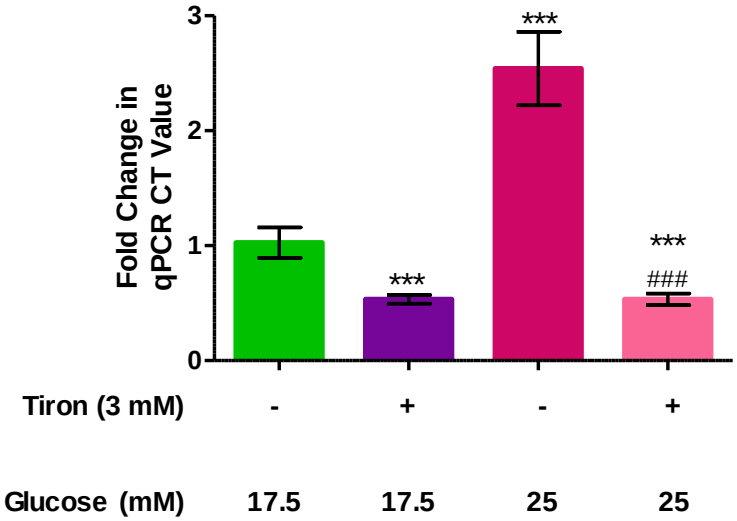

Figure 3.21. *The effects on mtDNA strand break formation with 72-hour treatment of both glucose (25 mM) and Tiron (3 mM) singularly and concomitantly. Tiron administered with 25 mM glucose for 72-hours offers protection against mtDNA strand breaks in HKC-8 cells. *** $p \leq 0.001$ vs. 17.5 mM glucose control; ### $p \leq 0.001$ vs. glucose 25 mM; n=9; mean±SEM, one-way ANOVA with Tukey post-hoc.*

3.4 Discussion

In this chapter, Tiron's protective capacity was initially observed against direct ROS application with H_2O_2. This was evidenced by the recovery of cell viability (Figure 3.6), significant protection against mtDNA strand break formation (Figure 3.7) and reduction of lipid peroxidation (Figure 3.9) following Tiron pre-treatment. Further to this, exposure to Tiron 24 hours prior to H_2O_2 treatment led to a reduction in intracellular ROS production as indicated by the oxidisation of DCF (Figure 3.8) and suggested that pre-treatment with Tiron can reduce the availability or generation of ROS required to perpetuate lipid peroxidation, induce mtDNA damage and compromise cell survival. This level of protection supports the findings of *Oyewole et al.* (2014), who found Tiron, also at 3 mM, provided a high degree of protection against 200 µM H_2O_2-induced damage in human dermal fibroblasts (HDFn).

Following confirmation that Tiron could elicit high levels of protection against direct ROS addition in HKC-8 cells, the use of Tiron was applied to a model of high glucose-induced ROS and associated damage, as has been observed in DM, to explore its protective effects in a clinically relevant model. As discussed previously, ROS have been implicated within DM and lead to cell dysfunction as well as secondary complications (Rains and Jain 2011). Additionally, a decreased concentration of endogenous antioxidants including SOD and catalase were identified in T2D patients, indicating the opportunity for antioxidant supplementation (Arif et al. 2010). For example, the endogenous antioxidant coenzyme Q has been found to be deficient in T2D and has been correlated with insulin resistance as well as the susceptibility to oxidative damage (Zhang et al. 2018, Hernández-Camacho et al. 2018). Treatment with MitoQ, a mitochondrially targeted variant of coenzyme Q, has shown a reduction of oxidative stress in leukocytes from T2D patients (Escribano-Lopez et al. 2016). Contrary to this, coenzyme Q supplementation has failed to reduce MDA and C-reactive protein levels, the latter of which is a biomarker of inflammation, despite improving blood glucose levels (Mezawa et al. 2012). This study evaluated supplementation within T2D patients with established and varied treatment regimens including statins (6 of 9 patients) and glucosidase inhibitors, which have been shown to reduce lipid peroxidation (Mezawa et al. 2012, Moon et al. 2014, Hadi et al. 2015, de Souza Bastos et al. 2016). Therefore, whilst the aim of the study was to investigate the clinical effects of coenzyme Q, the primary objective of improving glycaemic control in these patients was successful (Mezawa et al. 2012).

Whilst DM glycaemic control is fundamental to preventing secondary complications, this study did not evaluate the levels of coenzyme Q, which may have been improved in line with the deficiencies observed in T2D patients (Zhang et al. 2018, Hernández-Camacho et al. 2018) and indicated whether supplementation may have improved the defence against oxidative

stress outside of the biomarkers measured within the study. Such reduction in oxidative stress with supplementation may further prevent or delay the development of secondary diabetic complications as raised coenzyme Q levels have been shown to be protective in diabetic retinopathy (Ates et al. 2013) and reduce diabetic neuropathy symptoms in a murine model (Shi et al. 2013). Furthermore, the small sample size of patients within this study could have impacted the data, evident by the detection of some improvement in MDA levels but not significantly. In support of this, a trial of 50 patients demonstrated decreased plasma MDA as well as AGEs (Gholnari et al. 2017). However, similar results have been found in a larger T2D patient study, where coenzyme Q improved glycaemic control but had no significant effect on cholesterol, whilst another study in T2D patients also demonstrated no significant reduction in MDA levels with 8-week coenzyme Q supplementation (Zahedi et al. 2014, Moazen et al. 2015). As a result, it could be argued that measurement of lipid profile associated with glycaemic control does not reflect the reduction of oxidative stress with coenzyme Q despite evidence *in vitro* and in T1D rat models demonstrating increases in SOD and catalase as well as decreases in ROS levels (Modi et al. 2006, Tsuneki et al. 2007). In line with this, coenzyme Q supplementation over 12 weeks has shown improvements in total antioxidant capacity in T2D patients (Akbari Fakhrabadi et al. 2014), whilst patients with coronary artery disease experienced a decrease in MDA and increase in catalase and SOD activity (Lee et al. 2012). These studies indicate the potential role for coenzyme Q in oxidative stress reduction, which requires more clinical exploration and illustrates the need for evaluation of multiple oxidative stress related biomarkers, including lipid peroxidation, DNA damage, ROS-mediated inflammation and protein oxidation, to fully evaluate any potential therapeutic application. As a result, there is an opportunity to investigate the use of other exogenous supplements, such as Tiron, across multiple parameters to reduce oxidative stress associated with high glucose and oxidative stress in diabetic models, supported by the results demonstrating Tiron's efficacy in reducing mtDNA damage and lipid peroxidation caused by H_2O_2 exposure (Figures 3.6-3.9). *In vivo* research has already demonstrated that Tiron does not impair the efficacy of diabetic therapies whilst also reducing ROS production, indicating that it could be used as an adjunctive agent and may reduce the incidence of secondary complications (Domingo et al. 1992, Cheng et al. 2010).

Prior to examination of the effects of Tiron, concentrations of high glucose were optimised to induce both significant ROS induction as well as mtDNA strand break damage (Figure 3.14). This optimisation ranged from 25-150 mM and aimed to induce a qPCR fold change comparable to levels observed directly with H_2O_2 exposure. In fulfilment of this, it was found that whilst significant ROS was produced at 25 mM ($p \leq 0.001$) (Figure 3.14), 50 mM was required to induce significant ($p \leq 0.001$) mtDNA strand break damage (Figure 3.15). Oxidative

DNA damage has been identified within T2D patients in a 2012 study, which found that DNA damage within peripheral blood mononuclear cells (PBMC) of T2D patients was significantly higher than healthy controls, indicating an opportunity to reduce the incidence of damaged DNA (El-Wassef et al. 2012).

However, even at 150 mM, it was not possible to induce mtDNA strand break damage to the same extent as had been observed with H_2O_2, the aim of using such a concentration and despite cell viability being reduced to similar levels as Figure 3.4 (Figure 3.16). Published literature has also used higher concentrations of glucose to observe inhibition of cell compromise with test compounds, including *Rosmarinus officinalis* extract or thymoquinone, in models of diabetic neuropathy with decreases in cell viability observed with glucose similar to that contained within this thesis (Chen et al. 2016, Rashidipour et al. 2017). However, the need for a higher glucose concentration could be due to the mechanism by which ROS are formed following exposure to hyperglycaemia, it could also be suggested that 24-hour induction of ROS by glucose is not sufficient to generate ROS levels comparable to that observed with H_2O_2. The need for a longer period of exposure to high glucose has been shown by *Busik et al.* (2008), who demonstrated increased mitochondrial $O_2^{\cdot-}$ and DCF oxidation with prolonged incubation of glucose (25 mM) greater than 24 hours in human retinal epithelial cells. Therefore, the effect of Tiron could be assessed with a greater period of glucose exposure to understand if ROS generation could reach levels comparable to H_2O_2 and whether Tiron could provide the same high degree of protection. However, the absence of protection against 150 mM induced mtDNA strand breaks (97%) comparable to observed with 50 mM would indicate that Tiron may not be as efficient in ameliorating higher glucose-induced ROS levels.

Nevertheless, pre-treatment of HKC-8 cells with Tiron (3 mM) led to significant ($p \leq 0.001$) amelioration of glucose-induced damage, evidenced in the protection elicited against mtDNA strand break formation (Figure 3.17), as well the reduction of lipid peroxidation by 50% (50 mM) and 44% (150 mM), measured through MDA concentration (Figure 3.19). As previously observed with H_2O_2, the protection demonstrated against oxidative damage by Tiron was due to its ROS reducing action, evidenced by a decrease in DCF fluorescence intensity compared to the untreated control (Figure 3.18). Pre-treatment with Tiron led to a reduction of ROS induced by both 50 and 150 mM glucose treatment to 81 and 116% respectively, compared to 140 and 227% of ROS production respectively, when compared to the untreated control. Similar results have been observed by *Leloup et al.* (2009) with Trolox where treatment of islet cells with glucose (16.7mmol/l) for 30 minutes increased ROS production 3-fold. This was reduced with increasing concentrations of Trolox, as observed similarly in Figure 3.18 with

Tiron treatment (Leloup et al. 2009). Furthermore, treatment of high glucose incubated myocardial (H9c2) cells with the scavenger edaravone, demonstrated a reduction in ROS and cell death, with preservation of cell viability measured by MTT showing similar results to those obtained in this thesis with Tiron (Ji et al. 2016). For example, Tiron pre-treatment preserved viability at 83% when treated with 50 mM glucose, leading to a reduction of 72% compared to the results found by *Ji et al.* (2016) (Figure 3.16), where high glucose induced a decrease in cell viability of approximately 25%, versus 10% with edaravone treatment. Therefore, and in accordance with published literature, the reduction of glucose-induced damage is correlated with the amelioration of ROS as a result of Tiron supplementation (Figure 3.18). This finding directly builds upon findings of *Cheng et al.* (2010) who found Tiron to significantly reduce high glucose induced ROS in canine renal cells and streptozotocin T1D rats. In the rat model, Tiron's reduction of ROS, particularly after 7 days of dosing, indicates good tolerance *in vivo* as well as in multiple species (Cheng et al. 2010). As such, the work contained within this chapter demonstrates the efficacy of Tiron against glucose-induced ROS and associated damage in human cells within an *in vitro* model, with potential for clinical translation.

The inability of Tiron to reduce the ROS produced from 150 mM glucose exposure to baseline correlates with the mtDNA strand breaks measured despite Tiron treatment. This is demonstrated by the reduction of ROS to 116% from 227% with Tiron treatment as well as the reduced protection observed against lipid peroxidation, where MDA concentration was reduced by 44% from 443% (Figure 3.19) Consequently, it can be hypothesised that the availability of ROS exceeds the ROS reducing capability of Tiron in this model, which could warrant a greater length of Tiron pre-treatment for greater protection against the damage induced by high concentrations of glucose. Supporting this, an increase in ROS was measured after 150 mM glucose treatment compared to the untreated control even with Tiron (Figure 3.18). However, modelling the damage incurred following such a high dose of glucose successfully with Tiron, provides accessible modelling of damage that could accumulate from chronic hyperglycaemia but in a short-term assay. Concentrations of this magnitude have been used previously to model the polyol pathway as well as inducing biomarkers of oxidative stress such as inhibition of catalase and induction of the Nrf2/ARE pathway (Suzuki et al. 2004, Sango et al. 2006, Yang et al. 2016, Fouda and Abdel-Rahman 2017). Whilst Tiron was not as protective in this model as observed with H_2O_2 and 50 mM glucose exposure, it demonstrated a significantly ($p \leq 0.001$) high degree of protection and provides evidence for the use of Tiron against glucose-induced oxidative damage against grossly elevated concentrations of glucose. A study in T1D rats showed that treatment with perindopril, an ACE inhibitor, reduced ROS levels and the VEGF to pigment epithelium-derived factor (PEDF) ration, which has been shown to increase retinal damage, demonstrating that reduction of

ROS is of benefit even if levels are not ameliorated to baseline (Zheng et al. 2009). This indicates that although Tiron was not as protective against 150 mM glucose exposure, it could still be of benefit in the prevention of oxidative damage clinically, following *in vivo* modelling, with DNA damage and serum levels of MDA found significantly elevated within T2D patients compared to healthy counterparts, biomarkers of which have been alleviated with Tiron in this chapter (Arif et al. 2010).

Further to this, work is required to understand the role of Tiron within prevention or reduction of secondary diabetic complications. This requires evaluation of Tiron's influence on the polyol and hexosamine pathways as well as the production of AGEs and the activity of PKC. Tiron's modulation of these pathways may contribute to reducing ROS load but also alleviate secondary effects such as lipid accumulation caused by hexosamine pathway flux that may be susceptible to peroxidation (Sage et al. 2010). For example, it has been shown previously that pomegranate extracts have significant antioxidant activity and inhibit rat lens aldose reductase activity, the first enzyme to catalyse the formation of sorbitol, which could lead to the prevention of diabetic retinopathy and reduces sorbitol accumulation (Karasu et al. 2012). Aldose reductase activity is correlated with the production and accumulation of ROS within diabetic tissue. However, ROS interaction with aldehydes later converted to unreactive alcohols leads to an increase in aldose reductase activity, the catalytic enzyme of the reaction, and elevated sorbitol production (Brownlee 2005, Karasu et al 2012, Tang et al 2012). Therefore, it could be hypothesised that considering Tiron's glucose-induced ROS reducing ability (Figure 3.18), it may also influence the activity of aldose reductase as suggested by *Karasu et al.* (2012) with pomegranate extract. Tiron's influence on this pathway could be measured *in vitro* colourimetrically with commercially available kits measuring aldose reductase activity (BioVision, USA) or an ELISA to measure sorbitol dehydrogenase activity (Abcam, UK), an enzyme closer to the formation of sorbitol within the polyol pathway which has been shown to be reduced with vitamin E supplementation *in vivo* (Chan et al. 2008). Evaluating whether Tiron has an effect on the activity of these enzymes may indicate how Tiron elicits its reduction of oxidative damage, potentially through the reduction of aldose reductase activity and reducing the accumulation of sorbitol.

Any reduction of aldose reductase or sorbitol dehydrogenase could consequently decrease the accumulation of sorbitol as well as additional ROS formation, which would lead to a reduction in oxidative damage as well as complications such as retinopathy or vascular disease, both of which have been attributed to elevated sorbitol concentrations (Berrone et al. 2006, Williams and Ido 2012, Rhee and Kim 2018). Aldose reductase inhibitors, such as Epalrestat, are already widely used for diabetic complication management and have shown

improvements in diabetic nephropathy symptoms (Sharma and Sharma 2008, Hotta et al. 2012). Reduction of aldose reductase by resveratrol has already been shown *in vivo* and attributed to ROS reduction and Nrf2 pathway induction, the latter of which has been demonstrated with Tiron in this thesis and provides good premise for Tiron's potential modulation of the polyol pathway (Palsamy and Subramanian 2011). Supporting this, cinnamic acid has reduced ROS and MDA concentrations alongside decreased sorbitol in high glucose cultured H9c2 cells (Anupama et al. 2018). Tiron has previously demonstrated a reduction in aldose reductase activity within isolated ischaemic Sprague-Dawley rat hearts correlated with sorbitol concentration, indicating the potential action of Tiron in this pathway (Kaiserova et al. 2006). However, whilst *in vitro* investigation would directly evaluate Tiron's influence on this pathway, it would be better to observe this in multicellular or *in vivo* models such as streptozotocin-induced DM to model the downstream and longitudinal effects, including retinopathy or cardiovascular complications, and whether Tiron's influence on these pathways and other oxidative stress biomarkers such as ROS or lipid peroxidation are observed and whether prevention through modulation of these pathways is possible. For example, aldose reductase inhibition, such as could be achieved with Tiron, has been shown to attenuate cardiac ischaemia and failure *in vivo* (Calderone et al. 2010, Son et al. 2012).

Furthermore, the antioxidant α-cyano-4-hydroxycinnamic acid has demonstrated a reduction in the hexosamine pathway in hyperglycaemic (33 mM) rat cardiomyoblasts and was associated with ROS amelioration (Rajamani and Essop 2010). Similarly, Benfotiamine, an antioxidant supplement, has been shown to modulate the hexosamine pathway, measured by hexosamine pathway end-product UDP-GlcNAc (Buse 2006), intracellular AGE as well as PKC activity within an *in vivo* diabetic model (Hammes et al. 2003). As such, there is potential to evaluate Tiron in these pathways, which may elicit a pathway activity reducing effect when considering the amelioration of ROS evidenced with Tiron in figures 3.8 and 3.18. As indicated above, it would advantageous to model the effects of Tiron on hyperglycaemia-associated pathways in a multicellular or *in vivo* diabetic model by measuring multiple end-products such as AGEs or UDP-GlcNAc, which can be done fluorescently as described by *Namboori and Graham* (2008) or by ELISA (de Vos et al. 2016). Supporting this, Tiron has already been shown to reduce AGE formation following 2 weeks of dosing streptozotocin-induced T1D rats with 300mg/kg (Jiang et al. 2017). AGEs have been shown to increase vascular complications within T1D and T2D patients and therefore reduction of these, particularly modelled *in vivo*, could reduce secondary complications additional to Tiron's role in protecting against oxidative damage and reducing ROS and warrants further investigation before clinical expansion (Giacco and Brownlee 2010, Rhee and Kim 2018). However, Tiron has shown little influence on glycaemic control, the instigator of the activation of these pathways (Domingo et al. 1993).

Therefore, Tiron's influence on these pathways may only be applicable when activity is increased, such as during hyperglycaemia, and would only be in a modulator capacity rather than preventative. As such, more study is required within both diabetic and non-diabetic models to observe Tiron's influence on these pathways and whether it can be used therapeutically to reduce enzymatic activity, such as with aldose reductase, or the formation of damage inducing end-products such as UDP-GlcNAc and if this is warranted additional to suitable glycaemic control or if use is only suitable for secondary complications. This is an important consideration for Tiron's future in the treatment of DM within these pathways.

As highlighted above, Tiron pre-treatment did not offer reduction of lipid peroxidation to baseline in both the glucose and H_2O_2 ROS models. It could be suggested that increases in MDA perpetuate lipid peroxidation through the formation of other lipid radicals, contributing to a further increase in MDA concentration that cannot be adequately managed by Tiron pre-treatment (Coskun et al. 2005). The generation of other lipid radicals not measured in this thesis could contribute to protein oxidation as well as enzymatic damage. Previously, lipid peroxidation has been found to lead to mitochondrial dysfunction and facilitate electron leakage from the ETC, increasing the generation of ROS (Coskun et al. 2005, Kwiecien et al 2014). In support of the reduction of MDA by antioxidant supplementation, a 2011 study supplemented T1D children with vitamin E and found that MDA were significantly decreased after a three month course (Gupta et al. 2011). Furthermore, a 2017 study also studied the effect of the same 3 month supplementation on MDA concentrations in diabetic adults, finding it to significantly decrease with supplementation and demonstrating the active study of reducing this biomarker in DM patients (Chatziralli et al. 2017). Therefore, the study of Tiron's effects on MDA is in keeping with the current clinical interest in reducing this biomarker and the results demonstrated efficacy in its reduction, which could be applicable within the clinic when considered with the evidence that Tiron can significantly decrease metal toxicity induced MDA increases in rats *in vivo* (Morgan et al 2018).

However, considering the results in Figure 3.8 and 3.18 where pre-treatment with Tiron significantly reduced ROS levels in HKC-8 cells in all models of ROS generation, it is unlikely that excess ROS production is the cause of Tiron's inability to offer greater levels of protection against MDA concentration elevation. It can be postulated that the formation of other lipid radicals diverts Tiron's action away from decreasing the availability of MDA alone. It has been identified that MDA contributes to the formation of lipid hydroperoxides which, whilst more stable than other ROS, can potentiate lipid peroxidation during propagation stages and exacerbate oxidative stress, elevating MDA concentrations further (Ayala et al. 2014). As a result, Tiron could inadvertently reduce ROS produced by MDA rather than decreasing MDA

concentrations, rendering it less ‘protective’ as observed in this study. Elevated lipid hydroperoxides have been found to be in T2D patients compared to healthy controls (Patel et al. 2015). Unfortunately, due to the limiting non-specificity of the DCFDA assay, the type of ROS or radicals produced was not distinguishable, therefore the formation of other radical products was not apparent from the results of Figure 3.8 and 3.18. Accordingly, it would be beneficial to study Tiron’s effect on other biomarkers of lipid peroxidation as well as other ROS types to understand if Tiron is preferentially targeting ROS, or whether it is not as protective against products of lipid peroxidation. Lipid peroxidation within DM has been positively correlated with the development of DKD and has also been associated with systemic inflammation, evidenced by elevation in cytokines, regardless of how sufficiently DM is managed by patients (Kumawat et al 2013, de Souza Bastos et al 2016). Therefore, the use of Tiron to reduce lipid peroxidation, as evidenced in this chapter, could be advantageous in reducing the development of DM secondary complications and to understand how protective this is across other biomarkers of lipid peroxidation could inform Tiron’s incorporation into a DM treatment regime and whether this may also influence the inflammation observed by *de Souza Bastos et al.* (2016).

Resveratrol has been shown to offer a high degree of protection against MDA when supplemented for 2 weeks *in vivo*, resulting in an additional increase in endogenous antioxidants such as SOD (Schmatz et al. 2012). Thus, it could be suggested that Tiron treatment for 24 hours is not sufficient to minimise lipid peroxidation and associated damage. As such, it would be pertinent to study the effect of prolonged Tiron treatment on the prevention of lipid peroxidation. Studies have shown that MDA is increased by up to 167% in T2D patients compared to healthy controls, these increases are shown to contribute to the progression of DM and secondary complications; contributing to the stiffening of cardiovascular collagen, resulting in cross-link formation and collagenous tissue and contributing to the development of cardiovascular disease (Calderon-Salinas et al. 2011, Snedeker and Gautieri 2014, Dunér et al. 2015). For this reason, the use of supplements such as Tiron to reduce MDA, even by half as shown in this chapter, could prevent secondary cardiovascular disease, a complication found to be reduced by 42-46% with adequately controlled T1D and T2D in a Swedish study (Rawshani et al. 2017). Understanding the protection demonstrated by Tiron against lipid peroxidation could also widen its application outside of the treatment of DM towards the treatment of cancers or preventative actions, following the finding that MDA is elevated in individuals at higher risk of developing oral cancer compared to healthy individuals (Chole et al 2010).

Throughout this chapter, the question has arisen as to what effect prolonged supplementation may have on Tiron's efficacy against oxidative stress. Initially, it was shown that prolonged treatment of HKC-8 cells with Tiron led to significant ($p \leq 0.001$) reduction in cell viability to 70% after 48- and 72-hour treatment with Tiron (Figure 3.11). This indicated that prolonged treatment was detrimental when supplemented in healthy cells, with higher concentrations of Tiron for 24 hours already shown to reduce cell viability (Figure 3.2). Exogenous supplements have previously been shown to induce adverse effects independent of their antioxidant effects, including in possum renal proximal tubular cells where MitoQ induced acute mitochondrial swelling and indicating that new supplements require detailed scrutiny to understand their mechanism of action as well as any potential side-effects (Gottwald et al. 2018). Whilst negative effects of antioxidants have been shown in carcinoma cell lines, arguably a beneficial consequence such as instigating apoptosis in melanoma cells with NAC treatment (Yang et al. 2007), the compromise of 'normal' cell viability from prolonged antioxidant exposure leads to trepidation about its use as a preventative supplement.

However, Tiron has not demonstrated overtly toxic side effects previously, with it being shown on more than one occasion to have anti-apoptotic effects and supported by the high estimation of Tiron's LD50 in this cell line (Figure 3.2) (Kobayashi et al. 2005, Yang et al. 2007). In support of this, when HKC-8 cells were challenged with 5 mM H_2O_2, Tiron pre-treatment preserved cell viability to 63% compared to 49% in the H_2O_2 control, offering mild protection against ROS (Figure 3.11). When HKC-8 cells were treated with Tiron for 72 hours as well as glucose (25 mM) with daily replenishment in a model of Tiron use in poorly managed DM, Tiron also preserved mtDNA. This supports the hypothesis that despite eliciting decreases in cell viability with Tiron treatment alone, there was a provision of protection. The preservation of cell viability and mtDNA in this model indicates that daily supplementation of Tiron could be beneficial even where DM is insufficiently managed, whether this is influenced by diet or other factors, and suggests that incorporation within a treatment regime could result in a degree of protection against glucose-induced damage, reducing the risk of cellular dysfunction or the development of secondary complications. *Kapoor and Kakkar* (2012) also found that pre-, co- and post-treatment of rat hepatocytes exposed to high glucose (40 mM) with the flavonoid Morin significantly protected against ROS generation and apoptosis. These findings support the protection demonstrated by Tiron against high-glucose induced oxidative stress and highlight that antioxidant supplementation, regardless of timing, could be beneficially used to combat high glucose-mediated damage. The protective response of daily supplementation of Tiron into cells of human origin adds to published research where Tiron has been incorporated in murine and rat models with oxidative stress ameliorating effects and a reduction of

nephrotoxicity, demonstrating that Tiron could be a beneficial adjunctive therapy in reducing high glucose associated oxidative damage (Domingo et al. 1997, Morgan et al. 2017, 2018).

Figure 3.21 also found a significant ($p \leq 0.001$) increase in qPCR efficiency compared to the untreated control, indicating an enhanced protection against ROS, which could also occur from physiological processes. This level of ROS reduction corresponds to the increase in Nrf2 protein expression observed in Figure 3.13, where cells treated with Tiron for 48 and 120 hours demonstrated a significant ($p \leq 0.001$) increase in expression, indicating activation of the Nrf2/ARE pathway. *Oyewole et al.* (2014) previously hypothesised that Tiron's mechanism of action was independent of the Nrf2/ARE pathway following UVR exposure through an absence of hemeoxygenase-1 induction, a biomarker of Nrf2 activation, compared to Nrf2 inducing antioxidants curcumin and Resveratrol (Oyewole et al. 2014). This was true for the results obtained following Tiron treatment for 24 hours in HKC-8 cells (Figure 3.10).

On the other hand, this was not observed with 48 and 120 hours of treatment and could be due to its proposed production of a reactive semiquinone by-product (Taiwo 2008, Rodacka et al. 2014). Production of a reactive by-product from Tiron's action was eluded to in Figure 3.12, where treatment of Tiron for more than 48 hours saw a significant ($p \leq 0.001$) increase in ROS generation. However, when challenged with H_2O_2, used as a known inducer of Nrf2 expression rather than glucose which whilst an activator of Nrf2 has been correlated with downregulation as a result of high concentrations and diabetic complications such as peridonitis (Zhou et al. 2015, Sima et al. 2016, Li et al. 2018, Sireesh et al. 2018, Oh et al. 2019), Tiron demonstrated a reduction in ROS compared to the H_2O_2 control and indicating a level of ROS reduction. The generation of ROS in this manner provides an explanation for the resultant increase in Nrf2 protein expression in Figure 3.13, indicating that treatment with Tiron for more than 48 hours led to inhibition of Nrf2 ubiquitination by Keap1 and resulting in a potential increase in the endogenous antioxidant defence, through translation of Nrf2 targeted genes. As such, induction of the antioxidant response could provide an explanation for the enhanced qPCR efficiency identified in Figure 3.21.

Activation of the Nrf2 pathway through exogenous antioxidant supplementation has been previously shown in murine mesangial cells exposured to high-glucose (Li et al. 2011). *Li et al.* (2011) found that modulation of Nrf2 expression reduced ROS and MDA concentrations as well as transforming growth factor β1 (TGF-β1), which has been implicated heavily in the development of diabetic nephropathy. It could be suggested that Tiron may elicit the same response to TGF-β1 when considered alongside the successful modulation of ROS and MDA as well as induction of Nrf2 seen within this chapter in HKC-8 cells, findings correlating with *Li et al.* (2011), and whether this impacts the development of diabetic complications. Tiron's

influence on Nrf2 protein expression could also reduce the incidence of insulin resistance. A study in adult cardiomyocytes found that H_2O_2 treatment contributed to insulin resistance but also that insulin increased the transcriptional activity of Nrf2, which was subsequently reduced by an insulin resistance promoting PI3K inhibitor (Tan et al. 2011). Furthermore, Nrf2 activation has been evidenced *in vitro* to confer endothelial cell protection in hyperglycaemic environments, which can reduce the risk of atherosclerosis and has been shown also to reduce cardiomyopathy in a murine T2D model (Zakkar et al. 2009, He et al. 2011, Gu et al. 2017, Matzinger et al. 2018). Therefore, this indicates that Tiron may improve DM and insulin sensitivity through its influence on the Nrf2 pathway addition to ameliorating ROS to reduce oxidative damage and secondary complications such as atherosclerosis, which warrants further modelling *in vitro* and *in vivo*.

Whilst the target of antioxidants is to minimise oxidative stress and damage, it has been shown in *Drosophila* that overexpression of glutathione peroxidase inhibits hematopoietic progenitor cell differentiation; whilst reduction of SOD2 led to an increase in differentiation (Owusu-Ansah and Banerjee 2009). This cements the notion that whilst ROS are damage- and disease-inducing, they also play a beneficial role in signalling and maintaining cell viability. Accordingly, it is important to conduct extensive studies to explore off-target and unexpected toxicities, as highlighted by *Gottwald et al.* (2018). Consequently to elucidate Tiron's interaction with the Nrf2 pathway further, it would be imperative to evaluate the response of Tiron supplementation on down-stream signalling molecules within the ARE pathway as well as the influence on endogenous antioxidant concentration following pathway induction. Nevertheless, Tiron's induction of Nrf2 protein expression is an important finding for the widening application of Tiron.

Unfortunately, studies have suggested that Tiron has no effect on reducing blood glucose levels, a characteristic that has been identified in a 2003 study where supplementation of T1D rats with vitamin E or the cholesterol targeting antioxidant probucol had no influence on glucose concentrations (Koya et al. 2003, Cheng et al. 2010). Nevertheless, the results presented in this chapter support the use of Tiron within a clinically relevant model to reduce high glucose-induced oxidative damage through the direct reduction of mtDNA strand breaks and MDA concentration, both of which have been identified as biomarkers within DM patients (Giacco and Brownlee 2010). Accordingly, research must now be directed towards understanding potential interaction or efficacy compromises of Tiron incorporation into a glucose management therapeutic regimen in DM treatment. Currently, Tiron has not been shown to ablate the efficacy of metformin or the insulin mimetic effects of vanadate, providing

good premise for further therapeutic application (Domingo et al. 1992, Domingo et al. 1993, Cheng et al. 2010).

To conclude, the results presented in this chapter demonstrate the protective action of Tiron against both H_2O_2 and high glucose mediated ROS damage and demonstrating the specific amelioration of oxidative damage, including mtDNA strand breaks and lipid peroxidation, in HKC-8 cells. This protection was obtained through pre-treatment with Tiron for 24 hours as well as the supplementation of Tiron at the time of ROS induction, supported by a decrease in intracellular ROS production. This adds to the knowledge of Tiron's protective capabilities within a human cell line outside of the previously used skin and cancer cell models. It was also shown that prolonged exposure to Tiron could result in an increase in Nrf2 expression as a result of Tiron-induced ROS production, which could lead to the modulation of insulin resistance with further investigation. The development of a hyperglycaemia mimicking *in vitro* model, optimised for HKC-8 cells within this thesis, permitted the early evaluation of Tiron as a potentially therapeutic compound in the treatment of diabetes with positive results. Accordingly, the results presented in this chapter provide direction for future experimentation to understand Tiron's role within the amelioration of hyperglycaemia-induced damage, such as Tiron's influence on the polyol pathway and if this can be utilised to reduce the development of secondary complications as well as insulin sensitivity before translation *in vivo*.

Chapter 4 – The Effect of Tiron in Human Bronchial Epithelial Cells

4.1 Introduction

4.1.1 Oxidative Stress within Asthma and COPD

The lungs are vulnerable to oxidative stress due to both their function and exposure to environmental particulates and allergens (Park et al. 2009, Chan et al. 2017). Whilst other tissues are also readily exposed to oxidative stress, this is pre-emptively managed by endogenous mechanisms as highlighted by *Smith et al.* (2007), *Fukai et al.* (2011) and *Bandeira et al.* (2012). However, chronic exposure of the lungs to exogenous pollutants or allergens can lead to the accumulation of oxidative damage alongside the induction of inflammation, exacerbating oxidative stress, which can overwhelm the endogenous defence mechanisms (Li et al. 2003, Gowdy et al. 2010). Evidence to this, ROS have been identified within the lung as both environmentally stimulated and cell-mediated, such as through the induction and prolongation of inflammation (Waris and Ahsan 2006, Bolisetty and Jaimes 2013). This has been attributed to the development of chronic conditions such as asthma and COPD, which have also been correlated with an increase in ROS and decrease in cellular antioxidants within the lung leading to cellular damage and diminished lung function (Kirkham and Rahman 2006, Birben et al. 2012, Wiegman et al. 2015, Bathri et al. 2017).

As indicated in section 1.4.2, the occurrence of symptoms such as airway hyperactivity and chronic inflammation in asthma has been attributed to an increase in inflammatory cell activation, leading to an oxidative or respiratory burst (Sahiner et al. 2011, Nadeem et al. 2014). This leads to the acute release of ROS from cells, including macrophages and neutrophils, following increased uptake of O_2. Resultant generation of ROS in this manner is self-propagating leading to increased $O_2^{\cdot -}$ and H_2O_2 and the additional recruitment of cytokines, which without adequate ROS reduction by antioxidants results in the accumulation of oxidative damage, resulting in compromised organ functionality (Wedes et al. 2009, Nadeem et al. 2014).

In both asthma and COPD, it has been suggested that the excess ROS production is correlated with the severity of symptoms and disease, which has given rise to the suggestion that symptoms are not only associated with airway inflammation but also the oxidative burst and identifies a need for intervention such as with antioxidant supplementation (Louhelainen et al. 2008, Castaneda et al. 2017, Bajpai et al. 2017). Unsurprisingly, a reduction in

endogenous antioxidant availability has been identified within asthma and COPD patients, supporting the presence of oxidative stress but also highlighting the deficit of antioxidants available to reduce ROS and the associated damage (Yao et al. 2010, Rahman 2012, Elmasry et al. 2015, Qu et al. 2017).

Subsequently, as the incidence of chronic pulmonary conditions is growing, there is a need for investigation into strategies to reduce the severity of symptoms and inhibit the accumulation of oxidative damage to improve disease prognosis (Reddy 2011, Mukherjee et al. 2014, Adeloye et al. 2015). As a result, the examination of ROS-associated damage and the effect of antioxidant supplementation in a pulmonary model prior to clinical trials, is highly relevant and could provide insight into the use of antioxidants therapeutically.

Tiron (detailed in chapter 1) was used in bronchial epithelial cells to investigate its action as a ROS reducing agent in this model. Tiron has previously been studied with good tolerance within the lung both *in vitro* and *in vivo* largely in proof-of-concept models demonstrating the involvement of oxidative stress within pulmonary disease (Kondrikov et al. 2011, Davidovich et al. 2013, Han and Park 2009). However, whilst there has been evidence of Tiron's beneficial use in pulmonary models, such as by *Ling et al.* (2003) where Tiron inhibited mitochondrial dysfunction and apoptosis H460 lung carcinoma cells through ROS reduction, there has yet to be evidence of amelioration of specific oxidative damage in cells of human origin.

4.1.2 Aim

The aim of this study was to evaluate the protective potential of Tiron against cellular ROS in an *in vitro* model of pulmonary disease. In this chapter, human bronchial epithelial cells (BEAS-2B) were treated with Tiron and subjected to ROS induction directly and physiologically.

4.2 Methods

4.2.1 Cell Culture

Human bronchial epithelial cells (BEAS-2B) were used to assess the effect of Tiron within lung derived cells. This cell line was kindly provided by a colleague at Coventry University. BEAS-2B cells are an immortalised cell line through transfection with the AD12-SV40 virus. This cell line is a robust and frequently used model for the effects of oxidative stress and inflammation *in vitro* (Lechner et al. 1982, 1984, Reddel et al. 1995, Park and Park 2007, Park et al. 2008, Heng et al. 2010). Bronchial epithelial cells have been identified as key cells within both COPD and asthma, responsible for maintaining a barrier between exogenous antigens and the endogenous environment (Wang et al. 2008). Changes to these cells have been identified in both COPD and asthma and associated with a reduced regenerative capability as a result of alterations within the extracellular matrix (Erle and Sheppard 2014, Hedström et al. 2018). Modelling with these cells has also demonstrated a correlation between asthma-like inflammation and the generation of ROS and have correlated with results observed from donated primary cells, particularly asthmatic patients (Bucchieri et al. 2015). Therefore, these cells are a relevant model to explore the potential prevention of oxidative damage with Tiron exposure.

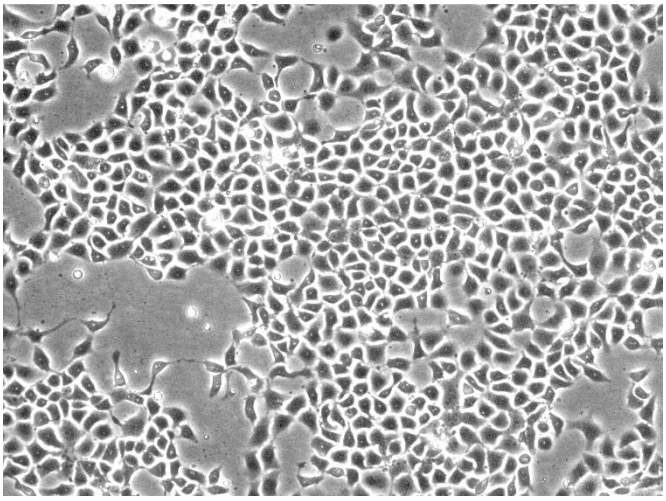

Figure 4.1. *The morphology of BEAS-2B cells during culture at 75% confluency, passage 7. Original figure obtained prior to cell passage using a TS100 eclipse microscope (Nikon, UK) and coolLED camera (Microtec, UK).*

BEAS-2B cells were cultured in bronchial epithelial cell growth medium (BEGM) (Lonza, UK) supplemented with the BEGM SingleQuots™ BulletKit™ which contained bovine pituitary extract, hydrocortisone, human epidermal growth factor, epinephrine, transferrin, insulin,

retinoic acid and trilodothyronine (Lonza, UK). Media was supplemented with penicillin:streptomycin (5U/ml) in place of the kit supplied gentamycin, as directed by the original cell line supplier (ATCC, UK). The cell culture routine as described in section 2.2.

4.2.2 Cell Treatment Protocol

4.2.2.1 The Induction of ROS

In accordance with section 2.3.1, BEAS-2B cells were cultured with H_2O_2 for 1 hour. H_2O_2 concentration was optimised following literature review where concentrations of 0.1 to 1mM have been used to model oxidative stress in BEAS-2B cells (Fujii et al 2002, Tsao and Yin 2017). For example, H_2O_2 (0.1-0.8 µM) significantly increased ROS, measured by DCFDA, and inflammatory mediator expression, such as IL-8, in BEAS-2B cells which was correlated with significant loss of cell viability (Khan et al 2014). Similarly, 1 mM H_2O_2 was used as a control for oxidative stress in BEAS-2B cells in understanding the role of H_2O_2 in zinc induced oxidative stress (Wages et al 2014). As such, a concentration range of 0.25-1 mM was optimised in both the cell viability and mtDNA strand break assays to obtain a suitable damage-inducing concentration without substantial cell loss caused by higher concentrations.

4.2.2.2 Hypoxia-Induced ROS Production

In the development of a model of physiologically induced ROS, the hypoxic conditions that BEAS-2B cells were subjected to was optimised to induce significant production of ROS which had the ability to generate measurable oxidative damage. Cells were cultured in hypoxic conditions (section 2.3.2.2) for 24 to 120 hours before assessment with the DCFDA and mtDNA strand break assays. In the assessment of the effect of Tiron on hypoxia-induced damage, BEAS-2B cells were pre-treated with Tiron for 24 hours before replacement with hypoxia-incubated BEGM media and hypoxic incubation.

4.2.2.3 Confirmation of Intracellular ROS Production

Production of intracellular ROS was evaluated as detailed in section 2.3.3 with the DCFDA assay (Abcam, UK). Measurement of DCF fluorescence through flow cytometry evaluated the use of Tiron against ROS-induction. Staining for the assessment of H_2O_2 treatment was conducted as in HKC-8 cells (section 3.2.2.3) whilst DCFDA stain at 20 µM was added to BEAS-2B cells following trypsinisation of hypoxia-incubated cells due to stain stability.

4.2.2.4 Treatment with Tiron

BEAS-2B cells were treated with Tiron as directed in section 2.4. Prolonged supplementation with Tiron was also observed with supplementation for up to 120 hours prior to removal from culture medium and commencement of the relevant ROS-induction protocol.

4.2.3 Assessment of ROS-Induced Damage and Data Analysis

All investigations into the effects of Tiron in reducing ROS-induced damage were conducted and analysed as detailed in chapter 2.

4.3 Results

4.3.1 The Effects of Increasing Tiron Concentrations on Cell Viability

BEAS-2B cells were treated with 24 hours with Tiron at 0-2 M to estimate Tiron's LD50 using the TOX-8 and SRB assays and is presented in Figure 4.2. A significant reduction ($p \leq 0.001$) in cell viability was observed between 50 mM and 2 M (50 mM: 85±0.54%; 2 M: 14±0.94%; vs. untreated control: 100±1.40%). The results obtained in the TOX-8 assay calculated the LD50 as 359 mM. This was confirmed with the SRB assay and calculated at 316 mM (50 mM: 85±1.36%; 2 M: 18±1.12%; vs. untreated control: 100±1.04%) (Figure 4.2B).

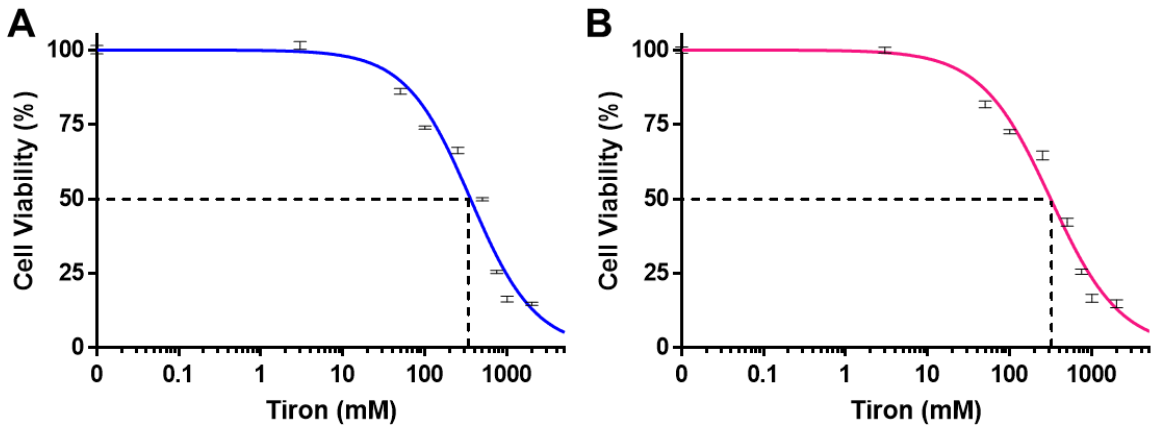

Figure 4.2. *The estimation of Tiron's LD50 in BEAS-2B cells after 24-hour treatment (0-2 M). The LD50, shown on each graph, was calculated by inverse log as 359 mM and 316 mM according to the TOX-8 (A) and SRB (B) assays. N=9 from 3 independent experiments; mean±SEM.*

Following treatment with Tiron 1-10 mM, used to identify a suitable test concentration of Tiron, there was no significant decrease in cell viability with 1-4 mM ($p \geq 0.05$) with the TOX-8 assay (Figure 4.3). Concentrations of 5 and 10 mM significantly ($p \leq 0.001$) decreased cell viability compared to the untreated control, indicating their unsuitability as test concentrations (5 mM: 96±0.38%; 10 mM: 93±0.84%; vs. 100±0.51%). These results were confirmed with the SRB assay (5 mM: 94±1.00%: 10 mM: 90±1.05%; vs. 100±0.43%)

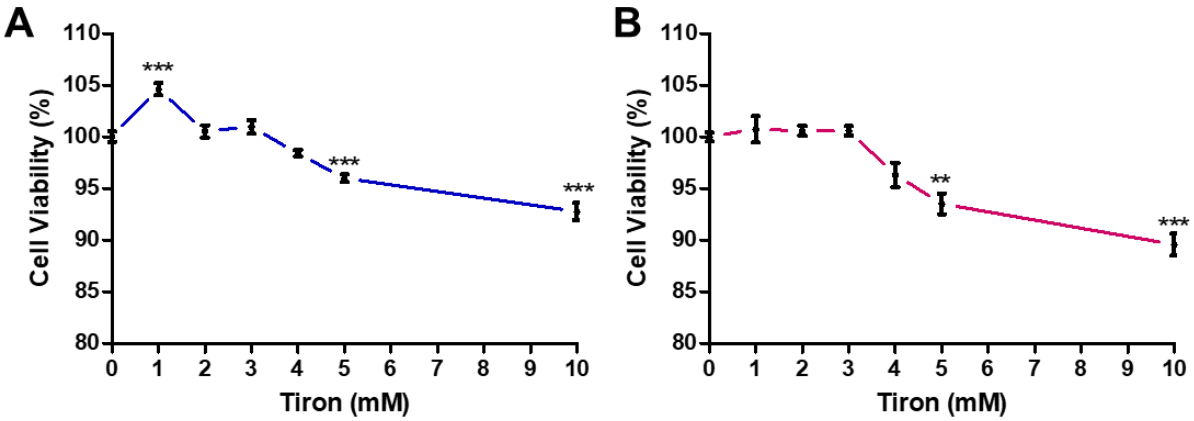

***Figure 4.3**. The effect of 24-hour treatment with Tiron (1-10 mM) in BEAS-2B cells on cell viability. Tiron reduced cell viability at 5 mM and greater in both the TOX-8 (A) and SRB assays (B). **, *** $p \leq 0.01$, 0.001 vs. untreated control; n=9 from 3 independent experiments; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

4.3.2 The Optimisation of H_2O_2-Induced ROS Damage Through Titration

Identification of a suitable H_2O_2 concentration which significantly reduced BEAS-2B cell viability was also assessed with the TOX-8 and SRB assays. As such, all concentrations of H_2O_2 (0.25-1 mM) showed induced a significant reduction in cell viability (H_2O_2 0.25 mM: 51±0.74%; 0.5 mM: 48±0.72%; 1 mM 48±1.53%; vs. untreated control: 100±0.31%). The results presented in Figure 4.4A were confirmed with the SRB assay (Figure 4.4B).

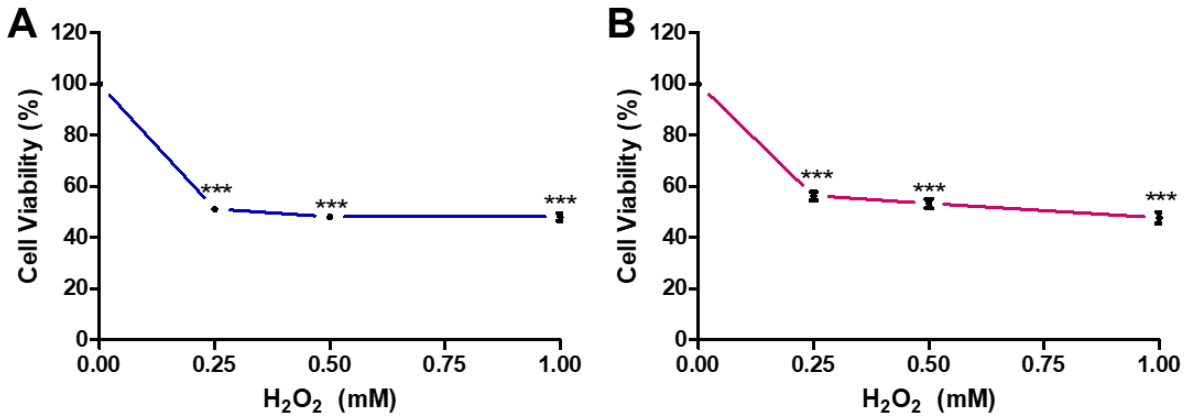

***Figure 4.4**. The effect of 1-hour H_2O_2 (0.25-1 mM) treatment on BEAS-2B cell viability. H_2O_2 significantly reduced cell viability at all concentrations in BEAS-2B cells according to the TOX-8 (A) and SRB (B) assays. *** p≤0.001 vs. untreated control; n=9 from 3 independent experiments; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

BEAS-2B cells were cultured for 24 hours before treatment with 0.25-1 mM H_2O_2 to identify a concentration at which significant mtDNA strand breaks were detectable by qPCR. A significant ($p \leq 0.001$) fold increase in CT value was observed at all concentrations of H_2O_2, with 0.25 and 0.5 mM H_2O_2 inducing the most significant fold change in CT (H_2O_2 0.25 mM: 3.14±0.10; 0.5 mM: 2.92±0.11; vs. untreated control: 1.01±0.03) (Figure 4.5). When considered with the results presented in Figure 4.4, a concentration of 0.25 mM H_2O_2 was taken forward.

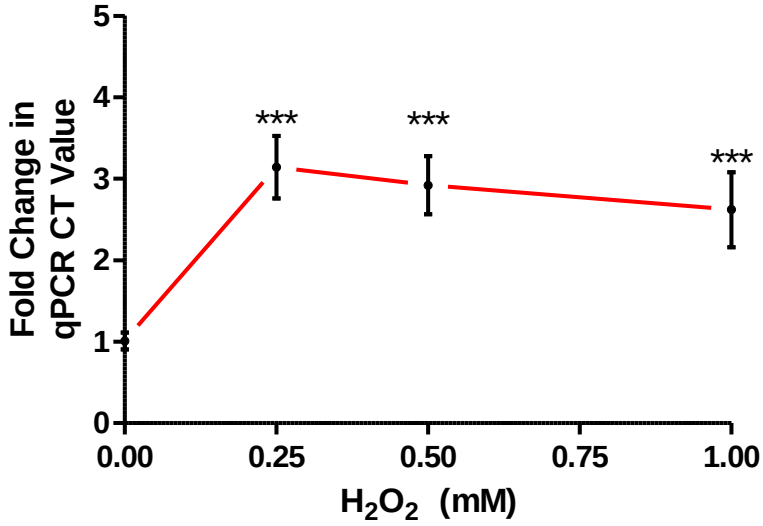

Figure 4.5. *The assessment of significant mtDNA strand break formation with H_2O_2 (0.25-1 mM) in BEAS-2B cells. H_2O_2 at all concentrations induces significant mtDNA strand break damage, through analysis of fold change in CT value using qPCR. *** $p \leq 0.001$ vs. untreated control; n=6; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

4.3.3 The Optimisation of Hypoxia-Induced Damage

The length of hypoxic culture (1% O_2) was optimised between 24 and 120 hours to ensure significant induction of intracellular ROS (Figure 4.6), determined fluorometrically by the DCFDA assay. Of these, 48 and 120 hours induced a significant increase in ROS as evidenced by increase in DCF fluorescence intensity compared to the untreated control (24 hours: 107±1.12%; 48 hours: 132±3.30%; 120 hours: 168±265%; vs. normoxic control (21% O_2): 100±1.23%).

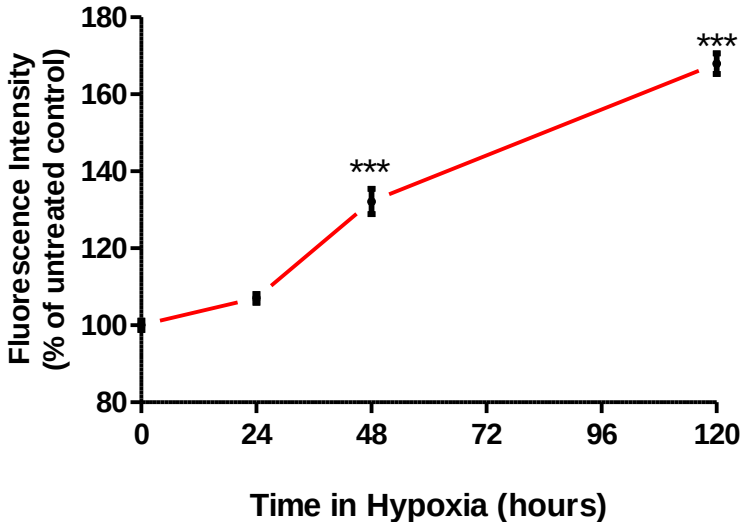

Figure 4.6. *The assessment of ROS production caused by prolonged culture in hypoxia. BEAS-2B cells subjected to hypoxic conditions (1% O_2) for 48 and 120 hours significantly increased intracellular ROS, which was not seen after 24 hours. *** p≤0.001 vs. normoxic control (21% O_2); n=6; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

No fold increase in CT value, indicative of increased mtDNA strand break damage, was observed at 24 and 48 hours of hypoxic culture, despite observing increased ROS production at 48 hours in Figure 4.13. However, prolonged incubation (120 hours) under hypoxic conditions did induce a significant ($p \leq 0.001$) reduction in CT value compared to the normoxic control (1.59±0.15 vs 1.05±0.06) (Figure 4.7).

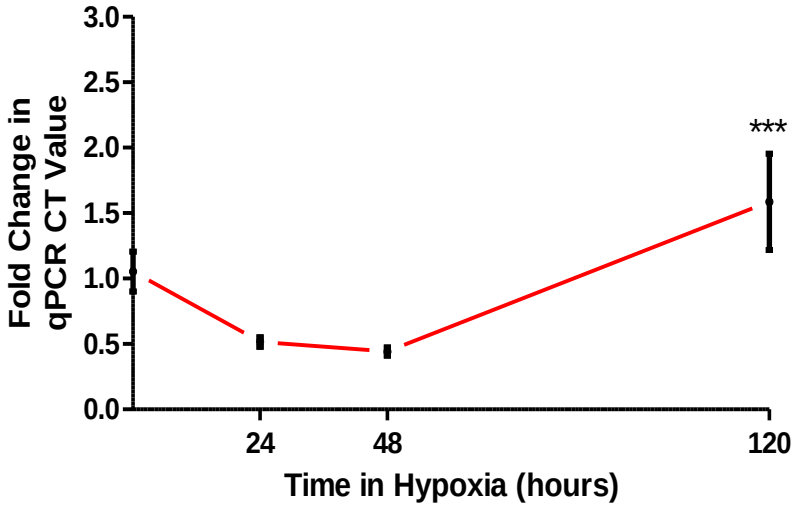

Figure 4.7. *The induction of mtDNA strand breaks with qPCR following prolonged exposure to hypoxic conditions. BEAS-2B cells subjected to hypoxic conditions for 120 hours significantly reduced qPCR efficiency, indicative of mtDNA strand break damage, not seen after 24 and 48 hours. *** $p \leq 0.001$ vs. normoxic control; n=6; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

4.3.4 The Recovery of ROS-Induced Cell Viability Decline with Tiron

4.3.4.1 The Effect of Tiron on H_2O_2-Induced Reduction of Cell Viability

Using 0.25 mM H_2O_2, the effect of Tiron pre-treatment on protecting against H_2O_2-induced damage in BEAS-2B cells was initially assessed by examining cell viability in the TOX-8 and SRB assays (Figure 4.8) and found to have significant ($p \leq 0.001$) effect on cytotoxicity with a significant ($p \leq 0.001$) difference between Tiron treated samples in the presence or absence of H_2O_2. Tiron (1-3 mM) pre-treatment led to a significant recovery in cell viability compared to the untreated control in the TOX-8 assay (Tiron 1 mM + H_2O_2: 72±0.79%; 3 mM + H_2O_2: 86±0.65%; vs. H_2O_2 0.25 mM: 52±0.35%). As has been previously documented in Figure 3.6, despite preserving cell viability across all concentrations, 3 mM Tiron offered the best protection compared to the H_2O_2 control with decreases in cell viability preservation corresponding to the decreases in viability observed in Figure 4.3 with high concentrations of Tiron (Tiron 4 mM+ H_2O_2: 70±0.41%; 5 mM + H_2O_2: 60±0.45%; vs H_2O_2: 52±0.35%). These results were confirmed with the SRB assay (Tiron 3 mM+ H_2O_2: 81±0.55%; vs. H_2O_2 0.25 mM: 53±1.19%) (Figure 4.8B).

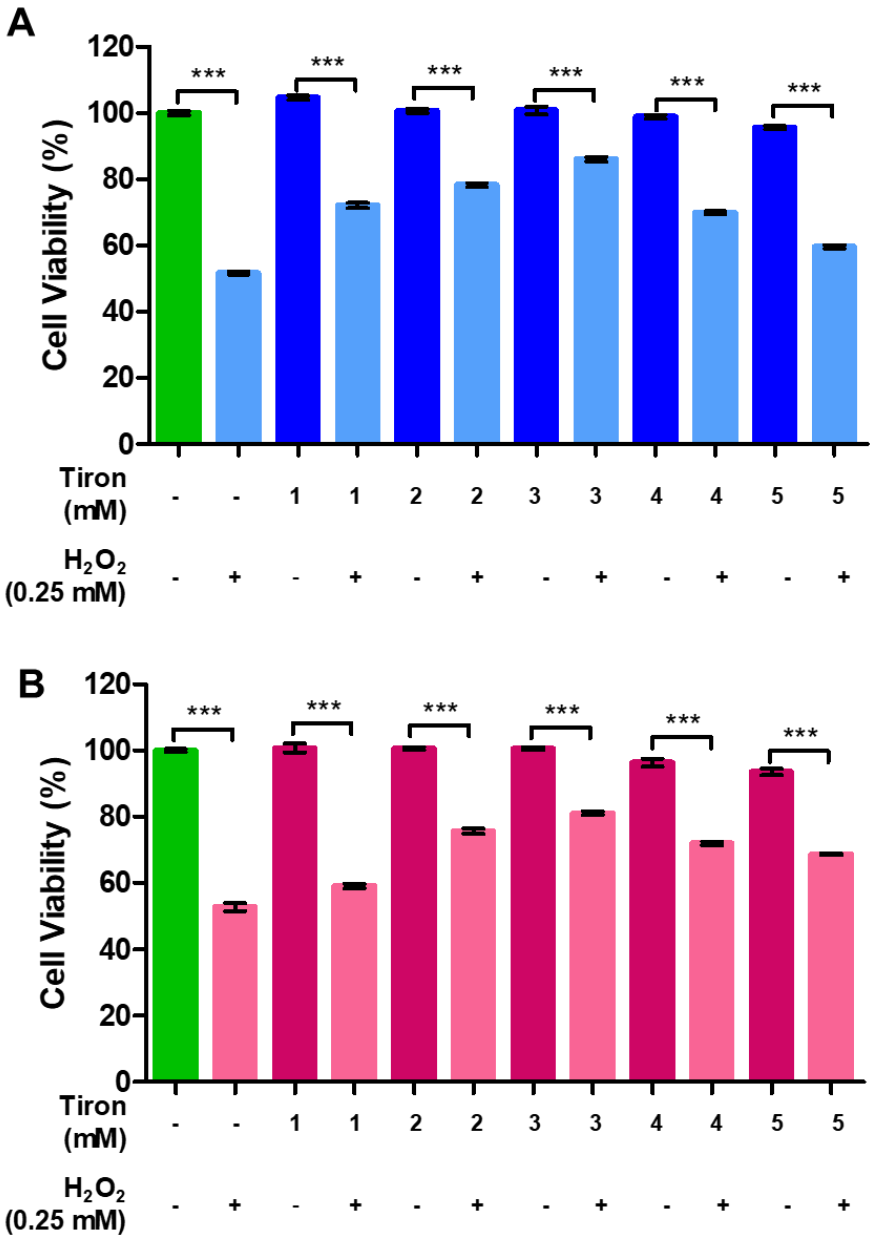

Figure 4.8. *The preservation of cell viability with Tiron (1-5 mM) pre-treatment followed by H_2O_2-0.25 mM) exposure. Tiron significantly improved cell viability at increasing concentrations in the presence of 0.25 mM H_2O_2 in BEAS-2B cells in both the TOX-8 (A) and SRB (B) assays. *** p≤0.001 significant difference with vs. without H_2O_2 treatment at each Tiron concentration; n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

4.3.4.2 The Effect of Tiron on Hypoxia-Induced Reduction of Cell Viability

Exposure of BEAS-2B cells to hypoxia for 120 hours significantly (p≤0.001) compromised cell viability compared to the normoxic control as determined by the TOX-8 and SRB assays

(58±1.11% vs. 100±2.73%) (Figure 4.9). No significant reduction in cell viability was observed following 24- and 48-hour hypoxic culture (24-hour: 99±2.73%; 48-hour: 96±3.40%; vs. 100±2.73%). These results corresponded with the absence of mtDNA strand break damage at these timepoints in Figure 4.6. Pre-treatment with Tiron for 24 hours prior to exposure to hypoxia for 120 hours significantly prevented cell viability loss (80±2.68% vs. 58±1.11%) (Figure 4.9A). These trends were also observed in the SRB assay (Tiron + 120-hour hypoxia: 79±1.29%; vs. 120-hour hypoxia: 66±1.59%; vs. normoxia: 100±1.21%) (Figure 4.9B).

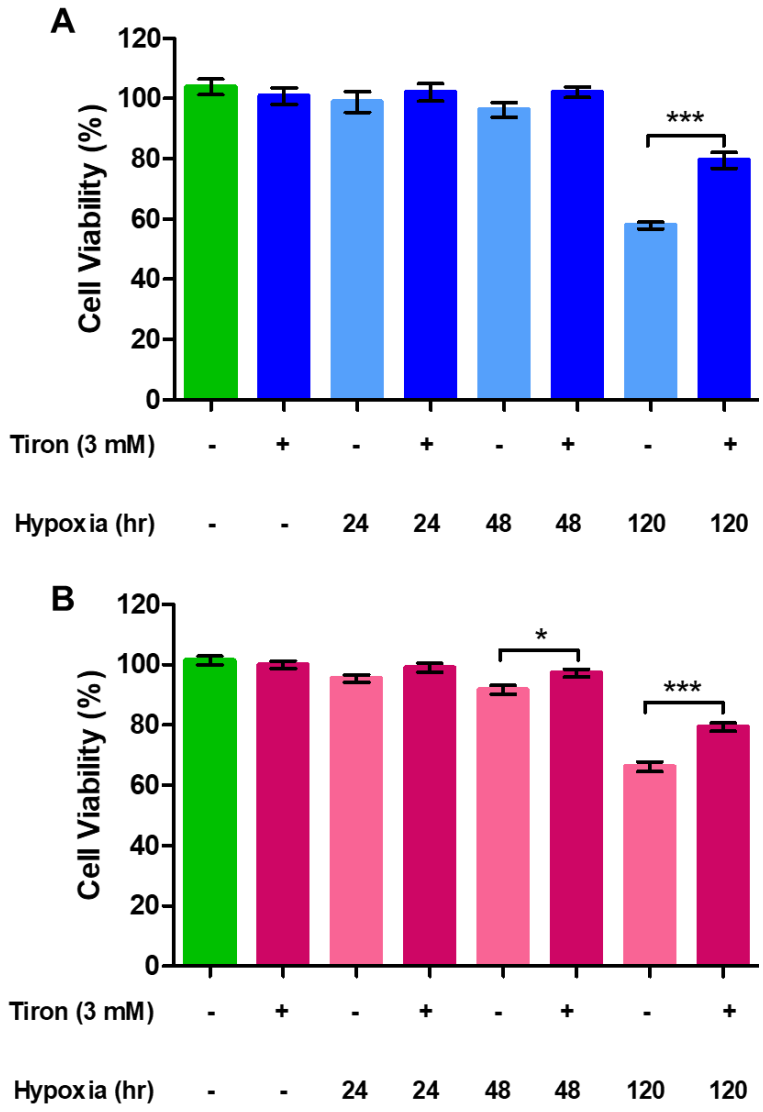

Figure 4.9. *The effect of Tiron (3 mM) pre-treatment on cell viability preservation following hypoxic culture (24-120 hours). Tiron pre-treatment preserved cell viability following 120 hours culture of BEAS-2B cells in both the TOX-8 (A) and SRB (B) assays. *, *** p≤0.05, 0.001 significant difference with and without Tiron treatment in hypoxia (1% O_2); n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

4.3.4.3 The Effect of Prolonged Treatment with Tiron on H_2O_2-Induced Reduction of Cell Viability

In this assay, BEAS-2B cells were treated with Tiron for 24-120 hours to assess the impact on cell viability of prolonged treatment and whether this still offered protection against H_2O_2 in the TOX-8 and SRB assays. Whilst treatment with Tiron (3 mM) for 48-120 hours, under normal cell culture conditions, induced a significant reduction in cell viability (Figure 4.10), it did demonstrate a preservation of viability following treatment with 0.25 mM H_2O_2 (Tiron 48 hours + H_2O_2: 83±3.27%; 72 hours + H_2O_2: 73±2.41%; 120 hours + H_2O_2: 62±2.02%; vs. H_2O_2: 51±0.70%). These results were confirmed with the SRB assay (Figure 4.10B).

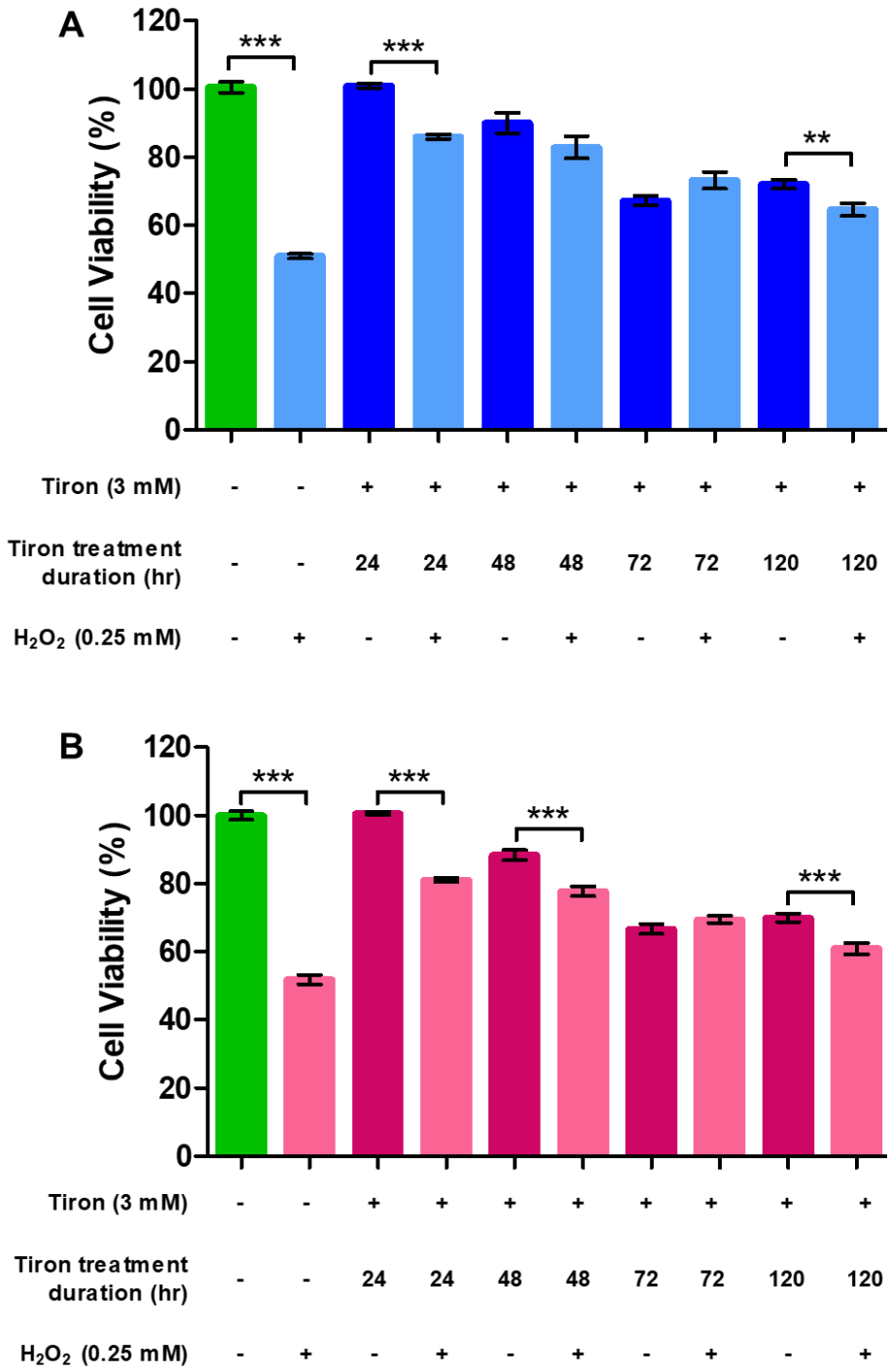

Figure 4.10. *The effect of prolonged treatment (48-120 hours) with Tiron on cell viability. Tiron treatment longer than 24 hours significantly reduced cell viability in both the TOX-8 (A) and SRB (B) assays. **, *** p≤0.01, 0.001 significant difference between H_2O_2 treated vs. untreated at each Tiron treatment duration; n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

4.3.5 The Effect of Tiron on Intracellular ROS Production

4.3.5.1 The Effect of Tiron Pre-Treatment on ROS Production

The effect of Tiron pre-treatment on ROS production induced by H_2O_2 treatment was assessed through measuring DCF fluorescence intensity by flow cytometry after 1 hour of H_2O_2 treatment. Significant ($p \leq 0.001$) increases in ROS were evidenced by increases in DCF fluorescence intensity measured by the DCFDA assay and compared to the untreated controls (H_2O_2 204±17.44%; vs.100±3.29%) (Figure 4.11). Compared to the H_2O_2 control, pre-treatment with 3 mM Tiron significantly reduced the fluorescence intensity (112±2.49% vs. 204±17.44%). Unlike in Figure 3.7, which detailed the effect on HKC-8 cells, Tiron treatment (both with and without H_2O_2) did not significantly reduce fluorescence intensity (Tiron: 109±4.46%; + H_2O_2: 112±2.49%; vs. 100±3.29%).

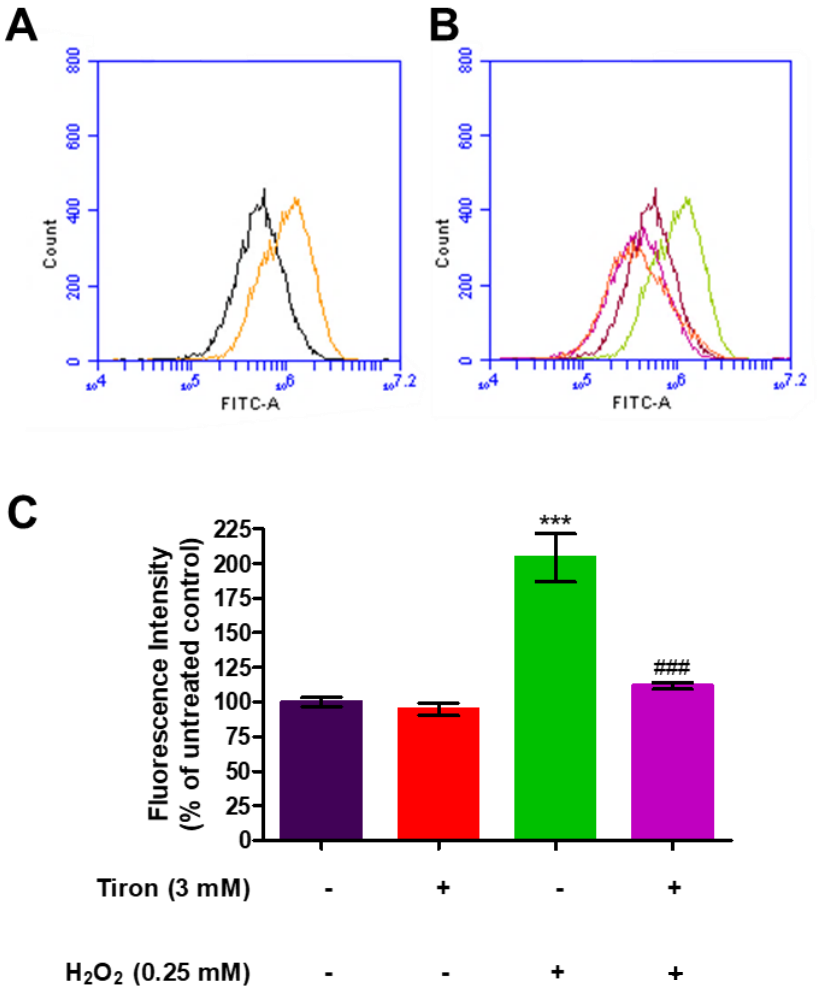

Figure 4.11. *The effects of Tiron (3 mM) on intracellular ROS with and without H_2O_2 (0.25 mM) treatment in the DCFDA assay by flow cytometry. (A) 0.25 mM H_2O_2 (orange) treatment significantly increased fluorescence intensity compared to untreated (black). (B) Tiron pre-treatment (red) significantly reduced ROS following H_2O_2 treatment (green) compared to the untreated (violet) and Tiron controls (purple). (C) Pre-treatment with Tiron significantly reduced fluorescence intensity following H_2O_2 treatment in BEAS-2B cells. The colours shown directly relate to Figure 4.11A and B. *** p≤0.001 vs. untreated control; ### p≤0.001 vs. H_2O_2; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

The effect of Tiron pre-treatment on hypoxia-induced ROS production was also assessed through measuring DCF fluorescence intensity by flow cytometry after 120 hours of hypoxic culture. In the prevention of ROS production, pre-treatment with Tiron significantly reduced fluorescence intensity (Tiron + hypoxia: 81±2.82%; vs. 221±2.83%) (Figure 4.12). These results corresponded with Figure 4.11. Furthermore, treatment with Tiron for 24 hours followed by 120 hours, in normal cell culture conditions (21% O_2), maintained baseline fluorescence intensity compared to the normoxic control (95±4.46%).

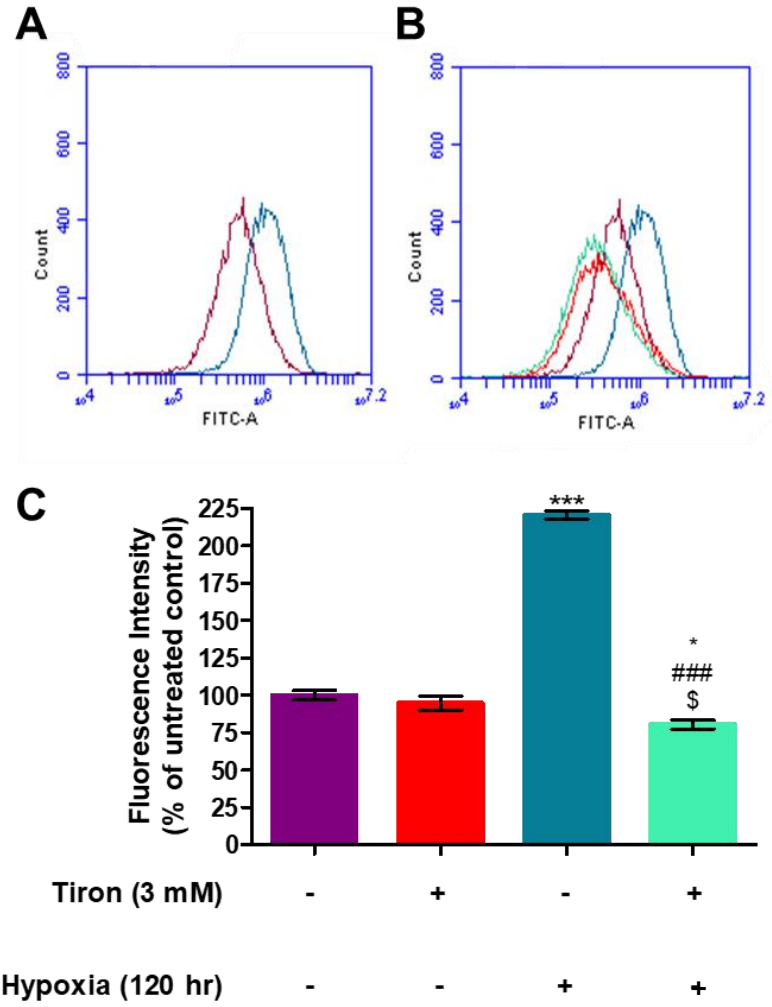

Figure 4.12. *The effect of Tiron on ROS production following hypoxic culture (1% O_2) and Tiron pre-treatment. (A) Significant production of ROS with 120 hours hypoxia (blue) vs. normoxia (21% O_2) (purple). (B) Tiron pre-treatment (green) inhibited hypoxia-induced ROS production (blue) vs. normoxia (purple) and Tiron controls (red). (C) Pre-treatment with Tiron significantly reduced fluorescence intensity following hypoxia treatment in BEAS-2B cells. The colours shown directly relate to Figures 4.12A-B. *, *** p≤0.05, 0.001 vs. normoxia control; ### p≤0.001 vs. Hypoxia; $ p≤0.05 vs. Tiron control; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

4.3.5.2 The Effect of Prolonged Treatment with Tiron on ROS Production

With prolonged treatment (48 to 120 hours) of Tiron in BEAS-2B cells, there was a significant fold increase in fluorescence intensity measured with the DCFDA assay (Tiron 48 hours: 117±2.45%; 120 hours: 123±2.48%; vs. untreated control: 100±0.68%; vs. Tiron 24 hours: 96±1.09%) (Figure 4.13). Despite increases in ROS production with Tiron treatment alone, a significant ($p \leq 0.001$) reduction in fluorescence intensity was measured following H_2O_2 treatment (Tiron 48 hours + H_2O_2: 140±3.48%; 120 hours: 148±2.26%; vs. 0.25 mM H_2O_2: 201±3.12%).

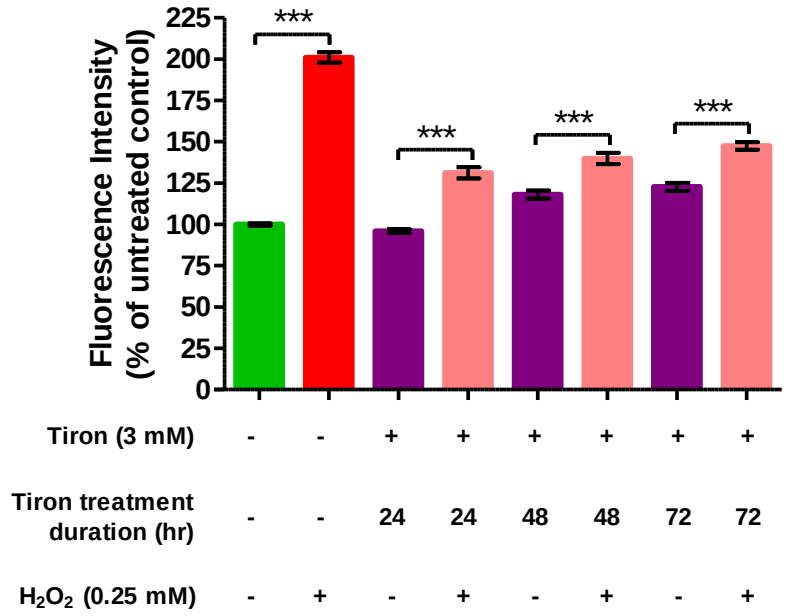

Figure 4.13. *The effects of prolonged treatment (48-120 hours) with Tiron on intracellular ROS production. Prolonged treatment significantly reduced H_2O_2-induced increases in fluorescence intensity measured fluorometrically with the DCFDA assay. *** $p \leq 0.001$ significant difference with vs. without H_2O_2 at each Tiron concentration; n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

4.3.6 The Prevention of MtDNA Strand Breaks with Tiron

4.3.6.1 The Prevention of H_2O_2-Induced MtDNA Strand Breaks with Tiron

Pre-treatment with Tiron (24 hours) significantly prevented mtDNA strand breaks at all concentrations (2-5 mM) (Figure 4.14). The most significant preservation of mtDNA was achieved with 3 mM Tiron compared to the untreated and H_2O_2 controls (Tiron 3 mM + H_2O_2: 0.90±0.02; vs. H_2O_2 0.25 mM: 4.52±0.06). Tiron at 4 and 5 mM did not preserve CT values to that of the untreated control but did significantly protect against H_2O_2-induced mtDNA damage as did Tiron at 2 mM (Tiron 2 mM + H_2O_2: 2.89±0.03; Tiron 4 mM + H_2O_2: 1.60±0.02; Tiron 5 mM + H_2O_2: 1.48±0.03; vs. H_2O_2 0.25 mM: 4.52±0.06). Treatment with 3 mM led to a significant ($p \leq 0.001$) fold increase in qPCR efficiency, measured as a fold decrease in CT value (Tiron 3 mM: 0.56±0.02 vs. 1.05±0.01).

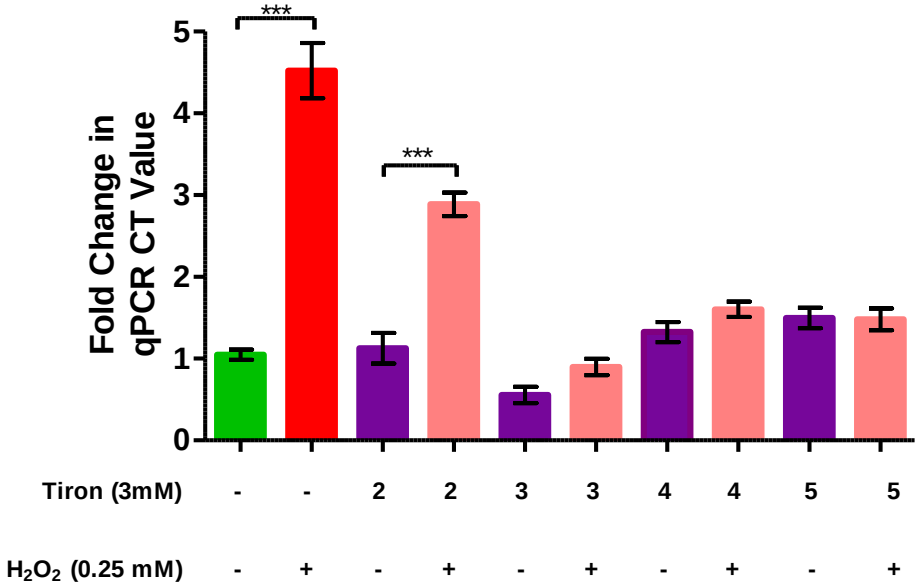

Figure 4.14. *The inhibition of mtDNA strand break formation with Tiron (2-5 mM) assessed by qPCR. Tiron pre-treatment offered significant ($p \leq 0.001$) protection against H_2O_2–induced mtDNA strand breaks in BEAS-2B cells at all concentrations. *** $p \leq 0.001$ significant difference with vs. without H_2O_2 at each Tiron concentration; $n \geq 6$; mean±SEM, two-way ANOVA with Bonferroni correction.*

4.3.6.2 The Inhibition of Hypoxia-Induced MtDNA Strand Break Formation with Tiron

In the preservation of mtDNA, Tiron pre-treatment (24 hours) was significantly protective against hypoxia-induced mtDNA strand breaks determined through qPCR analysis as a significant ($p \leq 0.001$) decrease compared to the hypoxia control (Tiron + hypoxia: 1.01±0.02; vs. hypoxia: 2.83±0.05).

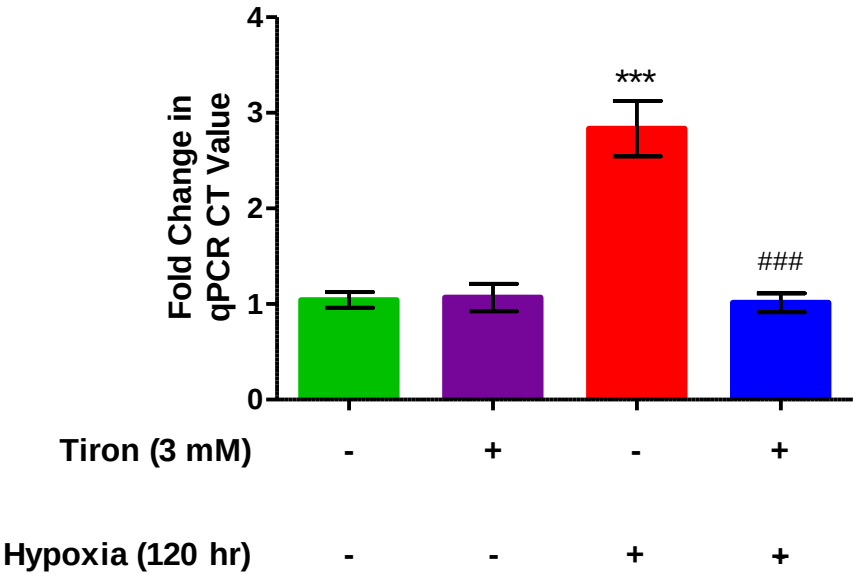

Figure 4.15. *The inhibition of hypoxia-induced mtDNA strand break formation with Tiron. Tiron pre-treatment (24 hours) offers significant ($p \leq 0.001$) protection against hypoxia–induced mtDNA strand breaks in BEAS-2B cells after 120 hours. *** $p \leq 0.001$ vs. normoxia (21% O_2); ### $p \leq 0.001$ vs. hypoxia (1% O_2); n=6; mean ±SEM, one-way ANOVA with Tukey post-hoc.*

4.3.6.3 The Effect of Co-treatment on Hypoxia-induced MtDNA Strand Breaks

Following the reduction of hypoxia-induced damage in BEAS-2B cells with Tiron pre-treatment for 24 hours, the effect of co-administration of Tiron and hypoxia was assessed, considered as a co-treatment. The effect of co-treatment on the prevention of mtDNA strand breaks was assessed by qPCR to provide primary evidence for the beneficial use of Tiron and is shown in Figure 4.16. Supplementation at the time of hypoxia induction offered considerable protection against strand break formation compared to the hypoxia control (0.26±0.01 vs. 2.49±0.04). Prolonged treatment with Tiron for 120 hours in normoxia also led to a significant ($p \leq 0.001$) fold decrease in qPCR CT value (0.42±0.01 vs. normoxia: 1.09±0.04).

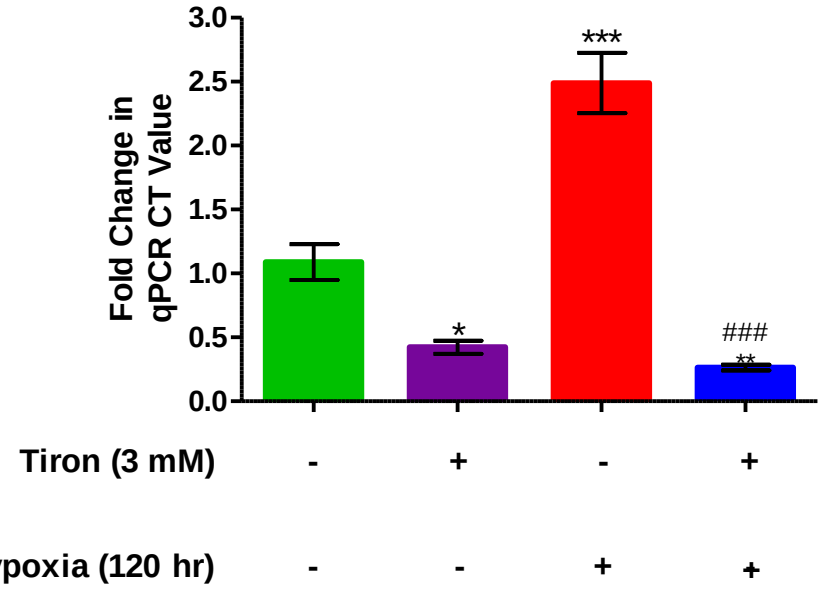

Figure 4.16. *The effects of Tiron on mtDNA strand break damage supplemented at the time of entry into hypoxia (120 hours). Tiron offered protection against hypoxia–induced mtDNA strand breaks in BEAS-2B cells when administered at the time of hypoxia (1% O_2) induction. *, **, *** $p \leq 0.05, 0.01, 0.001$ vs. normoxia (21% O_2); ### $p \leq 0.001$ vs. hypoxia; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

4.3.7 The Effect of Tiron on Lipid Peroxidation

4.3.7.1 The Effects of Tiron on H_2O_2-Induced Lipid Peroxidation

A significant (p≤0.001) reduction in MDA production of 36% was observed following 24-hour pre-treatment with Tiron compared to the H_2O_2 control measured colourimetrically (H_2O_2 0.25 mM: 186±2.70%; Tiron + H_2O_2: 120±4.75%). Treatment with Tiron without H_2O_2 led to a significant (p≤0.001) increase in MDA production not observed with HKC-8 cells in Figure 3.8 (117±3.57% vs. 100±0.58%) (Figure 4.17). Dissimilar to the results in Figures 4.14 and 4.15, Tiron was not restorative of baseline concentrations of MDA; this outcome is in keeping with the results in chapter 3.

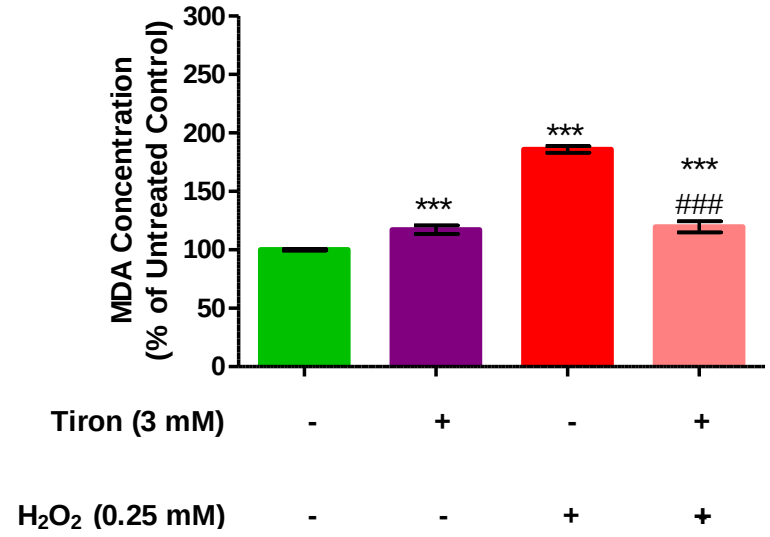

Figure 4.17. *The effect of Tiron on increased MDA concentration following H_2O_2 treatment (0.25 mM). Tiron demonstrated protection against H_2O_2-induced lipid peroxidation in BEAS-2B cells through the measurement of the MDA biomarker. *** p≤0.001 vs. control; ### p≤0.001 vs. H_2O_2; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

4.3.7.2 The Effects of Tiron on Hypoxia-Induced Lipid Peroxidation

Similar to Figure 4.17, Tiron pre-treatment led to a significant ($p \leq 0.001$) decrease in hypoxia-induced MDA production following 120-hour exposure to hypoxia (Tiron + Hypoxia: 139±3.18% vs. 262±3.18%) (Figure 4.18). Culture of BEAS-2B cells for 120 hours in hypoxia led to a greater increase in MDA production than observed with H_2O_2 (Figure 4.17). Tiron pre-treatment was unable to restore MDA concentration to that of the normoxic control whilst Tiron alone led to an increase greater than that observed in Figure 4.17 (Tiron: 132±2.14 vs. normoxic control: 100±0.58%).

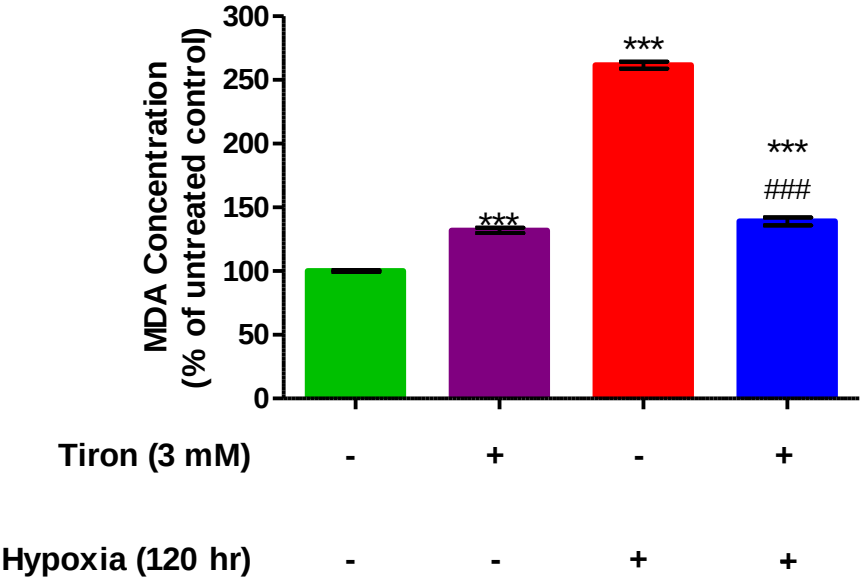

Figure 4.18. *The reduction of hypoxia-augmented MDA concentration with Tiron. Tiron demonstrated reduction of MDA following hypoxia (1% O_2) treatment for 120 hours in BEAS-2B cells. *** $p \leq 0.001$ vs. normoxia (21% O_2); ### $p \leq 0.001$ vs. hypoxia; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

4.3.8 The Effect of Tiron on Nrf2 Expression

4.3.8.1 The Effect of 24-Hour Treatment with Tiron on Nrf2 Expression

The protein expression of Nrf2 in BEAS-2B cells was qualitatively assessed by ELISA after 24-hour Tiron pre-treatment and treatment did not significantly ($p \geq 0.05$) augment Nrf2 protein expression (115±3.72% vs. untreated control 100±5.02%) (Figure 4.19). Treatment with 0.25 mM H_2O_2 for 1 hour significantly augmented ($p \leq 0.001$) Nrf2 expression (257±13.61% vs. 100±5.02%). Against this, Tiron did not stimulate an increase in Nrf2 expression (117±4.72% vs. 257±13.61%).

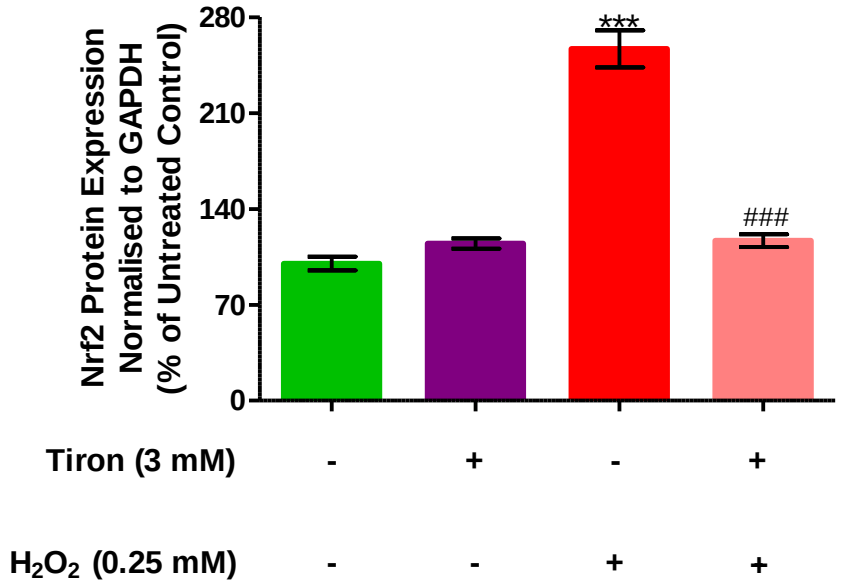

Figure 4.19 *The qualitative assessment of Nrf2 expression with Tiron (3 mM) and H_2O_2 (0.25 mM) treatment. Tiron did not augment Nrf2 expression in BEAS-2B cells following ROS induction by H_2O_2. *** $p \leq 0.001$ vs. untreated control; ### $p \leq 0.01$ vs. H_2O_2; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

4.3.8.2 The Expression of Nrf2 Following Prolonged Treatment with Tiron

Tiron treatment for longer than 24 hours significantly (p≤0.001) increased the protein expression of Nrf2 measured by ELISA (Tiron 48 hours: 158±10.23%; 120 hours: 188±6.99%; vs. untreated control: 100±5.02%) (Figure 4.13). In the protection of BEAS-2B cells against H_2O_2, Tiron pre-treatment for both durations led to a significant (p≤0.001) reduction in Nrf2 expression, indicating some level of independent ROS amelioration (Tiron 48 hours + H_2O_2: 165±10.77%; 120 hours + H_2O_2: 209±6.28%; vs. H_2O_2: 257±13.61%).

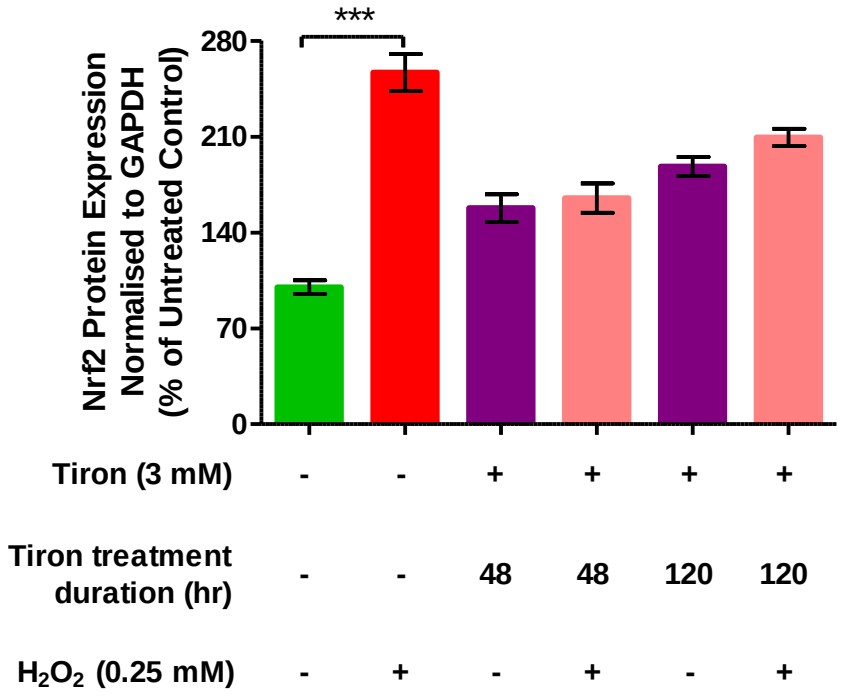

Figure 4.20. *The expression of Nrf2 following prolonged Tiron treatment (48-120 hours) by ELISA. Tiron induced Nrf2 expression in BEAS-2B cells following prolonged incubation both with and without ROS induction by H_2O_2. *** p≤0.001 significant difference with vs. without H_2O_2 at each Tiron treatment duration; n=9 from 2 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

4.4 Discussion

Tiron pre-treatment (3 mM) significantly ($p \leq 0.001$) preserved cell viability (Figure 4.8), reduced MDA concentration (Figure 4.14) and was significantly protective against mtDNA strand breaks (Figure 4.17) in lung epithelial (BEAS-2B) cells exposed to 0.25 mM H_2O_2. The high level of protection offered by Tiron was indicated to be as a result of ROS amelioration, observed as a reduction in DCF oxidation (Figure 4.11). H_2O_2-induced DNA damage and lipid peroxidation has been alleviated *in vitro* by pre-treatment of lung fibroblasts with the plant sourced and ROS scavenging antioxidant hyperoside (quercetin-3-O-galactoside) (Piao et al. 2008). The protection demonstrated by Tiron in BEAS-2B cells against H_2O_2 induced stress is supported by the work conducted in chapter 3 in addition to published literature highlighted in the previous chapter and demonstrates that the high level of protection achieved with Tiron is not cell-type specific. This is contrary to the variable responses observed with hyperoside, which was found to offer less protection within lung cells compare to other cell types, including melanocytes and adrenal medulla cells (Liu et al. 2005, Piao et al. 2008, Yang et al. 2016, Ye et al. 2017). Therefore, the consistent levels of protection offered by Tiron in multiple cell types (HKC-8 and BEAS-2B) suggests that it could supersede protection offered by antioxidants such as hyperoside to reduce oxidative stress to a high degree systemically (Fang et al. 2012, Oyewole et al. 2014). The protection offered by Tiron in BEAS-2B cells specifically could reduce the ROS that is being increasing identified within pulmonary disease (Ochs-Balcom et al. 2006, Bartoli et al. 2011). For example, mtDNA strand break damage has been correlated with increased ROS and ETC inefficiency in lung tissue and skeletal muscle cells isolated from COPD patients (Puente-Maestu et al. 2009, Pastukh et al. 2011). Similarly, breath condensates from asthmatic patients identified increased levels of MDA alongside ROS (Corradi et al. 2004, Bartoli et al. 2011). Therefore, observing the effects and tolerance of Tiron within pulmonary derived cells was integral to understanding the therapeutic possibilities with Tiron supplementation to treat ROS-associated pulmonary disease, including asthma or COPD, as well as adding to the knowledge of Tiron's protective capabilities within human cells derived from different tissues.

Evaluation of a range of Tiron concentrations, including the estimation of Tiron's LD50 at approximately 316-359mM (Figure 4.2), found that 3 mM demonstrated the highest level of cell viability and mtDNA preservation in BEAS-2B cells (Figure 4.3, 4.8 and 4.14). This finding agreed with results observed in HKC-8 cells (chapter 3) and previously by *Oyewole et al.* (2014). A test concentration range of 2-5 mM after estimation of the LD50 was within the range that has been used frequently with Tiron *in vitro* (0.1-10 mM) without adverse effects including in primary osteoblasts (Marinucci et al. 2018), and human embryonic kidney (HEK293) cells

(Liu et al. 2006). Tiron treatment for 24 hours has previously been shown reduce pesticide-induced ROS production in BEAS-2B cells at 10 mM, which is greater than used in this chapter (Alleva et al. 2016). Figure 4.3 found that 10 mM significantly ($p \leq 0.001$) reduced cell viability when supplemented alone, data of which was not presented by *Alleva et al.* (2016). Therefore, the findings of this chapter add to the evidence presented by *Alleva et al.* (2016) supporting Tiron's use but that whilst a higher concentration may reduce oxidative, a lower concentration such as 3 mM is preferential considering the reduction of cytotoxicity and mtDNA damage shown in Figures 4.3 and 4.8. A concentration range of 0.1-5 mM Tiron has also been used in primary human neutrophils, which demonstrated dose-dependent decreases in ROS production alongside Trolox (0.1-4 mM) and Tempol (0.1-5 mM) (Vorobjeva and Pinegin 2016). This indicated that a small concentration of Tiron can be effective in reducing oxidative stress across more cell types (Vorobjeva and Pinegin 2016). This suggests that lower doses of Tiron may still be influential in reducing oxidative stress. Whilst the efficacy of Tiron at lower concentrations was indicated within HKC-8 cells (Figure 3.7), this was not observed with BEAS-2B cells. This indicates that a higher concentration is required to provide similar levels of protection compared to kidney cells. This is a valuable insight prior to *in vivo* studies and indicates that ROS amelioration with the lung should be considered when determining dosing levels of Tiron to ensure that protection is offered to the similar levels as other tissues.

A range of concentrations have also been used to assess the protective capabilities of other antioxidant supplements, including vitamin E and curcumin. However, *Zhu et al.* (2014) found that concentrations as low as 40 µM0 µM curcumin and 80 µM0 µM vitamin E demonstrated a significant reduction in BEAS-2B cell viability to 20% after 24-hour treatment. In Figure 4.2, a 20% reduction in cell viability induced by Tiron was sustained after 24-hour treatment with 1M, suggesting a better tolerance of Tiron by BEAS-2B cells within this chapter compared to the findings of *Zhu et al.* (2014) and justifying its use at a 'higher' concentration. On the other hand, considerably lower doses of Resveratrol (10 µM) have been used protectively in BEAS-2B cells against paraquat-induced oxidative stress (He et al. 2012). However, this dose did not completely ameliorate ROS production (He et al. 2012). At 3 mM, Tiron has previously demonstrated a superior protective capacity compared to Resveratrol (5 µM) in HDFn cells as well as at 40 mM (vs Resveratrol at 1 mM) in propionic acidemia patient fibroblasts (Oyewole et al. 2014, Gallego-Villar et al. 2014). This provides evidence that, whilst Resveratrol concentrations are lower, the use of Tiron at 3 mM can be hypothesised to be preferential to Resveratrol due to its provision of higher levels of protection (Oyewole et al. 2014, Gallego-Villar et al. 2014).

It has been shown that persistent deprivation of oxygen, such as through hypoxia, leads to mitochondrial dysfunction. This leads to increases in the formation of ROS and resulting in oxidative damage, which has been supported by increased expression of endogenous antioxidants in a rat hypoxia model (Chou et al. 2009, Toth and Warfel 2017). As had been seen with H_2O_2 treatment, Tiron pre-treatment demonstrated significant protection against hypoxia-induced mtDNA strand break damage (Figure 4.15) alongside significantly ($p \leq 0.001$) reduced ROS availability (Figure 4.12), cytotoxicity (Figure 4.9) and lipid peroxidation (Figure 4.18). Hypoxia-induced double strand break DNA damage and MDA elevation has been previously correlated with ROS production in primary lung fibroblasts and rat lung homogenates. This was attenuated with antioxidant treatment, thus supporting the incidence of such damage alongside elevated ROS in BEAS-2B cells as well as the protection offered by Tiron (Bell et al. 2007, Purushothaman et al. 2011).

ROS occur in O_2 depleted environments due to ETC inefficiency resulting from cytochrome c's inability to readily accept electrons from complex III, leading to an abundance of unpaired electrons (Chandel et al. 2000, Toth and Warfel 2017). Such formation of ROS was first seen in BEAS-2B cells after 48 hours in hypoxia (Figure 4.6), with levels of ROS comparable to those seen with 0.25 mM H_2O_2 after 120 hours of hypoxia (221% vs. 204%; Figure 4.12 and 4.11 respectively). ROS readily stabilise HIF-1α during hypoxia through denaturation of prolyl hydroxylases, resulting in the accumulation of HIF-1α and upregulation of vascular endothelial growth factor (VEGF) and glycolysis, promoting O_2-independent ATP synthesis, sustaining cell viability and decreasing the reliance on OXPHOS as well as ROS formation (Guzy et al. 2005, Clanton 2007, Chan et al. 2009, Chua et al. 2010, Toth and Warfel 2017). This upregulation of glycolysis could account for the absence of mtDNA damage observed after 48 hours of hypoxic culture (Figure 4.7) and has also been observed in pulmonary artery cells, where exposure to hypoxia for 72 hours resulted in a significant increase in ROS generation not observed at 24 or 48 hours, despite an increase in H_2O_2 observed after 48-hours (Porter et al. 2014). This hypothesis of glycolytic-facilitated preservation is further supported by the finding in Figure 4.9 that hypoxic culture for 24 to 48 hours did not significantly reduce cell viability ($p \geq 0.05$). A similar event was observed in chapter 3 with HKC-8 cells whereby 25 mM glucose treatment induced significant intracellular ROS but did not lead to significant mtDNA strand break damage (Figure 3.14 and 3.15). Together, these demonstrate the activity of the endogenous antioxidant defence through which ROS-induced damage is initially abrogated. However, the increase in ROS generation after 48 hours does present an opportunity to use supplementation, such as with Tiron, to prevent the development of ROS-induced damage during hypoxia exposure such as during allergen-induced bronchoconstriction, which has been shown to lead to the development of alveolar hypoxia (Chaouat et al. 2008).

Reduced HIF-1α protein expression has been identified in COPD patient samples indicating a deficient adaptive mechanism to hypoxia, accounting for increases in oxidative stress in COPD and has been correlated with the development of pulmonary hypertension (Yasuo et al. 2011, To et al. 2012). Pulmonary hypertension in COPD, associated with hypoxia, has been correlated with elevated MDA and oxidative DNA damage (Bowers et al. 2004, Wong et al. 2013). Antioxidants have been shown to modulate hypoxia-induced ROS *in vivo* resulting in a decrease in pulmonary hypertension (Lachmanová et al. 2005, Jankov et al. 2008). Therefore, the results obtained with Tiron demonstrating the reduction of ROS, preservation of mtDNA and reduction of lipid peroxidation induced by hypoxia could be effective within the treatment of pulmonary disease with the potential to also reduce pulmonary hypertension.

It was shown that pre-treatment with Tiron significantly ($p \leq 0.001$) reduced MDA concentration by 36% against H_2O_2 and 47% following hypoxia (Figure 4.17 and 4.18). Serum levels of MDA have been found to be elevated during exacerbation of COPD and asthmatics, with higher levels correlating with hospitalisation frequency. Therefore any reduction of MDA, as is shown with Tiron, could be deemed beneficial in reducing oxidative stress within these patients (Ahmad et al. 2012, Voskresenska et al. 2015). However, with both hypoxia- and H_2O_2-induced MDA augmentation, Tiron did not restore levels back to baseline, suggesting that ROS-induced lipid peroxidation was not reduced to the same extent as seen with qPCR with Tiron. The absence of this level of MDA inhibition was also observed in the HKC-8 cell line (chapter 3). This indicates that Tiron is either unable to successfully ameliorate products of lipid peroxidation which could be as a result of their chemistry, a selectivity suggested with vitamin C and E previously (Niki 2014). This could then contribute to the cytotoxicity also observed in BEAS-2B cells, which was not adequately protected against by Tiron (Figure 4.8 and 4.9).

An alternative explanation could be that the self-generating mechanism of MDA and lipid peroxidation, resulting in the formation of other radicals, is greater than the action elicited by Tiron (Niedernhofer et al. 2003, Ayala et al. 2014). The inability of Tiron to maintain levels of lipid peroxidation to baseline in both HKC-8 and BEAS-2B cells indicates that this response is not a cell specific phenomenon.

In consideration of this, the reduction of ROS levels to below that of the untreated control following hypoxia and H_2O_2 treatment indicate that the levels of ROS do not correlate with the measured MDA increases. This has been shown in studies of protective compounds in other lung-derived cells alongside alterations in endogenous antioxidant concentrations, including a 2011 study of isolated rat lung cells subjected to H_2O_2 (Ganie et al. 2011, Liu et al. 2017). This study compared the protection elicited by *podophyllum hexandrum* plant and vitamin E but

also the responses across lung, kidney and liver cells. Reduction of MDA levels to baseline was only achieved in liver cells with vitamin E, which correlates with the results of this thesis in both HKC-8 and BEAS-2B cells (Ganie et al. 2011). Tiron has previously demonstrated partial amelioration of MDA production comparable to the existing treatment for asthma, dexamethasone, in a ovalbumin model of asthma (El-Sherbeeny et al. 2016). The finding that Tiron was not able to adequately prevent MDA concentration increases in BEAS-2B cells, unlike that achieved with mtDNA strand break prevention, is therefore in keeping with published literature (Ganie et al. 2011, El-Sherbeeny et al. 2016, Liu et al. 2017). Nevertheless, whilst not completely ameliorative of MDA, Tiron could still be used to reduce lipid peroxidation within pulmonary disease especially considering the decrease in antioxidant levels identified in such patients (Tug et al. 2004, Bajpai et al. 2017).

Considering that hypoxia incidence cannot always be pre-empted, such as in acute and severe allergic reactions or asthmatic exacerbations, it was of interest to observe if Tiron could provide protection against hypoxia without pre-treatment. When administered at the same time as BEAS-2B cell incubation in hypoxia, Tiron was significantly protective against mtDNA strand breaks as had been seen with pre-treatment (Figure 4.16). The high degree of protection offered by Tiron as a result of co-treatment is also supported by results obtained by *El-Sherbeeny et al*. (2016), who demonstrated that Tiron ameliorated established airway inflammation in mice. Therefore, the protection against ROS-induced mtDNA damage as well as ROS reduction shown in this thesis adds to, and supports, the evidence for Tiron's efficacy when administered at the time, or after in the case of *El-Sherbeeny et al*'s (2016) model, of ROS elevation. Considering this, further work should be conducted to observe if combined treatment of dexamethasone and Tiron could offer greater reductions in MDA biomarkers within asthmatic models, not modelled by *El-Sherbeeny et al*. (2016), and how this may affect other biomarkers of oxidative stress and asthma.

Furthermore, Tiron could be administered to reactively reduce ROS such as generated during allergen-induced airway hyperresponsiveness and bronchoconstriction. *Sook Cho et al.* (2004) have suggested similar with α-lipoic acid supplementation in ovalbumin treated mice. There is also potential to use Tiron to reduce ROS or other markers of oxidative stress with supplementation as measured in breath condensates clinically, such as reported by *Stefanska et al.* (2012), who found that nebulised apocynin significantly reduced H_2O_2 levels in asthmatics. It was suggested by *Nakamoto et al.* (2016) that elevated ROS within serum could be used to predict the incidence of severe asthmatic exacerbations, which could then ensure timely intervention. As such and with further experimentation, Tiron could be supplemented to reduce these levels, supported by the Figures 4.11 and 4.12. Therefore, the finding that Tiron

could be administered during ROS induction, such as is present within pulmonary disease, could widen the therapeutic use of Tiron in that, as opposed to preventative therapy, Tiron could be used acutely to manage ROS and minimise damage accumulation observed in patients with chronic disease, such as COPD (Maluf et al. 2007). These results also support the data in chapter 3, which demonstrated that co-administration of Tiron alongside glucose (25 mM) consecutively for 72 hours was significantly protective and further indicates the use of Tiron as a reactive therapy additional to its preventative role and expanding its potential.

It was indicated in Figure 4.13 that prolonged (≥48 hours) treatment with Tiron significantly ($p \leq 0.001$) increased ROS levels. However, when BEAS-2B cells were treated with H_2O_2 following prolonged treatment with Tiron, there was still a significant ($p \leq 0.001$) decrease in ROS levels, suggesting both the production and reduction of ROS by Tiron. *Taiwo* (2008) demonstrated that Tiron forms a semiquinone radical independent of $O_2^{\cdot-}$ availability, supporting the elevation of ROS in Figure 4.13, and that induction of ROS also enhances this formation. Therefore, it can be suggested that the production of ROS by Tiron contributes to the protection offered during co-treatment in a feed-forward mechanism (Figure 4.16). However, the decrease in cell viability (Figure 4.10) as a result of prolonged treatment suggests that supplementation with Tiron alone may elicit undesirable cytotoxicity, likely as a result of its ROS generation, and should only be considered when ROS generation is present such as during inflammation or exposure to low oxygen environments.

Further to this, *Wong et al.* (2015) found Tiron to enhance bradykinin-induced vasodilation through H_2O_2 production during hyperoxia. It was shown that Tiron reduced hyperoxia-induced $O_2^{\cdot-}$ resulting in the generation of H_2O_2, which was then ameliorated by co-treatment with catalase and highlighting the production of ROS as a result of Tiron's action (Wong et al. 2015). This could explain the protection of BEAS-2B cells after 120 hours of hypoxic exposure, particularly following Tiron's removal from cell culture medium after pre-treatment (Figure 4.15), in that the ROS formed as a result of Tiron's action could also induce the endogenous response, enhancing ROS reduction (Wong et al. 2015). In support of this, it was indicated that treatment with Tiron (≥48 hours) significantly increased Nrf2 protein expression.

Previously Tiron's action as a result of 24-hour pre-treatment was found to be independent of Nrf2 in a knockdown model in human dermal fibroblasts (Oyewole et al. 2014). This was supported by results obtained in Figure 4.19 where following treatment with H_2O_2 (0.25 mM), Tiron maintained comparable Nrf2 protein expression levels to the Tiron control. On the other hand, when BEAS-2B cells were treated with Tiron for 48-120 hours, the induction of Nrf2 protein expression (Figure 4.20) was correlated with the elevation of ROS seen in Figure 4.13. Increases in ROS-mediated Nrf2 expression have been evidenced previously in human and

mouse pulmonary fibroblasts alongside the downstream target gene NAD(P)H: quinone oxidoreductase 1 (NQO1) (Zucker et al. 2014, David et al. 2017). As such, it can be suggested that the protection offered by Tiron is as a result of ROS-mediated Nrf2 induction, which in turn would induce the expression of endogenous antioxidants. To support this hypothesis, the effect of Tiron supplementation on the expression of endogenous antioxidants such as SOD or catalase requires evaluation to understand if this is as a result of the elevated Nrf2 protein expression seen in Figure 4.20, with evidence *in vivo* indicating that Tiron maintains control levels of SOD in ovalbumin-induced asthma (El-Sherbeeny et al. 2016).

The use of exogenous Nrf2 inducers in pulmonary disease has yet to be extensively evaluated. However, evidence is emerging illustrating Nrf2 deficiencies within diseases such as COPD and asthma compared to control subjects (Li et al. 2013, Taha et al. 2014, Yamada et al. 2016). As such Tiron's independent mechanism of Nrf2 as a result of 24-hour treatment could be beneficial in conditions where Nrf2 activity is reduced and correlated with oxidative damage, such as COPD, but could also be used to enhance the reduction of oxidative stress potentially through endogenous antioxidant induction, which needs to be evidenced with further study (Mercado et al 2011, Duan et al. 2017).

In summary, the study of Tiron supplementation in BEAS-2B cells demonstrated a protective mechanism against ROS-induced damage. This was shown through reduction of oxidative damage including cell viability loss, decreases in lipid peroxidation and the prevention of mtDNA strand break formation and was found to be as a result of ROS reduction by Tiron in models of ROS induced directly by H_2O_2 and physiologically through hypoxic culture. The development of a BEAS-2B-specific model of hypoxia-induced ROS within this chapter permitted insight into the potential application of Tiron within pulmonary disease such as COPD or asthma, where hypoxia is encountered as a result of restricted airflow, leading to inflammation and oxidative stress. The preservation of BEAS-2B cells when Tiron was administered as both a pre- and co-treatment is an important finding, suggesting that Tiron could be used both preventatively and in response to acute situations known to induce ROS production. The findings of this chapter demonstrate the protective capabilities of Tiron build upon the evidence of Tiron's efficacy within human cell lines of different origins and future work must now be directed towards the application of Tiron in disease-relevant models to further support its expansion into clinical use.

Chapter 5– The Effect of Tiron in Human Astroglial Cells

5.1 Introduction

5.1.1 Oxidative Stress within Alzheimer's and Parkinson's Disease

The brain is one of the most vulnerable organs to oxidative stress and associated damage (Uttara et al. 2009). The incidence of oxidative damage within the brain is related to the abundance of localised transition metals (Uttara et al. 2009, Chakrabarti et al. 2011). This is further perpetuated by an absence of antioxidant enzymes relative to risk of oxidative stress (Uttara et al. 2009, Nayernia et al. 2014).

A correlation between oxidative stress and neurodegenerative diseases, such as AD and PD, has been identified *in vitro* and in patient samples, including 8OHdG and HNE (Lovell and Markesbery 2007, Hwang 2013, Selvakumar et al. 2014). A study of AD patient samples found elevated ROS production, measured by DCFDA, compared to healthy controls and when challenged with amyloid-β peptides, an increase in ROS, MDA concentration and apoptosis mediated by elevated caspase activity was evidenced (Cardoso et al. 2004). A study of PD patient cerebrospinal fluid found a reduced concentration of coenzyme Q10 as well as oxidised DNA, which is thought to contribute to the pathogenesis of PD through mitochondrial dysfunction (Isobe et al. 2010). Increased 8OHdG within SNc mitochondria have also been identified in PD alongside augmented formation of the dopamine oxidative by-product cysteinyl-DOPA (Alam et al. 2002, Miyazaki and Asanuma 2008, Dias et al. 2013). Oxidative damage to the DJ-1 protein, also known as PD protein 7, has been identified in PD and AD patients (Choi et al. 2006). Cellular models of DJ-1 knockdown have shown primary astrocyte impaired protection against oxidative stress as a result of mitochondrial dysfunction, thus corroborating the importance of protecting DJ-1 from oxidative damage (Larsen et al. 2011). As such, research is being directed towards the amelioration of ROS and associated oxidative damage to understand the relationship and reduce the incidence or progression of neurodegenerative disease.

There is a wealth of research indicating the benefits of antioxidant therapy for AD and PD *in vitro* and *in vivo* (Aksenov et al. 2001, Di Matteo and Esposito 2003, Wang et al. 2006, Manczak et al. 2010, Torres et al. 2011). MitoQ and resveratrol have both demonstrated a reduction in amyloid-β toxicity in a murine cell model of AD as well as mouse neuroblastoma cells (Manczak et al. 2010). A transgenic mouse model of AD also found MitoQ to attenuate

amyloid-β toxicity as well as reduce lipid peroxidation and caspase activity following 5 months of treatment (McManus et al. 2011). Curcumin has been shown to reduce α-synuclein-induced ROS generation, measured by DCFDA assay, in SH-SY5Y neuroblastoma cells (Wang et al. 2010). This study corresponded to the finding that α-synuclein overexpression in SH-SY5Y cells leads to elevation of ROS generation, compromising neuronal cell viability (Junn and Mouradian 2002). A 2017 study of rats administered with 6-hydroxydopamine, used to model PD, found that pre-treatment with olive leaf extraction, rich in antioxidants, restored antioxidant activity and reduced MDA concentrations (Ebrahimi and Moghaddam 2017).

There is also an abundance of clinical data examining various antioxidant-like compounds, many measuring improvements in cognitive outcomes (Adair et al. 2001, Morris et al. 2002, Rodacka et al. 2014). One of the first clinical trials to incorporate AD and antioxidant therapy, carried out in 1997, found that those treated with vitamin E alongside their conventional treatment and with vitamin E alone experienced a significant delay in AD symptoms (Sano et al. 1997). It was also found that patients at significant risk of developing AD experienced cognitive improvements, potentially delaying the onset of cognitive decline following 3-6 months of NAC supplementation (Adair et al. 2001). Furthermore, 3 month combined omega-3 fatty acid and vitamin E supplementation also showed improvements in antioxidant capacity, clinical PD disease rating and decreased C-reactive protein sensitivity, an inflammatory biomarker (Zhang et al. 2002, Taghizadeh et al. 2017).

Nevertheless, there has also been a number of studies that suggest that exogenous antioxidant supplementation has marginal or no benefit in AD and PD (Luchsinger et al. 2003, Boccardi et al. 2016). As a result, inconsistencies in clinical data inhibit the progression and investment in antioxidant research for neurodegenerative disease (Hermann 2016). This demonstrates a clear need for better understanding of therapeutic mechanisms of action to improve translation into humans.

Tiron, as detailed in chapter 1, is a free radical scavenger which has been shown to supersede some exogenously supplemented antioxidants, including Resveratrol (Rodacka et al. 2014, Oyewole et al. 2014). *Rodacka et al.* (2014) found that Tiron reduced GAPDH oxidation, a protein which interacts readily with amyloid-β precursor proteins within AD, through ROS reduction to a greater extent compared to Resveratrol and indicating that Tiron has the potential to be used protectively in neurodegenerative disease (Butterfield et al. 2010). Tiron has also been used *in vivo* within the brain, demonstrating permeability, good tolerance and blood brain barrier (BBB) protection (Mayhan 2000, Sharma et al. 2007). However, Tiron has yet to be evaluated in the prevention of oxidative damage within human-derived brain cells *in vitro*.

5.1.2 Aim

The aim of this study was to evaluate the use of Tiron as a protective agent against cellular ROS generated in astroglial cells *in vitro*. The human astroglial cell line, SVGp12, was used in models of H_2O_2 and hypoxia induced ROS, the latter of which was used to model physiologically derived ROS.

5.2 Methods

5.2.1 Cell Culture

Immortalised human foetal astrocyte (SVGp12) cells (ATCC, UK) are a cell line first characterised in 1985 (Henriksen et al. 2014). Derived from primary foetal brain cells, these cells were cultured in eagle's minimum essential medium (EMEM) (ATCC, UK) supplemented with 10% FBS and 1% penicillin:streptomycin (5U/ml).

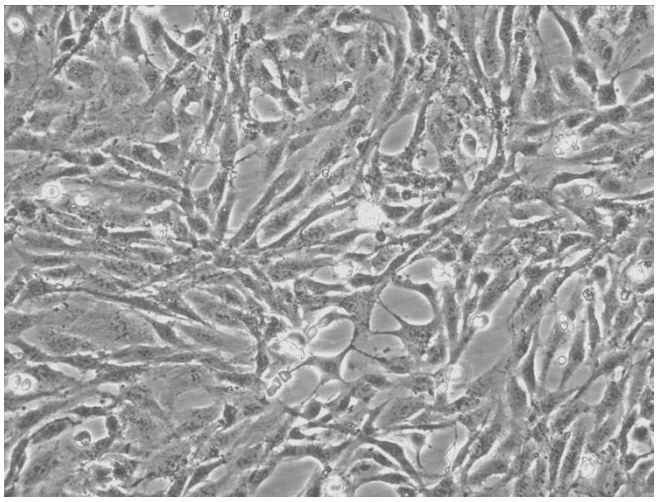

Figure 5.1. *The morphology of SVGp12 cells during culture at 90% confluency, passage 12. Original figure obtained prior to cell passage using a TS100 elipse microscope (Nikon, UK) and cooled camera (Microtec, UK).*

Astrocytes are one of the most abundant cell types within the brain and have been implicated in the pathology of AD and PD with evidence of astrogliosis and apoptosis found in clinical samples alongside oxidative stress (Kobayashi et al. 2002, Verkhratsky et al. 2015, Eugenín et al. 2016). Similar to BEAS-2B cells, SVGp12 cells have often been used in the study of oxidative stress, particularly in comparison to rodent primary or neuroblastoma cells (Lai et al. 2006, Smith et al. 2007). Together with the evidence of implication in neurodegenerative disease, the use of astrocytes to study the protective effects of Tiron against oxidative is highly relevant.

5.2.2 Cell Treatment Protocol

5.2.2.1 The Induction of ROS

A concentration range of 0.25-1 mM H_2O_2 was assessed in both the cell viability and mtDNA strand break assay, concentrations used in the BEAS-2B cells, with the aim to identify a concentration able to induce significant mtDNA damage (Kim et al. 2015). This protocol was detailed in section 2.3.1.

5.2.2.2 Induction of Hypoxia

Hypoxia was used to induce physiologically derived ROS as had been conducted in chapter 4. The length of SVGp12 exposure to hypoxic conditions was optimised to ensure a significant level of intracellular ROS was produced and that this was capable of causing substantial mtDNA strand breaks. In the assessment of Tiron's protective effect against hypoxia-induced damage, cells were pre-treated with Tiron before being placed in hypoxia-incubated BEGM media (24 hours).

5.2.2.3 Confirmation of Intracellular ROS Production

The generation of intracellular ROS by both hypoxia and H_2O_2 was evaluated using the DCFDA assay (Abcam, UK), which is detailed in section 2.3.3. During the H_2O_2 treatment protocol, SVGp12 cells were incubated with DCFDA reagent prior to induction of ROS. For hypoxia treated cells, 20 µM of DCFDA stain was added to SVGp12 cells following trypsinisation as a result of stain stability (manufacturer's recommendation).

5.2.2.4 Treatment with Tiron

Tiron was supplemented into culture as detailed in section 2.4. Furthermore, and in-line with chapters 3 and 4, the effect of prolonged exposure to Tiron was also observed for up to 120 hours before culture medium was replaced and ROS was induced.

Cell viability was assessed as following Tiron treatment as detailed in section 2.4 with the TOX-8 and SRB assays (Sigma-Aldrich, UK).

5.2.3 Assessment of ROS-Induced Damage and Data Analysis

The assessment of Tiron's protection against ROS-induced damage was assayed and analysed as detailed in chapter 2.

5.3 Results

5.3.1 The Effects of Increasing Tiron Concentrations on Cell Viability

The LD50 of Tiron in SVGp12 cells was estimated with the TOX-8 and SRB assays, consistent with chapters 3 and 4, where SVGp12 cells were treatment with 0-2 M Tiron for 24 hours (Figure 5.2). Tiron 50 mM to 2 M induced a significant ($p \leq 0.001$) reduction in cell viability compared to the untreated control (50 mM: 84±1.92%; 2 M: 8±0.60%; vs. 100±0.89%). As such, the LD50 was estimated at 261 mM. This was corroborated with the SRB assay where LD50 was 299 mM (50 mM: 86±1.19%; 2 M: 18±0.81%; vs. 100±0.40%).

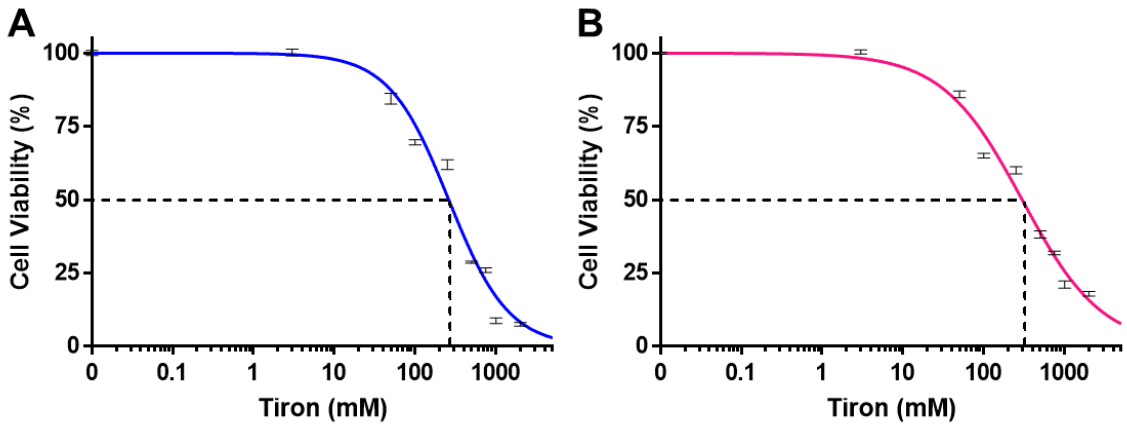

Figure 5.2. *The establishment of Tiron's LD50 in SVGp12 cells after 24-hour treatment (0-2M). The LD50 was calculated from the gradient and was found at 261 mM and 299mM, using the TOX-8 (A) and SRB (B) assays. N=9 from 3 independent experiments; mean±SEM.*

Treatment with Tiron at 1-10 mM for 24 hours was used to identify a suitable test concentration. No significant effect on cell viability was observed with 1-4 mM compared to the untreated control ($p \geq 0.05$) (Figure 5.3). The results obtained were comparable with Figures 3.3 and 4.3. Treatment with 5-10 mM led to a significant reduction in cell viability ($p \leq 0.001$), as seen in previous chapters (5 mM: 90±0.75%; 10 mM: 72±1.24%; vs. untreated control: 100±0.40%). These results were confirmed with the SRB assay (5 mM: 93±0.69%; 10 mM: 77±3.28%; vs. 100±0.62%).

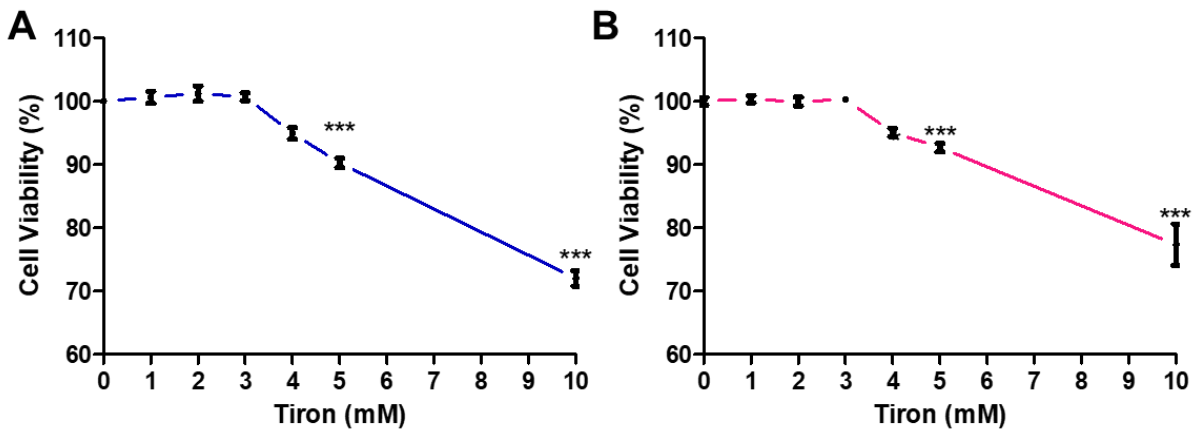

***Figure 5.3**. The effect of Tiron (1-10 mM) on cell viability after 24-hour treatment. Tiron reduced cell viability at 5-10 mM in SVGp12 cells in both the TOX-8 (A) and SRB assays (B). *** p≤0.001 vs. untreated control; n=9 from 3 independent experiments; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

5.3.2 The Optimisation of H_2O_2-Induced ROS Damage Through Titration

H_2O_2 treatment (0.25-1 mM) demonstrated a significant effect on cell viability in SVGp12 cells compared to the untreated control in the TOX-8 assay (H_2O_2 0.25 mM: 64±1.51%; 0.5 mM: 60±1.10%; 1 mM: 53±0.85%; vs. 100±1.56%) (Figure 5.4A). These results were confirmed with the SRB assay (0.25 mM: 54±2.92%; 0.5 mM: 51±2.38%; 1 mM: 51±2.57%; vs. 100±0.62%).

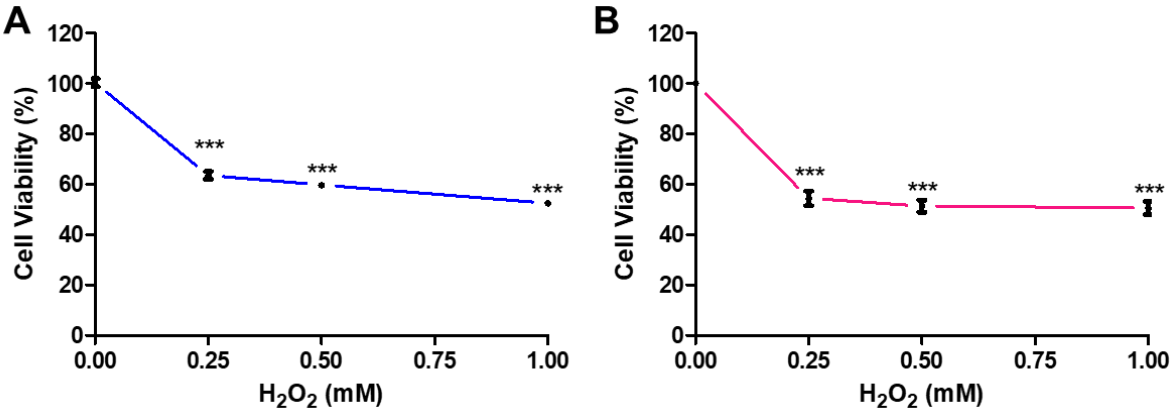

***Figure 5.4**. The effect of H_2O_2 (0.25-1 mM) treatment on cell viability in SVGp12 cells. H_2O_2 significantly reduced cell viability at all concentrations in both the TOX-8 (A) and SRB (B) assays. *** p≤0.001 vs. untreated control; n=9 from 3 independent experiments; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

SVGp12 cells cultured for 24 hours before treatment with H_2O_2, at all concentrations for 1-hour, led to a significant fold increase in CT value measured by qPCR and indicative of mtDNA strand breaks ($p \leq 0.001$) (Figure 5.5). Treatment with 0.5 mM H_2O_2 led to the greatest reduction in qPCR efficiency compared to 0.25 mM and 1 mM (0.25 mM: 2.93±0.10; 0.5 mM: 4.11±0.17; 1 mM: 3.30±0.11; vs. untreated control: 1.03±0.03). Therefore, this was selected as the most appropriate damage-inducing concentration for further experiments.

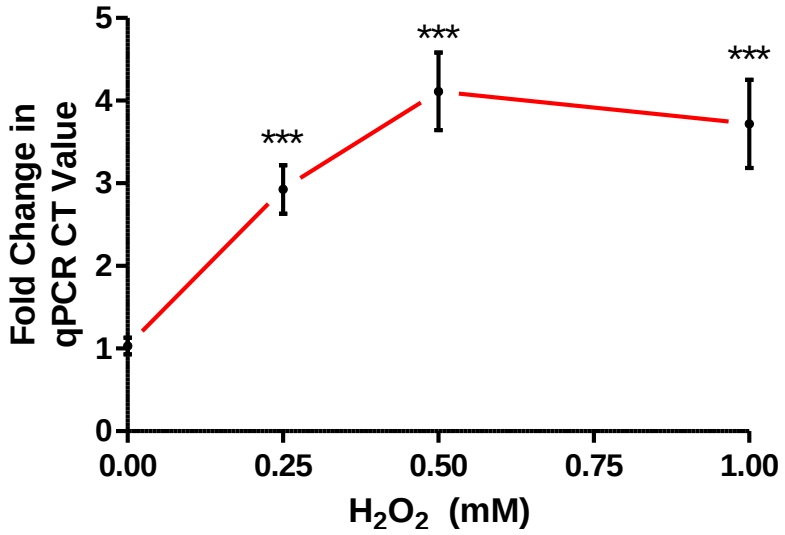

Figure 5.5. *The assessment of significant mtDNA strand break formation following H_2O_2 (0.25-1 mM) treatment. H_2O_2 at all concentrations induced significant damage in SVGp12 cells according to fold change in CT value from qPCR. *** $p \leq 0.001$ vs. untreated control; n=6; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

5.3.3 The Effect of Tiron on H_2O_2-Induced ROS Damage

5.3.3.1 The Preservation of Cell Viability with Tiron after H_2O_2 Treatment

The most suitable and protective concentration of Tiron was first optimised in the TOX-8 and SRB assays (Figure 5.6). Pre-treatment with Tiron at 1-5 mM for 24 hours before H_2O_2 treatment had a significant ($p \leq 0.001$) effect on cell viability. A significant difference between Tiron H_2O_2 treated and non-treated samples was also observed ($p \leq 0.001$) (Tiron 1 mM + H_2O_2: 73±0.49%; 3 mM+ H_2O_2: 91±1.35%; 5 mM + H_2O_2: 63±1.40%; vs. H_2O_2 52±0.35%). The SRB assay supported these findings also.

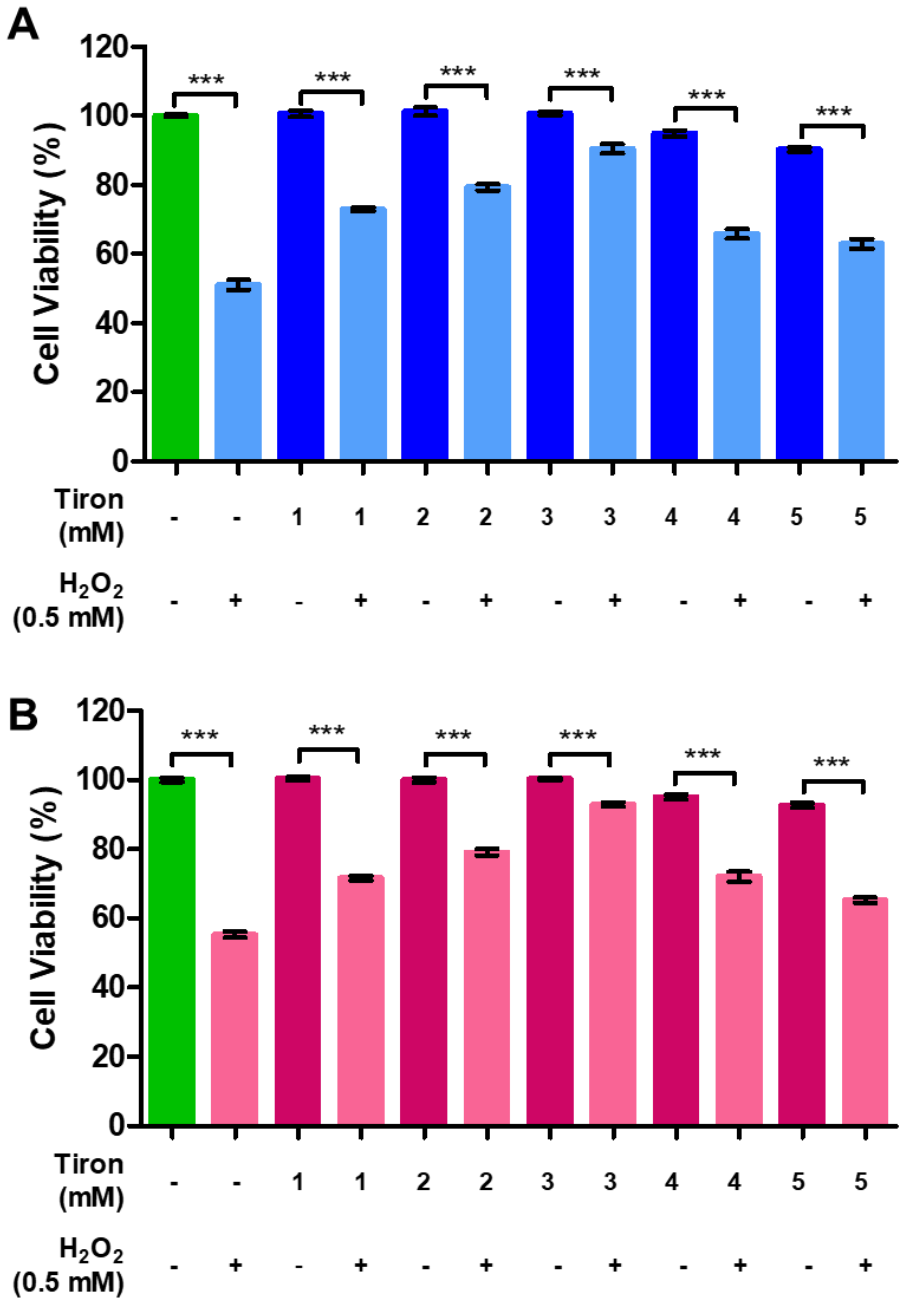

Figure 5.6. *The preservation of cell viability with Tiron (1-5 mM) pre-treatment followed by H_2O_2 exposure. Tiron significantly improved cell viability at increasing concentrations in the presence of 0.5 mM H_2O_2 in SVGp12 cells observed in both the TOX-8 (A) and SRB (B) assays. *** p≤0.001 significant difference with vs. without H_2O_2 at each Tiron concentration; n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

5.3.3.2 The Prevention of H_2O_2-Induced MtDNA Strand Breaks with Tiron

Pre-treatment with Tiron (2-5 mM) for 24 hours before the addition of H_2O_2 (0.5 mM) significantly ($p \leq 0.001$) preserved mtDNA determined by qPCR, similar to had been observed in Figure 5.6 in preservation of cell viability (H_2O_2 +; Tiron 2 mM: 1.48±0.05; 3 mM: 0.79±0.02; 4 mM: 0.75±0.02; 5 mM: 1.34±0.06; vs. H_2O_2 0.5 mM: 3.97±0.07) (Figure 5.7). As had been observed in chapters 3 and 4, treatment with 3 mM offered a high degree of protection against H_2O_2-induced strand breaks. Therefore, these results supported the comparable use of Tiron at 3 mM.

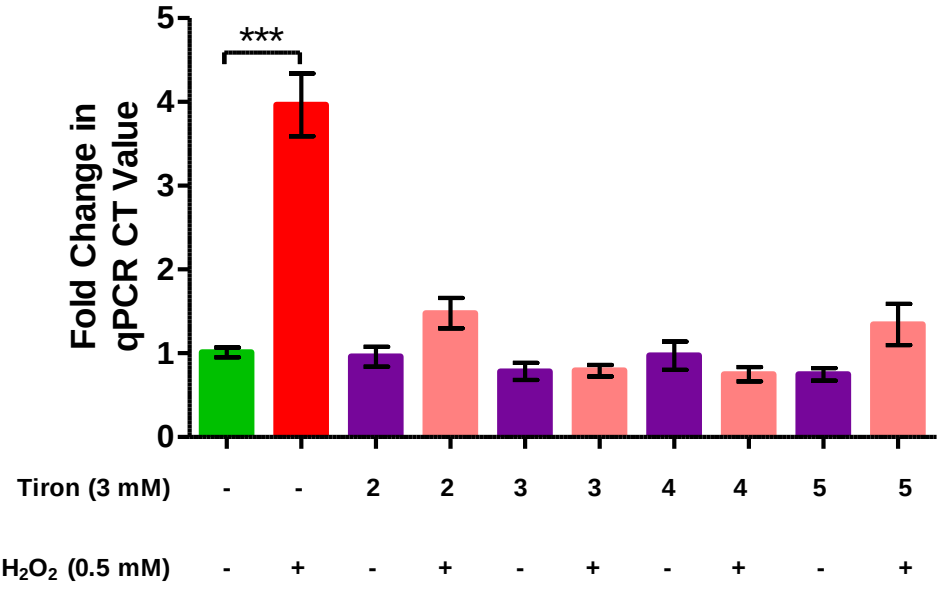

Figure 5.7. *The protection offered by Tiron (2-5 mM) against H_2O_2-induced mtDNA strand breaks. Tiron demonstrated significant protection against mtDNA strand breaks in SVGp12 cells at concentrations between 2 and 5 mM. *** $p \leq 0.001$ significant difference with vs. without H_2O_2; n=6; mean±SEM, two-way ANOVA with Bonferroni correction.*

5.3.3.3 The Effect of Tiron on H_2O_2-Induced ROS production

SVGp12 cells treated with 0.5 mM H_2O_2 significantly ($p \leq 0.001$) increased fluorescence intensity measured by flow cytometry in the DCFDA assay, indicative of intracellular ROS production (159±6.21% vs. untreated control: 100±2.06%). Pre-treatment with Tiron (3 mM) reduced this significantly compared to the H_2O_2 control and resulted in a decrease in fluorescence intensity below the untreated control ($p \leq 0.001$) (Tiron+ H_2O_2: 39±3.99%; vs. H_2O_2: 159±6.21%; vs. 100±2.06%) (Figure 5.8). Furthermore, treatment with Tiron without H_2O_2 significantly reduced fluorescence intensity greater than the baseline (59±5.51%). These results were also observed in the HKC-8 cells but not in BEAS-2B cells.

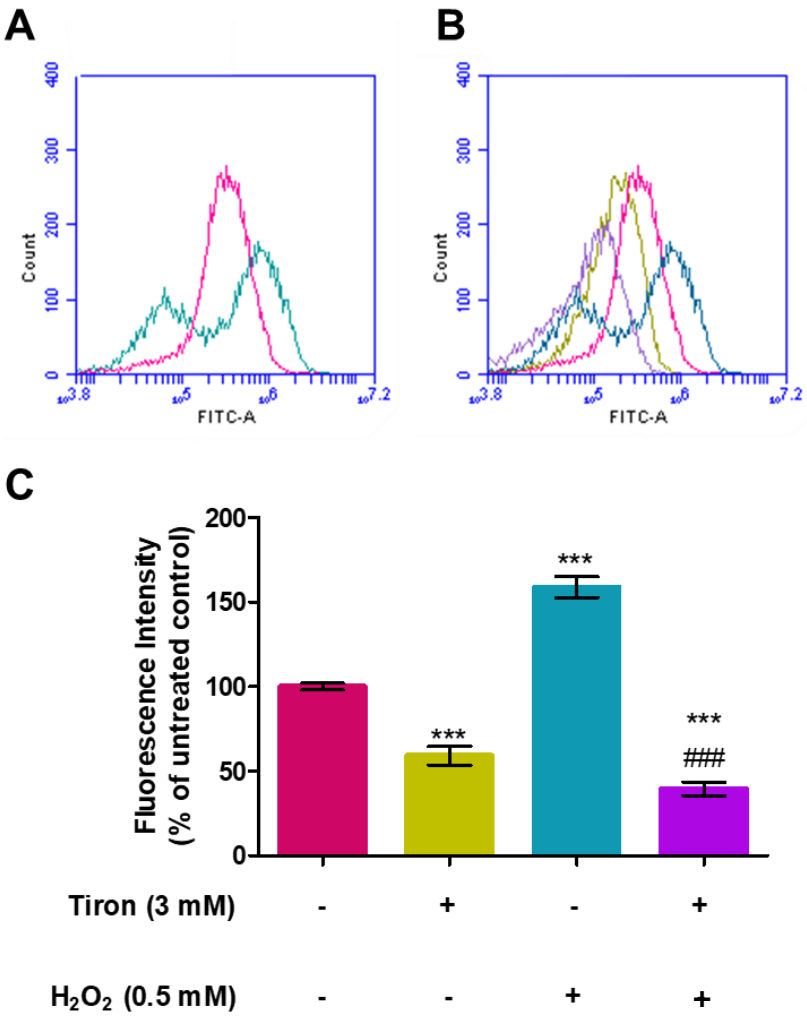

Figure 5.8. *The effects of Tiron (3 mM) on intracellular ROS production with and without H_2O_2 treatment (0.5 mM) in the DCFDA assay analysed by flow cytometry. (A) 0.5 mM H_2O_2 (blue) treatment significantly increased fluorescence intensity compared to untreated control (pink). (B) Tiron pre-treatment (purple) significantly reduced intensity following H_2O_2 treatment. (C) Tiron pre-treatment significantly reduced DCFDA fluorescence following H_2O_2 treatment in SVGp12 cells. The colours shown directly relate to Figure 5.8A and B. *** p≤0.001 vs. untreated control; ### p≤0.001 vs. H_2O_2; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

5.3.3.4 The Effects of Tiron on Lipid Peroxidation

Treatment of SVGp12 cells with 0.5 mM H_2O_2 significantly ($p \leq 0.001$) induced lipid peroxidation, measured by MDA (132±10.95% vs. untreated control: 100±2.31%). Tiron pre-treatment resulted in a significant ($p \leq 0.001$) decrease in MDA production compared to H_2O_2 (105±6.29% vs.132±10.95%). Unlike the results in Figures 3.8 and 4.8, Tiron pre-treatment did not significantly increase MDA concentration ($p \geq 0.05$) and was supported by an MDA reduction with Tiron alone (70±7.39% vs. 100±2.31%).

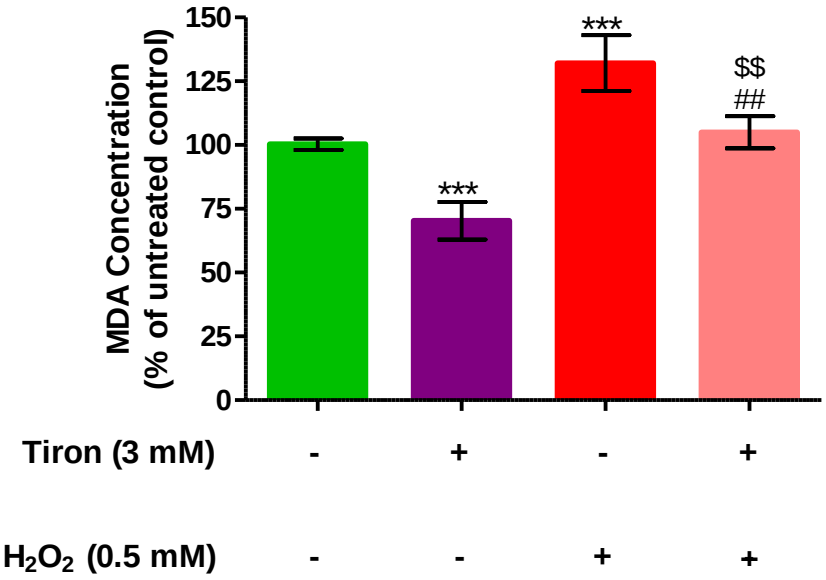

Figure 5.9. *The prevention of MDA concentration increases induced by H_2O_2 with Tiron pre-treatment. Tiron offered significant protection against H_2O_2-induced lipid peroxidation in SVGp12 cells through the measurement of the MDA biomarker. *** $p \leq 0.001$ vs. cell only control; ## $p \leq 0.01$ vs. H_2O_2; $$ $p \leq 0.01$ vs. Tiron; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

5.3.3.5 The Effect of 24-Hour Treatment with Tiron on Nrf2 Expression

As observed in chapters 3 and 4, SVGp12 cells treated with Tiron for 24 hours did not significantly increase Nrf2 protein expression as assessed by ELISA ($p \geq 0.05$) (Tiron 3 mM: 103±7.07%; vs. untreated control: 100±5.08%) (Figure 5.10). Furthermore, treatment with 0.5 mM H_2O_2 for 1 hour, after Tiron pre-treatment, did not significantly induce Nrf2 expression compared to the H_2O_2 control (Tiron + H_2O_2: 102±5.20%; vs. 336±13.27%). The absence of an increase in Nrf2 protein expression following H_2O_2 treatment after Tiron exposure was similar to results observed in HKC-8 cells.

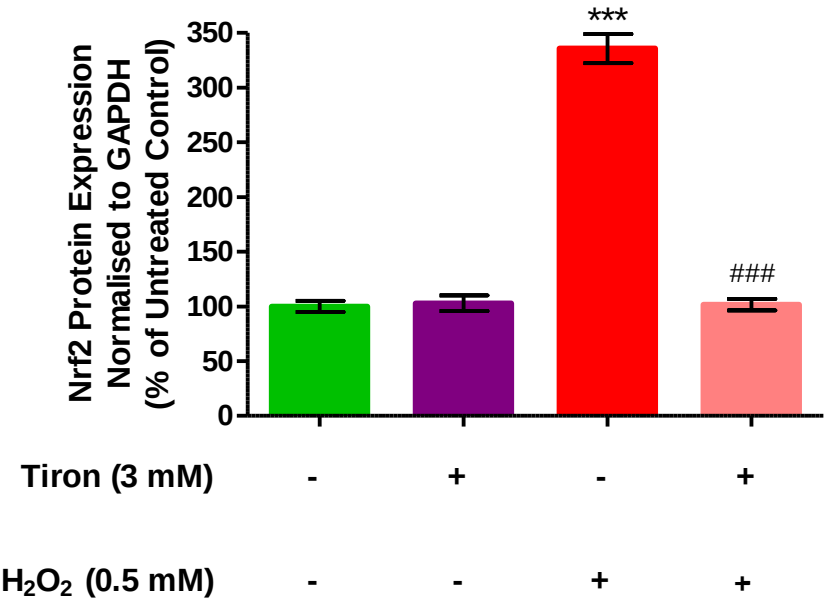

Figure 5.10. *The qualitative assessment of Nrf2 expression with Tiron (3 mM) after H_2O_2 (0.5 mM) treatment. Pre-treatment with Tiron did not induce an increase in Nrf2 expression in SVGp12 cells following ROS induction. *** p≤0.001 vs. cell only control; ### p≤0.001 vs. H_2O_2; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

5.3.4 The Effects of Prolonged Treatment with Tiron

5.3.4.1 The Effects of Prolonged Tiron Treatment on Cell Viability

The effect of 48-72 hour treatment on the cell viability of SVGp12 was assessed alone and with the addition of 0.5 mM H_2O_2 for 1-hour in the TOX-8 and SRB assays. Tiron significantly reduced cell viability compared to the untreated control in the TOX-8 assay after 48 or 72 hours of exposure (Tiron 48 hours: 48±3.34%; 72 hours: 32±1.59%; vs. 100±2.88%) (Figure 5.11). Pre-treatment with Tiron followed by H_2O_2 did not lead to a significant ($p \geq 0.05$) preservation of cell viability as had been observed in Figures 3.11 and 4.19 (Tiron 48 hours +

H_2O_2: 30±1.59%; 72 hours + H_2O_2: 28±1.23%; vs. H_2O_2: 60±1.10%). The SRB assay confirmed this trend.

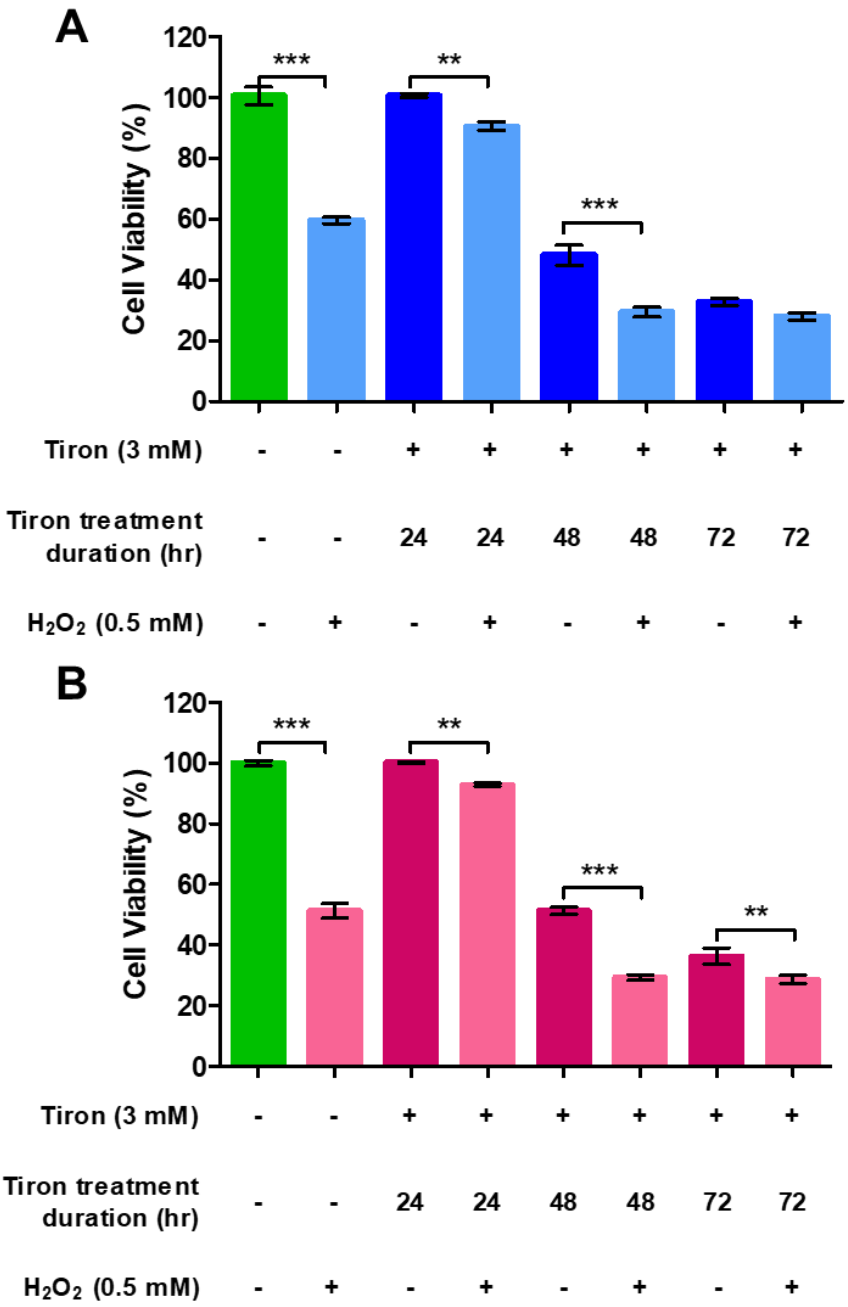

Figure 5.11. *The effect of prolonged treatment (48-72 hours) with Tiron (3 mM) on cell viability. Tiron treatment longer than 24 hours significantly reduced cell viability in both the TOX-8 (A) and SRB (B) assays. **,*** p≤0.01,0.001 significant difference with vs.without H_2O_2 at each Tiron treatment duration; n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

5.3.4.2 The Effects of Prolonged Treatment with Tiron on Intracellular ROS Production

Treatment of SVGp12 cells for 48 hours with Tiron did not increase fluorescence intensity fluorometrically, contrary to the negative effect on cell viability observed in Figure 5.11 (Tiron 48 hours: 99±2.23%; vs. untreated control: 100±2.93%). Treatment for 120 hours did increase fluorescence intensity significantly, indicative of ROS production ($p \leq 0.001$) (249±11.50%) (Figure 5.12). Prolonged treatment with Tiron did have a protective effect when SVGp12 cells were treated with H_2O_2, which was not indicated previously in Figure 5.11 (Tiron 48 hours + H_2O_2: 177±5.29%; 120 hours + H_2O_2: 276±8.66%; vs. H_2O_2: 513±13.97%).

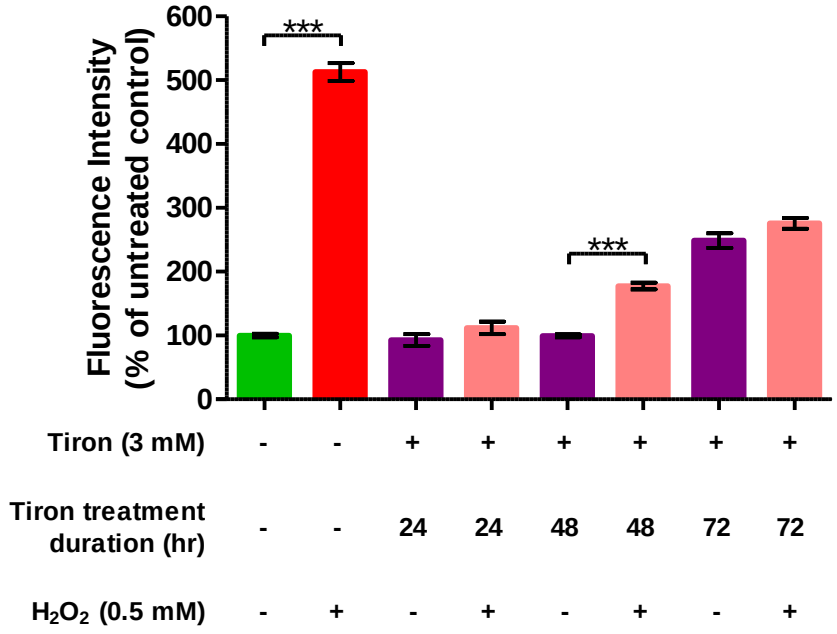

Figure 5.12. *The effects of prolonged treatment (48-120 hours) with Tiron on intracellular ROS production. Prolonged treatment significantly reduced H_2O_2-induced fluorescence intensity measured fluorometrically with the DCFDA assay in SVGp12 cells. *** $p \leq 0.001$ with vs. without H_2O_2 at each Tiron treatment duration; n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

5.3.4.3 The Expression of Nrf2 Following Prolonged Treatment with Tiron

Corresponding to results in Figure 5.12, supplementation with Tiron for 48 hours did not affect Nrf2 expression alone, measured by ELISA (Tiron 48 hours: 107±4.51%; vs. untreated control: 100±5.08%) (Figure 5.13). However, treatment with H_2O_2, induced significant Nrf2 expression ($p \leq 0.001$) (Tiron 48 hours + H_2O_2: 200±11.31; vs. H_2O_2 0.5 mM: 336±13.27%). As shown in Figures 3.13 and 4.20, 120-hour treatment with Tiron led to a significant ($p \leq 0.001$) increase in Nrf2 expression (Tiron 120 hours: 232±18.41%; vs. 100±5.08%). This was also seen in response to H_2O_2 treatment (Tiron 120 hours + H_2O_2: 245±18%; vs. H_2O_2 0.5 mM: 336±13.27%).

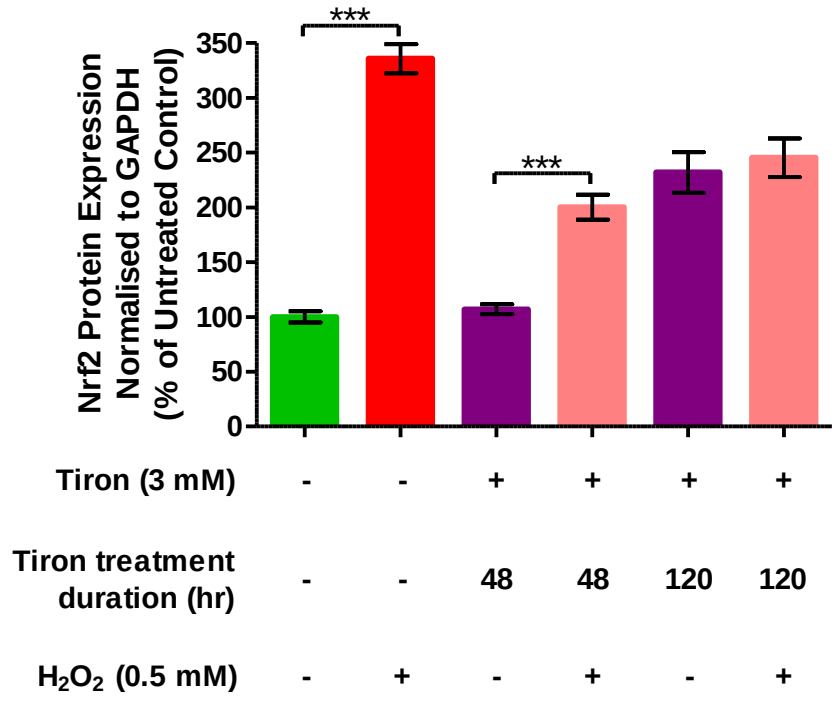

Figure 5.13. *The expression of Nrf2 following prolonged treatment with Tiron (48-120 hours) by ELISA. Tiron augmented Nrf2 expression in SVGp12 cells as a result of prolonged incubation with and without ROS induction by H_2O_2. *** $p \leq 0.001$ significant difference with vs. without H_2O_2 at each Tiron treatment duration; n=6 from 2 independent experiments; mean±SEM, two-way ANOVA with Bonferroni correction.*

5.3.5 The Optimisation of Hypoxia-Induced Damage

The induction of ROS through culture of SVGp12 cells in hypoxic conditions (1% O_2) was optimised over 1-48 hours in Figure 5.14. Exposure for 1-2 hours did not significantly increase fluorescence intensity fluorometrically in the DCFDA assay ($p \geq 0.05$). However, both 24 and

48 hours significantly induced a fold change in fluorescence intensity, indicative of ROS production (p≤0.001) (Hypoxia 24 hours: 222±16.34%; 48 hours: 294±11.32%; vs. normoxic (21% O_2) control: 100±5.62%). This was agreeable with published literature (Al Ahmad et al. 2012).

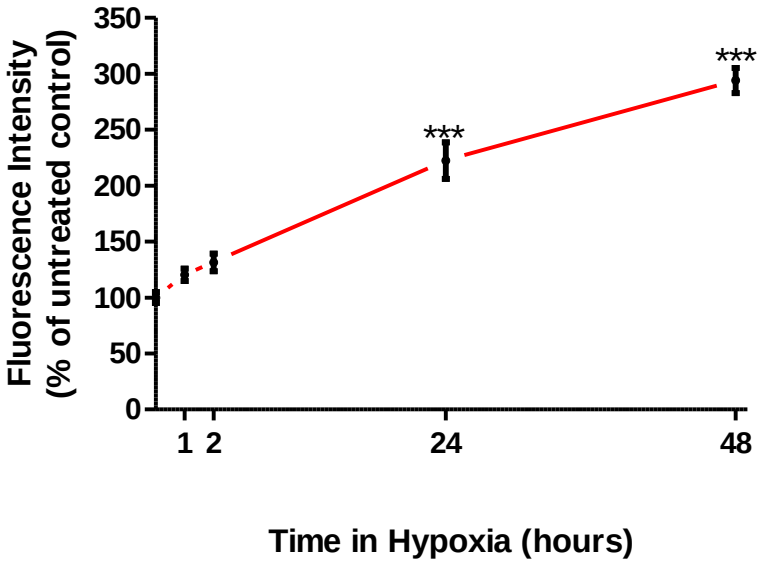

Figure 5.14. *The assessment of ROS production by prolonged culture in hypoxia. SVGp12 cells subjected to hypoxic conditions for 24 and 48 hours significantly increased intracellular ROS, not seen after 1 and 2 hours. *** p≤0.001 vs. normoxia (21% O_2); n=6; mean±SEM.*

The use of hypoxia to induce significant mtDNA strand breaks in SVGp12 cells was novel to this study. In contrast to results in Figure 5.14, SVGp12 cells cultured in hypoxia for 24 hours did not lead to a measurable induction of mtDNA strand break damage (p≥0.05) (Hypoxia 24 hours: 1.00±0.01; vs. normoxic control: 1.02±0.01). Shown in Figure 5.15, incubation for 48 hours led to a significant (p≤0.001) reduction in qPCR efficiency indicated by a fold reduction compared to the normoxic control (Hypoxia 48 hours:2.92±0.10). This was a shorter hypoxic incubation time than required in BEAS-2B cells (Figure 4.7).

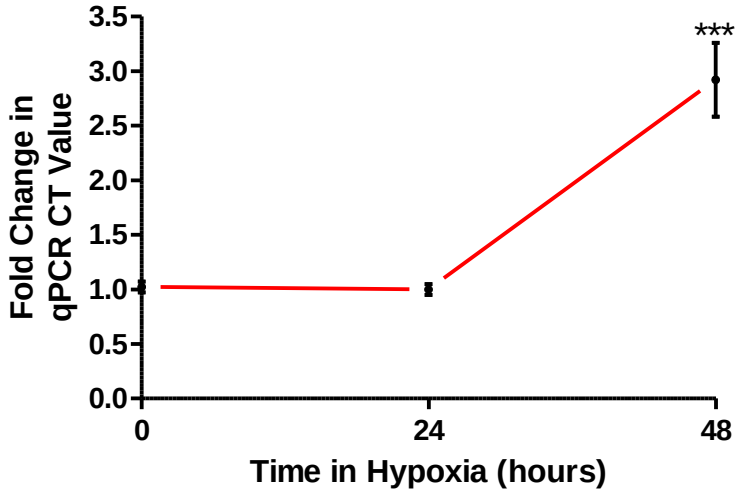

Figure 5.15. *The induction of significant mtDNA strand breaks with qPCR following hypoxic culture. SVGp12 cells subjected to hypoxic conditions (1% O_2) for 48 hours significantly reduced qPCR efficiency, indicative of mtDNA strand break damage. *** p≤0.001 vs. normoxia (21% O_2); n=6; mean±SEM, one-way ANOVA with Dunnett's post-hoc.*

5.3.6 The Effects of Tiron on Hypoxia-Induced ROS Damage

5.3.6.1 Preservation of Cell Viability with Tiron Pre-treatment Before Hypoxic Exposure

In agreement with Figure 5.14, a significant (p≤0.001) decrease in cell viability was observed after 24-hour culture in hypoxia (24 hours: 83±0.86%; 48 hours: 62±1.57%; vs. untreated control: 100±0.55%). Similar to Figure 4.10, pre-treatment for 24 hours with Tiron significantly preserved cell viability following hypoxic exposure (Tiron + hypoxia 24 hours: 94±1.56%; vs. hypoxia 24 hours: 83±0.86%) (Figure 5.16). This was also observed with pre-treatment following 48 hours of hypoxia (Tiron + hypoxia 48 hours: 93±1.83%; vs. hypoxia 48 hours: 62±1.57%). These results were replicated in the SRB assay (Hypoxia 24 hours: 82±0.90%; Tiron + hypoxia 24 hours: 95±0.87%; 48 hours: 71±1.00%; Tiron + 48 hours: 89±1.00%; vs. normoxia: 100±0.86%).

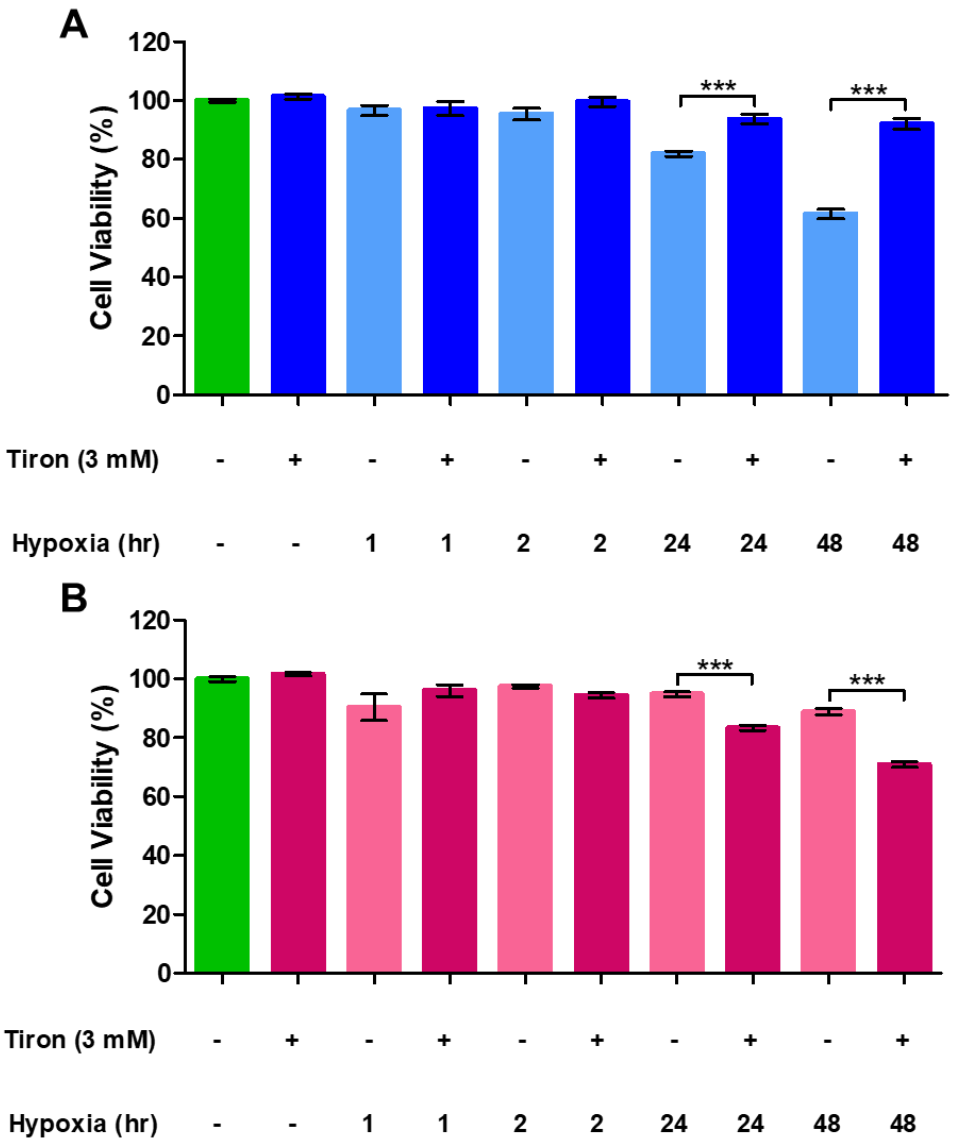

Figure 5.16. *The effect of Tiron (3 mM) on cell viability preservation following hypoxic (1% O_2) culture (1-48 hours). Hypoxia reduced cell viability at 24 and 48 hours in SVGp12 cells in both the TOX-8 (A) and SRB (B) assays but was recovered with Tiron pre-treatment. *** $p \leq 0.001$ significant difference with or without Tiron treatment during hypoxia; n=9 from 3 independent experiments; mean±SEM, two-way ANOVA with Bonferroni post-hoc.*

5.3.6.2 The Inhibition of Hypoxia-Induced MtDNA Strand Break Formation with Tiron

Pre-treatment with Tiron for 24 hours offered a high degree of protection against hypoxia-induced mtDNA strand breaks in SVGp12 cells, demonstrated by a significant fold decrease in CT value ($p \leq 0.001$) (Tiron + hypoxia: 0.79±0.02; vs. hypoxia : 3.35±0.08; vs. normoxia: 1.07±0.02). This was indicative of augmented qPCR efficiency in new strand formation (Figure 5.17).

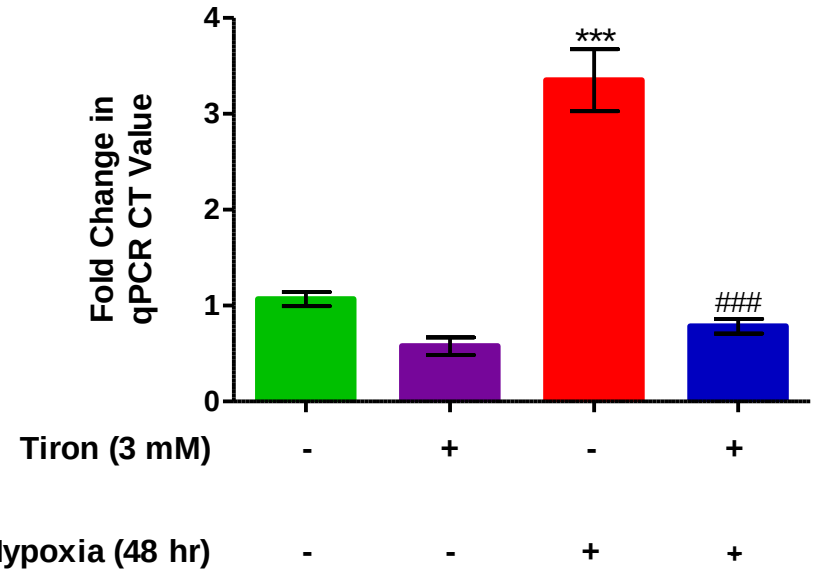

Figure 5.17. *The inhibition of hypoxia-induced (1% O_2) mtDNA strand break formation with Tiron pre-treatment. Tiron demonstrated significant protection against hypoxia-induced damage in SVGp12 cells after 48 hours. *** p≤0.001 vs. normoxia (21% O_2); ### p≤0.001 vs. hypoxia; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

5.3.6.3 The Effects of Tiron on Hypoxia-Induced ROS Production

Exposure of SVGp12 cells to hypoxia for 48 hours significantly ($p \leq 0.001$) increased fluorescence intensity, indicative of ROS production (133±3.38% vs. normoxia: 100±4.24%) (Figure 5.18). Pre-treatment with Tiron prior to hypoxia led to a significant reduction in intensity compared to the hypoxic control (32±2.52% vs. 133±3.38%). Treatment of SVGp12 cells with Tiron for 24 hours followed by 48-hour replacement of EMEM and culture in standard conditions also led to a significant decrease in intracellular ROS ($p \leq 0.001$) (40±2.83% vs. 100±3.10%). These results were similar to those obtained in the BEAS-2B cell line and Figure 5.8.

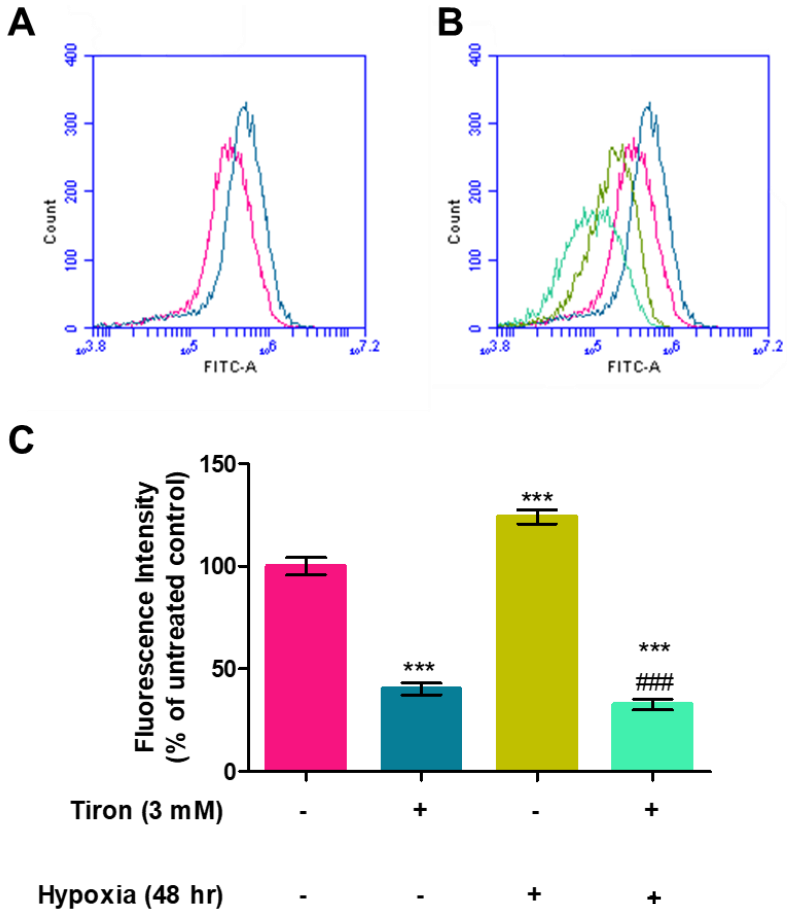

Figure 5.18. *The effect of Tiron on ROS production following hypoxic culture and Tiron pre-treatment. (A) Hypoxic culture for 48 hours (blue) caused significant increases in intracellular ROS vs. untreated control (pink). (B) Tiron treatment both with (turquoise) and without (green) hypoxia induction significantly reduced fluorescence intensity. (C) Pre-treatment with Tiron significantly reduced DCFDA fluorescence measured by flow cytometry following hypoxia treatment in SVGp12 cells. The colours shown directly relate to Figures 5.18A-B. *** p≤0.001 vs. normoxia (21% O_2); ### p≤0.001 vs. hypoxia (1% O_2); n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

5.3.6.4 The Effects of Tiron (3 mM) on Hypoxia-Induced MDA Increases

Tiron supplementation reduced the formation of MDA as a response to hypoxia significantly ($p \leq 0.001$) (Tiron + hypoxia: 117±4.16%; vs. hypoxia 48 hours: 314±0.96%; vs. normoxia: 100±0.58%) (Figure 5.19). Hypoxia induced a greater increase in MDA production compared to H_2O_2 in Figure 5.9. Similar to Figure 5.9, Tiron pre-treatment did not lead to a significant increase in MDA compared to the untreated control.

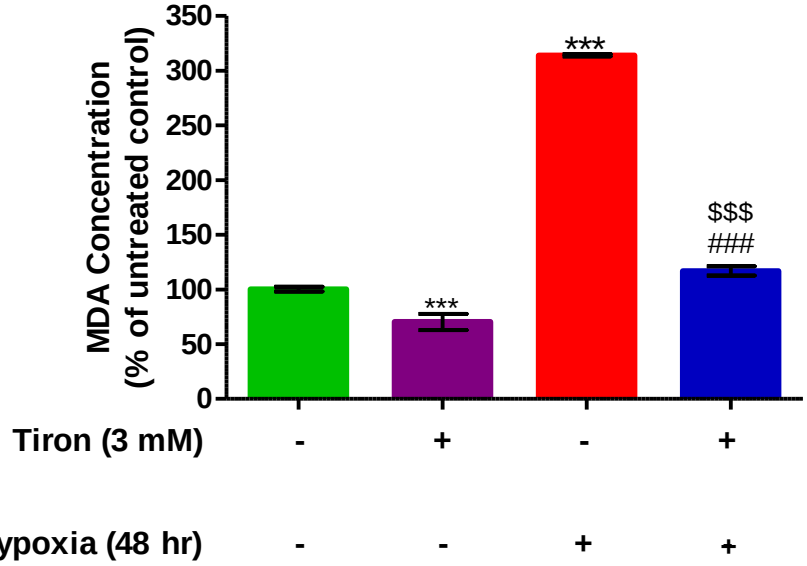

Figure 5.19. *The prevention of augmented MDA concentration with Tiron following hypoxia. Tiron offered protection against hypoxia-induced lipid peroxidation in SVGp12 cells through the measurement of the MDA biomarker. *** $p \leq 0.001$ vs. normoxic (21% O_2) control; ### $p \leq 0.001$ vs. hypoxia (1% O_2); $$$ $p \leq 0.001$ vs. Tiron; n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

5.3.7 The Effect of Co-treatment on Hypoxia-Induced ROS Damage

Shown in Figure 5.20, Tiron supplemented at the start of hypoxia induction for 48 hours offered significant ($p \leq 0.001$) protection against mtDNA damage, although not as protective as measured in Figure 5.17 (Tiron + hypoxia: 1.34±0.02 vs. hypoxia: 2.72±0.03). Tiron being present in cell culture medium for 48 hours without ROS induction caused no significant mtDNA damage ($p \geq 0.05$) (Tiron: 0.97±0.01; vs. untreated control: 0.93±0.02).

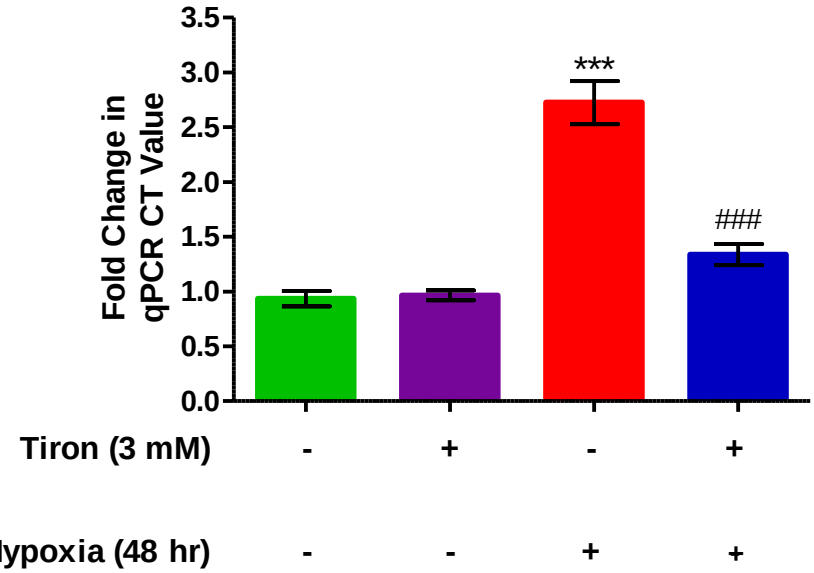

Figure 5.20. *The effects of Tiron on mtDNA strand break damage administered at the time of entry into hypoxia (48 hours). Tiron offered some protection against hypoxia–induced mtDNA strand breaks in SVGp12 cells when administered at the time of hypoxia induction. *** p≤0.05, 0.001 vs. normoxia (21% O_2); ### p≤0.001 vs. hypoxia (1% O_2); n=6; mean±SEM, one-way ANOVA with Tukey post-hoc.*

5.4 Discussion

In the prevention of H_2O_2 and hypoxia-induced damage, Tiron pre-treatment was found to be significantly protective ($p \leq 0.001$) against mtDNA strand breaks (Figure 5.7 and 5.17) and preserved MDA to near baseline levels (105 to 117% respectively) ($p \leq 0.01$) (Figure 5.9 and 5.19). The protection demonstrated by Tiron was also supported by preservation of cell viability (Figure 5.6 and 5.16), 91 to 94%, and a significant decrease ($p \leq 0.001$) in intracellular ROS compared to the H_2O_2 and hypoxia controls (39±3.99%; vs. H_2O_2: 159±6.21% and 32±2.52% vs. hypoxia: 133±3.38%) (Figure 5.8 and 5.18). These results demonstrate the protective effect elicited by Tiron against ROS-induced damage in astroglial cells. The protection achieved by Tiron was shown to be a ROS reducing action, evidenced by the reduction in DCF oxidation across all cell lines to levels comparable to the untreated control or below (35-112% vs untreated control). Similar results have also been shown in prostate cancer cells, where Tiron pre-treatment reduced lysophosphatidic acid-induced ROS levels to that of the untreated control, supporting the evidence for Tiron's ROS reducing mechanism (Lin et al. 2012). This was also shown in hippocampal embryonal cells with Tiron treatment reducing xanthine oxidase-induced ROS and demonstrating Tiron's efficacy in another brain-originating cell line (Genius et al. 2012). *In vivo*, Tiron treatment (471 mg/kg for 7 days) has also been shown to mitigate toxicity following radiation and manganese exposure through the reduction of H_2O_2 and MDA levels as evidenced within rat brains. Furthermore, it was shown that Tiron increased MnSOD and glutathione as well as ETC activity within these samples, resulting in increased concentration of coenzyme Q which maintained mitochondrial function and further contributed to the antioxidants effects of Tiron and demonstrating Tiron's efficacy within the brain (Abdel-Magied et al 2019).The results obtained in chapters 3 to 5 add to evidence from published literature suggesting that Tiron pre-treatment elicits a significant protective action, mediated by the reduction of ROS levels across different cell and tissue types, resulting in the reduction of ROS availability and oxidative damage.

The protection elicited by Tiron was achieved with a concentration of 3 mM, the same concentration that had been used throughout chapters 3 and 4. In SVGp12 cells, whilst all concentrations of Tiron demonstrated significant protection against H_2O_2-induced mtDNA damage ($p \leq 0.001$), the preservation of cell viability followed the same trend as observed in Figures 3.6 and 4.8 whereby 3 mM Tiron offered the highest cell viability (Figure 5.6) and with higher concentrations demonstrating cell viability decreases (Figure 5.3). This unanimous finding indicates that a concentration of 3 mM led to similar high degrees of protection across all cell lines assessed in this thesis and that this dose could provide consistently high levels of protection against ROS-induced damage independent of cell line origin. As has been stated

in previous chapters, a concentration of 3 mM also correlates with the published results of *Oyewole et al.* (2014), as well as another study which found Tiron to be protective against radiation-induced mtDNA damage in primary keratinocytes using the same qPCR methodology and indicating that 3 mM is also a beneficial concentration in skin-derived human cells (Hanna et al. 2018).

Additional to the supplementation of 3 mM Tiron in this thesis, the concentration of damage inducing H_2O_2 was optimised unique to each cell line to ensure similar but significant ($p \leq 0.001$) levels of damage in each chapter. In SVGp12 cells 0.5 mM H_2O_2 led to a fold change of 4.11 in qPCR CT value, indicative of significant mtDNA strand break damage (Figure 5.5) with similar fold changes observed in HKC-8 (4.89) and BEAS-2B cells (4.52). This demonstrates that the high degree of protection achieved by Tiron was comparable across similar levels of damage. Additionally, this concentration of H_2O_2 was not dissimilar to those used to induce ROS-damage in astrocytes previously, indicating the relevance of this concentration and the susceptibility of astrocytes to H_2O_2-induced damage at low concentrations (Fauconneau et al. 2002, Zhu et al. 2005, Park et al. 2012). Specifically, *Park et al.* (2012) identified that 0.5 mM significantly increased ROS as well as phosphorylation of STAT6 in primary rat astrocytes, leading to an inflammatory response. This study also found that H_2O_2 could be inhibited by the supplementation of catalase or NAC, corresponding to the results identified in this thesis with Tiron (Figure 5.8) and supporting its ROS reducing action (Park et al. 2012).

Whilst the results across cell lines in this thesis demonstrate a reproducible and high degree of protection by Tiron when assessing viability and DNA damage, there was a difference in the level of protection offered against MDA in SVGp12 cells. In Figure 5.9, it was shown that Tiron pre-treatment preserved MDA concentrations to near baseline levels. This was not observed in HKC-8 and BEAS-2B cells (Figure 3.9 and 4.17), despite a reduction in MDA concentration of 32 to 68% compared to the H_2O_2 control. This suggests that Tiron is more effective against lipid peroxidation in astrocytes than the pulmonary or renal cell lines. However, in this chapter and compared to the untreated control, H_2O_2 treatment in SVGp12 cells induced the smallest increase in MDA (132%) than had been seen in HKC-8 (149%) and BEAS-2B cells (186%). It has previously been suggested that the brain holds higher concentrations of PUFAs compared to other tissues, which can readily react with ROS and lead to the production of reactive aldehyde electrophiles such as MDA (Sultana et al. 2013, Shichiri 2014). Therefore, SVGp12 cells incurring the least augmentation in MDA concentration compared to the other cell lines was unexpected. For example, a study of Wistar rats exposed to an electromagnetic field of 1800MHz found that MDA levels were higher in

the brain in all treatment groups, including the control, compared to levels in the liver, kidney and blood (Bodera et al. 2015).

It could be hypothesised that there is an adaptive defence within astroglial cells, compared to other brain-derived cells, which may correlate with the lower concentration of MDA as a result of H_2O_2 treatment (Uttara et al. 2009, Saio et al. 2012). This has been evidenced when comparing the levels of MDA in neurons and astrocytes after treatment with an amyloid-β peptide, where at least double the concentration of MDA was measured in neurons, suggesting that they are a more vulnerable than astrocytes (Aguirre-Rueda et al. 2015). Additionally, it was shown that co-culture of neurons and astrocytes led to preservation of MDA levels when challenged with amyloid-β peptide, demonstrating the neuroprotective role of astrocytes and also the importance of preserving the viability of this cell type to ensure the efficacy of the endogenous defence (Desagher et al. 1996, Sidoryk-Wegrzynowicz et al. 2011). Therefore, experimentation in astrocytes for this assay could be a factor in the perceived attenuation of H_2O_2-induced MDA increase with Tiron greater than observed in other chapters of this thesis. It has been shown previously that astrocytes increase their antioxidant capacity in response to inflammation and oxidative stress *in vitro* which conveyed protection to adjacent neurons (van Horssen et al. 2008, Nijland et al. 2014). As such, more study is required to understand the induction of lipid peroxidation within the brain and what implication this may have within diseases which have recorded elevated biomarkers of lipid peroxidation (Chen et al. 2007, Yoshida et al. 2013). Oxidative stress including increased levels of MDA as well as SOD and catalase, have been correlated previously with cognitive decline in AD patients (Delibas et al. 2002, Torres et al. 2011, Talarowska et al. 2012). Oxidative damage, such as mtDNA strand breaks or adducts, have also been correlated with cognitive decline (Wang et al. 2006), with a recent study suggesting that biomarkers such as decreased glutathione levels could indicate cognitive decline (Hajjar et al. 2018). Despite accumulating evidence, it still remains unclear as to how oxidative stress impacts cognition. Nevertheless, work is required to understand the role in which Tiron could be used to reduce lipid peroxidation and whether this may delay cognitive deterioration through the reduction of oxidative stress. For example, a rat study has shown that supplementation with dietary antioxidants including curcumin significantly reduced MDA levels which resulted in improved memory, indicating a role for antioxidant supplementation in the reduction of cognitive deficits (Liaquat et al. 2016). Considering the results obtained with MDA throughout this thesis, it would also be of benefit to expand work with Tiron into the evaluation of HNE, another lipid peroxidation biomarker, to understand how Tiron interacts with other biomarkers of lipid peroxidation and further apply this to cognitive studies.

HNE has been found to be elevated within AD patients alongside MDA and amyloid-β deposits (Hardas et al. 2013, Rani et al. 2017). Levels of HNE have also been correlated with cognitive deterioration in AD compared to healthy controls (Keller et al. 2005, Butterfield et al. 2006) Tiron has already been shown to abrogate HNE increases in rat adrenal pheochromocytoma cells, indicating that it is not selective in MDA reduction (Lin et al. 2014). This is important in understanding the potential therapeutic role of Tiron in treatment of neurodegenerative diseases, which has been associated with both elevated levels of lipid peroxidation as well as deficiencies in function and even loss of astrocytes (de Farias et al. 2016). For example, lipid peroxidation has been found to precede amyloid-β plaque formation in transgenic mouse studies, emphasising the requirement to target oxidative damage and lipid peroxidation to reduce the manifestation of disease (Praticò et al. 2001). Similarly, significant increases in MDA plasma levels have been identified in PD patients (Sanyal et al. 2009) whilst HNE has been found to be localised within Lewy Bodies post-mortem and α-synuclein aggregates (Castellani et al. 2002, Qin et al. 2007). These published findings highlight the importance of the protective results obtained with Tiron pre-treatment against MDA increases in SVGp12 cells and indicate the therapeutic potential of Tiron within the treatment of neurodegenerative disease. This is further supported by evidence that Tiron is BBB permeable, therefore indicating the feasibility of translating these results *in vivo* (Sharma et al. 2007). In support of this, it has been previously demonstrated that Tiron does not alter BBB permeability, a barrier that is regulated in part by astrocytes, and protects against increases in permeability (Mayhan 2000). This could be exploited in the treatment of AD and PD as dysfunction of the BBB has been found to accelerate disease progression as well as neuroinflammation. This further highlights the importance of the protection that Tiron conveys within SVGp12 cells as presented in this chapter (Mayhan 2000, Desai et al. 2007).

To mimic the hypoxia observed in AD, which has been associated with increases in APP and amyloid-β plaque formations, a model of ROS induction as a result of hypoxia was developed (Austin et al. 2011, Kim et al. 2015, Zhang et al. 2018). Culture of SVGp12 cells in hypoxia for 48 hours was found to significantly ($p \leq 0.001$) induce intracellular ROS production and mtDNA strand breaks, not seen consistently after 24 hours (Figures 5.14 and 5.15). In the generation of intracellular ROS, both 24 and 48-hour incubation had a significant ($p \leq 0.001$) effect on DCF oxidation, demonstrated by an increase from 222% to 294% compared to the untreated control and supporting the formation of ROS as a result of hypoxic exposure (Figure 5.14). An increase in DCF oxidation has been seen in rat brain epithelial monolayers with increasing lengths of hypoxic incubation also and supporting the formation of hypoxia-induced ROS observed in Figure 5.14 (Al Ahmad et al. 2012). In the induction of mtDNA strand breaks however, there was a significant ($p \leq 0.001$) reduction of qPCR efficiency after 48 hours with a

fold change of 2.92 compared to 24 hours, thus showing that 24-hour exposure to hypoxia did not sufficiently induce mtDNA strand break damage compared to the untreated control (Figure 5.15). Also observed in BEAS-2B cells, significant ($p \leq 0.001$) ROS generation was induced within SVGp12 cells without a corresponding induction of mtDNA strand break damage. This suggested that insufficient ROS levels were generated to induce mtDNA damage compared to the levels induced by H_2O_2 in chapter 4. However, in SVGp12 cells, the level of ROS induced by 0.5 mM H_2O_2 were 159% compared to the untreated control (Figure 5.8) and contrary to this, the level of ROS induced following 24 hours of culture in hypoxia was greater at 222%, which suggests that ROS levels should have been elevated enough to induce mtDNA damage.

In Figure 5.16, it was shown that cell viability was significantly compromised as a result of hypoxic exposure for 24 hours ($p \leq 0.001$), supported by significantly elevated ROS levels detected in Figure 5.14. However, significant mtDNA damage was not detected at this timepoint, shown in Figure 5.15. What this could suggest is that the absence of mtDNA damage after 24 hours is as a result of the loss of cells, due to cytotoxicity, and that those sampled have maintained an undamaged or wild-type phenotype. As a result of this, it is probable that mtDNA strand breaks detected after 48 hours are due to damage incurred in the viable cells identified after 24 hours, due to mitochondrial heteroplasmicity. Mitochondrial heteroplasmicity, as introduced in section 1.3.1.2, is the accumulation of damaged or mutated alongside wild-type mtDNA without compromising cellular function (Malik and Czajka 2013, Wallace and Chalkia 2013). Therefore, it could be suggested that the results may be skewed towards thinking that damage occurs only after 48 hours of hypoxic exposure. However, damage is likely to have occurred earlier than 24 hours, but after at least 2 hours, correlating with the cytotoxicity observed in Figure 5.16 as well as the significant generation of ROS in Figure 5.14. As a result, the protection against hypoxia-induced damage achieved with Tiron pre-treatment could occur during the first 24 hours of hypoxic exposure, supported by the maintenance of cell viability at 95% in Figure 5.16 after 24 hours. The potential accumulation of hypoxia-induced damage before 24 hours of exposure aligns with the brain's reliance on O_2 and thus demonstrates that protection against such damage is paramount to preserving cellular viability, particularly in astroglial cells which are heavily relied upon for neuronal cell protection (Bélanger and Magistretti 2009, Cobley et al. 2018). Therefore, the protection achieved by Tiron, despite only being measured after 48 hours of hypoxic exposure, is important in the preservation of brain function and should be further evaluated *in vivo* to understand whether the level of protection is translatable.

Whilst SVGp12 cells were exposed to hypoxia for a shorter period (48 hours) compared to BEAS-2B cells (120 hours), the significant ($p \leq 0.001$) fold change in CT value was comparable

between the two tissues (3.35 vs 2.83-fold in BEAS-2B cells). This similar and significant level of damage permitted comparison of Tiron's protective action in the two cell lines, as had been done with H_2O_2. Tiron pre-treatment was found to be significantly protective against hypoxia-induced mtDNA strand breaks (Figure 5.17), as had also been seen with BEAS-2B cells, and the protection elicited by Tiron against hypoxia-induced damage was found to be ROS reducing. This is supported by Tiron's significant ($p \leq 0.001$) reduction of DCF fluorescence to 35% compared to the untreated control (Figure 5.18) and indicating a reduction in ROS generation through Tiron pre-treatment. *Chiu et al.* (2015) also found that antioxidant supplementation reduced hypoxia-induced ROS and supports Tiron's protective mechanism.

As had been observed following H_2O_2 treatment, Tiron pre-treatment significantly ($p \leq 0.001$) reduced MDA concentration as a result of 48-hour exposure to hypoxia (Figure 5.19). In corroboration with the results observed in chapter 3 and 4, Tiron failed to offer reduction to baseline levels following hypoxia-induced MDA formation but did reduce MDA concentration by 92% compared to the hypoxia control (117% vs. 314%). This was the greatest reduction observed with Tiron across this thesis as well as observing the greatest increase in MDA concentration. Unlike the results following H_2O_2, this increase in MDA concentration conforms to the hypothesis that the brain has a greater concentration of PUFA susceptible to peroxidation during oxidative stress and gives rise to the suggestion that a longer incubation with H_2O_2 may have elicited a greater increase in MDA formation, also offering explanation as to why Tiron was able to elicit a greater level of protection (Sultana et al. 2013, Shichiri 2014). This also supports the postulation that hypoxia-induced damage may occur earlier than, and subsequently induce, the formation of mtDNA strand breaks given the findings of significant ROS at 24 hours in Figure 5.14 and correlated reduced cell viability in Figure 5.16.

A correlation between increased MDA and the formation of mtDNA damage has been identified, with MDA considered to be the most mutagenic product of lipid peroxidation (Voulgaridou et al. 2011). Evidence to this, *an* increase in mtDNA damage following hypoxia in the hippocampus, cortex and cerebellum has been found in rats as well as an elevation of MDA (Lee et al. 2002). Accordingly, it has been hypothesised that elevation of both MDA and mtDNA damage is as a result of MDA's reactivity with DNA as well as its influence on ROS production, therefore demonstrating the consequence of excessive and unmanaged elevation in MDA concentration; warranting amelioration with antioxidants such as Tiron (Bono et al. 2010, Cline et al. 2010, Voulgaridou et al. 2011).

It has been shown previously that MDA is capable of inducing DNA damage including the mutagenic adduct M_1dG, which has been found in up to 100-fold higher concentrations in mtDNA than nDNA (Otteneder et al. 2006, Chan and Dedon 2010, Wauchope et al. 2018).

The formation of M_1dG adducts has also been postulated to contribute to mitochondrial dysfunction as well as being perpetuated by ROS (Otteneder et al. 2006, Chan and Dedon 2010, Wauchope et al. 2018). This indicates the importance of reducing ROS and MDA concertation by Tiron to limit the incidence of damage, as highlighted by the results in Figure 5.18 and 5.19 respectively. Therefore there is weight to the hypothesis that whilst it took 48 hours to detect significant mtDNA strand breaks in SVGp12 cells subjected to hypoxia, there could be damage-inducing increases in lipid peroxidation before the incidence of mtDNA damage, which may be detectable and it is the reactivity of lipid peroxidation products that may perpetuate the generation of ROS observed in Figure 5.14 and induce significant damage, such as mtDNA strand breaks. However, to support this hypothesis, the concentration of MDA as a result of 24-hour incubation in hypoxia would need to be assessed.

The brain has a heavy reliance on OXPHOS and the synthesis of ATP as a result of the high rate of O_2 consumption relative to the weight of the tissue and because of this, diminished O_2 can have dramatic consequences on brain function (Dong et al. 2012, Cobley et al. 2018). A decrease in OXPHOS can impair the ETC leading to inefficient ATP synthesis causing cell death and a catastrophic loss of neurons (Eltzschig and Carmeliet 2011, Gan and Johnson 2014, Dong et al. 2015, Terraneo et al. 2017, Cobley et al. 2018). As such, the brains reliance on O_2 makes it particularly sensitive to hypoxia, which has been shown in a murine model of hypoxia, demonstrating significant mitochondrial ROS production and ETC impairment (Ishii et al. 2017). This was also highlighted in Figure 5.14 of this chapter. Further contributing to the brain's sensitivity to O_2 depletion and oxidative stress is the reduced antioxidant defence compared to other tissues, which could be as a result of the frequency of ROS production within the brain due to its reliance on OXPHOS and O_2 consumption (Cobley et al. 2018). Whilst astrocytes have a higher antioxidant capacity than neurons, which is suggested to be maintained with age, glutathione has been identified as being up to 50% lower in neurons than other cell types and indicating that antioxidant supplementation could be employed to maintain brain health particularly in the event of astrocyte loss such as observed in neurodegenerative disease (Bélanger and Magistretti 2009, Liddell et al. 2010, Bell et al. 2015, Baxter and Hardingham 2016, Dossi et al. 2018). Considering the reduced antioxidant capacity with the brain, the effect of Tiron on Nrf2 protein expression was of interest to understand if supplementation could be used to reduce oxidative stress but also whether treatment could induce an endogenous defence to strengthen the ROS ameliorative action of Tiron.

Treatment for 24 and 48 hours with Tiron in SVGp12 cells without ROS induction, led to no significant ($p \geq 0.05$) increase in Nrf2 activity (Figure 5.10 and 5.13). Similarly, no augmentation of ROS production was measured through DCF oxidation (Figure 5.12), thus indicating that

Tiron treatment alone did not increase Nrf-2 expression and that Tiron's protection was elicited independently of the Nrf2 pathway. Treatment with Tiron for 24-hours in BEAS-2B and HKC-8 cells was also found to be independent of Nrf2 in the presence of ROS, as has been demonstrated previously with BEAS-2B and HDFn cells *in vitro* (Antognelli et al. 2014, Oyewole et al. 2014).

The same finding in SVGp12 cells with Tiron treatment after 48 hours also being independent of Nrf2 activity was not in keeping with previous chapters, illustrating the need to evaluate Tiron in different cell lines to assess its potential therapeutic application. The absence of measurable effect with Tiron, contrary to observed in other cell lines, could be due to Tiron's presence compensating for the lower antioxidant capacity suggested to be within this cell type (Baxter and Hardingham 2016, Cobley et al. 2018). Within neurodegenerative disease, it has been observed through analysis of patient samples that there is also a deficiency in Nrf2 which contributes to the induction of neuroinflammation and neuronal cell death (de Vries et al. 2008, Lastres-Becker et al. 2012). Therefore, the finding that Tiron is capable of protecting against ROS independent of Nrf2 supports its potential use therapeutically within neurodegenerative disease. If supplementation were to be reliant on the induction of the Nrf2 pathway in reducing oxidative stress, exogenous supplementation would be hindered by the deficiency. Thus, Tiron supplementation could be more effective in reducing ROS-induced damage within the brain than Nrf2-reliant antioxidants such as sulforaphane, which has been shown to elicit some beneficial effects in AD treatment (Tarozzi et al. 2013, Zhang et al. 2017).

However, the reduction in cell viability observed with Tiron treatment for 48 hours requires consideration as to its benefit in prolonged supplementation due to the cell viability reduction to 70% (Figure 5.11). It could be argued that Tiron supplementation alone could be detrimental to SVGp12 cell viability, as was also observed in HKC-8 and BEAS-2B, but that when supplemented where ROS are in excess, such as identified within PD and AD, cell viability may be preserved. This hypothesis is supported by the significant ($p \leq 0.001$) reduction in mtDNA strand break damage observed in Figure 5.20 and the absence of strand break induction in the Tiron only control as a result of supplementation with Tiron at the time of entry into hypoxia.

Tiron has previously demonstrated significant protection when administered after the induction of toxicity (Sharma et al. 2007). *Sharma et al*. (2007) dosed Sprague-Dawley rats daily with aluminium chloride for 10 weeks to induce toxicity before treating with Tiron, glutathione or the two combined for 7 days, 24 hours after the last dose of aluminium chloride. Tiron treatment led to significant reduction in endogenous antioxidant levels including catalase and SOD as well as a decrease in lipid peroxidation present within the brain, indicating a reduction in

oxidative stress by Tiron as well as Tiron's BBB permeability (Sharma et al. 2007). However, contrary to the results obtained with co-treatment in HKC-8 and BEAS-2B cells, Tiron was not as protective in SVGp12 cells. The absence of greater protection could be as a result of the significant reduction of cell viability to 30% with Tiron treatment for 48 hours and challenged with H_2O_2 compared to HKC-8 (63%) and BEAS-2B (83%) cells. Therefore, incidence of some mtDNA damage in SVGp12 during co-treatment with Tiron in hypoxia could be as a result of the previously discussed theory whereby the mtDNA samples obtained are not representative of the cell population due to the substantial loss of viability in Figure 5.11. Alternatively, it could be suggested that Tiron's protection as a result of co-treatment requires the induction of the Nrf2 pathway, which is supported by the results achieved in chapters 3 and 4 and also correlates with the results of Figure 5.13, whereby Tiron treatment alone did not lead to a significant induction of Nrf2 protein expression. It could be hypothesised that co-treatment of Tiron alongside ROS-induction diminishes the concentration available for cell preservation, leading to a reliance on endogenous antioxidant induction to increase or sustain the level of protection conveyed. In SVGp12 cells, the absence of Nrf2 induction could therefore correlate with the accumulation of damage when compared to Tiron's influence on Nrf2 protein expression observed in BEAS-2B and HKC-8 cells. Consequently, more work is required to evaluate the efficacy of Tiron co-treatment in SVGp12 cells as a result of a shorter exposure time as well as if the potential induction of Nrf2 with longer treatment with Tiron could influence the level of protection achieved. However, the latter proposal is limited by the influence this treatment may have on cell viability, indicated by the decrease is cell viability after 72-hour Tiron treatment in Figure 5.11. As such, co-treatment may not be as effective over longer periods in response to ROS-induced damage within astroglial cells.

Nonetheless, the finding that Tiron demonstrated a high degree of protection as a result of co-treatment with ROS-induction presents an opportunity to use Tiron within a neurodegenerative disease treatment regime to target the ROS produced as a result of disease manifestations. This is a potentially crucial finding as the diagnosis of neurodegenerative disease is often made following the observation of significant symptoms including declining cognition, bradykinesia or a tremor, which nullifies the use of Tiron as a preventative supplement, also suggested by the results in Figure 5.11 (Ellwanger et al. 2015, Hullinger and Puglielli 2016). In particular, mutations within PINK1 (PTEN-induced kinase 1) in PD have been found to lead to mitochondrial dysfunction through decreased ATP synthesis, reduced O_2 consumption as well as increased ROS production (Dias et al. 2013, Joe et al. 2018). Therefore using hypoxia to model the physiological induction of ROS as a result of diminished O_2 supply not only models the hypoxia observed in AD but also simulates mitochondrial dysfunction, which could be symptomatic of PINK1 mutations (Dias et al. 2013, Joe et al. 2018). As such, the protective

capabilities as a result of Tiron co-treatment present an opportunity to acutely reduce the consequences of the PINK1 mutation, which cannot be adequately prevented by their very nature; a direction for further research.

Whilst the results in chapters 3 and 4 suggested that Tiron could be used to prevent the development of secondary conditions or disease exacerbations associated with DM or asthma/COPD, this chapter suggests that Tiron's use in neurodegenerative disease would be primarily to delay the progression of disease. As a result, it would be pertinent to evaluate the use of Tiron within neurodegenerative disease specific models including the protective effects of Tiron against the toxicities associated with amyloid-β, tau phosphorylation or α-synuclein. As highlighted in section 5.1, there have been a number of studies that have demonstrated efficacy in the reduction of cognitive decline, inflammation and restoration of antioxidant capacity within neurodegenerative disease (Sano et al. 1997, Adair et al. 2001, Morris et al. 2002, NINDS NET-PD Investigators 2007, Taghizadeh et al. 2017). Therefore, the evidence suggesting that Tiron is effective when administered at the time of ROS induction should be taken further and evaluated in models of neurodegenerative disease. For example, it has been shown in a PD rat model that resveratrol is significantly neuroprotective through the reduction of mitochondrial swelling, TNF-α and COX2 after 6-hydroxydopamine-induced PD and demonstrating that antioxidants have been effective in neurodegenerative disease when supplemented after the onset of disease (Jin et al. 2008).

To conclude, the treatment of SVGp12 astrocytes *in vitro* with Tiron was found to be protective against mtDNA strand break damage and lipid peroxidation. This was following direct and indirect induction of ROS-mediated damage by H_2O_2 and hypoxia respectively and further demonstrated significant preservation of cell viability alongside a reduction in ROS generation as a result of both Tiron pre- and co-treatment. The comparative use of H_2O_2 induction of ROS in SVGp12 cells, as well as in chapters 3 and 4, demonstrates a high level of protection against oxidative damage with Tiron across all assessed tissues, which is promising for Tiron's potential therapeutic use. The results in chapter 3-5 supported Tiron's protective capabilities against ROS-induced damage and suggested that this mechanism was both independent and influenced the Nrf2 response pathway, depending on the length of treatment with Tiron, the latter of which has not been indicated previously. Overall the results presented in this chapter are congruent with those of chapter 3 and 4, supporting the protective action of Tiron as well as the tolerance across multiple tissues without significant adverse effects, highlighting the need for further research into the expansion of Tiron therapeutically.

Chapter 6 – General Discussion

6.1 General Discussion

This thesis aimed to evaluate the use of Tiron, a ROS reducing agent, protectively against oxidative damage and the reproducibility in different models of ROS induction in order to better understand the potential application of Tiron. This was investigated through the direct application of ROS with H_2O_2 or physiologically induced with hypoxia or high concentrations of glucose. The identification of different responses in each cell type within this thesis provided insight into the potential use of Tiron, with results demonstrating consistently significant degrees of protection across all cell lines. ROS-induced damage was measured though several endpoints including; intracellular ROS generation; cell viability reduction; mtDNA damage; and increases in MDA concentration. Physiologically relevant ROS induction was used to model disease specific to each cell line and provided better understanding as to the potential application of Tiron therapeutically. However, whilst Tiron demonstrated significant levels of protection against oxidative damage, some assays indicated that treatment with Tiron still incurred a degree of damage, such as in the amelioration of MDA, and that more work to understand Tiron's capabilities is required. Adding to this, the finding that Tiron's mechanism of action may be both independent and dependent on the Nrf2 pathway, whilst expanding on previously published literature (Oyewole et al. 2014) warrants significant expansion to understand the future benefits or consequences of Tiron's use therapeutically.

Within this thesis, it was demonstrated that a concentration of 3 mM Tiron elicited significant reductions in ROS-induced mtDNA strand breaks (Figures 3.7, 4.14 and 5.7) and high degrees of protection against lipid peroxidation (Figures 3.9, 3.19 4.17, 4.18, 5.9 and 5.19) in renal, pulmonary and astroglial cell lines as a result of 24-hour pre-treatment modelled with both directly applied and physiologically produced ROS. These results were supported by a preservation of cell viability offered by Tiron when challenged with ROS (Figures 3.16, 4.8 and 5.16). The results obtained in chapters 3-5 expand on work conducted by *Fang el al.* (2012) and *Oyewole et al.* (2014), who determined that Tiron could be used preventatively against the effects of UVR-induced oxidative damage in human skin cells and indicating that Tiron could be used to prevent premature skin aging and damage, in line with Denham Harman's theory of aging (Harman 1956, Oyewole and Birch-Machin 2015). Therefore, the work contained within this thesis adds to the knowledge of Tiron's protective capabilities and suggests that Tiron could be used beneficially against ROS-induced damage, both directly and indirectly sourced, in cell types additional to human skin cells.

However, whilst Tiron did demonstrate significant reduction of MDA levels as a result of pre-treatment in all models of ROS, levels were not consistently maintained at baseline compared to the controls, as had been observed with the qPCR and DCFDA assays. This has been observed *in vivo*, where 7-day Tiron treatment post radiation or manganese toxicity induction in rats led to a reduction in MDA but not to that of the controls (Abdel-Magied et al. 2019). The same was observed in rats with titanium-induced hepatotoxicity (Morgan et al. 2018) as well as a murine asthma model (El-Sherbeeny et al. 2016). Therefore, whilst the results were not as seen in other assays within this thesis, they are consistent with previously published literature demonstrating the potential use of Tiron to reduce MDA levels. However further experimentation is required to understand whether Tiron elicits greater effects on other lipid peroxidation biomarkers, such as HNE. Such investigations may provide insight into Tiron's potential use in the reduction of lipid peroxidation with implications in the treatment of diseases such as atherosclerosis (Niki 2014).

Another consideration is the reduction of cell viability observed with Tiron at concentrations 5 mM and above in all cell lines, albeit to 90%, indicating that Tiron concentration needs careful refinement before translation. Similar cytotoxic results were also observed with prolonged treatment, with viability reduced to 32% in SVGp12 cells after 72 hours (Figure 5.11). Whilst Tiron still demonstrated a significant degree of protection against mtDNA strand breaks after prolonged treatment, the reduction in viability warrants further investigation as results may have been influenced by the loss of cells as discussed in chapter 5. Considering the results in figures such as 5.12, the reduction in cell viability could be as a result of Tiron's formation of ROS after prolonged exposure (Taiwo 2008) and this may also apply at higher concentrations, therefore inducing a level of oxidative stress and limiting the frequency of dosing possible in clinic. This was in part indicated in figure 4.14 where mtDNA strand breaks were detected compared to the control at 5 mM. Antioxidants at high concentrations have been shown to compromise cell viability previously, including *epigallocatechin gallate* which demonstrated concentration dependent decreases in cell viability in normal human lung and skin cells and was suggested to be as a result of electron reduction as well as the potential increase in $O_2^{\bullet -}$, the latter hypothesised within this thesis with Tiron (Lu et al. 2013).

Administering Tiron in a single cell environment, rather than multicellular *in vivo*, does not adequately model how a drug may be metabolised and what concentration of the dose may reach that cell type, which could indicate that cell viability reduction results may be exaggerated *in vitro*; a limitation of this thesis. However, these results must be considered in future work when optimising the dose required to provide significant oxidative stress amelioration *in vivo*. Antioxidant-induced cytotoxicity within healthy individuals may

inadvertently elevate ROS levels through the induction of apoptosis or parylation (Alano et al. 2010, Wei and Yu 2016). Repeated administration without consideration of potential cytotoxicity may subsequently induce organ dysfunction and exacerbate the oxidative damage it was initially meant to target.

Conversely, the use of antioxidant compounds to beneficially induce apoptosis has been modelled within cancerous cell lines (Doughan and Dikalov 2007, Delmas et al. 2011, Zhang et al. 2015). For example, Tiron has been shown to potentiate the cytotoxic effects of magnolol, an autophagy inducer (Kumar et al. 2013), and also shown to induce apoptosis in human promyelotic leukaemia (HL-60) cells (Kim et al. 2006).Therefore, future work is required to understand whether the same cytotoxic effects in this thesis are observed *in vivo* and how this can be reduced or used depending on application.

The mechanism by which Tiron elicits its protection was consistently shown to be as a result of diminishing ROS in each chapter and cell line assessed, as demonstrated by reduced oxidation of DCF (Figure 3.8, 3.18, 4.11, 4.12, 5.8 and 5.18) compared to the H_2O_2, high glucose and hypoxia controls, which is supported by published literature identifying Tiron as metal chelating and ROS scavenging (Domingo et al. 1993, Supinski et al. 1999, Yamada et al. 2003, Han and Park 2009, Monticone et al. 2014). Some authors have also suggested that Tiron is intracellularly localised due to its superior levels of protection compared to extracellular antioxidants such as glutathione, demonstrated through DCF oxidation *in vitro* (Supinski et al. 1999, Silveira et al. 2003, McArdle et al. 2005).

The high degree of protection elicited by Tiron suggests that Tiron is capable of intracellular localisation to sustain protection against ROS-induced damage, as evidenced by the considerably high degree of protection measured 24 (Figure 3.17) and 120 hours (Figure 4.15) after its removal from cell culture medium compared to damage controls. If Tiron was not capable of such localisation, the removal of Tiron at the end of the 24-hour pre-treatment protocol would have likely resulted in the accumulation of ROS-induced damage. Similar responses have been shown in Flavonoids, where protection against H_2O_2 was maintained following removal from cell culture in caco-2 cells (Yokomizo and Moriwaki 2006). Mitochondrial-targeted vitamin E (MitoVit E) has been also shown to reduce hepatic oxidative stress within mice and to be 350-fold more potent than vitamin E supplementation (Mao et al. 2010). This suggests that mitochondrially-targeted antioxidants could be superior to supplementation of untargeted antioxidants. However, this requires more thorough investigation in light of the findings of *Gottwald et al.* (2018) and MitoQ toxicity, discussed in chapter 3. Nevertheless, Tiron's cell permeability is advantageous in its potential treatment of disease in that it could directly target the ROS produced by mitochondrial dysfunction as well

as reducing extracellular ROS and limiting the accumulation of damage. This is supported by a number of cell-permeable antioxidants which have demonstrated positive results in patients, including a study of coenzyme Q supplementation found to reduce lipid peroxidation and restore antioxidant capacity in T2D patients (Rodríguez-Carrizalez et al. 2016). The availability of these studies demonstrates the widening direction of this field of research into antioxidant amelioration in disease with cell-permeable antioxidants, something that should be explored with Tiron in the future.

As discussed throughout this thesis, oxidative stress and mitochondrial dysfunction has been demonstrated in numerous diseases. Therefore the results in the thesis, coupled with the work of *Oyewole et al.* (2014), *Morgan et al.* (2017, 2018) and *Ateyya et al.* (2016), the latter of which showed Tiron to be protective against damage in the lung, kidney and liver as well as reducing the effects of acute pancreatitis in rats, indicate the wider use of Tiron. Furthermore, the provision of protection for up to 120 hours after Tiron removal, as observed in chapter 4 in the reduction of ROS generation (Figure 4.12), mtDNA strand breaks (Figure 4.15) and MDA (Figure 4.18), could shape how frequently Tiron needs to be applied clinically and expands upon previous literature which has demonstrated Tiron's efficacy shortly after ROS-induction (mostly up to 24 hours) or termination of treatment (Shrivastava et al. 2007, Cheng et al. 2010, Shoeib et al. 2016, Morgan et al. 2018). It has been suggested in a phase I clinical trial, that the combination of antioxidant supplementation, such as ascorbic acid or α-tocopherol, with antiviral therapy could improve the efficacy of treatment within hepatitis C patients, a condition in which oxidative stress and lipid peroxidation has been shown to increase hepatic inflammation and necrosis (Melhem et al. 2005, Paracha et al. 2013). As such, future work should be directed towards the use of Tiron in other conditions where oxidative stress and mitochondrial dysfunction have been implicated such as hepatitis C as well as rheumatoid arthritis, schizophrenia, autism spectrum disorders, breast cancer and many more. Study in other ROS-related diseases would build upon evidence from this thesis as well as published literature, which has shown that Tiron is well tolerated and effective in multiple organs *in vitro* and *in vivo* (Melhem et al 2005, Salim 2014, Anglin 2016, Ateyya et al. 2016, Bergman and Ben-Shachar 2016, Hecht et al. 2016, Johar et al. 2016, Hollis et al. 2017, Morgan et al. 2018).

The high degree of protection and comparable results with 3 mM Tiron in each cell line, following the induction of similar levels of ROS-induced damage, is important in understanding the wider use of Tiron. By ensuring that levels of ROS damage were similar, as demonstrated by the induction of a fold change of 4.11 in qPCR CT value in SVGp12 cells compared to HKC-8 (4.89) and BEAS-2B cells (4.52) with different H_2O_2 concentrations, the results obtained evidence that Tiron's protective capabilities are independent of cell type and provide a good

premise for the use of Tiron protectively against oxidative stress in multiple tissues, adding to previously published evidence of Tiron's tolerance (Domingo et al 1992, El-Sherbeeny et al. 2016). As such, this could permit the use of Tiron in the treatment of diseases that are difficult to prevent such as T1D but could also be used in the treatment of myocardial ischaemia/reperfusion (I/R) injury, where the generation of ROS has been found to be a contributing factor both experimentally and clinically (Giannini et al. 2007, de Vries et al. 2013, Gonzalez-Montero et al. 2018, Zhou et al. 2018). H_2O_2 has been identified as the main source of ROS within I/R injury, a leading cause of cardiovascular disease-related death, with the use of an antioxidant prodrug (BRAP) eliciting anti-apoptotic and anti-inflammatory action as well as inhibition of oxidative stress (Lee et al 2015, Xia et al 2016). Tiron has already demonstrated cardioprotective properties within a mouse model of I/R injury through the reduction of $O_2^{\bullet-}$, demonstrating the wider application of Tiron (Jiao et al. 2009).

However, in conditions such as PD, where the aetiology is still widely unknown, modelling with Tiron could provide better understanding as to whether ROS are causative of disease or if they exacerbate symptoms and how this could be targeted (Sarrafchi et al. 2016, Puspita et al. 2017). Whilst the exact cause of inflammatory bowel disease (IBD) is still unknown, the role of oxidative stress in IBD has been evidenced with increased levels of MDA evidenced in patient biopsies as well as DNA oxidation (Alzoghaibi et al. 2007, Bouzid et al. 2013, Pereira et al. 2016). However, whether the presence of oxidative stress is causative of IBD or contributes to the manifestation of symptoms is unclear, requiring further investigation. Therefore, Tiron could be used to improve the understanding of the role of ROS within disease where the aetiology has yet to be precisely underpinned through *in vitro* or *in vivo* modelling additional to its therapeutic capacity. This would build upon published work that has used Tiron's ROS reducing action to understand the mechanism of anti-cancer agents such as Bortezomib, a ROS-producing proteasome inhibitor (Ling et al. 2003).

Across all chapters, it was shown that Tiron reduced levels of ROS, mtDNA strand break damage or MDA concentration to below that of the control when normalised. Whilst it has been suggested within this thesis that this could occur as a result of Tiron reducing physiologically produced ROS, which could have wider ROS mediated signalling issues and shown previously with two day Resveratrol treatment and H_2O_2 exposure in primary skeletal myotubes (Conlan et al. 2010), it could also be suggested that the culture conditions of these cell lines are a factor. Studies in immortalised cell lines provide homogeneity as well as economic benefits in evaluating cellular mechanisms and early pharmacological leads. However, *in vitro* culture is conducted in atmospheric O_2 (18-21%), levels which are up to 5-fold higher than experienced *in vivo*, where tissues are exposed to 2-9% as a result of inhaled O_2 dispersion, thus cell

culture conditions could be considered hyperoxic (Halliwell 2014, Jagannathan et al. 2016a). Immortalised cell lines do not experience growth inhibition at these high O_2 concentrations, which would be experienced by primary cells, eliminating the need to culture cell lines at differing O_2 levels (Brahimi-Horn and Pouyssegur 2007, Halliwell 2014). However, it has been indicated that there may be a level of oxidative stress that could be associated with culturing at high O_2 levels, which could result in the generation of damage (Martin-Romero et al 2008). It has been shown that high O_2 can exacerbate cellular response to inflammation of apoptosis as shown *in vitro* with doxorubicin or UVR treated kidney cells, indicating the influence O_2 may have on result presentation (Carrera et al 2010). This has also been shown to some extent with neural primary stem cells, where higher levels of ROS were observed in comparative immortalised cells, which could be related to the elevated mitochondrial activity, alongside greater levels of apoptosis and different levels of SOD and catalase induction, indicating a limitation of using immortalised compared to primary cell lines, which may be cultured at physiologically relevant O_2 levels (Madhavan et al. 2009).

As such, it could be suggested that the presence of Tiron within culture could reduce the culture-induced oxidative stress. This is indicated in Figures 3.7, 4.16 and 5.9 where Tiron increased qPCR efficiency in the formation of new mtDNA strands compared to the control, indicating that control samples may contain some degree of mtDNA damage. This was also shown in Figures 3.8, 4.12 and 5.18, which demonstrated a lower level of ROS through DCF oxidation with Tiron treated samples compared to the control. However, this phenomenon was not evident in all results presented in this thesis. It has been suggested that mitochondrial copy number may indicate oxidative DNA damage within samples before analysis (Qiu et al. 2013, Ji et al. 2014). Copy number reflects mitochondrial abundance within a cell, which is influenced by energy demands, and has been positively correlated with oxidative stress and indicative of mitochondrial dysfunction *in vitro* and within human tissue, peripheral blood monocytes and leukocytes (Liu et al. 2003, Wang et al. 2011, Qiu et al. 2013, Al-Kafaji and Golbahar 2013). Therefore, it could be suggested that control cells cultured at atmospheric O_2 within this thesis may have increased copy numbers compared to Tiron treated samples, indicating culture-related oxidative stress. However, whilst the 83bp qPCR assay did indicate mitochondrial copy number per sample, this was not normalised to a specific cell concentration but within the whole sample meaning it is not possible to evaluate if stress was evident in control samples versus Tiron retrospectively.

Therefore, further experiments are required to understand if cell culture conditions account for Tiron's perceived reduction of oxidative stress greater than control levels. To investigate this, cell lines could be cultured at O_2 levels that are physiologically relevant, such as approximately

16% for BEAS-2B cells, using a dedicated workstation with regulated O_2 (Keeley and Mann 2019). To monitor intracellular O_2, the MitoXpress Intra® Intracellular Oxygen Assay Kit (Agilent, USA) could be used, an assay that uses a probe to monitor intra- and peri-cellular O_2 levels measured fluorometrically (Chapple et al 2016). Assay under these conditions could elucidate if Tiron is reducing physiological ROS or that induced by 'normal' cell culture conditions and may demonstrate greater levels of ROS-induced oxidative damage as it has been suggested that some immortalised cell lines are more resistant to oxidative stress owing to their culture in 21% O_2 (Jagannathan et al 2016, Keeley and Mann 2019). Conversely, it has been shown that BEAS-2B cells cultured at 10% O_2 had a lower inflammatory response less susceptible to H_2O_2-induced toxicity than at 21%, suggesting that lower O_2 levels may preserve cell viability (Jagannathan et al. 2016b). In support of this, lymphocyte proliferation has been shown to be influenced by O_2 levels with greater activation at atmospheric O_2 coupled with a decrease glutathione, demonstrating a greater level of oxidative stress which could correlate with heightened inflammatory response (Atkuri et al. 2005, Atkuri et al. 2007). It could therefore be suggested that culture at lower O_2 levels may offer reduced levels of oxidative stress and subsequently higher concentrations of endogenous antioxidants capable of reducing oxidative stress and that potentially lowering the levels of O_2 through 'hypoxic' culture such as within this thesis, may improve the endogenous ROS defence and convey a degree of protection against oxidative stress potentially accounting for the prolonged period of culture required to induce significant mtDNA strand breaks (Figure 4.7 and 5.15). As such, maintaining cells in a lower O_2 atmosphere may provide more physiologically relevant measures of ROS production and amelioration. Consequently, to establish the most translatable results with Tiron, work should be conducted at physiological O_2 levels unique to each cell type to establish whether the results observed within this thesis are emulated at lower O_2. Consideration must also be applied to hypoxia experiments and what is determined hypoxic in each cell type to avoid significant cell loss, such as discussed in chapter 5 with SVGp12 cells, and to potentially yield more physiologically relevant results and appropriate modelling of hypoxia adaptation and the role in which Tiron plays, perhaps with evaluation of multiple O_2 levels to understand the effect of differing levels of O_2 reduction or exposure (Keeley and Mann 2019).

However, if results indicate that Tiron is capable of reducing physiological levels of ROS, this requires mechanistic evaluation as highlighted throughout this thesis. By their nature, it is impossible to differentiate between beneficial and 'harmful' ROS when utilising antioxidant supplementation (Dündar and Aslan 2000, Poljsak and Milisav 2012). Therefore, too much supplementation could lead to a reduction of ROS below physiologically beneficial levels, resulting in 'antioxidant stress'. This can impact negatively on physiological functions such as

response to injury and other cell signalling cascades; increasing the susceptibility to damage and disease. Therefore, it is important to refine antioxidant supplementation so that it does not impede biological function. For example, decreased ROS levels have been shown to impair the division of haematopoietic stem cells, reducing haematopoiesis and impacting their differentiation into monocytes, neutrophils and lymphocytes (Schieber and Chandel 2014, Oh and Nör 2015). This reduced differentiation as a result of decreased ROS availability, as could be hypothetically achieved with Tiron supplementation, could therefore result in a deficient immune response. Whilst Tiron has been shown to reduce inflammation within the treatment of asthma *in vivo*, including a study of ovalbumin-induced asthma in mice (El-Sherbeeny et al. 2016), there has yet to be a study determining how supplementation could affect an acute response to allergen-induced inflammation following routine treatment with Tiron.

The results obtained in Figures 3.12, 4.13 and 5.12 showed that prolonged supplementation of Tiron led to an increase in ROS generation without exogenous influence, which would not necessarily impact haematopoietic stem cell division as suggested by *Oh and Nör* (2015). What is unclear from this thesis is the effect that this increased availability of ROS has on the endogenous antioxidant concentration. Considering the corresponding induction of Nrf2 protein expression with prolonged supplementation (Figures 3.13, 4.20 and 5.13), it is likely that Tiron has an influence on the expression of endogenous antioxidants. As such, this could result in the reduction of ROS levels, such as seen in Figures 3.8, 3.18, 4.12, 5.8 and 5.18, to a level which could potentially suppress an inflammatory response. As such, extensive work is required to understand the effects of Tiron on antioxidant stress and the potential impact this could have on ROS-reliant physiological processes, something that would be best evaluated *in vivo* in order to utilise a multi-organ model.

However, the finding that Tiron could elicit significant levels of protection against oxidative damage when supplemented at the time of ROS indicates that Tiron could also be used in situations when ROS is acutely produced such as in conditions of decreased oxygen, encountered during high altitude training, or in cases of acetaminophen (paracetamol) overdose (Bakonyi and Radak 2004, Jaeschke and Bajt 2006, Saito et al. 2010, Quindry et al. 2015). The administration of intravenous NAC in acetaminophen poisoning was implemented by the Commission on Human Medicines in 2012 and has been found to be significantly beneficial in the reduction of hepatic damage (Bateman et al. 2014, Medicines and Healthcare products Regulatory Agency 2014, Buckley et al. 2016). The adoption of a treatment protocol using NAC demonstrates that exogenous supplementation of antioxidant-like compounds can have considerable beneficial effects and highlights the relevance of evaluating compounds for their therapeutic potential, such as with Tiron.

Singular dosing as required would also reduce the decrease in cell viability observed with prolonged supplementation in each cell line and could see Tiron used as a short-term preventative supplement, such as postulated by *Birch-Machin* and colleagues (2015), before known exposure to ROS including UV exposure. It could also be used promptly when excess ROS is measured as could be encountered during a hyperglycaemic episode. Use in this manner is supported by the results obtained with Tiron treatment at the time of ROS induction within this thesis and proposes a new treatment methodology within disease. This expands on the evidence that pre-treatment can be beneficial in preventing disease symptoms as has been shown *in vivo*, something that was also supported by work within this thesis (El-Sherbeeny et al. 2016, Morgan et al. 2017, 2018).

Also identified in this thesis, treatment with Tiron for greater than 48 hours alone resulted in the production of ROS. This correlated with the formation of a reactive semiquinone by-product by Tiron evidenced previously and indicated a degree of physiological ROS amelioration (Krishna et al. 1992, Silveira et al. 2003, Taiwo 2008). Semiquinone has been identified as a radical produced in the mechanism of action for doxorubicin, a chemotherapy agent, and also in the reduction of O_2 to $O_2^{\cdot-}$ (Nowicka et al. 2017, Sangomla et al. 2018). As such it is suggested that the generation of $O_2^{\cdot-}$ can be targeted either by endogenous antioxidants as well as remaining Tiron and indicating that Tiron's protective mechanism could be as a result of self-renewing activity. This was highlighted by the absence of mtDNA damage as well as reduced ROS production and lipid peroxidation in all chapters with Tiron pre-treatment. Therefore, the data within this thesis supports the production of a semiquinone or reactive by-product of Tiron but also adds to *Taiwo's* (2008) work, who only evidenced Tiron's activity in solution, by demonstrating the induction of ROS without exogenous stimulation consisting within different human cell types. This should be explored further to elucidate its precise mechanism and any implications this may have in signalling pathways, which could lead to unwarranted effects (Hybertson et al. 2011, Erlank et al. 2011). As such, it is suggested that the effects of Tiron on ROS generation are investigated, potentially through the detection of specific ROS including semiquinone over time or whether there is activation of ROS mediated pathways as a result of Tiron treatment as well as evaluating the effect on endogenous antioxidants levels all of which may help to understand how Tiron is capable of sustaining its protectivity.

The targeting of Nrf2 to restore oxidative balance is a research topic that is gaining momentum with the use of compounds such as resveratrol and curcumin, which have recently evidenced to activate Nrf2 in addition to their protective capabilities (Ben-Yehuda Greenwald et al. 2016, Duan et al. 2017). Therefore, the finding that Tiron increases Nrf2 protein expression following

prolonged exposure could expand its use, with further evaluation, to target the restoration of oxidative balance through the induction of Nrf2. Resveratrol has also demonstrated upregulation of Nrf2 activity through increased expression of heme oxygenase-1 (HO-1), which has been shown to have antioxidant properties as well as restore glutathione levels *in vitro* (Chen et al. 2005, Kode et al. 2008, Parfenova et al. 2012). Therefore, the potential use of Tiron in a similar capacity could widen its application, particularly considering that Tiron has exceeded the protection elicited by Resveratrol as evidenced in published literature (Lin et al. 2012, Oyewole et al. 2014, Rodacka et al. 2014).

A number of Nrf2 activators have been used within the treatment of disease including the use of sulforaphane supplementation (100 µMol per day for two weeks) in asthmatic patients, which ameliorated bronchoconstriction and improved pulmonary function in 27 of 45 patients (Brown et al. 2015). Therefore, there is the potential to exploit the Nrf2 inducive potential of Tiron, as shown in Figures 3.13, 4.20 and 5.13, for the restoration of antioxidant activity as well as alleviation of disease symptoms. However, current published literature has suggested that Tiron is independent of Nrf2 and suppresses its action when used as pre-treatment (≤24 hours), a finding that was also emanated with the pre-treatment studies of this thesis (Smith et al. 2012, Antognelli et al. 2018). Consequently, the indication that Tiron interacts with Nrf2 could be a significant finding. Whilst the potential mechanism is hypothesised in Figure 6.1, the precise interaction requires considerable evaluation in light of the absence of supporting published literature.

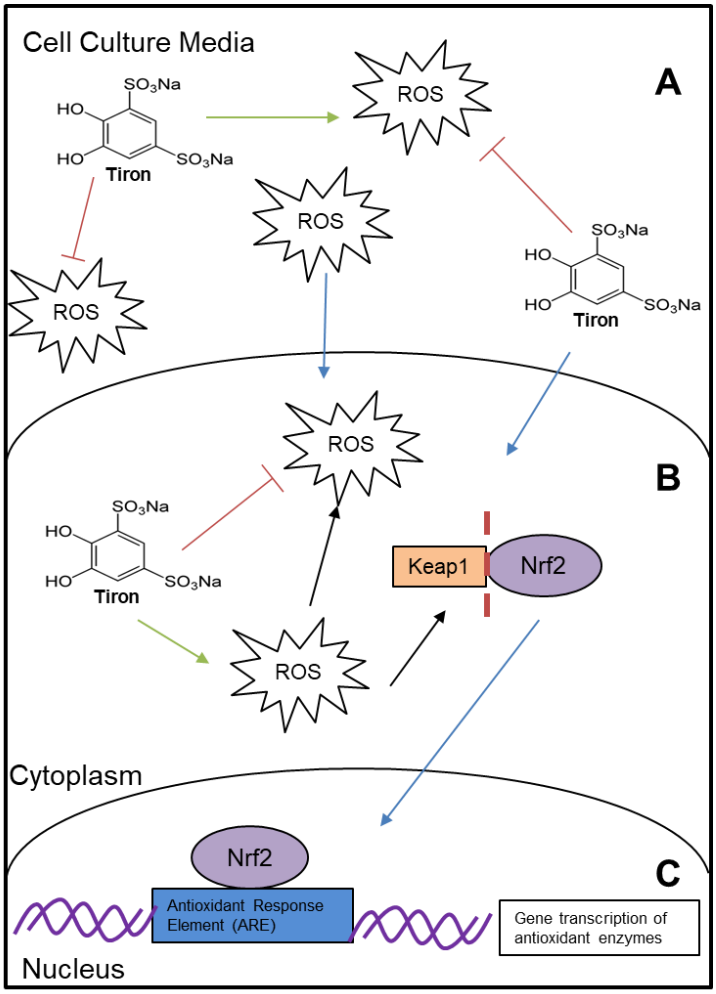

Figure 6.1 Postulated action elicited by Tiron. *(A) Tiron present within the cell culture medium has a direct effect on ROS, whether produced directly with H_2O_2 or indirectly through high glucose or hypoxic conditions. (B) Tiron enters the cell and ameliorates ROS generated within the cell. As proposed by Silveira et al (2003) and Taiwo (2008), Tiron's reactive by-product could react with other available ROS and have a neutralising effect. Similarly, this could also facilitate the dissociation of Keap1 from Nrf2. (C) Liberated Nrf2 can translocate into the nucleus, bind with the ARE and lead to the transcription of antioxidants and other detoxifying enzymes such as HO-1 and NQO1. An original figure.*

Accordingly, understanding the role in which Tiron interacts with the Nrf2 pathway is an important consideration for its use therapeutically and requires investigation, particularly with respect to Tiron's effect on antioxidant enzymes. This could indicate whether the action of Tiron renders these enzymes redundant or whether it supplements the response with time as suggested with prolonged treatment in this thesis. Furthermore, to elucidate Tiron's elevation of the Nrf2 pathway, it would be worthwhile to quantify Nrf2 activity across multiple timepoints during treatment in order to ascertain at which point Tiron 'loses' it's Nrf2 independence and

whether increasing concentrations of Tiron can more readily stimulate the Nrf2 pathway. This could be conducted by PCR or intracellular staining of downstream targets within the pathway and would elucidate whether Tiron could be used in a similar capacity to compounds such as sulforaphane (Chen et al. 2005, Hybertson et al. 2011). Both an independent action and influence on Nrf2 was identified with a cocktail of tocopherols supplemented over two weeks to wild-type and Nrf2 knockdown mice (Li et al. 2012). Interestingly, whilst the study demonstrated that tocopherols were able to elicit protection against inflammation and oxidative DNA damage independent of Nrf2, the reductions observed were less than those in the wild type. This indicated that there was some independence of Nrf2 but also the possibility of this cocktail to induce a greater response with Nrf2 (Li et al. 2012). A similar hypothesis can be postulated in this thesis with the protection with Tiron achieved both independently and inducive of Nrf2 across all three cell lines.

6.2 Limitations

In the evaluation of Tiron's protective capability, it was demonstrated within this thesis that Tiron offered high degrees of protection across all cell lines as a result of exposure to H_2O_2 as well as physiologically induced ROS. However, there are number of limitations within the study design and methodology, which require further work to improve understanding of both the mechanism and application of Tiron.

The DCFDA assay was used to detect ROS in all cell lines during optimisation of ROS-induction but also to evaluate the effect of Tiron supplementation. As such, it was demonstrated in Figures 3.8, 4.12 and 5.18 that supplementation of Tiron correlated with a reduction of ROS, evident through decreased fluorescence related to DCF oxidation. Whilst results were normalised against the untreated control to account for normal ROS availability, the DCFDA assay lacked the specificity to identify the type of ROS produced; a limitation of the assay. Therefore, these studies do not elucidate Tiron's mechanism of ROS reduction and should be supplemented with protocols that specifically measure the ROS being reduced, such as MitoSOX or Amplex®Red (Thermo Fisher Scientific, UK) which would indicate reactivity with $O_2^{\cdot-}$ and H_2O_2 respectively. Results from these assays would therefore indicate whether Tiron preferentially targets specific ROS, such as is the case for catalase. Irrespective of the non-specificity of the DCFDA assay, the results obtained facilitated optimisation of models of indirect ROS induction as well as indicating that Tiron's protective action was as a result of ROS reduction compared to relevant controls.

On the other hand, a limitation of the mtDNA strand break assessment is its specificity and the absence of recording any mutations, base oxidation or single strand breaks that occurred

as a result of ROS exposure. Oxidative DNA damage has been implicated within carcinogenesis, with 8OHdG identified in breast cancer patients alongside reduced SOD activity as well as in acute myeloid leukaemia, coupled with elevated MDA levels and negatively correlated with relapse frequency (Himmetoglu et al. 2009, Zhang et al. 2010). From the results presented in this thesis, it is not clear whether the significant protection against mtDNA strand breaks is due to Tiron's direct reduction of ROS or correlated with a reduction in mutation accumulation leading to strand breaks. Therefore, more investigation is required to assess the influence of Tiron supplementation on ROS-induced mutations or other DNA damage biomarkers which may also provide insight as to Tiron's use in reducing other biomarkers of DNA damage within disease, such as 8OHdG.

The same limitation is also attributed to the MDA assay, which was used to determine the response of Tiron to lipid peroxidation. The principle of the assay is to measure MDA-TBA adducts colourimetrically. However, TBA readily reacts with other by-products of lipid peroxidation which could limit the detection of MDA within this assay. Therefore, to supplement the results obtained in Figures 3.9, 4.17 and 5.9, measurement of other lipid peroxidation by-products should be conducted. Associated with this, the absence of measuring MDA levels as a result of Tiron prolonged treatment is also a limitation. Results were not obtained for MDA levels following 48 and 120 hour treatment of Tiron considering the inability for Tiron to consistently maintain MDA levels as a result of 24 hour pre-treatment (Figure 3.9 and 3.19), with Tiron raising MDA levels in BEAS-2B cells (Figures 4.17-4.18). However, this evaluation may have provided more insight as to whether greater lengths of Tiron treatment would be required to reduce MDA levels, which have been suggested previously with Resveratrol which demonstrated similar results to Tiron *in vitro* where amelioration of MDA to baseline levels was not observed after 24 hours but after 7 day supplementation (Pandey et al. 2015, Fu et al. 2018, Li et al. 2018). As suggested in previous chapters, the evaluation of other lipid peroxidation products, such as HNE or lipid hydroperoxides, could provide insight into whether Tiron is incapable of reducing the formation of MDA to baseline or whether the response extends to lipid peroxidation in general, the latter of which would be contrary to published evidence demonstrating Tiron's protection against HNE in cultured human saphenous veins (Joddar et al. 2015), and could be used to evaluate the effect of prolonged Tiron treatment on both biomarkers. However, previous evidence indicates that this may not be sufficient therefore, evaluation should be expanded to understand if co-treatment with another antioxidant may convey more efficient protection against MDA level increases (Morgan et al. 2018, Abdel-Magied et al. 2019). Nevertheless, the finding that Tiron does significantly reduce MDA levels, even if not completely, is important for its clinical potential and thus could be used

to reduce elevated MDA levels that have been observed in patients with ROS-associated diseases (Sanyal et al. 2009, Lorente et al. 2015, Smriti et al. 2016).

The qualitative assessment of Nrf2 protein expression is also a study limitation. The motivation behind this assessment was to evaluate if prolonged treatment with Tiron employed the endogenous defence to prevent oxidative damage, similar to observed with sulforaphane and potentially indicate that Tiron could be used as a preventative supplement (Tarozzi et al. 2013). This was in light of the results obtained in Figure 3.21 where repeated supplementation of Tiron over 72 hours was found to be significantly protective against ROS induced by daily replenishment of Glucose as well as the results obtained in Figures 3.13, 4.20 and 5.13 where combined treatment led to significant protection against oxidative damage. As such, Tiron was evaluated for its influence on Nrf2 protein expression and whether this may contribute to the protection provided. Previous literature has shown that Tiron has no effect on HO-1 with 24-hour pre-treatment, which was also partially indicated by the absence of Nrf2 protein expression after 24-hour Tiron treatment in this thesis (Oyewole et al. 2014). Results in this thesis indicated that, following prolonged exposure, Tiron has both a dependent and independent relationship with Nrf2. This was demonstrated by the reduction of H_2O_2-induced Nrf2 expression in HKC-8 cells treated with Tiron for 48 hours followed by 1 hour with H_2O_2 compared to the control (Figure 3.13). However, a limitation of this section of the study was the absence of testing Tiron's influence on Nrf2 in the presence of physiological ROS induction, such as with glucose or hypoxia. Whilst the results suggested that prolonged supplementation with Tiron could be used to induce the Nrf2 pathway potentially as a supplement, which may have an immunomodulatory effect similar to Aspirin which has been found to reduce inflammation and increase Nrf2 signalling in Sprague-Dawley rats (Wei et al. 2018), this thesis does not indicate how Tiron's influence on Nrf2 may be modulated in the presence of persistent ROS production such as in diabetes or hypoxia and requires further evaluation. As such, work is required to address this gap to build on this preliminary study indicating Tiron's potential activation of Nrf2 alone and whether this is a phenomenon experienced only in the absence of physiological ROS generation. In line with this, despite data obtained suggesting Tiron does influence Nrf2 protein expression, to better understand the mechanism of Tiron and its interaction with Nrf2, both alone and in the presence of ROS, it would have been useful to evaluate the expression of downstream targets of Nrf2, such as HO-1, NQO1 or other endogenous antioxidants. Nonetheless, whilst the mechanism of action of Tiron is still not fully underpinned, this thesis did suggest that Tiron may influence the Nrf2/ARE pathway in its ROS amelioration, but further work is required to clarify whether this is also evident in the presence of physiologically-induced ROS.

Another limitation of this study was that a second antioxidant-like compound was not assessed alongside Tiron. The aim of this thesis was to establish the potential use of Tiron within a number of human cell lines and whether these results were comparable and also reproducible within models of physiological ROS, directly adding to the work of *Oyewole et al.* (2014). As such, work was primarily focused on model optimisation and the effect of Tiron in said models and whilst it has been demonstrated that Tiron can be beneficial in preventing ROS-induced damage compared to the ROS controls, the response was not compared to another antioxidant. As such, comparison of these results with a second antioxidant, such as Resveratrol or MitoQ, is warranted to support Tiron's ROS ameliorative action, indicated in this thesis, but also identify if Tiron elicits a greater effect than other well studied antioxidants and could direct work towards therapeutic use. A second antioxidant may also provide more insight into Tiron's results, particularly in relation to MDA. For example, vitamin E demonstrated dose dependent lipid peroxidation reduction in lung surfactant whereas SOD had no effect but vitamin C, melatonin and ebselen required higher doses before a significant effect was observed (Bouhafs and Jarstrand 2002). Different combinations of these antioxidants were then found to offer better levels of amelioration of MDA and HNE levels suggesting that evaluation with a second antioxidant may have indicated whether Tiron's results were associated with the test antioxidant or limited by the assay performed and whether combined supplementation may have yielded a greater reduction in MDA (Bouhafs and Jarstrand 2002). Tiron has been previously evidenced alongside other antioxidants including Resveratrol, Tempol, Trolox and NAC, with results predominantly showing Tiron's level of ROS amelioration to be superior across multiple cell lines including neutrophils, adrenal pheochromocytoma, melanoma and dermal fibroblasts (Yamada et al. 2003, Yang et al. 2007, Rodacka et al. 2014, Vorobjeva and Pinegin 2016). However, whilst Tiron has shown superiority previously, the same cannot be hypothesised for the response within the cell lines evaluated within this thesis in the absence of a comparative antioxidant. Despite this, the results documented within this thesis demonstrate a clear role for Tiron in the reduction of ROS-induced damage, supported by the comparable results obtained in the three different human cell lines and add to previously published evidence in human skin cells as well as *in vivo* investigations (Pi et al. 2003, Fang et al. 2012, Oyewole et al. 2014, Shoeib et al. 2016).

Overall, the work presented in this thesis has contributed to the widening of Tiron's application in its reduction of ROS-associated damage *in vitro*. However, studies are required to understand Tiron's mechanism of action, including its effect on endogenous antioxidants, the immune system as well as signalling pathways to visualise any unexpected effects of Tiron supplementation which could hinder its translation clinically. Such investigations could be conducted using qPCR or flow cytometry protocols.

Currently, therapeutic regimes are predominantly targeted to treat or manage disease symptoms rather than ROS such as the use of analgesics, opioids or anti-inflammatory drugs within rheumatoid arthritis, where oxidative stress has been implicated within disease pathology alongside a diminished antioxidant response (Wilcox 2005, Miyazaki and Asanuma 2008, Phull et al. 2018). Therefore, Tiron should be applied to disease-specific models outside the scope of those incorporated within this thesis, such as evaluated by *El-Sherbeeny et al.* (2016) and *Shoeib et al.* (2016), to understand if the promising *in vitro* effects can be successfully modelled *in vivo* and if disease state may influence this before expansion into clinic. Expanding on this, it would also be important to model the effect Tiron has on the efficacy of established therapies so not to impede disease prognosis, with studies already indicating that Tiron does not negatively affect the response of metformin in a diabetic model (Domingo et al. 1992, Cheng et al. 2010).

6.3 Conclusion

To conclude, the antioxidant-like compound Tiron has demonstrated a consistently high level of protection against ROS-induced damage throughout this thesis. In the absence of *in vivo* modelling, this thesis optimised relevant models of physiologically-induced ROS in three different human cell lines which provided evidence that Tiron could be used beneficially in multiple cell lines and using the same concentration. This supports its use clinically and indicates that Tiron is capable of indiscriminately reducing ROS-associated damage. The investigations outlined within this thesis should now be built upon, as highlighted above and throughout this thesis, to expand the use of Tiron *in vivo* and in other disease-specific models in order to evaluate its therapeutic potential. This could lead to wider application into the treatment of ROS-implicated diseases, such as rheumatoid arthritis, hepatitis C or IBD. Overall, the findings of this thesis support the use of Tiron as a protective agent *in vitro* both as a result of pre- and co-treatment and indicates that this significant degree of protection could be advantageous within the treatment of ROS-related disease, building on previously published literature.

References

Abdel-Magied, N., Abdel-Aziz, N., Shedid, S.M., and Ahmed, A.G. (2019) 'Modulating effect of tiron on the capability of mitochondrial oxidative phosphorylation in the brain of rats exposed to radiation or manganese toxicity'. *Environmental Science and Pollution Research* [online] 26 (12), 12550-12562. available from <https://link.springer.com/article/10.1007%2Fs11356-019-04594-4> [25 May 2019]

Abramov, A.Y., Canevari, L., and Duchen, M.R. (2003) 'Changes in Intracellularly Calcium and Glutathione in Astrocytes as the Primary Mechanism of Amyloid Neurotoxicity'. *Journal of Neuroscience* [online] 23 (12), 5088-5095. available from <http://www.jneurosci.org/content/23/12/5088> [22 June 2019]

Adair, J.C., Knoefel, J.E., and Morgan, N. (2001) 'Controlled Trial of N-Acetylcysteine for Patients with Probable Alzheimer's Disease'. *Neurology* [online] 57 (8), 1515–1517. available from <http://www.neurology.org/cgi/doi/10.1212/WNL.57.8.1515> [27 March 2018]

Adeloye, D., Chua, S., Lee, C., Basquill, C., Papana, A., Theodoratou, E., Nair, H., Gasevic, D., Sridhar, D., Campbell, H., Chan, K.Y., Sheikh, A., Rudan, I., and Global Health Epidemiology Reference Group (GHERG) (2015) 'Global and Regional Estimates of COPD Prevalence: Systematic Review and Meta-Analysis.' *Journal of global health* [online] 5 (2), 020415. available from <http://www.ncbi.nlm.nih.gov/pubmed/26755942> [23 May 2018]

Adibhatia, R.M, and Hatcher, J.F. (2009) 'Lipid oxidation and peroxidation in CNS health and disease: from molecular mechanisms to therapeutic opportunities.' *Antioxidants & Redox Signalling* [online] 12 (1), 125-69. available from <https://www.ncbi.nlm.nih.gov/pubmed/19624272> [12 March 2019]

Agmon, E. and Stockwell, B.R. (2017) 'Lipid Homeostasis and Regulated Cell Death'. *Current Opinion in Chemical Biology* [online] 39, 83–89. available from <https://www.sciencedirect.com/science/article/pii/S1367593117300650> [25 April 2018]

Aguirre-Rueda, D., Guerra-Ojeda, S., Aldasoro, M., Iradi, A., Obrador, E., Ortega, A., Mauricio, M.D., Vila, J.M., and Valles, S.L. (2015) 'Astrocytes Protect Neurons from Aβ1-42 Peptide-Induced Neurotoxicity Increasing TFAM and PGC-1 and Decreasing PPAR-γ and SIRT-1.' *International Journal of Medical Sciences* [online] 12 (1), 48–56. available from <http://www.ncbi.nlm.nih.gov/pubmed/25552918> [24 October 2018]

Akbari Fakhrabadi, M., Zeinali Ghotrom, A., Mozaffari-Khosravi, H., Hadi Nodousham, H., and Nadjarzadeh, A. (2014) 'Effect of Coenzyme Q10 on Oxidative Stress, Glycemic Control and Inflammation in Diabetic Neuropathy: A Double Blind Randomized Clinical Trial'. Interational *Journal for Vitamin and Nutrition Research* [online] 84 (5-6), 252-60. available from <https://econtent.hogrefe.com/doi/abs/10.1024/0300-9831/a000211> [21 June 2019]

Al Ahmad, A., Gassman, M., and Ogunshola, O.O. (2012) 'Involvement of Oxidative Stress in Hypoxia-Induced Blood-Brain Barrier Breakdown'. *Microvascular Research* 84 (2), 222–225

Ahmad, A., Shameem, M., and Husain, Q. (2012) 'Relation of Oxidant-Antioxidant Imbalance with Disease Progression in Patients with Asthma.' *Annals of Thoracic Medicine* [online] 7 (4), 226–32. available from <http://www.ncbi.nlm.nih.gov/pubmed/23189100> [20 January 2019]

Ajiboye, O. and Scott, G. (1982) 'Mechanisms of Antioxidant Action: Stabilisation of Nitrile Rubber by Interpenetrating Networks Based on Thiol Adducts'. *Polymer Degradation and Stability* [online] 4 (6), 397–413. available from <https://www.sciencedirect.com/science/article/pii/014139108290012X> [25 April 2018]

Aksenov, M.Y., Aksenova, M.V., Butterfield, D.A., Geddes, J.W., and Markesbery, W.R. (2001) 'Protein Oxidation in the Brain in Alzheimer's Disease'. *Neuroscience* [online] 103 (2), 373–383. available from <https://www.sciencedirect.com/science/article/pii/S0306452200005807?via%3Dihub> [27 March 2018]

Alam, Z.I., Jenner, A., Daniel, S.E., Lees, A.J., Cairns, N., Marsden, C.D., Jenner, P., and Halliwell, B. (2002) 'Oxidative DNA Damage in the Parkinsonian Brain: An Apparent Selective Increase in 8-Hydroxyguanine Levels in Substantia Nigra'. *Journal of Neurochemistry* [online] 69 (3), 1196–1203. available from <http://doi.wiley.com/10.1046/j.1471-4159.1997.69031196.x> [7 April 2018]

Al-Kafaji, G., and Golbahar, J. (2013) ‘High Glucose-Induced Oxidative Stress Increases the Copy Number of Mitochondrial DNA in Human Mesangial Cells‘. *BioMed Research International* [online] 2013. available from <https://www.hindawi.com/journals/bmri/2013/754946/#B19> [22 June 2019]

Alano, C.C., Ying, W., and Swanson, R.A. (2004) 'Poly(ADP-ribose) Polymerase-1-mediated Cell Death in Astrocytes Requires NAD^+ Depletion and Mitochondrial Permeability Transition'. *The Journal of Biological Chemistry* [online] 279. available from <http://www.jbc.org/content/279/18/18895.long> [10 June 2019]

Alano, C.C., Garnier, P., Ying, W., Higashi, Y., Kauppinen, T,M., and Swanson, R.A. (2010) 'NAD^+ deplition is necessary and sufficient for PARP-1 - mediated neuronal death'. *Journal of Neuroscience* [online] 30 (8), available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2864043/> [10 June 2019]

Albuquerque, R.V., Malcher, N.S., Amado, L.L.,Coleman, M.D., dos Santos, D.C., Borges, R.S., Valente, S.A.S., Valente, V.C., and Monteiro, M.C. (2015) ‘In Vitro Protective Effect and Antioxidant Mechanism of Resveratrol Induced by Dapsone Hydroxylamine in Human Cells‘. *PLoS One* [online] 10 (8). available from <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0134768> [29 June 2019]

Alexeyev, M., Shokolenko, I., Wilson, G., and LeDoux, S. (2013) ‘The Maintenance of Mitochondrial DNA Integrity - Critical Analysis and Update’. *Cold Spring Harbor perspectives in biology* [online] 5 (5). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3632056/> [15 October 2016]

Allen, D., Harwood, S., Varagunam, M., Raftery, M.J., and Yaqoob, M.M. (2003) 'High glucose-induced oxidative stress causes apoptosis in proximal tubular epithelial cells and is mediated by multiple caspases.' *FASEB Journal* [online] 17 (8), 908-10. available from < https://www.ncbi.nlm.nih.gov/pubmed/12670885/>

Allen, S., Britton, J.R., and Leonardi-Bee, J.A. (2009) ‘Association between Antioxidant Vitamins and Asthma Outcome Measures: Systematic Review and Meta-Analysis’. *Thorax* [online] 64 (7), 610–619. available from <http://www.ncbi.nlm.nih.gov/pubmed/19406861> [26 March 2018]

Alleva, R., Manzella, N., Gaetani, S., Bacchetti, T., Bracci, Massimo, Ciarapica, V., Monaco, F., Borghi, Battista, Amati, Monica, Ferretti, G., and Tomasetti, M. (2018) ‘Mechanism Underlying the Effect of Long-Term Exposure to Low Dose of Pesticides on DNA Integrity’. *Environmental Toxicology* [online] 33 (4), 476–487. available from <https://onlinelibrary.wiley.com/doi/abs/10.1002/tox.22534> [19 March 2018]

Alleva, R., Manzella, N., Gaetani, S., Ciarapica, V., Bracci, M., Caboni, M.F., Pasini, F., Monaco, F., Amati, M., Borghi, B., and Tomasetti, M. (2016) ‘Organic Honey Supplementation Reverses Pesticide-Induced Genotoxicity by Modulating DNA Damage Response’. *Molecular Nutrition & Food Research* [online] 60 (10), 2243–2255. available from <http://doi.wiley.com/10.1002/mnfr.201600005> [15 April 2017]

de Alvaro, C., Teruel, T., Hernandez, R., and Lorenzo, M. (2004) ‘Tumor Necrosis Factor Alpha Produces Insulin Resistance in Skeletal Muscle by Activation of Inhibitor KappaB Kinase in a P38 MAPK-Dependent Manner.’ *The Journal of Biological Chemistry* [online] 279 (17), 17070–8. available from <http://www.ncbi.nlm.nih.gov/pubmed/14764603> [3 May 2018]

Alzoghaibi, M.A., Al Mofleh, I.A., and Al-Jebreen, A.M. (2007) 'Lipid peroxides in patients with inflammatory bowel disease.' *Saudi Journal of Gastroenterology* [online] 13 (4), 187-90. available from < http://www.saudijgastro.com/text.asp?2007/13/4/187/36750> [9 March 2019]

Andreyev, A.Y., Kushnareva, Y.E., Murphy, A.N., and Starkov, A.A. (2015) ‘Mitochondrial ROS Metabolism: 10 Years Later.’ *Biochemistry. Biokhimiia* [online] 80 (5), 517–31. available from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4511471&tool=pmcentrez&rendertype=abstract> [22 January 2016]

Angelelli, F., Chiavarino, B., Crestoni, M.E., and Fornarini, S. (2005) ‘Binding of gaseous Fe(III)-heme cation to model biological molecules: Direct association and ligand transfer reactions’. *Journal of the American Society for Mass Spectrometry* [online] 16 (4), 589-598. available from <https://www.sciencedirect.com/science/article/pii/S1044030505000693?via%3Dihub> [7 June 2019]

Anglin, R. (2016) ‘Mitochondrial Dysfunction in Psychiatric Illness.’ *Canadian Journal of Psychiatry. Revue canadienne de psychiatrie* [online] 61 (8), 444–5. available from <http://www.ncbi.nlm.nih.gov/pubmed/27412727> [16 February 2019]

Anoopkumar-Dukie, S., Carey, J.B., Conere, T., O’Sullivan, E., van Pelt, F.N., and Allshire, A. (2005) ‘Resazurin Assay of Radiation Response in Cultured Cells.’ *The British journal of radiology* 78 (934), 945–7.

Antognelli, C., Gambelunghe, A., Talesa, V.N., and Muzi, G. (2014) ‘Reactive Oxygen Species Induce Apoptosis in Bronchial Epithelial BEAS-2B Cells by Inhibiting the Antiglycation Glyoxalase I Defence: Involvement of Superoxide Anion, Hydrogen Peroxide and NF-KB’. *Apoptosis* [online] 19 (1), 102–116. available from <http://link.springer.com/10.1007/s10495-013-0902-y> [2 February 2017]

Antognelli, C., Trapani, E., Delle Monache, S., Perrelli, A., Fornelli, C., Retta, F., Cassoni, P., Talesa, V.N., and Retta, S.F. (2018) 'Data in Support of Sustained Upregulation of Adaptive Redox Homeostasis Mechanisms Caused by KRIT1 Loss-of-Function'. *Data in Brief* [online] 16, 929-938. available from <https://doi.org/10.1016/j.dib.2017.12.026> [18 February 2019]

Anupama, N., Preetha Rani, M.R., Shyni, G.L., and Raghu, K.G. (2018) ‘Glucotoxicity Results in Apoptosis in H9c2 Cells via Alteration in Redox Homeostasis Linked Mitochondrial Dynamics and Polyol Pathway and Possible Reversal with Cinnamic Acid’. *Toxicology in Vitro* [online] 53, 178–192. available from <http://www.ncbi.nlm.nih.gov/pubmed/30144576> [26 January 2019]

Araneda, O.F. and Tuesta, M. (2012) ‘Lung Oxidative Damage by Hypoxia’. *Oxidative Medicine and Cellular Longevity* [online] 2012, 1–18. available from <http://www.hindawi.com/journals/omcl/2012/856918/> [20 May 2017]

Araoye, E. and Ckless, K. (2016) 'Effects of High Fructose/Glucose on Nlrp3/Il1β Inflammatory Pathway'. *Journal of Young Investigators* [online] 31 (5), 25-30. available from <https://www.jyi.org/2016-november/2017/2/13/effects-of-high-fructoseglucose-on-nlrp3il1-inflammatory-pathway> [6 May 2018]

Arif, M., Islam, M.R., Waise, T.M.Z., Hassan, F., Mondal, S.I., and Kabir, Y. (2010) ‘DNA Damage and Plasma Antioxidant Indices in Bangladeshi Type 2 Diabetic Patients’. *Diabetes & Metabolism* [online] 36 (1), 51–57. available from <http://www.ncbi.nlm.nih.gov/pubmed/20036596> [16 December 2018]

Askarova, S., Yang, X., Sheng, W., Sun, G.Y., and Lee, J.C.-M. (2011) ‘Role of Aβ-Receptor for Advanced Glycation Endproducts Interaction in Oxidative Stress and Cytosolic Phospholipase A_2 Activation in Astrocytes and Cerebral Endothelial Cells.’ *Neuroscience* [online] 199, 375–85. available from <http://www.ncbi.nlm.nih.gov/pubmed/21978883> [11 January 2018]

Ates, O., Bilen, H., Keles, S., Alp, H.H., Keles, M.S., Yildirim, K., Ondas, O., Pinar, L.C., Civelekler, M., and Baykal, O. (2013) ‘Plasma coenzyme Q10 levels in type 2 diabetic patients with retinopathy’ *International Journal of Ophthalmology* [online] 6 (5), 675-679. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3808920/> [15 June 2019]

Ateyya, H., Wagih, H.M., and El-Sherbeeny, N.A. (2016) 'Effect of tiron on remote organ injury in rats with severe acute pancreatitis induced by L-arginine'. *Naunyn-Schmiedeberg's Archives of Pharmacology* [online] 389 (8), 873-85. available from <https://link.springer.com/article/10.1007%2Fs00210-016-1250-6> [12 January 2017]

Atkuri, K.R., Herzenberg, L.A., and Herzenberg, L.A. (2005) ‘Culturing at atmospheric oxygen levels impacts lymphocyte function*’. Proceedings of the National Academy of Sciences of the United States of America* [online] 102 (10), 3756-59. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC553335/> [25 June 2019]

Atkuri, K.R., Herzenberg, L.A., Niemi, A.K., Cowan, T. and Herzenberg, L.A. (2007) ‘Importance of culturing primary lymphocytes at physiological oxygen levels'. *Proceedings of the National Academy of Sciences of the United States of America* [online] 104 (11), 4547-4552. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1838638> [25 June 2019]

Austin, B.P., Nair, V.A., Meier, T.B., Xu, G., Rowley, H.A., Carlsson, C.M., Johnson, S.C., and Prabhakaran, V. (2011) ‘Effects of Hypoperfusion in Alzheimer’s Disease.’ *Journal of Alzheimer’s disease : JAD* [online] 26 (3), 123–33. available from <http://www.ncbi.nlm.nih.gov/pubmed/21971457> [11 November 2018]

Ayala, A., Muñoz, M.F., and Argüelles, S. (2014) ‘Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal.’ *Oxidative Medicine and Cellular Longevity* [online] 2014, 360438. available from <http://www.ncbi.nlm.nih.gov/pubmed/24999379> [27 July 2016]

Azizi, G., Navabi, S.S., Al-Shukaili, A., Seyedzadeh, M.H., Yazdani, R., and Mirshafiey, A. (2015) ‘The Role of Inflammatory Mediators in the Pathogenesis of Alzheimer’s Disease.’ *Sultan Qaboos University Medical Journal* [online] 15 (3), e305-16. available from <http://www.ncbi.nlm.nih.gov/pubmed/26357550> [13 October 2016]

Bae, E.J., Ho, D.H., Park, E., Jung, J.W., Cho, K., Hong, J.H., Lee, H.J., Kim, K.P., and Lee, S.J. (2013) ‘Lipid Peroxidation Product 4-Hydroxy-2-Nonenal Promotes Seeding-Capable Oligomer Formation and Cell-to-Cell Transfer of α-Synuclein’. *Antioxidants & Redox Signaling* [online] 18 (7), 770–783. available from <http://www.ncbi.nlm.nih.gov/pubmed/22867050> [16 February 2019]

Baek, K.J., Cho, J.Y., Rosenthal, P., Alexander, L.E.C., Nizet, V., and Broide, D.H. (2013) ‘Hypoxia Potentiates Allergen Induction of HIF-1α, Chemokines, Airway Inflammation, TGF-B1, and Airway Remodeling in a Mouse Model’. *Clinical Immunology* [online] 147 (1), 27–37. available from<https://www.sciencedirect.com/science/article/pii/S1521661613000399#bb0005> [24 August 2018]

Baek, S.M., Kwon, C.H., Kim, J.H., Woo, J.S., Jung, J.S., and Kim, Y.K. (2003) ‘Differential Roles of Hydrogen Peroxide and Hydroxyl Radical in Cisplatin-Induced Cell Death in Renal Proximal Tubular Epithelial Cells’. *Journal of Laboratory and Clinical Medicine* [online] 142 (3), 178–186. available from <http://www.sciencedirect.com/science/article/pii/S0022214303001112> [8 May 2017]

Bajpai, J., Prakash, V., Kant, S., Verma, A.K., Srivastava, A., Bajaj, D.K., Ahmad, M.K., and Agarwal, A. (2017) ‘Study of Oxidative Stress Biomarkers in Chronic Obstructive Pulmonary Disease and Their Correlation with Disease Severity in North Indian Population Cohort.’ *Lung India: Official Organ of Indian Chest Society* [online] 34 (4), 324–329. available from <http://www.ncbi.nlm.nih.gov/pubmed/28671162> [9 November 2017]

Bakonyi, T. and Radak, Z. (2004) 'High Altitude and Free Radicals' *Journal of Sports Science & Medicine* [online] 3 (2), 64-9. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3899533/> [23 March 2018]

Balteau, M., Tajeddine, N., de Meester, C., Ginion, A., Des Rosiers, C., Brady, N.R., Sommereyns, C., Horman, S., Vanoverschelde, J.-L., Gailly, P., Hue, L., Bertrand, L., and Beauloye, C. (2011) ‘NADPH Oxidase Activation by Hyperglycaemia in Cardiomyocytes Is Independent of Glucose Metabolism but Requires SGLT1’. *Cardiovascular Research* [online] 92 (2), 237–246. available from <http://www.ncbi.nlm.nih.gov/pubmed/21859816> [21 April 2018]

Bandeira, S. de M., Guedes, G. da S., da Fonseca, L.J.S., Pires, A.S., Gelain, D.P., Moreira, J.C.F., Rabelo, L.A., Vasconcelos, S.M.L., and Goulart, M.O.F. (2012) ‘Characterization of Blood Oxidative Stress in Type 2 Diabetes Mellitus Patients: Increase in Lipid Peroxidation and SOD Activity.’ *Oxidative Medicine and Cellular Longevity* [online] 2012, 819310. available from <http://www.ncbi.nlm.nih.gov/pubmed/23259029> [10 February 2018]

Barreiro, J.C., Capelato, M.D., Martin-Neto, L., Christian, H., and Hansen, B. (2007) ‘Oxidative decomposition of atrazine by a Fenton-like reaction in a H2O2/ferrihydrite system.’ *Water Research* [online] 41 (1), 55-62. available from <https://www.sciencedirect.com/science/article/abs/pii/S0043135406005343> [07 Jun 2019]

Bartels, K., Grenz, A., and Eltzschig, H.K. (2013) ‘Hypoxia and Inflammation Are Two Sides of the Same Coin.’ *Proceedings of the National Academy of Sciences of the United States of America* [online] 110 (46), 18351–2. available from <http://www.ncbi.nlm.nih.gov/pubmed/24187149> [20 May 2017]

Bartoli, M.L., Novelli, F., Costa, F., Malagrinò, L., Melosini, L., Bacci, E., Cianchetti, S., Dente, F.L., Di Franco, A., Vagaggini, B., and Paggiaro, P.L. (2011) ‘Malondialdehyde in Exhaled Breath Condensate as a Marker of Oxidative Stress in Different Pulmonary Diseases.’ *Mediators of inflammation* [online] 2011, 891752. available from <http://www.ncbi.nlm.nih.gov/pubmed/21772668> [24 February 2017]

Bateman, D.N., Dear, J.W., Thanacoody, H.K.R., Thomas, S.H.L., Eddleston, M., Sandilands, E.A., Coyle, J., Cooper, J.G., Rodriguez, A., Butcher, I., Lewis, S.C., Vliegenthart, A.D.B., Veiraiah, A., Webb, D.J., and Gray, A. (2014) ' Reduction of adverse effects from intravenous acetylcysteine treatment for paracetamol poisoning: a randomised controlled trial.' *The Lancet* [online] 383 (9918), 697-704. available from <https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(13)62062-0/fulltext?rss%3Dyes> [9 March 2019]

Bathri, R., Bose, P., Gujar, V.S., and Kumar, L. (2017) 'The Role of ROS in COPD Progression and Therapeutic Strategies'. *Reactive Oxygen Species* 4 (10), 1–14.

Baxter, P.S. and Hardingham, G.E. (2016) *Adaptive Regulation of the Brain's Antioxidant Defences by Neurons and Astrocytes*. [online] available from <http://dx.doi.org/10.1016/j.freeradbiomed.2016.06.027> [19 August 2018]

Baumann, M.U., Zamudio, S., and Illsley, N.P. (2007) 'Hypoxic upregulation of glucose transporters in BeWo choriocarcinoma cells is mediated by hypoxia-inducible factor-1'. *American Journal of Physiology-Cell Physiology* [online] 293 (1). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4497554/> [16 June 2019]

Bedad, S.K., and Nearati, P. (2015) 'Effect of Resveratrol on the Pharmacokinetics of Carbamazepine in Healthy Human Volunteers'. *Phytotherapy Research* [online] 29, 701-6. available from <https://onlinelibrary.wiley.com/doi/abs/10.1002/ptr.5302> [29 June 2019]

Bélanger, M. and Magistretti, P.J. (2009) 'The Role of Astroglia in Neuroprotection.' *Dialogues in Clinical Neuroscience* [online] 11 (3), 281–95. available from <http://www.ncbi.nlm.nih.gov/pubmed/19877496> [12 November 2018]

Bell, E.L., Klimova, T.A., Eisenbart, J., Schumacker, P.T., and Chandel, N.S. (2007) 'Mitochondrial Reactive Oxygen Species Trigger Hypoxia-Inducible Factor-Dependent Extension of the Replicative Life Span during Hypoxia.' *Molecular and Cellular Biology* [online] 27 (16), 5737–45. available from <http://www.ncbi.nlm.nih.gov/pubmed/17562866> [21 January 2019]

Bell, K.F.S., Al-Mubarak, B., Martel, M.-A., McKay, S., Wheelan, N., Hasel, P., Márkus, N.M., Baxter, P., Deighton, R.F., Serio, A., Bilican, B., Chowdhry, S., Meakin, P.J., Ashford, M.L.J., Wyllie, D.J.A., Scannevin, R.H., Chandran, S., Hayes, J.D., and Hardingham, G.E. (2015) 'Neuronal Development Is Promoted by Weakened Intrinsic Antioxidant Defences Due to Epigenetic Repression of Nrf2'. *Nature Communications* [online] 6 (1), 7066. available from <http://www.nature.com/articles/ncomms8066> [19 August 2018]

Ben-Yehuda Greenwald, M., Ben-Sasson, S., Bianco-Peled, H., and Kohen, R. (2016) 'Skin Redox Balance Maintenance: The Need for an Nrf2-Activator Delivery System'. *Cosmetics* [online] 3 (1), 1. available from <http://www.mdpi.com/2079-9284/3/1/1> [14 February 2017]

Bergman, O. and Ben-Shachar, D. (2016) 'Mitochondrial Oxidative Phosphorylation System (OXPHOS) Deficits in Schizophrenia: Possible Interactions with Cellular Processes.' *Canadian Journal of Psychiatry* [online] 61 (8), 457–69. available from <http://www.ncbi.nlm.nih.gov/pubmed/27412728> [16 February 2019]

Berman, A.Y., Motechin, R.A., Wiesenfeld, M.Y., and Holz, M.K. (2017) 'The Therapeutic Potential of Resveratrol: A Review of Clinical Trials'. *NPJ Precision Oncology* [online] 1 (1), 35. available from <http://www.nature.com/articles/s41698-017-0038-6> [4 May 2018]

Berrone, E., Beltramo, E., Solimine, C., Ape, A.U., and Porta, M. (2006) 'Regulation of Intracellular Glucose and Polyol Pathway by Thiamine and Benfotiamine in Vascular Cells Cultured in High Glucose.' *The Journal of Biological Chemistry* [online] 281 (14), 9307–13. available from <http://www.ncbi.nlm.nih.gov/pubmed/16452468> [13 April 2018]Betti, M., Ambrogini, P., Minelli, A., Floridi, A., Lattanzi, D., Ciuffoli, S., Bucherelli, C., Prospero, E., Frontini, A., Santarelli, L., Baldi, E., Benetti, F., Galli, G., and Cuppini, R. (2011) 'Maternal dietary loads of α-tocopherol depress protein kinase C signaling and synaptic plasticity in rat postnatal developing hippocampus and promote permanent deficits in adult offspring'. *Journal of Nutritional Biochemistry* [online] 22 (1), 60-70. available from <https://www.sciencedirect.com/science/article/pii/S0955286310000082> [29 June 2019]

Betti, M., Minelli, A., Canonico, B., Castaldo, P., Magi, S., Aisa, M.C., Piroddi, M., Di Tomaso, V., and Galli, F. (2006) 'Antiproliferative Effects of Tocopherols (Vitamin E) on Murine Glioma C6 Cells: Homologue-Specific Control of PKC/ERK and Cyclin Signaling.' *Free Radical Biology & Medicine* [online] 41 (3), 464–72. available from <http://www.sciencedirect.com/science/article/pii/S0891584906002553> [7 June 2015]

Bhatti, F.Y., Mehmood, A., Latief, N., Zhara, S., Cho, H., Khan, S.N., and Riazuddin, S. (2017) 'Vitamin E protects rat mesenchymal stem cells against hydrogen peroxide-induced oxidative stress in vitro and improves their therapeutic potential in surgically-induced rat model of osteoarthritis'. *Osteoarthritis and Cartilage* [online] 25 (2), 321-33. available from <https://www.sciencedirect.com/science/article/pii/S1063458416302941> [29 June 2019]

Bickers, D.R. and Athar, M. (2006) 'Oxidative Stress in the Pathogenesis of Skin Disease.' *The Journal of Investigative Dermatology* 126 (12), 2565–2575

Birben, E., Sahiner, U.M., Sackesen, C., Erzurum, S., and Kalayci, O. (2012) 'Oxidative Stress and Antioxidant Defense.' *The World Allergy Organization Journal* [online] 5 (1), 9–19. available from <http://www.ncbi.nlm.nih.gov/pubmed/23268465> [30 January 2017]

Birch-Machin, M.A., Russell, E. V, and Latimer, J.A. (2013) 'Mitochondrial DNA Damage as a Biomarker for Ultraviolet Radiation Exposure and Oxidative Stress.' *The British Journal of Dermatology* [online] 169, 9–14. available from <http://www.ncbi.nlm.nih.gov/pubmed/23786615>

Bisht, K., Wagner, K., and Bulmer, A.C. (2010) 'Curcumin, Resveratrol and Flavonoids as Anti-Inflammatory, Cyto- and DNA-Protective Dietary Compounds'. *Toxicology* 278, 88–100

Bjelakovic, G., Nikolova, D., Gluud, L.L., Simonetti, R.G., and Gluud, C. (2012) 'Antioxidant Supplements for Prevention of Mortality in Healthy Participants and Patients with Various Diseases'. in *Cochrane Database of Systematic Reviews* [online] ed. by Bjelakovic, G. Chichester, UK: John Wiley & Sons, Ltd. available from <http://doi.wiley.com/10.1002/14651858.CD007176.pub2> [24 February 2017]

Block, K.I., Koch, A.C., Mead, M.N., Tothy, P.K., Newman, R.A., and Gyllenhaal, C. (2007) 'Impact of Antioxidant Supplementation on Chemotherapeutic Efficacy: A Systematic Review of the Evidence from Randomized Controlled Trials'. *Cancer Treatment Reviews* [online] 33 (5), 407–418. available from <http://www.ncbi.nlm.nih.gov/pubmed/17367938> [16 February 2019]

Boccardi, V., Baroni, M., Mangialasche, F., and Mecocci, P. (2016) 'Vitamin E Family: Role in the Pathogenesis and Treatment of Alzheimer's Disease'. *Alzheimer's & Dementia: Translational Research & Clinical Interventions* [online] 2 (3), 182–191. available from <https://www.sciencedirect.com/science/article/pii/S2352873716300324> [27 March 2018]

Bodera, P., Stankiewicz, W., Antkowiak, B., Paluch, M., Kieliszek, J., Sobiech, J., and Niemcewicz, M. (2015) 'Influence of Electromagnetic Field (1800 MHz) on Lipid Peroxidation in Brain, Blood, Liver and Kidney in Rats'. *International Journal of Occupational Medicine and Environmental Health* [online] 28 (4), 751–759. available from <http://www.ncbi.nlm.nih.gov/pubmed/26216313> [24 October 2018]

Bohgaki, T., Bohgaki, M., and Hakem, R. (2010) 'DNA Double-Strand Break Signaling and Human Disorders.' *Genome Integrity* [online] 1 (1), 15. available from <http://www.genomeintegrity.com/content/1/1/15>

Bolignano, D., Cernaro, V., Gembillo, G., Baggetta, R., Buemi, M., and D'Arrigo, G. (2017) 'Antioxidant Agents for Delaying Diabetic Kidney Disease Progression: A Systematic Review and Meta-Analysis'. *PLOS ONE* [online] 12 (6), e0178699. available from <https://dx.plos.org/10.1371/journal.pone.0178699> [30 November 2018]

Bolisetty, S. and Jaimes, E. (2013) 'Mitochondria and Reactive Oxygen Species: Physiology and Pathophysiology'. *International Journal of Molecular Sciences* [online] 14 (3), 6306–6344. available from <http://www.mdpi.com/1422-0067/14/3/6306/> [11 January 2017]

Bono, R., Romanazzi, V., Munnia, A., Piro, S., Allione, A., Ricceri, F., Guarrera, S., Pignata, C., Matullo, G., Wang, P., Giese, R.W., and Peluso, M. (2010) 'Malondialdehyde-Deoxyguanosine Adduct Formation in Workers of Pathology Wards: The Role of Air Formaldehyde Exposure.' *Chemical Research in Toxicology* [online] 23 (8), 1342–8. available from <http://www.ncbi.nlm.nih.gov/pubmed/20707408> [19 August 2018]

de Bont, R. and van Larebeke, N. (2004) ‘Endogenous DNA Damage in Humans: A Review of Quantitative Data’. *Mutagenesis* [online] 19 (3), 169–185. available from <http://mutage.oxfordjournals.org/content/19/3/169.long>

Booth, H.D.E., Hirst, W.D., and Wade-Martins, R. (2017) ‘The Role of Astrocyte Dysfunction in Parkinson’s Disease Pathogenesis’. *Trends in Neurosciences* [online] 40 (6), 358-70. available from <https://www.cell.com/trends/neurosciences/fulltext/S0166-2236(17)30068-1> [17 June 2019]

Bossmann, S.H., Oliveros, E., Kantor, M., Niebler, S., Bonfill, A., Shahin, N., Worner, M., and Braun, A.M. (2004) ‘New insights into the mechanisms of the thermal Fenton reactions occurring using different iron(II)-complexes’. *Water Science and Technology: a journal of the International Association on Water Pollution Research* [online] 49 (4), 75-80. [7 June 2019]

Bouayed, J. and Bohn, T. (2010) ‘Exogenous Antioxidants—Double-Edged Swords in Cellular Redox State: Health Beneficial Effects at Physiologic Doses versus Deleterious Effects at High Doses’. *Oxidative Medicine and Cellular Longevity* [online] 3 (4), 228. available from <http://www.ncbi.nlm.nih.gov/pubmed/20972369> [17 March 2018]

Bouhafs, R. K. L., and Jarstrand, C. (2002) 'Effects of Antioxidants on Surfactant Peroxidation by Stimulated Human Polymorphonuclear Leukocytes'. *Free Radical Research* [online] 36 (7), 727–734. available from <https://www.tandfonline.com/doi/abs/10.1080/10715760290032593?journalCode=ifra20> [28 June 2019]

Bouzid, D., Gargouri, B., Mansour, R.B., Amouri, A., Tahri, N., Lassoued, S., and Masmoudi, H. (2013) ' Oxidative stress markers in intestinal mucosa of Tunisian inflammatory bowel disease patients.' *Saudi Journal of Gastroenterology* [online] 19 (3), 131-5. available from <https://www.ncbi.nlm.nih.gov/pubmed/23680711> [9 March 2019]

Bowers, R., Cool, C., Murphy, R.C., Tuder, R.M., Hopken, M.W., Flores, S.C., and Voelkel, N.F. (2004) ‘Oxidative Stress in Severe Pulmonary Hypertension’. *American Journal of Respiratory and Critical Care Medicine* [online] 169 (6), 764–769. available from <http://www.ncbi.nlm.nih.gov/pubmed/14701708> [22 January 2019]

Bowman, A., Martinez-Levasseur, L.M., Acevedo-Whitehouse, K., Gendron, D., and Birch-Machin, M.A. (2013) ‘The Simultaneous Detection of Mitochondrial DNA Damage from Sun-Exposed Skin of Three Whale Species and Its Association with UV-Induced Microscopic Lesions and Apoptosis’. *Mitochondrion* [online] 13 (4), 342–349. available from <http://dx.doi.org/10.1016/j.mito.2013.04.003>

Brahimi-Horn, M.C., and Pouysseguar, J. (2007) ‘Oxygen, a source of life and stress’. *FEBS Letters* [online] 581 (19). available from <https://febs.onlinelibrary.wiley.com/doi/full/10.1016/j.febslet.2007.06.018> [20 June 2019]

Broe, M., Kril, J., and Halliday, G.M. (2004) ‘Astrocytic degeneration relates to the severity of disease in frontotemporal dementia’. *Brain* [online] 127 (10), 2214-20. available from <https://academic.oup.com/brain/article/127/10/2214/404478> [18 June 2019]

Brown, R.H., Reynolds, C., Brooker, A., Talalay, P., and Fahey, J.W. (2015) ‘Sulforaphane Improves the Bronchoprotective Response in Asthmatics through Nrf2-Mediated Gene Pathways’. *Respiratory Research* [online] 16 (1), 106. available from <http://respiratory-research.biomedcentral.com/articles/10.1186/s12931-015-0253-z> [20 October 2018]

Brownlee, M. (2005) ' The Pathobiology of Diabetic Complications; A Unifying Mechanism'. *Diabetes* [online] 54 (6), 1615-25. available from <http://diabetes.diabetesjournals.org/content/54/6/1615.article-info> [24 December 2018]

Brunner, D., Frank, J., Appl, H., Schöffl, H., Pfaller, W., and Gstraunthaler, G. (2010) ‘Serum-Free Cell Culture: The Serum-Free Media Interactive Online Database.’ *ALTEX* 27 (1), 53–62

Brustovetsky, N., LaFrance, R., Purl, K.J., Brustovetsky, T., Keene, C.D., Low, W.C., and Dubinsky, J.M. (2005) ‘Age-dependent changes in the calcium sensitivity of striatal mitochondria in mouse models of Huntington's Disease’. *Journal of Neurochemistry* [online] 93 (6), 1361-70. available from <https://onlinelibrary.wiley.com/doi/full/10.1111/j.1471-4159.2005.03036.x> [21 June 2019]

Bucchieri, F., Marino Gammazza, A., Pitruzzella, A., Fucarino, A., Farina, F., Howarth, P., Holgate, S.T., Zummo, G., and Davies, D.E. (2015) 'Cigarette Smoke Causes Caspase-Independent Apoptosis of Bronchial Epithelial Cells from Asthmatic Donors.' *PLOS ONE* [online] 10 (3), e0120510. available from <http://www.ncbi.nlm.nih.gov/pubmed/25793769> [22 May 2018]

Buckley, N.A., Dawson, A.H., and Isbister, G.K. (2016) 'Treatments for paracetomol poisoning.' *The BMJ* [online] 353. available from < https://www.bmj.com/content/353/bmj.i2579> [9 March 2019]

Bullón, P., Román-Malo, L., Marín-Aguilar, F., Alvarez-Suarez, J.M., Giampieri, F., Battino, M., and Cordero, M.D. (2015) 'Lipophilic Antioxidants Prevent Lipopolysaccharide-Induced Mitochondrial Dysfunction through Mitochondrial Biogenesis Improvement'. *Pharmacological Research* [online] 91, 1–8. available from <http://linkinghub.elsevier.com/retrieve/pii/S1043661814001686>

Burnett, E. and Penn, L. (2013) 'Mycoplasma Detection and Elimination'. *Biofiles* [online] 8 (18), 1. available from <http://www.sigmaaldrich.com/technical-documents/articles/biofiles/mycoplasma-detection-and-elimination.html> [21 August 2017]

Buse, M.G. (2006a) 'Hexosamines, Insulin Resistance, and the Complications of Diabetes: Current Status.' *American journal of physiology. Endocrinology and Metabolism* [online] 290 (1). available from <http://www.ncbi.nlm.nih.gov/pubmed/16339923> [20 May 2017]

Busik, J. V., Mohr, S., and Grant, M.B. (2008) 'Hyperglycemia-Induced Reactive Oxygen Species Toxicity to Endothelial Cells Is Dependent on Paracrine Mediators'. *Diabetes* [online] 57 (7), 1952–1965. available from <http://www.ncbi.nlm.nih.gov/pubmed/18420487> [15 December 2018]

Butterfield, D.A., Hardas, S.S., and Lange, M.L.B. (2010) 'Oxidatively Modified Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) and Alzheimer's Disease: Many Pathways to Neurodegeneration'. *Journal of Alzheimer's Disease* [online] 20 (2), 369–393. available from <http://www.ncbi.nlm.nih.gov/pubmed/20164570> [14 December 2018]

Calderone, V., Testai, L., Martelli, A., La Motta, C., Sartini, S., Da Settimo, F., Breschi, M.C. (2010) 'Anti-ischaemic activity of an antioxidant aldose reductase inhibitor on diabetic and non-diabetic rat hearts'. *Journal of Pharmacy and Pharmacology* [online] 62 (1), 107-13. available from <https://onlinelibrary.wiley.com/doi/abs/10.1211/jpp.62.01.0012> [24 June 2019]

Calderon-Salinas, J.V., Munoz-Reyes, E.G., Guerrero-Romero, J.F., Rodriguez-Moran, M., Bracho-Riquelme, R.L., Carrera-Garcia, M.A., and Quintanar-Escorza, M.A. (2011) 'Eryptosis and Oxidative Damage in Type 2 Diabetic Mellitus Patients with Chronic Kidney Disease'. *Molecular and Cellular Biochemistry* [online] 357 (1–2), 171–179. available from <https://link.springer.com/article/10.1007%2Fs11010-011-0887-1> [9 August 2017]

Callapina, M., Zhou, J., Schmid, T., Kö, R., and Brüne, B. (2005a) 'NO Restores HIF - 1a Hydroxylation during Hypoxia : Role of Reactive Oxygen Species'. *Free Radical Biology & Medicine* [online] 39, 925–936. available from <https://www.sciencedirect.com/science/article/pii/S0891584905002881?via%3Dihub> [26 July 2017]

Camello Almaraz, C., Gomez Pinilla, P., Pozo, M., and Camello, P. (2006) 'Mitochondrial Reactive Oxygen Species and Ca2+ Signaling'. *American Journal of Physiology Cell physiology* [online] 291 (5), C1082–C1088. available from <http://link3.kesli.or.kr:3410/cmclib?sid=Entrez%3APubMed&id=pmid%3A16760264%5Cnhttp://ajpcell.physiology.org/content/ajpcell/291/5/C1082.full.pdf>

Carbonell, T. and Rama, R. (2009) 'Respiratory Hypoxia and Oxidative Stress in the Brain. Is the Endogenous Erythropoietin an Antioxidant?' *Current Chemical Biology* 3, 238–252

Cardoso, S.M., Santana, I., Swerdlow, R.H., and Oliveira, C.R. (2004) 'Mitochondria Dysfunction of Alzheimer's Disease Cybrids Enhances Aβ Toxicity'. *Journal of Neurochemistry* [online] 89 (6), 1417–1426. available from <http://doi.wiley.com/10.1111/j.1471-4159.2004.02438.x> [19 August 2018]

Carocho, M. and Ferreira, I.C.F.R. (2013) 'A Review on Antioxidants, Prooxidants and Related Controversy: Natural and Synthetic Compounds, Screening and Analysis Methodologies and Future Perspectives.' *Food and Chemical Toxicology : an International Journal Published for the British Industrial Biological Research Association* [online] 51, 15–25. available from <http://www.ncbi.nlm.nih.gov/pubmed/23017782> [20 August 2014]

Carrera, S., de Verdier, P.J., Khan, Z., Zhao, B., Mahale, A., Bowman, K.J., Zainol, M., Jones, G.D.D., Lee, S.W., Aaronson, S.A., and Macip, S. (2010) 'Protection of Cells in Physiological Oxygen Tensions against DNA Damage-Induced Apoptosis'. *Journal of Biological Chemistry* [online] 285 (10), 13658-13665. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2859528/> [29 May 2019]

Carter, S.F., Schoell, M., Almkvist, O., Wall, A., Engler, H., Langstroem, B., and Norberg, A. (2012) ' Evidence for Astrocytosis in Prodromal Alzheimer Disease Provided by 11C-Deuterium-L-Deprenyl: A Multitracer PET Paradigm Combining 11C-Pittsburgh Compound B and 18F-FDG'. *The Journal of Nuclear Medicine* [online] 53 (1), 37-46. available from <http://jnm.snmjournals.org/content/53/1/37.long> [18 June 2019]

Castaneda, O.A., Lee, S.-C., Ho, C.-T., and Huang, T.-C. (2017) 'Macrophages in Oxidative Stress and Models to Evaluate the Antioxidant Function of Dietary Natural Compounds'. *Journal of Food and Drug Analysis* [online] 25 (1), 111–118. available from <https://www.sciencedirect.com/science/article/pii/S1021949816301752> [8 March 2018]

Castellani, R.J., Perry, G., Siedlak, S.L., Nunomura, A., Shimohama, S., Zhang, J., Montine, T., Sayre, L.M., and Smith, M.A. (2002) 'Hydroxynonenal adducts indicate a role for lipid peroxidation in neocortical and brainstem Lewy bodies in humans'. *Neuroscience Letters* [online] 319 (1), 25-8. available from <https://www.sciencedirect.com/science/article/abs/pii/S0304394001025149?via%3Dihub> [21 June 2019]

Catalá, A. (2009) ' Lipid peroxidation of membrane phospholipids generates hydroxy-alkenals and oxidized phospholipids active in physiological and/or pathological conditions.' Chemistry and Physics of Lipids [online] 157 (1), 1-11. available from <https://www.sciencedirect.com/science/article/pii/S0009308408003708> [12 March 2019]

Ceriello, A. and Motz, E. (2004) 'Is Oxidative Stress the Pathogenic Mechanism Underlying Insulin Resistance, Diabetes, and Cardiovascular Disease? The Common Soil Hypothesis Revisited.' *Arteriosclerosis, Thrombosis, and Vascular Biology* [online] 24 (5), 816–23. available from <http://www.ncbi.nlm.nih.gov/pubmed/14976002> [7 January 2018]

Chakrabarti, S., Munshi, S., Banerjee, K., Thakurta, I.G., Sinha, M., and Bagh, M.B. (2011) 'Mitochondrial Dysfunction during Brain Aging: Role of Oxidative Stress and Modulation by Antioxidant Supplementation.' *Aging and Disease* [online] 2 (3), 242–56. available from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3295058&tool=pmcentrez&rendertype=abstract> [31 May 2016]Chan, A.W.H., Ho, Y.S., Chung, S.K., and Chung, S.S.M. (2008) 'Synergistic effect of osmotic and oxidative stress in slow-developing cataract formation'. *Experimental Eye Research* [online] 87 (5), 454-61. available from <https://www.sciencedirect.com/science/article/pii/S0014483508002637?via%3Dihub> [24 June 2019]

Chan, S.W. and Dedon, P.C. (2010) 'The Biological and Metabolic Fates of Endogenous DNA Damage Products.' *Journal of Nucleic Acids* [online] 2010, 929047. available from <http://www.ncbi.nlm.nih.gov/pubmed/21209721> [14 November 2018]

Chan, S.Y., Zhang, Y.-Y., Hemann, C., Mahoney, C.E., Zweier, J.L., and Loscalzo, J. (2009) 'Article MicroRNA-210 Controls Mitochondrial Metabolism during Hypoxia by Repressing the Iron-Sulfur Cluster Assembly Proteins ISCU1/2'. *Cell Metabolism* [online] 10, 273–284. available from < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2759401/> [4 November 2018]

Chan, T.K., Tan, W.S.D., Peh, H.Y., and Wong, W.S.F. (2017) 'Aeroallergens Induce Reactive Oxygen Species Production and DNA Damage and Dampen Antioxidant Responses in Bronchial Epithelial Cells.' *Journal of Immunology* [online] 199 (1), 39–47. available from <http://www.ncbi.nlm.nih.gov/pubmed/28526682> [15 July 2018]

Chandel, N.S., McClintock, D.S., Feliciano, C.E., Wood, T.M., Melendez, J.A., Rodriguez, A.M., and Schumacker, P.T. (2000) 'Reactive Oxygen Species Generated at Mitochondrial Complex III Stabilize Hypoxia-Inducible Factor-1alpha during Hypoxia: A Mechanism of O2 Sensing.' *The Journal of Biological Chemistry* [online] 275 (33), 25130–8. available from <http://www.ncbi.nlm.nih.gov/pubmed/10833514> [21 April 2018]

Chang, D., Feng, L.-G., Xu, F.-J., Kuang, H.-Y., and Lu, M.-J. (2007) 'Oxidative Damage to DNA and Its Relationship With Diabetic Complications'. Biomedical and Environmental Sciences [online] 20, 160–163. available from <https://pdfs.semanticscholar.org/b9f4/a1898c99c781cca3f006d4b18f0bee3501b5.pdf> [6 May 2018]

Chaouat, A., Naeije, R., and Weitzenblum, E. (2008) 'Pulmonary Hypertension in COPD.' *The European Respiratory Journal* [online] 32 (5), 1371–85. available from <http://www.ncbi.nlm.nih.gov/pubmed/18978137> [2 November 2018]

Chapple, S.J., Keeley, T.P., Mastronicola, D., Arno, M., Vizcay-Barrena, G., Fleck, R., Siow, R.C.M., and Mann, G.E. (2016) 'Bach1 differentially regulates distinct Nrf2-dependent genes in human venous and coronary artery endothelial cells adapted to physiological oxygen levels'. *Free Radical Biology and Medicine* [online] 92, 152-162. available from <https://www.sciencedirect.com/science/article/pii/S0891584915011661?via%3Dihub> [30 May 2019]

Chatziralli, I.P., Theodossiadis, G., Dimitriadis, P., Charalambidis, M., Agorastos, A., Migkos, Z., Platogiannis, N., Moschos, M.M., Theodossiadis, P., and Keryttopoulos, P. (2017) 'The Effect of Vitamin E on Oxidative Stress Indicated by Serum Malondialdehyde in Insulin-Dependent Diabetes Mellitus Patients with Retinopathy'. *The Open Ophthalmology Journal* [online] 11 (1), 51–58. available from <http://benthamopen.com/ABSTRACT/TOOPHTJ-11-51> [13 July 2018]

Chauhan, A., Gu, F., Essa, M.M., Wegiel, J., Kaur, K., Brown, W.T., and Chauhan, V. (2011) 'Brain Region-Specific Deficit in Mitochondrial Electron Transport Chain Complexes in Children with Autism'. *Journal of Neurochemistry* [online] 117 (2), 209–220. available from <http://doi.wiley.com/10.1111/j.1471-4159.2011.07189.x> [16 February 2019]

Chen, C.-Y., Jang, J.-H., Li, M.-H., and Surh, Y.-J. (2005) 'Resveratrol Upregulates Heme Oxygenase-1 Expression via Activation of NF-E2-Related Factor 2 in PC12 Cells'. *Biochemical and Biophysical Research Communications* [online] 331 (4), 993–1000. available from <https://www.sciencedirect.com/science/article/pii/S0006291X05007424> [11 March 2018]

Chen, C., Arjomandi, M., Balmes, J., Tager, I., and Holland, N. (2007) 'Effects of Chronic and Acute Ozone Exposure on Lipid Peroxidation and Antioxidant Capacity in Healthy Young Adults.' *Environmental Health Perspectives* [online] 115 (12), 1732–7. available from <http://www.ncbi.nlm.nih.gov/pubmed/18087591> [10 August 2017]

Chen, C., Pore, N., Behrooz, A., Ismail-Beigi, F., and Maity, A. (2001) 'Regulation of Glut1 MRNA by Hypoxia-Inducible Factor-1. Interaction between H-Ras and Hypoxia.' *The Journal of Biological Chemistry* [online] 276 (12), 9519–25. available from <http://www.ncbi.nlm.nih.gov/pubmed/11120745> [15 December 2018]

Chen, J., Liu, B., Yuan, J., Yang, J., Zhang, J., An, Y., Tie, L., Pan, Y., and Li, X. (2012) 'Atorvastatin Reduces Vascular Endothelial Growth Factor (VEGF) Expression in Human Non-Small Cell Lung Carcinomas (NSCLCs) via Inhibition of Reactive Oxygen Species (ROS) Production'. *Molecular Oncology* [online] 6 (1), 62–72. available from <http://www.ncbi.nlm.nih.gov/pubmed/22153388> [22 January 2019]

Chen, K.-H., Hung, C.-C., Hsu, H.-H., Jing, Y.-H., Yang, C.-W., and Chen, J.-K. (2011) 'Resveratrol Ameliorates Early Diabetic Nephropathy Associated with Suppression of Augmented TGF-β/Smad and ERK1/2 Signaling in Streptozotocin-Induced Diabetic Rats'. *Chemico-Biological Interactions* [online] 190 (1), 45–53. available from <https://www.sciencedirect.com/science/article/pii/S0009279711000573> [4 May 2018]

Chen, L., Li, B., Chen, B., Shao, Y., Luo, Q., Shi, X., and Chen, Y. (2016) 'Thymoquinone Alleviates the Experimental Diabetic Peripheral Neuropathy by Modulation of Inflammation'. Scientific Reports [online] 6. available from <https://www.nature.com/articles/srep31656> [21 June 2019

Chen, P.C., Vargas, M.R., Pani, A.K., Smeyne, R.J., Johnson, D.A., Kan, Y.W., and Johnson, Y.A. (2009) 'Nrf2-mediated neuroprotection in the MPTP mouse model of Parkinson's disease: Critical role for the astrocyte'. *Proceedings of the National Academy of Sciences of the United States of America* [online] 106 (8), 2933-38. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2650368/> [22 June 2019]

Chen, T., He, J., Shen, L., Fang, H., Nie, H., Jin, T., Wei, X., Xin, Y., Jiang, Y., Li, H., Chen, G., Lu, J., and Bai, Y. (2011) 'The mitochondrial DNA 4,977-bp deletion and its implication in copy number alteration in colorectal cancer'. *BMC Medical Genetics* [online] 12 (8). available from <https://bmcmedgenet.biomedcentral.com/articles/10.1186/1471-2350-12-8> [15 June 2019]

Chen, Y., McMillan-Ward, E., Kong, J., Israels, S.J., and Gibson, S.B. (2007) 'Mitochondrial Electron-Transport-Chain Inhibitors of Complexes I and II Induce Autophagic Cell Death Mediated by Reactive Oxygen Species'. *Journal of Cell Science* 120 (23)

Cheng, M.-F., Chen, L.-J., and Cheng, J.-T. (2010) 'Decrease of Klotho in the Kidney of Streptozotocin-Induced Diabetic Rats.' *Journal of biomedicine & biotechnology* [online] 2010, 513853. available from <http://www.ncbi.nlm.nih.gov/pubmed/20625492> [7 May 2018]

Cheng, X., Siow, R.C.M., and Mann, G.E. (2011) 'Impaired Redox Signaling and Antioxidant Gene Expression in Endothelial Cells in Diabetes: A Role for Mitochondria and the Nuclear Factor-E2-Related Factor 2-Kelch-Like ECH-Associated Protein 1 Defense Pathway'. *Antioxidants & Redox Signaling* [online] 14 (3), 469–487. available from <http://www.liebertonline.com/doi/abs/10.1089/ars.2010.3283>

Chiu, S.-C., Lin, Y.-J., Huang, S.-Y., Lien, C.-F., Chen, S.-P., Pang, C.-Y., Lin, J.-H., and Yang, K.-T. (2015) 'The Role of Intermittent Hypoxia on the Proliferative Inhibition of Rat Cerebellar Astrocytes'. *PLOS ONE* [online] 10 (7), e0132263. available from <https://dx.plos.org/10.1371/journal.pone.0132263> [25 October 2018]

Cho, N.H., Shaw, J.E., Karuranga, S., Huang, Y., da Rocha Fernandes, J.D., Ohlrogge, A.W., and Malanda, B. (2018) 'IDF Diabetes Atlas: Global Estimates of Diabetes Prevalence for 2017 and Projections for 2045'. *Diabetes Research and Clinical Practice* [online] 138, 271–281. available from <https://www.sciencedirect.com/science/article/pii/S0168822718302031> [4 September 2018]

Choi, J., Sullards, M.C., Olzmann, J.A., Rees, H.D., Weintraub, S.T., Bostwick, D.E., Gearing, M., Levey, A.I., Chin, L.-S., and Li, L. (2006) 'Oxidative Damage of DJ-1 Is Linked to Sporadic Parkinson and Alzheimer Diseases'. *Journal of Biological Chemistry* [online] 281 (16), 10816–10824. available from <http://www.jbc.org/lookup/doi/10.1074/jbc.M509079200> [19 August 2018]

Chole, R.H., Patil, R.N., Basak,A., Palandukar, K., and Bhowate, R. (2010) ' Estimation of serum malondialdehyde in oral cancer and precancer and its association with healthy individuals, gender, alcohol, and tobacco abuse.' *Journal of Cancer Research and Therapeutics* [online] 6 (4). 487-91. available from <http://www.cancerjournal.net/text.asp?2010/6/4/487/77106> [01 March 2019]

Chong, S.J.F., Low, I.C.C., and Pervaiz, S. (2014) 'Mitochondrial ROS and Involvement of Bcl-2 as a Mitochondrial ROS Regulator.' *Mitochondrion* [online] 19 Pt A, 39–48. available from <http://www.sciencedirect.com/science/article/pii/S1567724914000877> [17 December 2015]

Chou, T.-F., Ma, M.-C., Tsai, C.-P., and Chen, C.-F. (2009) 'Enhancement of Superoxide Dismutase Activity in Rat Lungs after Hypoxic Preconditioning.' *The Chinese Journal of Physiology* [online] 52 (5 Suppl), 376–83. available from <http://www.ncbi.nlm.nih.gov/pubmed/20359128> [22 January 2019]

Chow, H.H.S., Garland, L., Hsu, C.H., Vining, D.R., Chew, W.M., Miller, J.A., Perloff, M., Crowell, J.A., and Alberts, D. (2010) 'Resveratrol Modulates Drug and Carcinogen Metabolising Enzymes in a Healthy Volunteer Study'. *Cancer Prevention Research* [online] 3 (9), 1168-75. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2933312/> [29 June 2019]

Chua, Y.L., Dufour, E., Dassa, E.P., Rustin, P., Jacobs, H.T., Taylor, C.T., and Hagen, T. (2010) 'Stabilization of Hypoxia-Inducible Factor-1alpha Protein in Hypoxia Occurs Independently of Mitochondrial Reactive Oxygen Species Production.' *The Journal of Biological Chemistry* [online] 285 (41), 31277–84. available from <http://www.ncbi.nlm.nih.gov/pubmed/20675386> [4 November 2018]

Chung, S.S.M., Ho, E.C.M., Lam, K.S.L., and Chung, S.K. (2003) 'Contribution of Polyol Pathway to Diabetes-Induced Oxidative Stress'. *Journal of the American Society of Nephrology* [online] 14, 233–236. available from <http://www.jasn.org/cgi/doi/10.1097/01.ASN.0000077408.15865.06> [14 June 2018]

Clanton, T.L. (2007) 'Hypoxia-Induced Reactive Oxygen Species Formation in Skeletal Muscle'. *Journal of Applied Physiology* [online] 102 (6), 2379–2388. available from <http://www.physiology.org/doi/10.1152/japplphysiol.01298.2006> [22 January 2019]

Clément, M.-V. and Pervaiz, S. (2001) ‘Intracellular Superoxide and Hydrogen Peroxide Concentrations: A Critical Balance That Determines Survival or Death’. *Redox Report* [online] 6 (4), 211–214. available from <http://www.tandfonline.com/action/journalInformation?journalCode=yrer20> [26 August 2018]

Cline, S.D., Lodeiro, M.F., Marnett, L.J., Cameron, C.E., and Arnold, J.J. (2010) ‘Arrest of Human Mitochondrial RNA Polymerase Transcription by the Biological Aldehyde Adduct of DNA, M1dG.’ *Nucleic Acids Research* [online] 38 (21), 7546–57. available from <http://www.ncbi.nlm.nih.gov/pubmed/20671026> [19 August 2018]

Clissold, F.J., Clark, X., Savage, T., and Simpson, S.J. (2018) ‘A Rapid, Precise and Low-Cost Method to Quantify Silicon for the Determination of Food Intake and Utilisation for Insect Herbivores’. *Austral Entomology* [online] available from <http://doi.wiley.com/10.1111/aen.12335> [29 April 2018]

Cobley, J.N., Fiorello, M.L., and Bailey, D.M. (2018) ‘13 Reasons Why the Brain Is Susceptible to Oxidative Stress’. *Redox Biology* [online] 15, 490–503. available from <https://www.sciencedirect.com/science/article/pii/S2213231718300041> [19 August 2018]

Colica, C., Di Renzo, L., Trombetta, D., Smeriglio, A., Bernardini, S., Cioccoloni, G., Costa de Miranda, R., Gualtieri, P., Sinibaldi Salimei, P., and De Lorenzo, A. (2017) ‘Antioxidant Effects of a Hydroxytyrosol-Based Pharmaceutical Formulation on Body Composition, Metabolic State, and Gene Expression: A Randomized Double-Blinded, Placebo-Controlled Crossover Trial‘. *Oxidative Medicine and Cellular Longevity* [online] 2017. available from <https://www.hindawi.com/journals/omcl/2017/2473495/> [29 June 2019]

Comhair, S.A.A., Ricci, K.S., Arroliga, M., Lara, A.R., Dweik, R.A., Song, W., Hazen, S.L., Bleecker, E.R., Busse, W.W., Chung, K.F., Gaston, B., Hastie, A., Hew, M., Jarjour, N., Moore, W., Peters, S., Teague, W.G., Wenzel, S.E., Erzurum, S.C., Heart, for the S.A.R.P. of the N., Lung, and Institute†, and B. (2005) ‘Correlation of Systemic Superoxide Dismutase Deficiency to Airflow Obstruction in Asthma.’ *American Journal of Respiratory and Critical Care Medicine* [online] 172 (3), 306–13. available from <http://www.ncbi.nlm.nih.gov/pubmed/15883124> [17 January 2017]

Conlan, X.A., Stupka, N., McDermott, G.P., Barnett, N.W., and Francis, P.S. (2010) ‘Correlation between Acidic Potassium Permanganate Chemiluminescence and in Vitrocell Culture Assay: Physiologically Meaningful Antioxidant Activity’. *Analytical Methods* [online] 2 (2), 171–173. available from <http://xlink.rsc.org/?DOI=B9AY00242A> [6 May 2018]

Cooley, J.C. and Lunte, C.E. (2011) ‘Detection of Malondialdehyde in Vivo Using Microdialysis Sampling with CE-Fluorescence.’ *Electrophoresis* [online] 32 (21), 2994–9. available from <http://www.ncbi.nlm.nih.gov/pubmed/22034011> [16 June 2017]

Corradi, M., Pignatti, P., Manini, P., Andreoli, R., Goldoni, M., Poppa, M., Moscato, G., Balbi, B., Mutti, A., Corradi, P.M., Manini, R., Andreoli, M., Poppa, G., Moscato, B., and Balbi, A.M. (2004) ‘Comparison between Exhaled and Sputum Oxidative Stress Biomarkers in Chronic Airway Inflammation’. *Europe Respiratory Journal* [online] 24, 1011–1017. available from <https://pdfs.semanticscholar.org/ad15/0f9bf4f917593c5a396ef2312d15291d1ecb.pdf> [9 November 2017]

Coskun, O., Kanter, M., Korkmaz, A., and Oter, S. (2005) ‘Quercetin, a Flavonoid Antioxidant, Prevents and Protects Streptozotocin-Induced Oxidative Stress and β-Cell Damage in Rat Pancreas’. *Pharmacological Research* [online] 51 (2), 117–123. available from <https://www.sciencedirect.com/science/article/pii/S1043661804001434> [13 July 2018]

Cui, H., Huang, J., Lu, M., Zhang, Q., Qin, W., Zhao, Y., Lu, X., Zhang, J., Xi, Z., and Li, R. (2019) ‘Antagonistic effect of vitamin E on nAl2O3-induced exacerbation of Th2 and Th17-mediated allergic asthma via oxidative stress‘. *Environmental Pollution* [online]. Available from <https://www.sciencedirect.com/science/article/pii/S0269749119310309> [29 June 2019]

Cummins, N.W., and Badley, A.D. (2010) 'Mechanism of HIV-associated lymphocyte apoptosis: 2010.' *Cell Death & Disease* [online] 1 (11), 99. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3032328/>

Czajka, A., Ajaz, S., Gnudi, L., Parsade, C.K., Jones, P., Reid, F., and Malik, A.N. (2015) ‘Altered Mitochondrial Function, Mitochondrial DNA and Reduced Metabolic Flexibility in Patients With Diabetic Nephropathy’. *EBioMedicine* [online] 2 (6), 499–512. available from <http://linkinghub.elsevier.com/retrieve/pii/S2352396415000961> [4 September 2017]

David, J.A., Rifkin, W.J., Rabbani, P.S., and Ceradini, D.J. (2017) ‘The Nrf2/Keap1/ARE Pathway and Oxidative Stress as a Therapeutic Target in Type II Diabetes Mellitus.’ *Journal of Diabetes Research* [online] 2017, 4826724. available from <http://www.ncbi.nlm.nih.gov/pubmed/28913364> [21 June 2018]

Davidovich, N., DiPaolo, B.C., Lawrence, G.G., Chhour, P., Yehya, N., and Margulies, S.S. (2013) ‘Cyclic Stretch–Induced Oxidative Stress Increases Pulmonary Alveolar Epithelial Permeability’. *American Journal of Respiratory Cell and Molecular Biology* [online] 49 (1), 156–164. available from <http://www.atsjournals.org/doi/abs/10.1165/rcmb.2012-0252OC> [4 April 2018]

Davis, J.M., Parad, R.B., Michele, T., Allred, E., Price, A., Rosenfeld, W., and North American Recombinant Human CuZnSOD Study Group (2003) ‘Pulmonary Outcome at 1 Year Corrected Age in Premature Infants Treated at Birth with Recombinant Human CuZn Superoxide Dismutase.’ *Pediatrics* [online] 111 (3), 469–76. available from <http://www.ncbi.nlm.nih.gov/pubmed/12612223> [26 April 2018]

De la Lastra, C.A. and Villegas, I. (2007) ‘Resveratrol as an Antioxidant and Pro-Oxidant Agent: Mechanisms and Clinical Implications’. *Biochemical Society Transactions* [online] 35 (5), 1156–1160. available from <http://biochemsoctrans.org/lookup/doi/10.1042/BST0351156> [17 February 2019]

De Saedeleer, C.J., Copetti, T., Porporato, P.E., Verrax, J., Feron, O., and Sonveaux, P. (2012) ‘Lactate Activates HIF-1 in Oxidative but Not in Warburg-Phenotype Human Tumor Cells’. *PLoS One* [online] 7 (10). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3474765/> [16 June 2019]

Del Rey, M.J., Valin, A., Usategui, A., Garcia-Herrero, C.M., Sanchez-Arago, M., Cuezva, J.M., Galindo, M., Bravo, B., Canete, J.D., Blanco, F.J., Criado, G., and Pablos, J.L. (2017) ‘Hif-1α Knockdown Reduces Glycolytic Metabolism and Induces Cell Death of Human Synovial Fibroblasts Under Normoxic Conditions‘. *Scientific Reports* [online] 7. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5473902/> [16 June 2019]

Delibas, N., Ozcankaya, R., and Altuntas I. (2002) ‘Clinical importance of erythrocyte malondialdehyde levels as a marker for cognitive deterioration in patients with dementia of Alzheimer type: a repeated study in 5-year interval‘. *Clinical Biochemistry* [online] 35 (2), 137-41. available from <https://www.sciencedirect.com/science/article/pii/S0009912002002874?via%3Dihub> [21 June 2019]

Delmas, D., Solary, E., and Latruffe, N. (2011) 'Resveratrol, a Phytochemical Inducer of Multiple Cell Death Pathways: Apoptosis, Autophagy and Mitotic Catastrophe'. *Current Medicinal Chemistry* [online] 18 (8). available from <http://www.eurekaselect.com/73552/article>

Desagher, S., Glowinski, J., and Premont, J. (1996) ‘Astrocytes Protect Neurons from Hydrogen Peroxide Toxicity’. *The Journal of Neuroscience* 16 (8), 2553–2562

Desai, B.S., Monahan, A.J., Carvey, P.M., and Hendey, B. (2007) ‘Blood – Brain Barrier Pathology in Alzheimer ’ s and Parkinson ’ s Disease : Implications for Drug Therapy’. *Review Literature And Arts Of The Americas* 16 (32), 285–299

Devasagayam, T.P. a, Tilak, J.C., Boloor, K.K., Sane, K.S., Ghaskadbi, S.S., and Lele, R.D. (2004) ‘Free Radicals and Antioxidants in Human Health: Current Status and Future Prospects.’ *The Journal of the Association of Physicians of India* 52 (October), 794–804

Devi, L., Raghavendran, V., Prabhu, B.M., Avadhani, N.G., and Anandatheerthavarada, H.K. (2008) ‘Mitochondrial Import and Accumulation of Alpha-Synuclein Impair Complex I in Human Dopaminergic Neuronal Cultures and Parkinson Disease Brain.’ *The Journal of Biological Chemistry* [online] 283 (14), 9089–100. available from <http://www.ncbi.nlm.nih.gov/pubmed/18245082> [25 February 2018]

Dewitz, C., McEachern, E., Shin, S., Akong, K., Nagle, D.G., Broide, D.H., Akuthota, P., and Crotty Alexander, L.E. (2017) 'Hypoxia-Inducible Factor-1α Inhibition Modulates Airway Hyperresponsiveness and Nitric Oxide Levels in a BALB/c Mouse Model of Asthma'. *Clinical Immunology* [online] 176, 94–99. available from <http://linkinghub.elsevier.com/retrieve/pii/S152166161630537X> [8 September 2017]

Diabetes UK (2017) *Facts and Stats* [online] available from <https://www.diabetes.org.uk/about_us/news/diabetes-prevalence-statistics> [23 April 2016]

Dias, V., Junn, E., and Mouradian, M.M. (2013a) 'The Role of Oxidative Stress in Parkinson's Disease.' *Journal of Parkinson's disease* [online] 3 (4), 461–91. available from <http://www.ncbi.nlm.nih.gov/pubmed/24252804> [17 January 2017]

Dias, V., Junn, E., and Mouradian, M.M. (2013b) 'The Role of Oxidative Stress in Parkinson's Disease.' *Journal of Parkinson's disease* [online] 3 (4), 461–91. available from <http://www.ncbi.nlm.nih.gov/pubmed/24252804> [14 August 2017]

di Domenico, A., Carola, G., Calatayud, C., Pons=Espinal, M., Munoz, J.P., Richaud-Patin, Y., Fernandez-Carasa, I., Gut, M., Faella, A., Parameswaran, J., Soriano, J., Ferrer, I., Tolosa, E., Zorzano, A., Cuervo, A.M., Raya, A., and Consiglio, A. (2019) 'Patient-Specific iPSC-Derived Astrocytes Contribute to Non-Cell-Autonomous Neurodegeneration in Parkinson's Disease'. *Stem Cell Reports* [online] 12 (2), 213-229. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6372974/> [22 June 2019]

Domingo, J.L., Gomez, M., Sanchez, D.J., Llobet, J.M., and Keen, C.L. (1992) 'Tiron Administration Minimizes the Toxicity of Vanadate but Not Its Insulin Mimetic Properties in Diabetic Rats'. *Life Sciences* [online] 50 (18), 1311–1317. available from <http://linkinghub.elsevier.com/retrieve/pii/002432059290281S> [25 March 2018]

Domingo, J.L., de la Torre, A., Bellés, M., Mayayo, E., Llobet, J.M., and Corbella, J. (1997) 'Comparative Effects of the Chelators Sodium 4,5-Dihydroxybenzene-1,3-Disulfonate (Tiron) and Diethylenetriaminepentaacetic Acid (DTPA) on Acute Uranium Nephrotoxicity in Rats'. *Toxicology* [online] 118 (1), 49–59. available from <http://linkinghub.elsevier.com/retrieve/pii/S0300483X96035895> [4 September 2017]

Domingo, J.L., Sanchez, D.J., Gomez, M., Llobet, J.M., and Corbella, J. (1993) 'Oral Vanadate and Tiron in Treatment of Diabetes Mellitus in Rats: Improvement of Glucose Homeostasis and Negative Side-Effects.' *Veterinary and Human Toxicology* [online] 35 (6), 495–500. available from <http://www.ncbi.nlm.nih.gov/pubmed/8303815> [25 March 2018]

Dong, D., Yu, J., Wu, Y., Fu, N., Villela, N.A., and Yang, P. (2015) 'Maternal Diabetes Triggers DNA Damage and DNA Damage Response in Neurulation Stage Embryos through Oxidative Stress'. *Biochemical and Biophysical Research Communications* [online] 467 (2), 407–412. available from < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4618162/ > [5 September 2017]

Dong, K., Ni, H., Wu, M., Tang, Z., Halim, M., and Shi, D. (2016) 'ROS-Mediated Glucose Metabolic Reprogram Induces Insulin Resistance in Type 2 Diabetes'. *Biochemical and Biophysical Research Communications* [online] 476 (4), 204–211. available from <http://www.ncbi.nlm.nih.gov/pubmed/27207834> [7 October 2017]

Dong, Y., Chen, X., Liu, Y., Shu, Y., Chen, T., Xu, L., Li, M., and Guan, X. (2018) 'Do low-serum vitamin E levels increase the risk of Alzheimer disease in older people? Evidence from a meta-analysis of case-control studies'. *International Journal of Geriatric Psychiatry* [online] 33 (2), 257-63. available from <https://onlinelibrary.wiley.com/doi/abs/10.1002/gps.4780> [29June 2019]

Doughan, A.K., and Dikalov, S.I. (2007) ' Mitochondrial redox cycling of mitoquinone leads to superoxide production and cellular apoptosis'. Antioxidant Redox Signalling [online] 9 (11), 1825-36. available from <https://www.liebertpub.com/doi/abs/10.1089/ars.2007.1693> [22 June 2019]

Dossi, E., Vasile, F., and Rouach, N. (2018) 'Human Astrocytes in the Diseased Brain'. *Brain Research Bulletin* [online] 136, 139–156. available from <https://www.sciencedirect.com/science/article/pii/S0361923017300825#sec0070> [12 November 2018]

Drechsel, D.A. and Patel, M. (2008) 'Role of Reactive Oxygen Species in the Neurotoxicity of Environmental Agents Implicated in Parkinson's Disease'. *Free Radical Biology and Medicine* [online] 44 (11), 1873–1886. available from <https://www.sciencedirect.com/science/article/pii/S0891584908001032#bib20> [28 March 2018]

Du, L., Chen, J., and Xing, Y. (2017) 'Eupatilin Prevents H2O2-Induced Oxidative Stress and Apoptosis in Human Retinal Pigment Epithelial Cells'. *Biomedicine & Pharmacotherapy* 85, 136–140

Du, X.L., Edelstein, D., Rossetti, L., Fantus, I.G., Goldberg, H., Ziyadeh, F., Wu, J., and Brownlee, M. (2000) 'Hyperglycemia-Induced Mitochondrial Superoxide Overproduction Activates the Hexosamine Pathway and Induces Plasminogen Activator Inhibitor-1 Expression by Increasing Sp1 Glycosylation.' *Proceedings of the National Academy of Sciences of the United States of America* [online] 97 (22), 12222–6. available from <http://www.ncbi.nlm.nih.gov/pubmed/11050244> [21 April 2018]

Du, Y., Miller, C.M., and Kern, T.. (2003) 'Hyperglycemia Increases Mitochondrial Superoxide in Retina and Retinal Cells'. *Free Radical Biology and Medicine* [online] 35 (11), 1491–1499. available from <https://www.sciencedirect.com/science/article/pii/S0891584903005665?via%3Dihub> [25 March 2018]

Duan, L., Li, J., Ma, P., Yang, X., and Xu, S. (2017) 'Vitamin E Antagonizes Ozone-Induced Asthma Exacerbation in Balb/c Mice through the Nrf2 Pathway'. *Food and Chemical Toxicology* [online] 107, 47–56. available from <http://linkinghub.elsevier.com/retrieve/pii/S0278691517303332> [10 August 2017]

Dumont, M., Ho, D.J., Calingasan, N.Y., Xu, H., Gibson, G., and Beal, M.F. (2009) 'Mitochondrial Dihydrolipoyl Succinyltransferase Deficiency Accelerates Amyloid Pathology and Memory Deficit in a Transgenic Mouse Model of Amyloid Deposition'. *Free Radical Biology and Medicine* [online] 47 (7), 1019–1027. available from <http://linkinghub.elsevier.com/retrieve/pii/S0891584909004110> [22 August 2017]

Dündar, Y. and Aslan, R. (2000) 'Antioxidative Stress'. *Eastern Journal Of Medicine* [online] 5 (2), 45–47. available from <http://www.eastjmed.org/jvi.aspx?pdir=ejm&plng=eng&un=EJM-92819&look4=> [7 May 2018]

Dunér, P., Gonçalves, I., Grufman, H., Edsfeldt, A., To, F., Nitulescu, M., Nilsson, J., and Bengtsson, E. (2015) 'Increased Aldehyde-Modification of Collagen Type IV in Symptomatic Plaques – A Possible Cause of Endothelial Dysfunction'. *Atherosclerosis* [online] 240 (1), 26–32. available from <https://www.sciencedirect.com/science/article/pii/S0021915015001343> [17 July 2018]

Dworski, R. (2000) 'Oxidant Stress in Asthma.' *Thorax* [online] 55, S51-3. available from <http://www.ncbi.nlm.nih.gov/pubmed/10992559> [26 March 2018]

Dysken, M.W., Sano, M., Asthana, S., Vertrees, J.E., Pallaki, M., Llorente, M., Love, S., Schellenberg, G.D., McCarten, J.R., Malphurs, J., Prieto, S., Chen, P., Loreck, D.J., Trapp G., Bakshi, R.S., Mintzer, J.E., Heidebrink, J.L., Vidal-Cardona, A., Arroyo, L.M., Cruz, AR14, Zachariah, S., Kowall, N.W., Chopra, M.P., Craft, S., Thielke, S., Turvey, C.L., Woodman, C17, Monnell, K.A., Gordon, K., Tomaska, J., Segal, Y., Peduzzi, P.N., and Guarino, P.D. (2014) 'Effect of vitamin E and memantine on functional decline in Alzheimer disease: the TEAM-AD VA cooperative randomized trial'. *JAMA* [online] 311)1), 33-44. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4109898/> [29 June 2019]

Ebrahimi, A. and Moghaddam, A.H. (2017) 'The Effect of Olive Leaf Methanolic Extract on Hippocampal Antioxidant Biomarkers in an Animal Model of Parkinson's Disease'. *Journal of Basic & Clinical Pathophysiology* [online] 5 (2), 9–14. available from <http://jbcp.shahed.ac.ir/article_538.html> [19 August 2018]

Van Eck, M.,Hoekstra, M., Hildebrand, R.B., Yaong, Y., Stengel, D., Kruijt, J.K., Sattler, W., Tietge, U.J., Ninio, E., Van Berkel, T.J., and Pratico, D. (2007) ' '. Arteriosclerotic, Thrombosis, and Vascular Biology [online] 27 (11), 2413-9. available from <https://www.ncbi.nlm.nih.gov/pubmed/17717299> [22 June 2019]

El-Sherbeeny, N.A., Hassan, Z.A., and Ateyya, H. (2016) 'Tiron Ameliorates Oxidative Stress and Inflammation in a Murine Model of Airway Remodeling'. *International Immunopharmacology* 39, 172–180

El-Wassef, M., El-Saeed, G.S.M., El-Tokhy, S.E., Raslan, H.M., Tawfeek, S., Siam, I., and Salem, S.. (2012) 'Oxidative DNA Damage in Patients with Type 2 Diabetes Mellitus'. *Diabetologia Croatica* [online] 41 (4), 121–127. available from <http://www.idb.hr/diabetologia/12no4-2.pdf> [6 May 2018]

Elliott, H.R., Samuels, D.C., Eden, J.A., Relton, C.L., and Chinnery, P.F. (2008) 'Pathogenic Mitochondrial DNA Mutations Are Common in the General Population'. *The American Journal of Human Genetics* [online] 83 (2), 254-260. available from <https://www.sciencedirect.com/science/article/pii/S0002929708004023> [11 June 2019]

Ellwanger, J.H., Molz, P., Dallemole, D.R., Pereira dos Santos, A., Müller, T.E., Cappelletti, L., Gonçalves da Silva, M., Rech Franke, S.I., Prá, D., and Pêgas Henriques, J.A. (2015) 'Selenium Reduces Bradykinesia and DNA Damage in a Rat Model of Parkinson's Disease'. *Nutrition* [online] 31 (2), 359–365. available from <http://linkinghub.elsevier.com/retrieve/pii/S0899900714003438> [20 August 2017]

Elmasry, S.A., Al-Azzawi, M.A., Ghoneim, A.D., Nasr, M.Y., and AboZaid, M.M.. (2015) 'Role of Oxidant–antioxidant Imbalance in the Pathogenesis of Chronic Obstructive Pulmonary Disease'. *Egyptian Journal of Chest Diseases and Tuberculosis* [online] 64 (4), 813–820. available from <http://www.sciencedirect.com/science/article/pii/S0422763815300534> [9 November 2017]

Eltzschig, H.K. and Carmeliet, P. (2011) 'Hypoxia and Inflammation.' *The New England Journal of Medicine* [online] 364 (7), 656–65. available from <http://www.ncbi.nlm.nih.gov/pubmed/21323543> [20 May 2017]

Enomoto, N., Koshikawa, N., Gassmann, M., Hayashi, J.-I., and Takenaga, K. (2002) 'Hypoxic Induction of Hypoxia-Inducible Factor-1α and Oxygen-Regulated Gene Expression in Mitochondrial DNA-Depleted HeLa Cells'. *Biochemical and Biophysical Research Communications* [online] 297 (2), 346–352. available from <https://www.sciencedirect.com/science/article/pii/S0006291X02021861> [21 April 2018]

Erlank, H., Elmann, A., Kohen, R., and Kanner, J. (2011) 'Polyphenols Activate Nrf2 in Astrocytes via H2O2, Semiquinones, and Quinones'. *Free Radical Biology and Medicine* [online] 51 (12), 2319–2327. available from <https://www.sciencedirect.com/science/article/pii/S0891584911006083?via%3Dihub> [24 February 2018]

Erle, D.J. and Sheppard, D. (2014) 'The Cell Biology of Asthma.' *The Journal of cell biology* [online] 205 (5), 621–31. available from <http://www.ncbi.nlm.nih.gov/pubmed/24914235> [8 April 2018]

Eruslanov, E. and Kusmartsev, S. (2010) 'Identification of ROS Using Oxidized DCFDA and Flow-Cytometry.' *Methods in Molecular Biology* [online] 594, 57–72. available from <http://www.ncbi.nlm.nih.gov/pubmed/20072909> [28 September 2016]

Escribano-Lopez, I., Diaz-Morales, N., Rovira-Llopis, S., Martinez de Maranon, A., Orden, S., Alvarez, A., Banuls, C., Rocha, M., Murphy, M.P., and Hernandez-Mijares, A. (2016) 'The mitochondria-targeted antioxidant MitoQ modulates oxidative stress, inflammation and leukocyte-endothelium interactions in leukocytes isolated from type 2 diabetic patients'. *Redox Biology* [online] 10, 200-05. available from <https://www.sciencedirect.com/science/article/pii/S2213231716301641> [21 June 2019]

Esterbauer, H. (1993) 'Cytotoxicity and Genotoxicity of Lipid-Oxidation Products'. *The American Journal of Clinical Nutrition* [online] 57 (5), 779–786. available from <https://academic.oup.com/ajcn/article/57/5/779S/4715981> [25 April 2018]

Eugenín, J., Vecchiola, A., Murgas, P., Arroyo, P., Cornejo, F., and von Bernhardi, R. (2016) 'Expression Pattern of Scavenger Receptors and Amyloid-β Phagocytosis of Astrocytes and Microglia in Culture Are Modified by Acidosis: Implications for Alzheimer's Disease'. *Journal of Alzheimer's Disease* [online] 53 (3), 857–873. available from <http://www.medra.org/servlet/aliasResolver?alias=iospress&doi=10.3233/JAD-160083> [8 January 2018]

Evans, J.L., Goldfine, I.D., Maddux, B.A., and Grodsky, G.M. (2003) 'Are Oxidative Stress-Activated Signaling Pathways Mediators of Insulin Resistance and Beta-Cell Dysfunction?' *Diabetes* [online] 52 (1), 1–8. available from <http://www.ncbi.nlm.nih.gov/pubmed/12502486> [7 January 2018]

Fader, C.M., Aguilera, M.O., and Colombo, M.I. (2012) ' ATP is released from autophagic vesicles to the extracellular space in a VAMP7-dependent manner.' *Autophagy* [online] 8 (12), 1741-56. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3541285/>

Fakhruddin, S., Alanazi, W., and Jackson, K.E. (2017) 'Diabetes-Induced Reactive Oxygen Species: Mechanism of Their Generation and Role in Renal Injury'. *Journal of Diabetes Research* [online] 2017, 1–30. available from <https://www.hindawi.com/journals/jdr/2017/8379327/> [24 September 2017]

Fan, L.M., Douglas, G., Bendall, J.K., McNeill, E., Crabtree, M.J., Hale, A.B., Li, J.M., McAteer, M.A., Schneider, J.E., Choudhury, R.P., and Channon, K.M. (2014) 'Endothelial Cell-Specific Reactive Oxygen Species Production Increases Susceptibility to Aortic Dissection'. *Circulation* 129 (25), 2661–2672

Fang, Y., Hu, X.-H., Jia, Z.-G., Xu, M.-H., Guo, Z.-Y., and Gao, F.-H. (2012) 'Tiron Protects against UVB-Induced Senescence-like Characteristics in Human Dermal Fibroblasts by the Inhibition of Superoxide Anion Production and Glutathione Depletion'. *Australasian Journal of Dermatology* [online] 53 (3), 172–180. available from <http://www.ncbi.nlm.nih.gov/pubmed/22734867> [24 August 2017]

de Farias, C.C., Maes, M., Bonifácio, K.L., Bortolasci, C.C., de Souza Nogueira, A., Brinholi, F.F., Matsumoto, A.K., do Nascimento, M.A., de Melo, L.B., Nixdorf, S.L., Lavado, E.L., Moreira, E.G., and Barbosa, D.S. (2016) 'Highly Specific Changes in Antioxidant Levels and Lipid Peroxidation in Parkinson's Disease and Its Progression: Disease and Staging Biomarkers and New Drug Targets'. *Neuroscience Letters* [online] 617, 66–71. available from <http://linkinghub.elsevier.com/retrieve/pii/S0304394016300787> [24 August 2017]

Farina, N., Llewellyn, D., Mgekn, I., and Tabet, N. (2017) 'Vitamin E for Alzheimer's dementia and mild cognitive impairment'. *Cochrane Database Systematic Reviews* [online] 2017 (4). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6478142/> [29 June 2019]

Farrukh, M.R., Nissar, U. a., Afnan, Q., Rafiq, R. a., Sharma, L., Amin, S., Kaiser, P., Sharma, P.R., and Tasduq, S. a. (2014) 'Oxidative Stress Mediated Ca2+ Release Manifests Endoplasmic Reticulum Stress Leading to Unfolded Protein Response in UV-B Irradiated Human Skin Cells'. *Journal of Dermatological Science* [online] 75 (1), 24–35. available from <http://dx.doi.org/10.1016/j.jdermsci.2014.03.005>

Fauconneau, B., Petegnief, V., Sanfeliu, C., Piriou, A., and Planas, A.M. (2002) 'Induction of Heat Shock Proteins (HSPs) by Sodium Arsenite in Cultured Astrocytes and Reduction of Hydrogen Peroxide-Induced Cell Death'. *Journal of Neurochemistry* [online] 83 (6), 1338–1348. available from <http://doi.wiley.com/10.1046/j.1471-4159.2002.01230.x> [22 January 2018]

Favaloro, B., Allocati, N., Graziano, V., Di Ilio, C., and De Laurenzi, V. (2012) 'Role of Apoptosis in disease.' *Aging* [online] 4 (5), 330-49. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3384434/> [29 September 2014]

Figueiredo, P.A., Powers, S.K., Ferreira, R.M., Appell, H.J., and Duarte, J.A. (2009) 'Aging Impairs Skeletal Muscle Mitochondrial Bioenergetic Function'. *The Journals of Gerontology Series A: Biologial Sciences and Medical Sciences* [online] 64A (1), 21-33. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2691197/> [15 June 2019]

Fiore, G., and Capasso, A. (2008) 'Effects of Vitamin E and C on Placental Oxidative Stress: An In Vitro Evidence for the Potential Therapeutic or Prophylactic Treatment of Preeclampsia'. *Medicinal Chemistry* [online] 4 (6), 526-30. available from <http://www.eurekaselect.com/83385/article> [29 June 2019]

Fitzpatrick, A.M., Park, Y., Brown, L.A.S., and Jones, D.P. (2014) 'Children with Severe Asthma Have Unique Oxidative Stress-Associated Metabolomic Profiles.' *The Journal of Allergy and Clinical Immunology* 133 (1), 258-61.e1–8

Flynn, J.M. and Melov, S. (2013) 'SOD2 in Mitochondrial Dysfunction and Neurodegeneration.' *Free radical biology & medicine* [online] 62, 4–12. available from <http://www.ncbi.nlm.nih.gov/pubmed/23727323> [23 July 2016]

Forbes, J.M., Coughlan, M.T., and Cooper, M.E. (2008) 'Oxidative Stress as a Major Culprit in Kidney Disease in Diabetes.' *Diabetes* [online] 57 (6), 1446–54. available from <http://www.ncbi.nlm.nih.gov/pubmed/18511445> [6 December 2015]

Forkink, M., Basit, F., Teixeira, J., Swarts, H.G., Koopman, W.J.H., and Willems, P.H.G.M. (2015) 'Complex I and Complex III Inhibition Specifically Increase Cytosolic Hydrogen Peroxide Levels without Inducing Oxidative Stress in HEK293 Cells'. *Redox Biology* [online] 6, 607–616. available from <https://www.sciencedirect.com/science/article/pii/S2213231715001147> [14 June 2018]

Fouda, M.A. and Abdel-Rahman, A.A. (2017) 'Endothelin Confers Protection against High Glucose-Induced Neurotoxicity via Alleviation of Oxidative Stress.' *The Journal of Pharmacology and Experimental Therapeutics* [online] 361 (1), 130–139. available from <http://www.ncbi.nlm.nih.gov/pubmed/28179472> [20 June 2018]

Fowler, M.J. (2011) 'Microvascular and Macrovascular Complications of Diabetes'. *Clinical Diabetes* [online] 29 (3), 116–122. available from <http://clinical.diabetesjournals.org/cgi/doi/10.2337/diaclin.29.3.116> [7 January 2018]

Frade, J., Pope, S., Schmidt, M., Dringen, R., Barbosa, R., Pocock, J., Laranjinha, J., and Heales, S. (2008) 'Glutamate induces release of glutathione from cultured rat astrocytes – a possible neuroprotective mechanism?'. *Journal of Neurochemistry* [online] 105, 1144-52. available from <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1471-4159.2008.05216.x> [22 June 2019]

Fu, S., Lv, R., Wang, L., Hou, H., Liu, H., and Shao, S. (2018) 'Resveratrol, an antioxidant, protects spinal cord injury in rats by suppressing MAPK pathway'. *Saudi Journal of Biological Sciences* [online] 25 (2), 259-66. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5815991/> [22 June 2019]

Fuchs-Tarlovsky, V. (2013) 'Role of Antioxidants in Cancer Therapy'. *Nutrition* [online] 29 (1), 15–21. available from <http://dx.doi.org/10.1016/j.nut.2012.02.014>

Fujii, Y., Tomita, K., Sano, H., Yamasaki, A., Hitsuda, Y., Adcock, I.M., and Shimizu, E. (2002) 'Dissociation of DNA Damage and Mitochondrial Injury Caused by Hydrogen Peroxide in SV-40 Transformed Lung Epithelial Cells.' *Cancer cell international* [online] 2 (1), 16. available from <http://www.ncbi.nlm.nih.gov/pubmed/12495439> [1 August 2017]

Fukai, T. and Ushio-Fukai, M. (2011) 'Superoxide Dismutases: Role in Redox Signaling, Vascular Function, and Diseases.' *Antioxidants & Redox Signaling* [online] 15 (6), 1583–606. available from <http://www.ncbi.nlm.nih.gov/pubmed/21473702> [25 April 2017]

Fukui, K., Nakamura, K., Shirai, M., Hirano, A., Takatsu, H., and Urano, S. (2015) 'Long-Term Vitamin E-Deficient Mice Exhibit Cognitive Dysfunction via Elevation of Brain Oxidation'. *Journal of Nutritional Science and Vitaminology* [online] 61 (5), 362-8. available from <https://www.jstage.jst.go.jp/article/jnsv/61/5/61_362/_article> [29 June 2019]

Gallego-Villar, L., Pérez, B., Ugarte, M., Desviat, L.R., and Richard, E. (2014) 'Antioxidants Successfully Reduce ROS Production in Propionic Acidemia Fibroblasts'. *Biochemical and Biophysical Research Communications* [online] 452 (3), 457–461. available from <http://linkinghub.elsevier.com/retrieve/pii/S0006291X14015149>

Gan, L. and Johnson, J.A. (2014) 'Oxidative Damage and the Nrf2-ARE Pathway in Neurodegenerative Diseases'. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* [online] 1842 (8), 1208–1218. available from <https://www.sciencedirect.com/science/article/pii/S0925443913003669#bb0295> [24 February 2018]

Ganie, S., Haq, E., Hamid, A., Masood, A., Zargar, M., Author, C., and Afzal Zargar, M. (2011) 'Long Dose Exposure of Hydrogen Peroxide (H_2O_2) in Albino Rats and Effect of Podophyllum Hexandrum on Oxidative Stress'. *European Review for Medical and Pharmacological Sciences* [online] 15 (8), 906–15. available from <http://www.europeanreview.org/wp/wp-content/uploads/1013.pdf> [11 February 2018]

Gao, J.J., Xu, K.H., Tang, B., Yin, L.L., Yang, G.W., and An, L.G. (2007) 'Selective Detection of Superoxide Anion Radicals Generated from Macrophages by Using a Novel Fluorescent Probe'. *FEBS Journal* [online] 274 (7), 1725–1733. available from <http://doi.wiley.com/10.1111/j.1742-4658.2007.05720.x> [7 April 2018]

Garcia-Contreras, C., Vazquez-Gomez, M., Barbero, A., Pesantez, J.L., Zinellu, A., Berlinguer, F., Gonzalez-Anover, P., Gonzalez, J., Ecinas, T., Torres-Rovira, L., Nunez, Y., Ballesteros, J., Ayuso, M., Astiz, S., Isabel, B., Ovilo, C., and Gonzalez-Bulnes, A. (2019) 'Polyphenols and IUGR Pregnancies: Effects of Maternal Hydroxytyrosol Supplementation on Placental Gene Expression and Fetal Antioxidant Status, DNA-Methylation and Phenotype'. *International Journal of Molecular Science* [online] 20 (5), 1187. available from <https://www.mdpi.com/1422-0067/20/5/1187> [29 June 2019]

Garcia-Larsen, V., Amigo, H., Bustos, P., Bakolis, I., Rona, R., Garcia-Larsen, V., Amigo, H., Bustos, P., Bakolis, I., and Rona, R.J. (2015) 'Ventilatory Function in Young Adults and Dietary Antioxidant Intake'. *Nutrients* [online] 7 (4), 2879–2896. available from <http://www.mdpi.com/2072-6643/7/4/2879> [8 November 2018]

García-Redondo, A.B., Briones, A.M., Beltrán, A.E., Alonso, M.J., Simonsen, U., and Salaices, M. (2009) 'Hypertension Increases Contractile Responses to Hydrogen Peroxide in Resistance Arteries through Increased Thromboxane A2, Ca2+, and Superoxide Anion Levels.' *The Journal of Pharmacology and Experimental Therapeutics* [online] 328 (1), 19–27. available from <http://www.ncbi.nlm.nih.gov/pubmed/10734155> [14 December 2018]

Gebert, N., Joshi, A.S., Kutik, S., Becker, T., McKenzie, M., Guan, X.L., Mooga, V.P., Stroud, D.A., Kulkarni, G., Wenk, M.R., Rehling, P., Meisinger, C., Ryan, M.T., Wiedemann, N., Greenberg, M.L., and Pfanner, N. (2009) 'Mitochondrial Cardiolipin Involved in Outer-Membrane Protein Biogenesis: Implications for Barth Syndrome'. *Current Biology* [online] 19 (2), 2133-2139. available from <https://www.sciencedirect.com/science/article/pii/S0960982209019824> [12 June 2019]

Genius, J., Geiger, J., Bender, A., Möller, H.-J., Klopstock, T., and Rujescu, D. (2012) 'Creatine Protects against Excitoxicity in an In Vitro Model of Neurodegeneration'. *PLOS ONE* [online] 7 (2), e30554. available from <https://dx.plos.org/10.1371/journal.pone.0030554> [5 December 2018]

Geraldes, P. and King, G.L. (2010) 'Activation of Protein Kinase C Isoforms and Its Impact on Diabetic Complications.' *Circulation Research* [online] 106 (8), 1319–31. available from <http://www.ncbi.nlm.nih.gov/pubmed/20431074> [21 April 2018]

Gheith, O., Farouk, N., Nampoory, N., Halim, M.A., and Al-Otaibi, T. (2016) 'Diabetic Kidney Disease: World Wide Difference of Prevalence and Risk Factors.' *Journal of Nephropharmacology* [online] 5 (1), 49–56. available from <http://www.ncbi.nlm.nih.gov/pubmed/28197499> [24 September 2017]

Gholnari, T., Aghadavod, E., Soleimani, A., Hamidi, G.A., Sharifi, N., and Asemi, Z. (2018) 'The Effects of Coenzyme Q10 Supplementation on Glucose Metabolism, Lipid Profiles, Inflammation, and Oxidative Stress in Patients With Diabetic Nephropathy: A Randomized, Double-Blind, Placebo-Controlled Trial' *Journal of the American College of Nutrition* [online] 37 (3), 188-193. available from <https://www.tandfonline.com/doi/abs/10.1080/07315724.2017.1386140> [15 June 2019]

Giacco, F. and Brownlee, M. (2010) 'Oxidative Stress and Diabetic Complications'. *Circulation Research* [online] 107 (9). available from <http://www.ncbi.nlm.nih.gov/pubmed/21030723> [22 June 2016]

Giannini, C., Lombardo, F., Currò, F., Pomilio, M., Bucciarelli, T., Chiarelli, F., and Mohn, A. (2007) 'Effects of High-Dose Vitamin E Supplementation on Oxidative Stress and Microalbuminuria in Young Adult Patients with Childhood Onset Type 1 Diabetes Mellitus'. *Diabetes/Metabolism Research and Reviews* [online] 23 (7), 539–546. available from <http://doi.wiley.com/10.1002/dmrr.717> [30 November 2018]

Gilkerson, R., Bravo, L., Garcia, I., Gaytan, N., Herrera, A., Maldonado, A., and Quintanilla, B. (2013) 'The Mitochondrial Nucleoid: Integrating Mitochondrial DNA into Cellular Homeostasis.' *Cold Spring Harbor Perspectives in Biology* [online] 5 (5), a011080. available from <http://www.ncbi.nlm.nih.gov/pubmed/23637282> [23 January 2017]

Gilkerson, R.W. and Schon, E.A. (2008) 'Nucleoid Autonomy: An Underlying Mechanism of Mitochondrial Genetics with Therapeutic Potential.' *Communicative & Integrative Biology* 1 (1), 34–36

Godic, A., Poljšak, B., Adamic, M., and Dahmane, R. (2014) 'The Role of Antioxidants in Skin Cancer Prevention and Treatment.' *Oxidative Medicine and Cellular Longevity* [online] 2014, 860479. available from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3984781&tool=pmcentrez&rendertype=abstract>

Gonzalez-Hunt, C.P., Rooney, J.P., Ryde, I.T., Anbalagan, C., Joglekar, R., and Meyer, J.N. (2016) 'PCR-Based Analysis of Mitochondrial DNA Copy Number, Mitochondrial DNA Damage, and Nuclear DNA Damage.' *Current Protocols in Toxicology* [online] 67, 20.11.1-20.11.25. available from <http://www.ncbi.nlm.nih.gov/pubmed/26828332> [19 June 2018]

González-Montero, J., Brito, R., Gajardo, A. I., and Rodrigo, R. (2018). 'Myocardial reperfusion injury and oxidative stress: Therapeutic opportunities'. *World Journal of Cardiology* [online] 10(9), 74-86. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6189069/> [9 March 2019]

González, R., Ballester, I., López-Posadas, R., Suárez, M.D., Zarzuelo, A., Martínez-Augustin, O., and Medina, F.S. De (2011) 'Effects of Flavonoids and Other Polyphenols on Inflammation'. *Critical Reviews in Food Science and Nutrition* [online] 51 (4), 331–362. available from <http://www.tandfonline.com/doi/abs/10.1080/10408390903584094> [30 January 2017]

Good, P.F., Werner, P., Hsu, A., Olanow, C.W., and Perl, D.P. (1996) 'Evidence of Neuronal Oxidative Damage in Alzheimer's Disease.' *The American Journal of Pathology* [online] 149 (1), 21–8. available from <http://www.ncbi.nlm.nih.gov/pubmed/8686745> [27 March 2018]

Gorman, G.S., Chinnery, P.F., DiMauro, S., Hirano, M., Koga, Y., McFarland, R., Suomalainen, A., Thorburn, D.R., Zeviani, M., and Turnbull, D.M. (2016) 'Mitochondrial Diseases'. *Nature Reviews Disease Primers* [online] 2. available from <https://www.nature.com/articles/nrdp201680> [15 June 2019]

Gottwald, E.M., Duss, M., Bugarski, M., Haenni, D., Schuh, C.D., Landau, E.M., and Hall, A.M. (2018) 'The Targeted Anti-Oxidant MitoQ Causes Mitochondrial Swelling and Depolarization in Kidney Tissue.' *Physiological Reports* [online] 6 (7), e13667. available from <http://www.ncbi.nlm.nih.gov/pubmed/29611340> [19 December 2018]

Gough, D.R. and Cotter, T.G. (2011) 'Hydrogen Peroxide: A Jekyll and Hyde Signalling Molecule'. *Cell Death & Disease* [online] 2 (10), e213–e213. available from <http://www.nature.com/articles/cddis201196> [26 August 2018]

Gowdy, K.M., Krantz, Q.T., King, C., Boykin, E., Jaspers, I., Linak, W.P., and Gilmour, M.I. (2010) 'Role of Oxidative Stress on Diesel-Enhanced Influenza Infection in Mice.' *Particle and Fibre Toxicology* [online] 7, 34. available from <http://www.ncbi.nlm.nih.gov/pubmed/21092162> [10 August 2017]

Grady, J.P., Pickett, S.J., Ng, Y.S., Alston, C.L., Blakely, E.L., Hardy, S.A., Feeney, C.L., Bright, A.A., Schaefer, A.M., Gorman, G.S., McNally, R.J.Q., Taylor, R.W., Turnbull, D.M., and McFarland, R. (2018) 'mtDNA heteroplasmy level and copy number indicate disease burden in m.3243A>G mitochondrial disease'. *EMBO Molecular Medicine* [online] 10 (6). available from <http://embomolmed.embopress.org/content/10/6/e8262#sec-15> [15 June 2019]

Gross, J.L., De Azevedo, M.J., Silveiro, S.P., Canani, H., Caramori, M.L., and Zelmanovitz, T. (2005) 'Diabetic Nephropathy: Diagnosis, Prevention, and Treatment'. *Diabetes Care* [online] 28, 176–188. available from <http://care.diabetesjournals.org/content/28/1/164.full-text.pdf> [7 January 2018]

Grunewald, A., Rygiel, K.A., Hepplewhite, P.D., Morris, C.M., Picard, M., and Turnbull, D.M. (2016) 'Mitochondrial DNA Depletion in Respiratory Chain–Deficient Parkinson Disease Neurons'. *Annals of Neurobiology* [online] 79, 366–378. available from <https://pdfs.semanticscholar.org/04a7/bdfcca20d2dac93c8c9f5f567f3852e273f2.pdf> [21 August 2017]

Gu, J., Cheng, Y., Wu, H., Kong, L., Wang, S., Xu, Z., Zhang, Z., Tan, Y., Keller, B.B., Zhou, H., Wang, Y., Xu, Z., and Cai, L. (2017) ' Metallothionein Is Downstream of Nrf2 and Partially Mediates Sulforaphane Prevention of Diabetic Cardiomyopathy.' *Diabetes* [online] 66 (2), 529-42. available from <http://diabetes.diabetesjournals.org/content/66/2/529> [02 January 2019]

Gu, X.-L., Long, C.-X., Sun, L., Xie, C., Lin, X., and Cai, H. (2010) 'Astrocytic Expression of Parkinson's Disease-Related A53T Alpha-Synuclein Causes Neurodegeneration in Mice.' *Molecular Brain* [online] 3, 12. available from <http://www.ncbi.nlm.nih.gov/pubmed/20409326> [7 August 2017]

Gubandru, M., Margina, D., Tsitsimpikou, C., Goutzourelas, N., Tsarouhas, K., Ilie, M., Tsatsakis, A.M., and Kouretas, D. (2013) 'Alzheimer's Disease Treated Patients Showed Different Patterns for Oxidative Stress and Inflammation Markers'. *Food and Chemical Toxicology* [online] 61, 209–214. available from < https://www.sciencedirect.com/science/article/pii/S0278691513004687?via%3Dihub> [27 March 2018]

Guimaraes, E.L., Empsen, C., Geerts, A., and van Grunsven, L.A. (2010) 'Advanced glycation end products induce production of reactive oxygen species via the activation of NADPH oxidase in murine hepatic stellate cells'. *Journal of Hepatology* [online] 52 (3), 389-97. available from <https://www.ncbi.nlm.nih.gov/pubmed/20133001/> [26 June 2019]

Guo, S., Yao, Q., Ke, Z., Chen, H., Wu, J., and Liu, C. (2015) 'Resveratrol Attenuates High Glucose-Induced Oxidative Stress and Cardiomyocyte Apoptosis through AMPK'. *Molecular and Cellular Endocrinology* [online] 412, 85–94. available from <https://www.sciencedirect.com/science/article/pii/S0303720715003056> [14 October 2018]

Gupta, A., Bhatt, M.L.B., and Misra, M.K. (2009) 'Lipid Peroxidation and Antioxidant Status in Head and Neck Squamous Cell Carcinoma Patients'. *Oxidative Medicine and Cellular Longevity* 2 (2), 68–72

Gupta, P., Jain, V., Pareek, A., Kumari, P., Singh, R., Agarwal, R., and Sharma, V. (2017) 'Evaluation of Effect of Alcoholic Extract of Heartwood of Pterocarpus Marsupium on in Vitro Antioxidant, Anti-Glycation, Sorbitol Accumulation and Inhibition of Aldose Reductase Activity'. *Journal of Traditional and Complementary Medicine* [online] 7 (3), 307–314. available from <https://www.sciencedirect.com/science/article/pii/S2225411016302814>

Gupta, R.K., Patel, A.K., Shah, N., Chaudhary, A.K., Jha, U.K., Yadav, U.C., Gupta, P.K., and Pakuwal, U. (2014) 'Oxidative Stress and Antioxidants in Disease and Cancer: A Review.' *Asian Pacific Journal of Cancer Prevention : APJCP* [online] 15 (11), 4405–9. available from <http://www.ncbi.nlm.nih.gov/pubmed/24969860>

Gupta, S., Sharma, T.K., Kaushik, G.G., and Shekhawat, V.P.S. (2011) 'Vitamin E Supplementation May Ameliorate Oxidative Stress in Type 1 Diabetes Mellitus Patients.' *Clinical Laboratory* [online] 57 (5–6), 379–86. available from <http://www.ncbi.nlm.nih.gov/pubmed/21755829> [13 July 2018]

Gupta, S.C., Hevia, D., Patchva, S., Park, B., Koh, W., and Aggarwal, B.B. (2012) 'Upsides and Downsides of Reactive Oxygen Species for Cancer: The Roles of Reactive Oxygen Species in Tumorigenesis, Prevention, and Therapy.' *Antioxidants & Redox Signaling* [online] 16 (11), 1295–322. available from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3324815&tool=pmcentrez&rendertype=abstract> [6 May 2015]

Guzy, R.D., Hoyos, B., Robin, E., Chen, H., Liu, L., Mansfield, K.D., Simon, M.C., Hammerling, U., and Schumacker, P.T. (2005) 'Mitochondrial Complex III Is Required for Hypoxia-Induced ROS Production and Cellular Oxygen Sensing'. *Cell Metabolism* [online] 1 (6), 401–408. available from <https://www.sciencedirect.com/science/article/pii/S1550413105001397> [16 October 2018]

Hadi, N.R., Abdelhussein, M.A., Rudha, A.R.M., Jamil, D.A., and Hal-Aubaidy, H.A. (2015) 'Simvastatin Use in Patients with Type 2 Diabetes Mellitus: The Effects on Oxidative Stress' *Oman Medical Journal* [online] 30 (4), 237-240. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4561641/> [15 June 2019]

Hajjar, I., Hayek, S.S., Goldstein, F.C., Martin, G., Jones, D.P., and Quyyumi, A. (2018) 'Oxidative stress predicts cognitive decline with aging in healthy adults: an observational study'. *Journal of Neuroinflammation* [online] 15 (17). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5771063/> [21 June 2019]

Hakim, F. and Pflueger, A. (2010) 'Role of Oxidative Stress in Diabetic Kidney Disease.' *Medical Science Monitor : International Medical Journal of Experimental and Clinical Research* 16 (2)

Halliday, G.M., and Stevens, C.H. (2011) 'Glia: initiators and progressors of pathology in Parkinson's disease'. *Movement disorders: official journal of the Movement Disorder Society* [online] 26 (1), 6-17. available from <https://onlinelibrary.wiley.com/doi/abs/10.1002/mds.23455> [22 June 2019]

Halliwell, B. (2013) ‘The Antioxidant Paradox: Less Paradoxical Now?’ *British Journal of Clinical Pharmacology* [online] 75 (3), 637–44. available from <http://www.ncbi.nlm.nih.gov/pubmed/22420826> [23 October 2016]

Halliwell, B. (2014) ‘Cell Culture, Oxidative Stress, and Antioxidants: Avoiding Pitfalls.’ *Biomedical Journal* [online] 37 (3), 99-105. available from <http://biomedj.cgu.edu.tw/pdfs/2014/37/3/images/BiomedJ_2014_37_3_99_128725.pdf> [30 May 2019]

Hammes, H.P., Du, X., Eldestein, D., Taguchi, T., Matsumura, T., Ju, Q., Lin, J., Bierhaus, A., Nawroth, P.,Hannak,D., Neumaier, M., Bergfeld, R., Giardino,I., and Brownlee, M. (2003) ‘Benfotiamine blocks three major pathways of hyperglycemic damage and prevents experimental diabetic retinopathy’. *Nature Medicine* [online] 9 (3), 294-99. available from <https://www.nature.com/articles/nm834> [24 June 2019]

Han, H.J., Lee, Y.J., Park, S.H., Lee, J.H., and Taub, M. (2005) ‘High Glucose-Induced Oxidative Stress Inhibits Na+/Glucose Cotransporter Activity in Renal Proximal Tubule Cells’. *American Journal of Physiology - Renal Physiology* 288 (5), 988–996

Han, Y.H. and Park, W.H. (2009a) ‘Tiron, a ROS Scavenger, Protects Human Lung Cancer Calu-6 Cells against Antimycin A-Induced Cell Death.’ *Oncology Reports* [online] 21 (1), 253–61. available from <http://www.ncbi.nlm.nih.gov/pubmed/19082470> [8 May 2017]

Han, Y.H. and Park, W.H. (2009b) ‘Tiron, a ROS Scavenger, Protects Human Lung Cancer Calu-6 Cells against Antimycin A-Induced Cell Death.’ *Oncology Reports* [online] 21 (1), 253–61. available from <http://www.ncbi.nlm.nih.gov/pubmed/19082470> [9 October 2018]

Hanna, R., Crowther, J.M., Bulsara, P.A., Wang, X., Moore, D.J., and Birch-Machin, M.A. (2018) ‘Optimised Detection of Mitochondrial DNA Strand Breaks’. *Mitochondrion* [online] available from <http://linkinghub.elsevier.com/retrieve/pii/S1567724917303458> [11 May 2018]

Hardas, S.S., Sultana, R., Clark, A.M., Beckett, T.L., Szweda, L.I., Murphy, M.P., and Butterfield, D.A. (2013) ‘Oxidative modification of lipoic acid by HNE in Alzheimer disease brain’. *Redox Biology* [online] 1 (1),80-85. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3757677/> [21 June2019]

Harman, D. (1956) ‘Aging: A Theory Based on Free Radical and Radiation Chemistry’. *Journal of Gerontology* [online] 11 (3), 298–300. available from <http://www.uccs.edu/Documents/rmelamed/harman_1956_13332224.pdf> [19 April 2017]

Hartung, D., Sarai, M., Petrov, A., Kolodgie, F., Narula, N., Verjans, J., Virmani, R., Reutelingsperger, C., Hofstra, L., and Narula, J. (2005) 'Resolution of Apoptosis in Atherosclerotic Plaque by Dietary Modification and Statin Therapy.' *Journal of Nuclear Medicine* [online] 46 (12), 2051-6. available from <http://jnm.snmjournals.org/content/46/12/2051.full.pdf> [11 March 2019]

Hatefi, Y. and Hanstein, W.G. (1970) ‘Lipid Oxidation in Biological Membranes’. *Archives of Biochemistry and Biophysics* [online] 138 (1), 73–86. available from <http://linkinghub.elsevier.com/retrieve/pii/0003986170902869> [5 May 2017]

He, M., Siow, R.C.M., Sugden, D., Gao, L., Cheng, X., and Mann, G.E. (2011) ‘Induction of HO-1 and Redox Signaling in Endothelial Cells by Advanced Glycation End Products: A Role for Nrf2 in Vascular Protection in Diabetes’. *Nutrition, Metabolism and Cardiovascular Diseases* [online] 21 (4), 277–285. available from <https://www.sciencedirect.com/science/article/pii/S0939475309003172#bib18> [25 March 2018]

He, X., Wang, L., Szklarz, G., Bi, Y., and Ma, Q. (2012) ‘Resveratrol Inhibits Paraquat-Induced Oxidative Stress and Fibrogenic Response by Activating the Nuclear Factor Erythroid 2-Related Factor 2 Pathway’. *The Journal of Pharmacology and Experimental Therapeutics* [online] 342 (1). available from <http://dx.doi.org/10.1124/jpet.112.194142.> [2 November 2018]

Hecht, F., Pessoa, C.F., Gentile, L.B., Rosenthal, D., Carvalho, D.P., and Fortunato, R.S. (2016) 'The role of oxidative stress on breast cancer development and therapy.' *Tumour Biology* [online] 37 (4), 4281-91. availablefrom <https://link.springer.com/article/10.1007/s13277-016-4873-9> [8 Aug 2017]

Hedström, U., Hallgren, O., Öberg, L., DeMicco, A., Vaarala, O., Westergren-Thorsson, G., and Zhou, X. (2018) 'Bronchial Extracellular Matrix from COPD Patients Induces Altered Gene Expression in Repopulated Primary Human Bronchial Epithelial Cells'. *Scientific Reports* [online] 8 (1), 3502. available from <http://www.nature.com/articles/s41598-018-21727-w> [8 April 2018]

Heng, B.C., Zhao, X., Xiong, S., Woei Ng, K., Yin-Chiang Boey, F., and Say-Chye Loo, J. (2010) 'Toxicity of Zinc Oxide (ZnO) Nanoparticles on Human Bronchial Epithelial Cells (BEAS-2B) Is Accentuated by Oxidative Stress'. *Food and Chemical Toxicology* [online] 48 (6), 1762–1766. available from <http://linkinghub.elsevier.com/retrieve/pii/S0278691510002371> [10 August 2017]

Henriksen, S., Tylden, G.D., Dumoulin, A., Sharma, B.N., Hirsch, H.H., and Rinaldo, C.H. (2014) 'The Human Fetal Glial Cell Line SVG P12 Contains Infectious BK Polyomavirus.' *Journal of Virology* [online] 88 (13), 7556–68. available from <http://www.ncbi.nlm.nih.gov/pubmed/24760884> [19 March 2018]

Hermann, D.M. (2016) 'Insufficient Evidence for Vitamin E in Alzheimer's Disease'. *Alzheimer's & Dementia: Translational Research & Clinical Interventions* [online] 2 (3), 199–201. available from <https://www.sciencedirect.com/science/article/pii/S2352873716300336?_rdoc=1&_fmt=high&_origin=gateway&_docanchor=&md5=b8429449ccfc9c30159a5f9aeaa92ffb&dgcid=raven_sd_recommender_email> [7 April 2018]

Hernández-Camacho, J.D., Bernier, M., López-Lluch, G., and Navas, P. (2018) 'Coenzyme Q10 Supplementation in Aging and Disease.' *Frontiers in Physiology* [online] 9, 44. available from <http://www.ncbi.nlm.nih.gov/pubmed/29459830> [30 November 2018]

Herrera, F., Sainz, R.M., Mayo, J.C., Martin, V., Antolin, I., and Rodriguez, C. (2001) 'Glutamate induces oxidative stress not mediated by glutamate receptors or cystine transporters: protective effect of melatonin and other antioxidants'. *Journal of Pineal Research* [online] 31 (4), 536-62. available from < https://onlinelibrary.wiley.com/doi/abs/10.1034/j.1600-079X.2001.310411.x?sid=nlm%3Apubmed> [22 June 2019]

Himmetoglu, S., Dincer, Y., Ersoy, Y.E., Bayraktar, B., Celik, V., and Akcay,T. (2009) 'DNA oxidation and antioxidant status in breast cancer.' *Journal of Investigative Medicine* [online] 57 (6), 720-3. available from < http://jim.bmj.com/cgi/pmidlookup?view=long&pmid=19494787> [9 March 2019]

Hoey, S.E., Williams, R.J., and Perkinton, M.S. (2009) 'Synaptic NMDA receptor activation stimulates alpha-secretase amyloid precursor protein processing and inhibits amyloid-beta production'. *Journal of Neuroscience* [online] 29 (14), 4442-60. available from <http://www.jneurosci.org/content/29/14/4442.long> [18 June 2019]

Hollis, F., Kanellopoulos, A.K., and Bagni, C. (2017) 'Mitochondrial Dysfunction in Autism Spectrum Disorder: Clinical Features and Perspectives'. *Current Opinion in Neurobiology* [online] 45, 178–187. available from <https://www.sciencedirect.com/science/article/pii/S0959438817300764?via%3Dihub> [16 February 2019]

Holmström, K.M. and Finkel, T. (2014) 'Cellular Mechanisms and Physiological Consequences of Redox-Dependent Signalling.' *Nature reviews. Molecular Cell Biology* [online] 15 (6), 411–21. available from <http://www.ncbi.nlm.nih.gov/pubmed/24854789>

van Horssen, J., Schreibelt, G., Drexhage, J., Hazes, T., Dijkstra, C.D., van der Valk, P., and de Vries, H.E. (2008) 'Severe oxidative damage in multiple sclerosis lesions coincides with enhanced antioxidant enzyme expression'. *Free Radical Biology & Medicine* [online] 45 (12), 1729-37. available from <https://www.sciencedirect.com/science/article/pii/S0891584908005455?via%3Dihub> [21 June 2019]

Horvath, S.E., and Daum, G. (2013) 'Lipids of mitochondria' *Progress in Lipid Research* [online] 52 (4), 590-614. available from <https://www.sciencedirect.com/science/article/pii/S0163782713000519> [12 June 2019]

Hotta, N., Kawamori, R., Fukuda, M., and Shigeta, Y. (2012) 'Long-term clinical effects of epalrestat, an aldose reductase inhibitor, on progression of diabetic neuropathy and other microvascular complications: multivariate epidemiological analysis based on patient background factors and severity of diabetic neuropathy'. Diabetic Medicine [online] 29 (12), 1529-1533. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3533175/> [17 June 2019]

Van Houten, B., Woshner, V., and Santos, J.H. (2006) 'Role of Mitochondrial DNA in Toxic Responses to Oxidative Stress'. *DNA Repair* 5 (2), 145–152

Huerta-Yepez, S., Baay-Guzman, G.J., Garcia-Zepeda, R., Hernandez-Pando, R., Vega, M.I., Gonzalez-Bonilla, C., and Bonavida, B. (2008) '2-Methoxyestradiol (2-ME) Reduces the Airway Inflammation and Remodeling in an Experimental Mouse Model'. *Clinical Immunology* [online] 129 (2), 313–324. available from <https://www.sciencedirect.com/science/article/pii/S1521661608007304> [27 August 2018]

Hullinger, R. and Puglielli, L. (2016) 'Molecular and Cellular Aspects of Age-Related Cognitive Decline and Alzheimer's Disease'. *Behavioural Brain Research* [online] 2016. available from <http://linkinghub.elsevier.com/retrieve/pii/S0166432816302741>

Hunter, S., Jung, D., Di Guilo, R.T., and Meyer, J.. (2010) 'The QPCR Assay for Analysis of Mitochondrial DNA Damage, Repair, and Relative Copy Number'. *Methods* [online] 51 (4), 444–451. available from <http://www.sciencedirect.com/science/article/pii/S1046202310000484> [10 November 2013]

Hurrle, S. and Hsu, W.H. (2017) 'The Etiology of Oxidative Stress in Insulin Resistance'. *Biomedical Journal* [online] 40 (5), 257–262. available from <https://www.sciencedirect.com/science/article/pii/S2319417017301506> [3 May 2018]

Hwang, O. (2013) 'Role of Oxidative Stress in Parkinson's Disease.' *Experimental Neurobiology* [online] 22 (1), 11–7. available from <http://www.ncbi.nlm.nih.gov/pubmed/23585717> [14 August 2017]

Hybertson, B.M., Gao, B., Bose, S.K., and McCord, J.M. (2011) 'Oxidative Stress in Health and Disease: The Therapeutic Potential of Nrf2 Activation'. *Molecular Aspects of Medicine* [online] 32 (4–6), 234–246. available from <https://www.sciencedirect.com/science/article/pii/S0098299711000501?via%3Dihub> [11 March 2018]

Hyrsova, L., Vanduchova, A., Dusek, J., Smutny, T., Carazo, A., Maresova, V., Trejtnar, F., Barta, P., Anzenbacher, P., Dvorak, Z., Pavek, P. (2019) 'Trans-resveratrol, but not other natural stilbenes occurring in food, carries the risk of drug-food interaction via inhibition of cytochrome P450 enzymes or interaction with xenosensor receptors'. *Toxicology Letters* [online] 300, 81-91. available from <https://www.sciencedirect.com/science/article/pii/S037842741831988X> [29 June 2019]

Inoguchi, T., Li, P., Umeda, F., Yu, H.Y., Kakimoto, M., Imamura, M., Aoki, T., Etoh, T., Hashimoto, T., Naruse, M., Sano, H., Utsumi, H., and Nawata, H. (2000) 'High Glucose Level and Free Fatty Acid Stimulate Reactive Oxygen Species Production through Protein Kinase C--Dependent Activation of NAD(P)H Oxidase in Cultured Vascular Cells.' *Diabetes* [online] 49 (11), 1939–45. available from <http://www.ncbi.nlm.nih.gov/pubmed/11078463> [21 April 2018]

Ishii, T., Takanashi, Y., Sugita, K., Miyazawa, M., Yanagihara, R., Yasuda, K., Onouchi, H., Kawabe, N., Nakata, M., Yamamoto, Y., Hartman, P.S., and Ishii, N. (2017) 'Endogenous Reactive Oxygen Species Cause Astrocyte Defects and Neuronal Dysfunctions in the Hippocampus: A New Model for Aging Brain.' *Aging Cell* [online] 16 (1), 39–51. available from <http://www.ncbi.nlm.nih.gov/pubmed/27623715> [12 November 2018]

Isobe, C., Abe, T., and Terayama, Y. (2010) 'Levels of Reduced and Oxidized CoenzymeQ-10 and 8-Hydroxy-2 -Deoxyguanosine in the Cerebrospinal Fluid of Patients with Living Parkinson's Disease Demonstrate That Mitochondrial Oxidative Damage and/or Oxidative DNA Damage Contributes to the Neurodegenerative Process'. *Neuroscience Letters* [online] 469, 159–163. available from <https://ac.els-cdn.com/S0304394009015560/1-s2.0-S0304394009015560-main.pdf?_tid=7e1e7f58-0073-4f16-b06f-e70207a727e4&acdnat=1534702141_1c12915012ce9cdf7c3131bde8a68c27> [19 August 2018]

Jagannathan, L., Cuddapah, S., and Costa, M. (2016a) Oxidative stress under ambient and physiological oxygen tension in tissue culture'. *Current Pharmacology Reports* [online] 2 (2), 64-72. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4809260/> [29 May 2019]

Jagannathan, L., Jose, C.C., Arita, A., Kluz, T., Sun, H., Zhang, X., Yao, Y., Kartashov, A.V., Barski, A., Costa, M., and Cuddapah, S. (2016b) 'Nuclear Factor κB1/RelA Mediates Inflammation in Human Lung Epithelial Cells at Atmospheric Oxygen Levels'. *Journal of Cellular Physiology* [online] 231 (7), 1611-20. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4845657/> [30 May 2019]

James, S.J., Cutler, P., Melnyk, S., Jernigan, S., Janak, L., Gaylor, D.W., and Neubrander, J.A. (2004) 'Metabolic Biomarkers of Increased Oxidative Stress and Impaired Methylation Capacity in Children with Autism'. *The American Journal of Clinical Nutrition* [online] 80 (6), 1611–1617. available from <http://www.ncbi.nlm.nih.gov/pubmed/15585776> [16 February 2019]

Jankov, R.P., Kantores, C., Pan, J., and Belik, J. (2008) 'Contribution of Xanthine Oxidase-Derived Superoxide to Chronic Hypoxic Pulmonary Hypertension in Neonatal Rats'. *American Journal of Physiology-Lung Cellular and Molecular Physiology* [online] 294 (2), L233–L245. available from <http://www.ncbi.nlm.nih.gov/pubmed/18083771> [22 January 2019]

Jena, N.R. (2012) 'DNA Damage by Reactive Species: Mechanisms, Mutation and Repair'. *Journal of Biosciences* 37 (3), 503–17

Ji, J., Sharma, V., Qi, S., Guarch, M.E., Zhao, P., Luo, Z., Fan, W., Wang, Y., Mbabaali, F., Neculai, D., Esteban, M.A., McPherson, J.D., and Batada, N.N (2014) ' Antioxidant Supplementation Reduces Genomic Aberrations in Human Induced Pluripotent Stem Cells'. Stem Cell Reports [online] 2 (1), 44-51. available from https://linkinghub.elsevier.com/retrieve/pii/S221367111300129X> [30 May 2019]

Ji, L., Liu, Y., Zhang, Y., Chang, W., Gong, J., Wei, S., Li, X., and Qin, L. (2016) 'The Antioxidant Edaravone Prevents Cardiac Dysfunction by Suppressing Oxidative Stress in Type 1 Diabetic Rats and in High-Glucose-Induced Injured H9c2 Cardiomyoblasts'. *Canadian Journal of Physiology and Pharmacology* [online] 94 (9), 996–1006. available from <http://www.nrcresearchpress.com/doi/10.1139/cjpp-2015-0587> [15 December 2018]

Jiang, H., Zhu, Y. Sen, Xu, H., Sun, Y., and Li, Q.F. (2010) 'Inflammatory Stimulation and Hypoxia Cooperatively Activate HIF-1α in Bronchial Epithelial Cells: Involvement of PI3K and NF-KB'. *American Journal of Physiology - Lung Cellular and Molecular Physiology* [online] 298 (5). available from <http://ajplung.physiology.org/content/298/5/L660.long> [3 August 2017]

Jiang, P., Zhang, D., Qiu, H., Yi, X., Zhang, Y., Cao, Y., Zhao, B., Xia, Z., and Wang, C. (2017) 'Tiron Ameliorates High Glucose-Induced Cardiac Myocyte Apoptosis by PKCδ-Dependent Inhibition of Osteopontin'. *Clinical and Experimental Pharmacology and Physiology* [online] 44 (7), 760–770. available from <http://doi.wiley.com/10.1111/1440-1681.12762> [14 July 2018]

Jiao, X.Y., Gao, E., Yuan, Y., Wang, Y., Lau, W.B., Koch, W., Ma, X.Y., and Tao, L. (2009) ' INO-4885 [5,10,15,20-Tetra[N-(benzyl-4′-carboxylate)-2-pyridinium]-21H,23H-porphine Iron(III) Chloride], a Peroxynitrite Decomposition Catalyst, Protects the Heart against Reperfusion Injury in Mice'. The Journal of Pharmacology and Experimental Therapeutic [online] 328 (3), 777-84. avaliable from < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2663530/> [15 October 2015]

Jimoh, A., Tanko, Y., Ahmed, A., Mohammed, A., and Ayo, J.O. (2018) 'Resveratrol Prevents High-Fat Diet-Induced Obesity and Oxidative Stress in Rabbits'. *Pathophysiology* [online] available from <https://www.sciencedirect.com/science/article/pii/S0928468018300385> [14 October 2018]

Jin, F., Wu, Q., Lu, Y.-F., Gong, Q.-H., and Shi, J.-S. (2008) 'Neuroprotective Effect of Resveratrol on 6-OHDA-Induced Parkinson's Disease in Rats'. *European Journal of Pharmacology* [online] 600 (1–3), 78–82. available from <https://www.sciencedirect.com/science/article/pii/S0014299908010248> [25 November 2018]

Jin, X., Yao, T., Zhou, Z., Zhu, J., Zhang, S., Hu, W., and Shen, C. (2015) 'Advanced Glycation End Products Enhance Macrophages Polarization into M1 Phenotype through Activating RAGE/NF-KB Pathway.' *BioMed Research International* [online] 2015, 732450. available from <http://www.ncbi.nlm.nih.gov/pubmed/26114112> [14 June 2018]

Jing, Y., Liu, L., Jiang, Y., Zhu, Y., Guo, N.L., Barnett, J., Rojanasakul, Y., Agani, F., and Jiang, B. (2012) 'Cadmium Increases HIF-1 and VEGF Expression through ROS, ERK, and AKT Signaling Pathways and Induces Malignant Transformation of Human Bronchial Epithelial Cells'. *Toxicological Sciences* [online] 125 (1), 10-19. available from <https://academic.oup.com/toxsci/article/125/1/10/1667369> [16 June 2019]

Joddar, B., Firstenberg, M.S., Reen, R.K., Varadharaj, S., Khan, M., Childers, R.C., Zweier, J.L., and Gooch, K.J. (2015) 'Arterial Levels of Oxygen Stimulate Intimal Hyperplasia in Human Saphenous Veins via a ROS-Dependent Mechanism'. *PLOS ONE* [online] 10 (3), e0120301. available from <https://dx.plos.org/10.1371/journal.pone.0120301> [17 February 2019]

Joe, E.-H., Choi, D.-J., An, J., Eun, J.-H., Jou, I., and Park, S. (2018) 'Astrocytes, Microglia, and Parkinson's Disease.' *Experimental Neurobiology* [online] 27 (2), 77–87. available from <http://www.ncbi.nlm.nih.gov/pubmed/29731673> [12 November 2018]

Johar, R., Sharma, R., Kaur, A., and Mukherjee, T.K. (2015) 'Role of Reactive Oxygen Species in Estrogen Dependent Breast Cancer Complication.' *Anti-cancer Agents in Medicinal Chemistry* 16(2), 190-9.

Johnson, W.M., Wilson-Delfosse, A.L., and Mieyal, J.J. (2012) 'Dysregulation of Glutathione Homeostasis in Neurodgenerative Diseases'. *Nutrients* [online] 4 (10), 1399–1440. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3497002/>

Junn, E. and Mouradian, M.M. (2002) 'Human α-Synuclein over-Expression Increases Intracellular Reactive Oxygen Species Levels and Susceptibility to Dopamine'. *Neuroscience Letters* [online] 320 (3), 146–150. available from <https://www.sciencedirect.com/science/article/pii/S0304394002000162?via%3Dihub> [19 August 2018]

Kachhawa, K., Agrawl, D., Rath, B., and Kumar, S. (2017) 'Association of Lipid Abnormalities and Oxidative Stress with Diabetic Nephropathy'. *Journal of Integrative Nephrology and Andrology* [online] 4 (1), 3–9. available from <http://www.journal-ina.com/temp/JIntegrNephrolAndrol413-3431164_093151.pdf> [20 March 2017]

Kaiserova, K., Srivastava, S., Hoetker, J.D., Awe, S.O., Tang, X.L., Cai, J., and Bhatnagar, A. (2006) 'Redox Activation of Aldose Reductase in the Ischemic Heart'. *Journal of Biological Chemistry* [online] 281, 15110-20. available from <http://www.jbc.org/content/281/22/15110.full.html#F4> [24 June 2019]

Kanazawa, H. (2007) 'Role of Vascular Endothelial Growth Factor in the Pathogenesis of Chronic Obstructive Pulmonary Disease.' *Medical Science Monitor : International Medical Journal of Experimental and Clinical Research* [online] 13 (11), RA189-195. available from <http://www.ncbi.nlm.nih.gov/pubmed/17968307> [22 January 2019]

Kandikattu, H.K., Deep, S.N., Razack, S., Amruta, N., Prasad, D., and Khanum, F. (2017) 'Hypoxia Induced Cognitive Impairment Modulating Activity of Cyperus Rotundus'. *Physiology & Behavior* [online] 175, 56–65. available from <http://linkinghub.elsevier.com/retrieve/pii/S0031938417300860> [22 August 2017]

Kaneto, H., Xu, G., Song, K.H., Suzuma, K., Bonner-Weir, S., Sharma, A., and Weir, G.C. (2001) 'Activation of the Hexosamine Pathway Leads to Deterioration of Pancreatic Beta-Cell Function through the Induction of Oxidative Stress.' *The Journal of Biological Chemistry* [online] 276 (33), 31099–104. available from <http://www.ncbi.nlm.nih.gov/pubmed/11390407> [21 April 2018]

Kanti Das, T., Wati, M.R., and Fatima-Shad, K. (2015) 'Oxidative Stress Gated by Fenton and Haber Weiss Reactions and Its Association With Alzheimer's Disease'. *Archives of Neuroscience* [online] 2 (2). available from <http://www.archneurosci.com/?page=article&article_id=20078> [28 September 2016]

Kapoor, R. and Kakkar, P. (2012) 'Protective Role of Morin, a Flavonoid, against High Glucose Induced Oxidative Stress Mediated Apoptosis in Primary Rat Hepatocytes.' *PLOS ONE* [online] 7 (8), e41663. available from <http://www.ncbi.nlm.nih.gov/pubmed/22899998> [7 May 2018]

Karasu, C., Cumaoğlu, A., Gürpinar, A.R., Kartal, M., Kovacikova, L., Milackova, I., and Stefek, M. (2012) 'Aldose Reductase Inhibitory Activity and Antioxidant Capacity of Pomegranate Extracts.' *Interdisciplinary Toxicology* [online] 5 (1), 15–20. available from <http://www.ncbi.nlm.nih.gov/pubmed/22783144> [16 December 2018]

Keeley, T. and Mann, G.E. (2019) Defining Physiological Normoxia for Improved Translation of Cell Physiology to Animal Models and Humans'. *Physiological Reviews* [online] 99 (1), 161-234. available from<https://www.physiology.org/doi/abs/10.1152/physrev.00041.2017?rfr_dat=cr_pub%3Dpubmed&url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&journalCode=physrev> [15 May 2019]

Keglowich, L., Baraket, M., Tamm, M., and Borger, P. (2014) ‘Hypoxia Exerts Dualistic Effects on Inflammatory and Proliferative Responses of Healthy and Asthmatic Primary Human Bronchial Smooth Muscle Cells.’ *PLOS ONE* [online] 9 (2), e89875. available from <http://www.ncbi.nlm.nih.gov/pubmed/24587090> [24 June 2018]

Keller, J.N., Schmitt, F.A., Scheff, S.W., Ding, Q., Chen, Q., Butterfield, D.A., and Markesbery, W.R. (2005) ‘Evidence of increased oxidative stress in subjects with mild cognitive impairment’. *Neurology* [online] 64 (7). available from <https://n.neurology.org/content/64/7/1152.long> [21 June 2019]

Khalid, S., Afzal, N., Khan, J.A., Hussain, Z., Qureshi, A.S., Anwar, H., and Jamil, Y. (2018) ‘Antioxidant resveratrol protects against copper oxide nanoparticle toxicity in vivo’. *Naunyn-Schmiedeberg’s Archives of Pharmacology* [online] 391 (10), 1053-62. available from <https://link.springer.com/article/10.1007/s00210-018-1526-0> [29 June 2019]

Khan, Y.M., Kirkham, P., Barnes, P.J., and Adcock, I.M. (2014) 'Brd4 is Essential for IL-1B-induced Inflammation in Human Airway Epithelial Cells'. PLoS One [online] 9 (4). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3997389/> [13 June 2019]

Khansari, N., Shakiba, Y., and Mahmoudi, M. (2009) ‘Chronic Inflammation and Oxidative Stress as a Major Cause of Age-Related Diseases and Cancer’. *Recent Patents on Inflammation & Allergy Drug Discovery* 3, 73–80

Kietzmann, T. and Görlach, A. (2005) ‘Reactive Oxygen Species in the Control of Hypoxia-Inducible Factor-Mediated Gene Expression’. *Seminars in Cell & Developmental Biology* [online] 16 (4–5), 474–486. available from <https://www.sciencedirect.com/science/article/pii/S1084952105000510#bib102> [21 April 2018]

Kilarkaje, N., Al-Hussaini, H., and Al-Bader, M.M (2014) 'Diabetes-induced DNA damage and apoptosis are associated with poly (ADP ribose) polymerase 1 inhibition in the rat testis.' *European Journal of Pharmacology* [online] 737, 29-40. available from <https://www.sciencedirect.com/science/article/pii/S0014299914003586?via%3Dihub> [10 March 2017]

Kim, D.K., Kim, H.S., Kim, A.-R., Kim, J.H., Kim, B., Noh, G., Kim, H.S., Beaven, M.A., Kim, Y.M., and Choi, W.S. (2013) ‘DJ-1 Regulates Mast Cell Activation and IgE-Mediated Allergic Responses.’ *The Journal of Allergy and Clinical Immunology* [online] 131 (6), 1653–62. available from <http://www.ncbi.nlm.nih.gov/pubmed/23182168> [22 May 2018]

Kim, G.H., Kim, J.E., Rhie, S.J., and Yoon, S. (2015) ‘The Role of Oxidative Stress in Neurodegenerative Diseases’. *Experimental Neurobiology* [online] 24 (4), 325. available from <https://synapse.koreamed.org/DOIx.php?id=10.5607/en.2015.24.4.325> [19 June 2017]

Kim, H., Hwang, J.-S., Woo, C.-H., Kim, E.-Y., Kim, T.-H., Cho, K.-J., Seo, J.-M., Lee, S.-S., and Kim, J.-H. (2008) ‘TNF-α-Induced up-Regulation of Intercellular Adhesion Molecule-1 Is Regulated by a Rac-ROS-Dependent Cascade in Human Airway Epithelial Cells’. *Experimental and Molecular Medicine* [online] 40 (2), 167. available from <http://www.nature.com/doifinder/10.3858/emm.2008.40.2.167> [22 May 2018]

Kim, I. and He, Y.-Y. (2014) ‘Ultraviolet Radiation-Induced Non-Melanoma Skin Cancer: Regulation of DNA Damage Repair and Inflammation’. *Genes & Diseases* [online] 1 (2), 188–198. available from <http://linkinghub.elsevier.com/retrieve/pii/S2352304214000178>

Kim, J.S., Cho, E.W., Chung, H.W. and Kim, I.G. (2006) 'Effect of Tiron, 4,5-dihydroxy-1,3-benzene disulfonic acid, on human promyelotic HL-60 leukemia cell differentiation and death'. *Toxicology* [online] 223 (1-2), 36-45. available from <https://www.sciencedirect.com/science/article/pii/S0300483X06001521?via%3Dihub> [23 June 2019]

Kim, K.S., Yoo, H.Y., Park, K.S., Kim, J.K., Zhang, Y., and Kim, S.J. (2011) ‘Differential Effects of Acute Hypoxia on the Activation of TRPV1 by Capsaicin and Acidic PH’. *Journal of Physiological Sciences* [online] 62 (2), 93–103. available from <https://link.springer.com/article/10.1007%2Fs12576-011-0185-4> [26 July 2017]

Kirkham, P. and Rahman, I. (2006) ‘Oxidative Stress in Asthma and COPD: Antioxidants as a Therapeutic Strategy’. *Pharmacology & Therapeutics* [online] 111 (2), 476–494. available from <http://linkinghub.elsevier.com/retrieve/pii/S0163725805002767> [10 August 2017]

Klein, E.A., Thompson, I.M., Tangen, C.M., Crowley, J.J., Lucia, M.S., Goodman, P.J., Minasian, L.M., Ford, L.G., Parnes, H.L., Gaziano, J.M., Karp, D.D., Lieber, M.M., Walther, P.J., Klotz, L., Parsons, J.K., Chin, J.L., Darke, A.K., Lippman, S.M., Goodman, G.E., Meyskens, F.L., and Baker, L.H. (2011) ‘Vitamin E and the Risk of Prostate Cancer The Selenium and Vitamin E Cancer Prevention Trial (SELECT)’. *JAMA* [online] 306 (14), 1549-56. available from <https://jamanetwork.com/journals/jama/article-abstract/1104493> [29 June 2019]

Klopstock, T., Metz, G., Yu-Wai-Man, P., Buechner, B., Gallenmueller, C., Bailie, M., Nwali, N., Griffiths, P.G., von Livonius, B., Reznicek, L., Rouleau, J., Coppard, N., Meier, T., and Chinnery, P.F. (2013) ‘Persistence of the treatment effect of idebenone in Leber’s hereditary optic neuropathy’. *Brain* [online] 136 (2). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3572931/> [15 June 2019]

Kobayashi, E., Nakano, M., Kubota, K., Himuro, N., Mizoguchi, S., Chikenji,T., Otani, M., Mizue, Y., Nagaishi, K., and Fujimiya, M. (2018) ‘Activated forms of astrocytes with higher GLT-1 expression are associated with cognitive normal subjects with Alzheimer pathology in human brain’. *Scientific Reports* [online] 8. available from <https://www.nature.com/articles/s41598-018-19442-7#Sec2> [21 June 2019]

Kobayashi, K., Hayashi, M., Nakano, H., Fukutani, Y., Sasaki, K., Shimazaki, M., and Koshino, Y. (2002) ‘Apoptosis of Astrocytes with Enhanced Lysosomal Activity and Oligodendrocytes in White Matter Lesions in Alzheimer’s Disease’. *Neuropathology and Applied Neurobiology* [online] 28 (3), 238–251. available from <http://doi.wiley.com/10.1046/j.1365-2990.2002.00390.x> [7 August 2017]

Kobayashi, N., Delano, F.A., and Schmid-Schönbein, G.W. (2005) 'Oxidative Stress Promotes Endothelial Cell Apoptosis and Loss of Microvessels in the Spontaneously Hypertensive Rats'. *Arteriosclerosis, Thrombosis and Vascular Biology* [online] 25(10), 2114-21 available from < https://www.ahajournals.org/doi/full/10.1161/01.ATV.0000178993.13222.f2?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub%3Dpubmed> [19 December 2018]

Kode, A., Rajendrasozhan, S., Caito, S., Yang, S.-R., Megson, I.L., and Rahman, I. (2008) ‘Resveratrol Induces Glutathione Synthesis by Activation of Nrf2 and Protects against Cigarette Smoke-Mediated Oxidative Stress in Human Lung Epithelial Cells’. *American Journal of Physiology-Lung Cellular and Molecular Physiology* [online] 294 (3), L478–L488. available from <http://www.physiology.org/doi/10.1152/ajplung.00361.2007> [25 August 2018]

Koistinaho, M., Lin, S., Wu, X., Esterman, M., Koger, D., Hanson, J., Higgs, R., Liu, F., Malkani, S., Bales, K.R. and Paul, S.M. (2004) ‘Apolipoprotein E promotes astrocyte colocalization and degradation of deposited amyloid-β peptides‘. *Nature Medicine* [online] 10, 719-26. available from <https://www.nature.com/articles/nm1058> [18 June 2019]

Kolesarova, A., Capcarova, M., Maruniakova, N., Lukac, N., Ciereszko, R.E., and Sirotkin, A.V. (2012) ‘Resveratrol inhibits reproductive toxicity induced by deoxynivalenol‘. *Journal of Environment Science and Health, Part A* [online] 47 (9), 1329-34. available from <https://www.tandfonline.com/doi/abs/10.1080/10934529.2012.672144?journalCode=lesa20> [29 June 2019]

Komethi, M., Othman, N., Ismail, H., and Sasidharan, S. (2012) ‘Comparative Study on Natural Antioxidant as an Aging Retardant for Natural Rubber Vulcanizates’. *Journal of Applied Polymer Science* [online] 124 (2), 1490–1500. available from <http://doi.wiley.com/10.1002/app.35160> [25 April 2018]

Kondrikov, D., Caldwell, R.B., Dong, Z., and Su, Y. (2011) ‘Reactive Oxygen Species-Dependent RhoA Activation Mediates Collagen Synthesis in Hyperoxic Lung Fibrosis’. *Free Radical Biology and Medicine* [online] 50 (11), 1689–1698. available from <https://www.sciencedirect.com/science/article/pii/S0891584911001924> [8 April 2018]

Kong, X., Thimmulappa, R., Craciun, F., Harvey, C., Singh, A., Kombairaju, P., Reddy, S.P., Remick, D., and Biswal, S. (2011) 'Enhancing Nrf2 Pathway by Disruption of Keap1 in Myeloid Leukocytes Protects against Sepsis'. *American Journal of Respiratory and Critical Care Medicine* [online] 184 (8), 928–938. available from <http://www.atsjournals.org/doi/abs/10.1164/rccm.201102-0271OC> [23 November 2016]

Kortekaas, R., Leenders, K.L., van Oostrom, J.C.H., Vaalburg, W., Bart, E.J.L., Willemsen, T.M., and Hendrikse, N.H. (2005) 'Blood-brain barrier dysfunction in parkinsonian midbrain in vivo'. Annals of Neurology [online] 57 (2), 176-9. available from <https://www.semanticscholar.org/paper/Blood-brain-barrier-dysfunction-in-parkinsonian-in-Kortekaas-Leenders/4c416834d25a513fac9ef774dcf6db7c0de5a872> [18 June 2019]

Koya, D., Hayashi, K., Kitada, M., Kashiwagi, A., Kikkawa, R., and Haneda, M. (2003) 'Effects of Antioxidants in Diabetes-Induced Oxidative Stress in the Glomeruli of Diabetic Rats.' *Journal of the American Society of Nephrology : JASN* [online] 14 (8 Suppl 3), S250-3. available from <http://www.ncbi.nlm.nih.gov/pubmed/12874441> [7 January 2018]

Kranenburg, A.R., de Boer, W.I., Alagappan, V.K.T., Sterk, P.J., and Sharma, H.S. (2005) 'Enhanced Bronchial Expression of Vascular Endothelial Growth Factor and Receptors (Flk-1 and Flt-1) in Patients with Chronic Obstructive Pulmonary Disease'. *Thorax* [online] 60 (2), 106–113. available from <http://www.ncbi.nlm.nih.gov/pubmed/15681497> [22 January 2019]

Krishna, C.M., Liebmann, J.E., Kaufman, D., DeGraff, W., Hahn, S.M., McMurry, T., Mitchell, J.B., and Russo, A. (1992) 'The Catecholic Metal Sequestering Agent 1,2-Dihydroxybenzene-3, 5-Disulfonate Confers Protection against Oxidative Cell Damage'. *Archives of Biochemistry and Biophysics* [online] 294 (1), 98–106. available from <http://linkinghub.elsevier.com/retrieve/pii/000398619290142J> [24 August 2017]

Krishnan, K.J., Ratnaike, T.E., De Gruyter, H.L.M., Jaros, E., and Turnbull, D.M. (2012) 'Mitochondrial DNA deletions cause the biochemical defect observed in Alzheimer's disease'. *Neurobiology of Aging* [online] 33 (9), 2210-14. available from <https://www.sciencedirect.com/science/article/pii/S0197458011003241?via%3Dihub#bib7> [15 June 2019]

Kryscio, R.J., Abner, E.K., Caban-Holt, A., Lovell, M., Goodman, P., Darke, A.K., Yee, M., Crowley, J., and Schmitt, F.A. (2017) 'Association of Antioxidant Supplement Use and Dementia in the Prevention of Alzheimer's Disease by Vitamin E and Selenium Trial (PREADViSE)'. *JAMA Neurology* [online] 74 (5), 567-73. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5506489/> [29 June 2019]

Kuchibhotla, K.V., Lattarulo, C., Hyman, B.T., and Bacskai, B.J. (2010) 'Synchronous Hyperactivity and Intracellular Calcium Waves in Astrocytes in Alzheimer Mice'. *Science* [online] 323 (5918), 1211-15. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2884172/> [17 June 2019]

Kumar, S., Kumar, A., Pathania, A.S., Guru, S.K., Jada, S., Sharma, P.R., Bhushan, S., Saxena, A.K., Kumar, H.M.S., and Malik, F. (2013) 'Tiron and trolox potentiate the autophagic cell death induced by magnolol analog Ery5 by activation of Bax in HL-60 cells'. *Apoptosis* [online] 18 (5), 605-17. available from <https://link.springer.com/article/10.1007/s10495-013-0805-y> [23 June 2019]

Kumar Nirala, S., Bhadauria, M., Upadhyay, A.K., Mathur, R., and Mathur, A. (2009) 'Reversal of Effects of Intra Peritoneally Administered Beryllium Nitrate by Tiron and CaNa 3 DTPA Alone or in Combination with α-Tocopherol'. *Indian Journal of Experimental Biology* [online] 47, 955–963. available from <http://nopr.niscair.res.in/bitstream/123456789/6725/1/IJEB 47(12) 955-963.pdf> [24 August 2017]

Kumawat, M., Sharma, T.K., Singh, I., Singh, N., Ghalaut, V.S., Vardey, S.K., and Shankar, V. (2013) ' Antioxidant Enzymes and Lipid Peroxidation in Type 2 Diabetes Mellitus Patients with and without Nephropathy.' *North American Journal of Medical Sciences* [online] 5 (3), 213-19. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3632026/> [11 July 2018]

Kussmaul, L. and Hirst, J. (2006) ‘The Mechanism of Superoxide Production by NADH:Ubiquinone Oxidoreductase (Complex I) from Bovine Heart Mitochondria.’ *Proceedings of the National Academy of Sciences of the United States of America* [online] 103 (20), 7607–12. available from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1472492&tool=pmcentrez&rendertype=abstract> [15 December 2015]

Kwiecien, S., Jasnos, K.M., Magierowski, M., Sliwowski, Z., Pajdoi,R., Brozozowski, B., Mach, T., Wojcik, D. and Dbrzozowski, T. (2014) 'Lipid Peroxidation, Reactive Oxygen, Species and Antioxidative Factors in the Pathogenesis of Gastric Mucosal Lesions and Mechanism of Protection Against Oxidative Stress-Induced Gastric Injury'. *Journal of Physiology and Pharamcology* [online] 65 (5), 613–622. available from < http://www.jpp.krakow.pl/journal/archive/10_14/pdf/613_10_14_article.pdf> [13 July 2018]

Labbé, A., Garand, C., Cogger, V.C., Paquet, E.R., Desbiens, M., Le Couteur, D.G., and Lebel, M. (2011) ‘Resveratrol Improves Insulin Resistance Hyperglycemia and Hepatosteatosis But Not Hypertriglyceridemia, Inflammation, and Life Span in a Mouse Model for Werner Syndrome’. *The Journals of Gerontology: Series A* [online] 66A (3), 264–278. available from <http://www.ncbi.nlm.nih.gov/pubmed/20974729> [17 February 2019]

Lachmanová, V., Hniličková, O., Povýšilová, V., Hampl, V., and Herget, J. (2005) ‘N-Acetylcysteine Inhibits Hypoxic Pulmonary Hypertension Most Effectively in the Initial Phase of Chronic Hypoxia’. *Life Sciences* [online] 77 (2), 175–182. available from <http://www.ncbi.nlm.nih.gov/pubmed/15862602> [22 January 2019]

Lagouge, M. and Larsson, N.-G. (2013) ‘The Role of Mitochondrial DNA Mutations and Free Radicals in Disease and Ageing.’ *Journal of Internal Medicine* [online] 273 (6), 529–43. available from <http://www.ncbi.nlm.nih.gov/pubmed/23432181> [25 September 2018]

Lai, J.S., Zhao, C., Warsh, J.J., and Li, P.. (2006) ‘Cytoprotection by Lithium and Valproate Varies between Cell Types and Cellular Stresses’. *European Journal of Pharmacology* 539 (1–2), 18–26

Lamarche, B.J., Orazio, N.I., and Weitzman, M.D. (2010) ‘The MRN Complex in Double-Strand Break Repair and Telomere Maintenance.’ *FEBS letters* [online] 584 (17), 3682–95. available from <http://www.ncbi.nlm.nih.gov/pubmed/20655309> [10 December 2016]

Larsen, N.J., Ambrosi, G., Mullett, S.J., Berman, S.B., Hinkle, D.. (2011) ‘DJ-1 KNOCK-DOWN IMPAIRS ASTROCYTE MITOCHONDRIAL FUNCTION’. *Neuroscience* [online] 196, 251–264. available from <https://ac.els-cdn.com/S0306452211009572/1-s2.0-S0306452211009572-main.pdf?_tid=90795c05-e1a5-419c-9062-b4a58b01ea07&acdnat=1534701077_662c0cfe1fa81d77ae21cab5d8974372> [19 August 2018]

Lastres-Becker, I., Ulusoy, A., Innamorato, N.G., Sahin, G., Rábano, A., Kirik, D., and Cuadrado, A. (2012) ‘α-Synuclein Expression and Nrf2 Deficiency Cooperate to Aggravate Protein Aggregation, Neuronal Death and Inflammation in Early-Stage Parkinson’s Disease’. *Human Molecular Genetics* [online] 21 (14), 3173–3192. available from <https://academic.oup.com/hmg/article-lookup/doi/10.1093/hmg/dds143> [25 August 2017]

Lechner, J.F., Haugen, A., McClendon, I.A., and Shamsuddin, A.M. (1984) ‘Induction of Squamous Differentiation of Normal Human Bronchial Epithelial Cells by Small Amounts of Serum’. *Differentiation* [online] 25 (1–3), 229–237. available from <http://linkinghub.elsevier.com/retrieve/pii/S0301468111616125> [8 April 2018]

Lechner, J.F., Haugen, A., McClendon, I.A., and Ward Pettis, E. (1982) ‘Clonal Growth of Normal Adult Human Bronchial Epithelial Cells in a Serum-Free Medium’. *In Vitro* [online] 18 (7), 633–642. available from <http://link.springer.com/10.1007/BF02796396> [8 April 2018]

Lee, B.J., Huang, Y.C., Chen, S.J., and Lin, P.T. (2012) ‘Coenzyme Q10 supplementation reduces oxidative stress and increases antioxidant enzyme activity in patients with coronary artery disease‘. *Nutrition* [online] 28 (3), 250-5. available from <https://www.sciencedirect.com/science/article/abs/pii/S0899900711002000?via%3Dihub> [21 June 2019]

Lee, D., Park, S., Bae,S., Jeong, D., Park, M., Kang, C., Yoo, W., Samad, M.A., Ke, Q., Khang, G., and Kang, P.M. (2015) 'Hydrogen peroxide-activatable antioxidant prodrug as targeted therapeutic agent for ischemia-reperfusion injury'. *Scientific Reports* [online] 5 (16592). available from < https://www.nature.com/articles/srep16592> [9 March 2019]

Lee, H.-M., Wang, C., Hu, Z., Greeley, G.H., Makalowski, W., Hellmich, H.L., and Englander, E.W. (2002) 'Hypoxia Induces Mitochondrial DNA Damage and Stimulates Expression of a DNA Repair Enzyme, the Escherichia Coli MutY DNA Glycosylase Homolog (MYH), in Vivo, in the Rat Brain.*' Journal of Neurochemistry* [online] 80 (5). available from <https://onlinelibrary.wiley.com/doi/pdf/10.1046/j.0022-3042.2002.00774.x> [19 August 2018]

Lee, H.B., Yu, M.R., Song, J.S., and Ha, H. (2004) 'Reactive Oxygen Species Amplify Protein Kinase C Signaling in High Glucose-Induced Fibronectin Expression by Human Peritoneal Mesothelial Cells'. *Kidney International* [online] 65 (4), 1170–1179. available from <http://linkinghub.elsevier.com/retrieve/pii/S0085253815498254> [21 April 2018]

Leloup, C., Tourrel-Cuzin, C., Magnan, C., Karaca, M., Castel, J., Carneiro, L., Colombani, A.-L., Ktorza, A., Casteilla, L., and Pénicaud, L. (2009) 'Mitochondrial Reactive Oxygen Species Are Obligatory Signals for Glucose-Induced Insulin Secretion.' *Diabetes* [online] 58 (3), 673–81. available from <http://www.ncbi.nlm.nih.gov/pubmed/19073765> [30 June 2016]

Li, F., Sonveaux, P., Rabbani, Z.N., Liu, S., Yan, B., Huang, Q., Vjaskovic, Z., Dewhirst, M.W., and Li, C. (2007) 'Regulation of HIF-1α Stability through S-nitrosylation'. *Molecular Cell* [online] 26 (1), 63-74.available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2905600/> [16 June 2019]

Li, G., Lee, M.-J., Liu, A.B., Yang, Z., Lin, Y., Shih, W.J., and Yang, C.S. (2012) 'The Antioxidant and Anti-Inflammatory Activities of Tocopherols Are Independent of Nrf2 in Mice'. *Free Radical Biology and Medicine* [online] 52 (7), 1151–1158. available from <https://www.sciencedirect.com/science/article/pii/S0891584911012305?via%3Dihub> [25 August 2018]

Li, H., Wang, F., Zhang, L., Cao, Y., Liu, W., Hao, J., Liu, Q., and Duan, H. (2011) 'Modulation of Nrf2 Expression Alters High Glucose-Induced Oxidative Stress and Antioxidant Gene Expression in Mouse Mesangial Cells'. *Cellular Signalling* [online] 23 (10), 1625–1632. available from <https://www.sciencedirect.com/science/article/pii/S0898656811001537?via%3Dihub> [19 December 2018]

Li, H.L., Yan, Z., Ke, Z.P., Tian, X.F., Zhong, L.L., Lin, Y.T., Xu, Y., and Zheng, D.H. (2018) ' IGFBP2 is a potential biomarker in acute kidney injury (AKI) and resveratrol-loaded nanoparticles prevent AKI'. *Oncotarget* [online] 9 (93), 36551-60. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6290963/> [22 June 2019

Li, J., Johnson, D., Calkins, M., Wright, L., Svendsen, C., and Johnson, J. (2004) 'Stabilization of Nrf2 by TBHQ Confers Protection against Oxidative Stress-Induced Cell Death in Human Neural Stem Cells'. *Toxicological Sciences* [online] 83 (2), 313–328. available from <https://academic.oup.com/toxsci/article-lookup/doi/10.1093/toxsci/kfi027> [9 February 2017]

Li, M., Schroeder, R., Ni, S., Madea, B., and Stoneking, M. (2014) 'Extensive tissue-related and allele-related mtDNA heteroplasmy suggests positive selection for somatic mutations'. *PNAS* [online] 112 (8), 2491-2496. available from <https://www.pnas.org/content/pnas/112/8/2491.full.pdf> [11 June 2019]

Li, N., Sioutas, C., Cho, A., Schmitz, D., Misra, C., Sempf, J., Wang, M., Oberley, T., Froines, J., and Nel, A. (2003) 'Ultrafine Particulate Pollutants Induce Oxidative Stress and Mitochondrial Damage.' *Environmental Health Perspectives* [online] 111 (4), 455–60. available from <http://www.ncbi.nlm.nih.gov/pubmed/12676598> [19 March 2018]

Li, W., Wang, Y., and Irini, A. (2014) 'Effect of pH and H2O2 dosage on catechol oxidation in nano-Fe3O4 catalyzing UV–Fenton and identification of reactive oxygen species'. *Chemical Engineering Journal* [online] 244, 1-8. available from <https://www.sciencedirect.com/science/article/pii/S138589471400028X> [7 June 2019]

Li, X., Fang, P., Mai, J., Choi, E.T., Wang, H., and Yang, X. (2013) 'Targeting Mitochondrial Reactive Oxygen Species as Novel Therapy for Inflammatory Diseases and Cancers.' *Journal of Hematology & Oncology* [online] 6, 19. available from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3599349&tool=pmcentrez&rendertype=abstract> [16 December 2014]

Li, X. and Heyer, W.-D. (2008) 'Homologous Recombination in DNA Repair and DNA Damage Tolerance.' *Cell Research* [online] 18 (1), 99–113. available from <http://www.ncbi.nlm.nih.gov/pubmed/18166982> [10 December 2016]

Li, X., Sun, X., Zhang, X., Mao, Y., Ji, Y., Shi, L., Cai, W., Wang, P., Wu, G., Gan, X., and Huang, S. (2018) ' '. Oxidaitve Medicine and Cellular Longevity [online] 2018. available from <https://www.hindawi.com/journals/omcl/2018/9421019/#B10> [22 June 2019]

Li, Y.-J., Kawada, T., and Azuma, A. (2013) 'Nrf2 Is a Protective Factor against Oxidative Stresses Induced by Diesel Exhaust Particle in Allergic Asthma.' *Oxidative Medicine and Cellular Longevity* [online] 2013, 323607. available from <http://www.ncbi.nlm.nih.gov/pubmed/23738037> [20 October 2018]

Li, Y., Zhou, Q., Pei, C., Liu, B., Li, M., Fang, L., Sun, Y., Li, Y., Meng, S., Klöting, I., Morcos, M., Kobayashi, S., Hirata, K., Kawashima, S., Itabe, H., Hayashi, Y., Imajoh-Ohmi, S., Itoh, H., and Yokoyama, M. (2016) 'Hyperglycemia and Advanced Glycation End Products Regulate MiR-126 Expression in Endothelial Progenitor Cells'. *Journal of Vascular Research* [online] 53 (1–2), 94–104. available from <http://www.karger.com/?doi=10.1159/000448713> [25 April 2017]

Liao, C.-Y., Chung, C.-H., Wu, C.-C., Lin, F.-H., Tsao, C.-H., Wang, C.-C., and Chien, W.-C. (2017) 'Protective Effect of N-Acetylcysteine on Progression to End-Stage Renal Disease: Necessity for Prospective Clinical Trial'. *European Journal of Internal Medicine* [online] 44, 67–73. available from <https://www.sciencedirect.com/science/article/pii/S0953620517302558> [14 October 2018]

Liaquat, L., Haider, S., Batool, Z., Suleman, A., Gul, N., and Jabbar, S. (2016) 'Memory enhancement and reduction in lipid peroxidation by dietary antioxidants in brain'. *Pakistan Journal of Biochemistry and Molecular Biology* [online] 49 (3), 53-59. available from <http://pjbmb.org.pk/images/PJBMBArchive/2016/PJBMB_49_3_Sep_2016/01.pdf> [21 June 2019]

Liddell, J.R., Robinson, S.R., Dringen, R., and Bishop, G.M. (2010) 'Astrocytes Retain Their Antioxidant Capacity into Advanced Old Age'. *Glia* [online] 58 (12), n/a-n/a. available from <http://doi.wiley.com/10.1002/glia.21024> [12 November 2018]

Liemburg-Apers, D.C., Willems, P.H.G.M., Koopman, W.J.H., and Grefte, S. (2015) 'Interactions between Mitochondrial Reactive Oxygen Species and Cellular Glucose Metabolism.' *Archives of toxicology* [online] 89 (8), 1209–26. available from <http://www.ncbi.nlm.nih.gov/pubmed/26047665> [15 December 2018]

Lim, K.H., Kim, K.Y., and Chang, Y. (2007) 'Investigations of the Molecular Mechanism of Metal-Induced Aβ (1−40) Amyloidogenesis.' Biochemistry [online] 46 (47), 13523-32. available from <https://pubs.acs.org/doi/10.1021/bi701112z> [7 June 2019]

Lin, C.-E., Chen, S.-U., Lin, C.-C., Chang, C.-H., Lin, Y.-C., Tai, Y.-L., Shen, T.-L., and Lee, H. (2012) 'Lysophosphatidic Acid Enhances Vascular Endothelial Growth Factor-C Expression in Human Prostate Cancer PC-3 Cells'. *PLOS ONE* [online] 7 (7), e41096. available from <https://dx.plos.org/10.1371/journal.pone.0041096> [18 November 2018]

Lin, C.-J., Lee, C.-C., Shih, Y.-L., Lin, T.-Y., Wang, S.-H., Lin, Y.-F., and Shih, C.-M. (2012) 'Resveratrol Enhances the Therapeutic Effect of Temozolomide against Malignant Glioma in Vitro and in Vivo by Inhibiting Autophagy'. *Free Radical Biology and Medicine* [online] 52 (2), 377–391. available from <https://www.sciencedirect.com/science/article/pii/S0891584911011646> [25 March 2018]

Lin, M.-H., Yen, J.-H., Weng, C.-Y., Wang, L., Ha, C.-L., and Wu, M.-J. (2014) 'Lipid Peroxidation End Product 4-Hydroxy-Trans-2-Nonenal Triggers Unfolded Protein Response and Heme Oxygenase-1 Expression in PC12 Cells: Roles of ROS and MAPK Pathways'. *Toxicology* [online] 315, 24–37. available from <https://www.sciencedirect.com/science/article/pii/S0300483X1300320X> [20 November 2018]

Lin, N., Zhang, H., and Su, Q. (2012) 'Advanced Glycation End-Products Induce Injury to Pancreatic Beta Cells through Oxidative Stress'. *Diabetes & Metabolism* [online] 38 (3), 250–257. available from <http://linkinghub.elsevier.com/retrieve/pii/S1262363612000298> [13 July 2018]

Ling, Y.-H., Liebes, L., Zou, Y., and Perez-Soler, R. (2003) 'Reactive Oxygen Species Generation and Mitochondrial Dysfunction in the Apoptotic Response to Bortezomib, a Novel Proteasome Inhibitor, in Human H460 Non-Small Cell Lung Cancer Cells.' *The Journal of Biological Chemistry* [online] 278 (36), 33714–23. available from <http://www.ncbi.nlm.nih.gov/pubmed/12821677> [8 May 2017]

Lissoni, P. (2007) 'Biochemotherapy with Standard Chemotherapies plus the Pineal Hormone Melatonin in the Treatment of Advanced Solid Neoplasms'. *Pathologie Biologie* [online] 55 (3–4), 201–204. available from <http://www.ncbi.nlm.nih.gov/pubmed/17446010> [16 February 2019]

Liu, B.-H., Wu, T.-S., Yu, F.-Y., and Su, C.-C. (2006) 'Induction of Oxidative Stress Response by the Mycotoxin Patulin in Mammalian Cells'. *Toxicological Sciences* [online] 95 (2), 340–347. available from <https://academic.oup.com/toxsci/article-lookup/doi/10.1093/toxsci/kfl156> [2 November 2018]

Liu, C.-S., Tsai, C.-S., Kuo, C.-L., Chen, H.-W., Lii, C.-K., Ma, Y.-S., & Wei, Y.-H. (2003). 'Oxidative Stress-related Alteration of the Copy Number of Mitochondrial DNA in Human Leukocytes'. *Free Radical Research* [online] 37(12), 1307–1317. available from <https://www.tandfonline.com/doi/abs/10.1080/10715760310001621342?journalCode=ifra2> [30 May 2019]

Liu, H., Qiu, H., Yang, J., Ni, J., and Le, W. (2016) 'Chronic Hypoxia Facilitates Alzheimer's Disease through Demethylation of γ-Secretase by Downregulating DNA Methyltransferase 3b'. *Alzheimer's & Dementia* [online] 12 (2), 130–143. available from <http://www.sciencedirect.com/science/article/pii/S1552526015002174> [22 August 2017]

Liu, Q., Gheorghiu, L., Drumm, M., Clayman, R., Eidelman, A., Wszolek, M.F., Olumi, A., Feldman, A., Wang, M., Marcar, L., Citrin, D.E., Wu, C.L., Benes, C.H., Efstathiou, J.A., and Willers, H. (2018) ' PARP-1 inhibition with or without ionizing radiation confers reactive oxygen species-mediated cytotoxicity preferentially to cancer cells with mutant TP53.' *Oncogene* [online] 37 (21), 2793-805. available from <https://www.ncbi.nlm.nih.gov/pubmed/29511347> [11 March 2019]

Liu, X., Liu, H., Zhai, Y., Li, Y., Zhu, X., and Zhang, W. (2017) 'Laminarin Protects against Hydrogen Peroxide-Induced Oxidative Damage in MRC-5 Cells Possibly via Regulating NRF2.' *PeerJ* [online] 5, e3642. available from <http://www.ncbi.nlm.nih.gov/pubmed/28785522> [11 February 2018]

Liu, Z., Tao, X., Zhang, C., Lu, Y., and Wei, D. (2005) 'Protective Effects of Hyperoside (Quercetin-3-o-Galactoside) to PC12 Cells against Cytotoxicity Induced by Hydrogen Peroxide and Tert-Butyl Hydroperoxide'. *Biomedicine & Pharmacotherapy* [online] 59 (9), 481–490. available from <https://www.sciencedirect.com/science/article/pii/S0753332205001770> [21 January 2019]

Lochhead, J.J., McCaffrey, G., Quigley, C.E., Finch, J., DeMarco, K.M., Nametz, N., and Davis, T.P. (2010) 'Oxidative Stress Increases Blood–Brain Barrier Permeability and Induces Alterations in Occludin during Hypoxia–Reoxygenation'. *Journal of Cerebral Blood Flow & Metabolism* [online] 30 (9), 1625–1636. available from <http://journals.sagepub.com/doi/10.1038/jcbfm.2010.29> [20 May 2017]

Lönn, M.E., Dennis, J.M., and Stocker, R. (2012) 'Actions of Antioxidants in the Protection against Atherosclerosis'. *Free Radical Biology and Medicine* 53 (4), 863–884

Lorente, L., Martín, M.M., Abreu-González, P., Ramos, L., Argueso, M., Solé-Violán, J., Riaño-Ruiz, M., and Jiménez, A. (2015) 'Serum Malondialdehyde Levels in Patients with Malignant Middle Cerebral Artery Infarction Are Associated with Mortality.' *PLOS ONE* [online] 10 (5), e0125893. available from <http://www.ncbi.nlm.nih.gov/pubmed/25933254> [18 April 2017]

Louhelainen, N., Myllärniemi, M., Rahman, I., and Kinnula, V.L. (2008) 'Airway Biomarkers of the Oxidant Burden in Asthma and Chronic Obstructive Pulmonary Disease: Current and Future Perspectives.' *International Journal of Chronic Obstructive Pulmonary Disease* [online] 3 (4), 585–603. available from <http://www.ncbi.nlm.nih.gov/pubmed/19281076> [17 January 2017]

Louhelainen, N., Rytilä, P., Haahtela, T., Kinnula, V.L., and Djukanović, R. (2009) 'Persistence of Oxidant and Protease Burden in the Airways after Smoking Cessation'. *BMC Pulmonary Medicine* [online] 9 (1), 25. available from <http://bmcpulmmed.biomedcentral.com/articles/10.1186/1471-2466-9-25> [24 February 2017]

Lovell, M.A. and Markesbery, W.R. (2007) 'Oxidative Damage in Mild Cognitive Impairment and Early Alzheimer's Disease'. *Journal of Neuroscience Research* [online] 85 (14), 3036–3040. available from <http://doi.wiley.com/10.1002/jnr.21346> [19 August 2018]

Lovell, M.A., Xie, C., and Markesbery, W.R. (2001) 'Acrolein is increased in Alzheimer's disease brain and is toxic to primary hippocampal cultures'. *Neurobiology of Aging* [online] 22 (2), 187-94. available from <https://www.sciencedirect.com/science/article/abs/pii/S0197458000002359?via%3Dihub> [20 June 2019]

Lowes, D.A., Webster, N.R., Murphy, M.P., and Galley, H.F. (2013) 'Antioxidants That Protect Mitochondria Reduce Interleukin-6 and Oxidative Stress, Improve Mitochondrial Function, and Reduce Biochemical Markers of Organ Dysfunction in a Rat Model of Acute Sepsis.' *British Journal of Anaesthesia* [online] 110 (3), 472–80. available from <http://www.ncbi.nlm.nih.gov/pubmed/23381720> [13 October 2016]

Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S.Y., AlMazroa, M.A., Alvarado, M., Anderson, H.R., Anderson, L.M., Andrews, K.G., Atkinson, C., Baddour, L.M., Barker-Collo, S., Bartels, D.H., Bell, M.L., Benjamin, E.J., Bennett, D., Bhalla, K., Bikbov, B., Abdulhak, A. Bin, Birbeck, G., Blyth, F., Bolliger, I., Boufous, S., Bucello, C., Burch, M., Burney, P., Carapetis, J., Chen, H., Chou, D., Chugh, S.S., Coffeng, L.E., Colan, S.D., Colquhoun, S., Colson, K.E., Condon, J., Connor, M.D., Cooper, L.T., Corriere, M., Cortinovis, M., de Vaccaro, K.C., Couser, W., Cowie, B.C., Criqui, M.H., Cross, M., Dabhadkar, K.C., Dahodwala, N., De Leo, D., Degenhardt, L., Delossantos, A., Denenberg, J., Des Jarlais, D.C., Dharmaratne, S.D., Dorsey, E.R., Driscoll, T., Duber, H., Ebel, B., Erwin, P.J., Espindola, P., Ezzati, M., Feigin, V., Flaxman, A.D., Forouzanfar, M.H., Fowkes, F.G.R., Franklin, R., Fransen, M., Freeman, M.K., Gabriel, S.E., Gakidou, E., Gaspari, F., Gillum, R.F., Gonzalez-Medina, D., Halasa, Y.A., Haring, D., Harrison, J.E., Havmoeller, R., Hay, R.J., Hoen, B., Hotez, P.J., Hoy, D., Jacobsen, K.H., James, S.L., Jasrasaria, R., Jayaraman, S., Johns, N., Karthikeyan, G., Kassebaum, N., Keren, A., Khoo, J.-P., Knowlton, L.M., Kobusingye, O., Koranteng, A., Krishnamurthi, R., Lipnick, M., Lipshultz, S.E., Ohno, S.L., Mabweijano, J., MacIntyre, M.F., Mallinger, L., March, L., Marks, G.B., Marks, R., Matsumori, A., Matzopoulos, R., Mayosi, B.M., McAnulty, J.H., McDermott, M.M., McGrath, J., Memish, Z.A., Mensah, G.A., Merriman, T.R., Michaud, C., Miller, M., Miller, T.R., Mock, C., Mocumbi, A.O., Mokdad, A.A., Moran, A., Mulholland, K., Nair, M.N., Naldi, L., Narayan, K.M.V., Nasseri, K., Norman, P., O'Donnell, M., Omer, S.B., Ortblad, K., Osborne, R., Ozgediz, D., Pahari, B., Pandian, J.D., Rivero, A.P., Padilla, R.P., Perez-Ruiz, F., Perico, N., Phillips, D., Pierce, K., Pope, C.A., Porrini, E., Pourmalek, F., Raju, M., Ranganathan, D., Rehm, J.T., Rein, D.B., Remuzzi, G., Rivara, F.P., Roberts, T., De León, F.R., Rosenfeld, L.C., Rushton, L., Sacco, R.L., Salomon, J.A., Sampson, U., Sanman, E., Schwebel, D.C., Segui-Gomez, M., Shepard, D.S., Singh, D., Singleton, J., Sliwa, K., Smith, E., Steer, A., Taylor, J.A., Thomas, B., Tleyjeh, I.M., Towbin, J.A., Truelsen, T., Undurraga, E.A., Venketasubramanian, N., Vijayakumar, L., Vos, T., Wagner, G.R., Wang, M., Wang, W., Watt, K., Weinstock, M.A., Weintraub, R., Wilkinson, J.D., Woolf, A.D., Wulf, S., Yeh, P.-H., Yip, P., Zabetian, A., Zheng, Z.-J., Lopez, A.D., and Murray, C.J. (2012) 'Global and Regional Mortality from 235 Causes of Death for 20 Age Groups in 1990 and 2010: A Systematic Analysis for the Global Burden of Disease Study 2010'. *The Lancet* [online] 380 (9859), 2095–2128. available from <https://www.sciencedirect.com/science/article/pii/S0140673612617280> [14 October 2018]

Lu, L.Y., Ou, N., and Lu, Q.B. (2013) 'Antioxidant Induces DNA Damage, Cell Death and Mutagenicity in Human Lung and Skin Normal Cells'. *Scientific Reports* [online] 3. available from <https://www.nature.com/articles/srep03169#discussion> [23 June 2019]

Lu, Y., Wang, B., Shi, Q., Wang, X., Wang, D., and Zhu, L. (2016) 'Brusatol inhibits HIF-1 signaling pathway and suppresses glucose uptake under hypoxic conditions in HCT116 cells'. *Scientific Reports* [online] 6. available from <https://www.nature.com/articles/srep39123> [16 June 2019]

Luchsinger, J.A., Tang, M.-X., Shea, S., and Mayeux, R. (2003) 'Antioxidant Vitamin Intake and Risk of Alzheimer Disease'. *Archives of Neurology* [online] 60 (2), 203. available from <http://archneur.jamanetwork.com/article.aspx?doi=10.1001/archneur.60.2.203> [27 March 2018]

Luo, X., Li, R., and Yan, L.J. (2015) 'Roles of Pyruvate, NADH, and Mitochondrial Complex I in Redox Balance and Imbalance in β Cell Function and Dysfunction'. *Journal of Diabetes Research* [online] 2015. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4629043/> [26 June 2019]

Ma, T., Tan, M.-S., Yu, J.-T., and Tan, L. (2014) 'Resveratrol as a Therapeutic Agent for Alzheimer's Disease.' *BioMed Research International* [online] 2014, 350516. available from <http://www.ncbi.nlm.nih.gov/pubmed/25525597> [23 July 2016]

Madhavan, L., Ourednik, V., and Ourednik, J. (2009) 'Increased "Vigilance" of Antioxidant Mechanisms in Neural Stem Cells Potentiates Their Capability to Resist Oxidative Stress'. *Stem Cells* [online] 24 (9). available from <https://stemcellsjournals.onlinelibrary.wiley.com/doi/full/10.1634/stemcells.2006-0018> [24 June 2019]

Makino, A., Skelton, M.M., Zou, A.P., and Cowley, A.W. (2003) 'Increased Renal Medullary H2O2 Leads to Hypertension'. *Hypertension* 42 (1), 25–30

Malik, A.N. and Czajka, A. (2013) 'Is Mitochondrial DNA Content a Potential Biomarker of Mitochondrial Dysfunction?' *Mitochondrion* [online] 13 (5), 481–492. available from <http://dx.doi.org/10.1016/j.mito.2012.10.011>

Malik, A.N., Shahni, R., Rodriguez-de-Ledesma, A., Laftah, A., and Cunningham, P. (2011) 'Mitochondrial DNA as a Non-Invasive Biomarker: Accurate Quantification Using Real Time Quantitative PCR without Co-Amplification of Pseudogenes and Dilution Bias'. *Biochemical and Biophysical Research Communications* [online] 412 (1), 1–7. available from <http://dx.doi.org/10.1016/j.bbrc.2011.06.067>

Maluf, S.W., Mergener, M., Dalcanale, L., Costa, C.C., Pollo, T., Kayser, M., da Silva, L.B., Pra, D., and Teixeira, P.J.Z. (2007) 'DNA Damage in Peripheral Blood of Patients with Chronic Obstructive Pulmonary Disease (COPD)'. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* [online] 626 (1), 180–184. available from <http://www.sciencedirect.com/science/article/pii/S1383571806003615> [8 September 2017]

Manczak, M., Mao, P., Calkins, M.J., Cornea, A., Reddy, A.P., Murphy, M.P., Szeto, H.H., Park, B., and Reddy, P.H. (2010) 'Mitochondria-Targeted Antioxidants Protect Against Amyloid-β Toxicity in Alzheimer's Disease Neurons'. *Journal of Alzheimer's Disease* [online] 20 (s2), S609–S631. available from <http://www.medra.org/servlet/aliasResolver?alias=iospress&doi=10.3233/JAD-2010-100564> [27 March 2018]

Mangialasche, F., Kivipelto, M., Mecocci, P., Rizzuto, D., Palmer, K., Winblad, B., and Fratiglioni, L. (2010) 'High plasma levels of vitamin E forms and reduced Alzheimer's disease risk in advanced age'. *Journal of Alzheimer's Disease* [online] 20 (4), 1029-37. available from <https://content.iospress.com/articles/journal-of-alzheimers-disease/jad091450> [29 June 2019]

Mantzavinos, D. (2003) 'Removal of Benzoic Acid Derivatives from Aqueous Effluents by the Catalytic Decomposition of Hydrogen Peroxide.' *Process Safety and Environmental Protection* [online] 81 (2), 99-106. available from <https://www.sciencedirect.com/science/article/pii/S0957582003710738> [7 June 2019]

Mao, G., Kraus, G.A., Kim, I., Spurlock, M.E., Bailey, T.B., Zhang, Q., and Beitz, D.C. (2010) 'A Mitochondria-Targeted Vitamin E Derivative Decreases Hepatic Oxidative Stress and Inhibits Fat Deposition in Mice'. *The Journal of Nutrition* [online] 140 (8), 1425–1431. available from <http://www.ncbi.nlm.nih.gov/pubmed/20554905> [29 December 2018]

Marinucci, L., Balloni, S., Fettucciari, K., Bodo, M., Talesa, V.N., and Antognelli, C. (2018) 'Nicotine Induces Apoptosis in Human Osteoblasts via a Novel Mechanism Driven by H2O2 and Entailing Glyoxalase 1-Dependent MG-H1 Accumulation Leading to TG2-Mediated NF-KB Desensitization: Implication for Smokers-Related Osteoporosis'. *Free Radical Biology and Medicine* [online] 117, 6–17. available from <https://www.sciencedirect.com/science/article/pii/S0891584918300273> [2 November 2018]

Martin-Romero, F.J., Miguel-Lasobras, E.M., Dominguez-Arroyo, J.A., Gonzalez-Carrera, E., and Alvarez, I.S. (2008) 'Contribution of culture media to oxidative stress and its effect on human oocytes.' *Reproductive Biomedicine Online* [online] 17 (5), 652-61. available from <https://www.rbmojournal.com/article/S1472-6483(10)60312-4/pdf> [29 May 2019]

Mason, R.P., Shoemaker, W.J., Shajenko, L., Chambers, T.E., and Herbette, L.G. (1992) ' Evidence for changes in the Alzheimer's disease brain cortical membrane structure mediated by cholesterol.' *Neurobiology of Aging* [online] 13 (3), 413-19. available from <https://www.sciencedirect.com/science/article/pii/019745809290116F> [12 March 2019]

Mathebula, S.D. (2015) 'Polyol Pathway: A Possible Mechanism of Diabetes Complications in They Eye'. *African Vision and Eye Health* 74 (13)

Matough, F.A., Budin, S.B., Hamid, Z.A., Alwahaibi, N., and Mohamed, J. (2012) 'The Role of Oxidative Stress and Antioxidants in Diabetic Complications.' *Sultan Qaboos University Medical Journal* [online] 12 (1), 5–18. available from <http://www.ncbi.nlm.nih.gov/pubmed/22375253> [25 March 2018]

Di Matteo, V. and Esposito, E. (2003) 'Biochemical and Therapeutic Effects of Antioxidants in the Treatment of Alzheimer ' s Disease , Parkinson ' s Disease , and Amyotrophic Lateral Sclerosis'. *Current Drug Targets - CNS & Neurological Disorders* [online] 2, 95–107. available from <https://www.researchgate.net/profile/Vincenzo_Di_Matteo/publication/7137729_Biochemical_and_Therapeutic_Effects_of_Antioxidants_in_the_Treatment_of_Alzheimers_Disease_Parkinsons_Disease_and_Amyotrophic_Lateral_Sclerosis/links/0912f5118e8109c537000000.pdf> [27 March 2018]

Matzinger, M., Fischhuber, K., Heiss, E.H. (2018) 'Activation of Nrf2 signaling by natural products-can it alleviate diabetes?' *Biotechnology Advances* [online] 6(1), 1738-67. available from <https://www.sciencedirect.com/science/article/pii/S0734975017301672#bb0760>

Mayhan, W.G. (2000) 'Nitric Oxide Donor-Induced Increase in Permeability of the Blood–brain Barrier'. *Brain Research* [online] 866 (1–2), 101–108. available from <http://linkinghub.elsevier.com/retrieve/pii/S000689930002254X> [4 April 2018]

McArdle, F., Pattwell, D.M., Vasilaki, A., McArdle, A., and Jackson, M.J. (2005) 'Intracellular Generation of Reactive Oxygen Species by Contracting Skeletal Muscle Cells'. *Free Radical Biology and Medicine* [online] 39 (5), 651–657. available from <http://linkinghub.elsevier.com/retrieve/pii/S0891584905002030> [24 August 2017]

McBryde, W.A.E. (1964) 'A spectrophotometric Reexamination of the Spectra and Stabilities of the Iron (III)-Tiron Complexes'. *Canadian Journal of Chemistry* [online] 42 (8), 1917–1927. available from <http://www.nrcresearchpress.com/doi/abs/10.1139/v64-284> [5 May 2017]

McManus, M.J., Murphy, M.P., and Franklin, J.L. (2011) 'The Mitochondria-Targeted Antioxidant MitoQ Prevents Loss of Spatial Memory Retention and Early Neuropathology in a Transgenic Mouse Model of Alzheimer's Disease.' *The Journal of Neuroscience : the Official Journal of the Society for Neuroscience* [online] 31 (44), 15703–15. available from <http://www.ncbi.nlm.nih.gov/pubmed/22049413> [19 August 2018]

Mecocci, P., Cherubini, A., Beal, M.F., Cecchetti, R., Chionne, F., Polidor, M.C., Romano, G., and Senin, U. (1996) 'Altered mitochondrial membrane fluidity in AD brain.' *Neuroscience Letters* [online] 207 (2), 129-32. available from <https://www.sciencedirect.com/science/article/pii/030439409612509X> [12 March 2019]

Mecocci, P. and Polidori, M.C. (2012) 'Antioxidant Clinical Trials in Mild Cognitive Impairment and Alzheimer's Disease'. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* [online] 1822 (5), 631–638. available from <https://www.sciencedirect.com/science/article/pii/S0925443911002298#s0020> [25 February 2018]

Medicines and Healthcare products Regulatory Agency (2014) 'Treating paracetamol overdose with intravenous acetylcysteine: new guidance.' *Drug Safety Update September 2012* [online], 6 (2). available from <https://www.gov.uk/drug-safety-update/treating-paracetamol-overdose-with-intravenous-acetylcysteine-new-guidance> [9 March 2019]

Melhem, A., Stern, M., Shibolet, O., Israeli, E., Ackerman, Z., Pappo, O., Hemed, N., Rowe, M., Ohana, H., Zabrecky, G., Cohen, R., and Ilan, Y. (2005) 'Treatment of chronic hepatitis C virus infection via antioxidants: results of a phase I clinical trial.' *Journal of Clinical Gastroenterology* [online] 39 (8), 737-742. available from < https://insights.ovid.com/pubmed?pmid=16082287> [9 March 2019]

Mercado,N., Thimmulappa, R., Thomas, C.M., Fenwick, P.S., Chana, K.K., Donnelly, L.E., Biswal, S., Ito, K. and Barnes, P.J. (2011) 'Decreased histone deacetylase 2 impairs Nrf2 activation oxidative stress.' *Biochemical and Biophysical Research Communications* [online] 406 (2), 292-8. available from <https://www.ncbi.nlm.nih.gov/pubmed/21320471> [8 February 2019]

Mezawa, M., Takemoto, M., Onishi, S., Ishibashi, R., Ishikawa, T., Yamaga, M., Fujimoto, M., Okabe, E., He, P., Kobayashi, K., and Yokote, K. (2012) 'The Reduced Form of Coenzyme Q10 Improves Glycemic Control in Patients with Type 2 Diabetes: An Open Label Pilot Study'. *BioFactors* [online] 38 (6), 416–421. available from <http://doi.wiley.com/10.1002/biof.1038> [30 November 2018]

Mihalas, B.P., De Iuliis, G.N., Redgrove, K.A., McLaughlin, E.A., and Nixon, B. (2017) 'The Lipid Peroxidation Product 4-Hydroxynonenal Contributes to Oxidative Stress-Mediated Deterioration of the Ageing Oocyte.' *Scientific Reports* [online] 7 (1), 6247. available from <http://www.ncbi.nlm.nih.gov/pubmed/28740075> [5 August 2017]

Min, K. and Ebeler, S.E. (2009) 'Quercetin Inhibits Hydrogen Peroxide-Induced DNA Damage and Enhances DNA Repair in Caco-2 Cells'. *Food and Chemical Toxicology* [online] 47 (11), 2716–2722. available from <http://dx.doi.org/10.1016/j.fct.2009.07.033>

Min, S., Jian-Bo, L., Hong-Man, Z., Ling-Fei, Y., Min, X., and Jia-Wei, C. (2012) 'Neuritin is expressed in Schwann cells and down-regulated in apoptotic Schwann cell under hyperglycaemia' *Nutritional Neuroscience* [online] 15 (6), 264-270. available from <https://www.tandfonline.com/doi/abs/10.1179/1476830512Y.0000000022?journalCode=ynns20> [21 June 2019]

Mitchell, P. (1961) 'Coupling of Phosphorylation to Electron and Hydrogen Transfer by a Chemi-Osmotic type of Mechanism'. *Nature* [online] 191, 141-48. available from <https://www.nature.com/articles/191144a0> [8 June 2019]

Mitchell, P. (2011) 'Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. 1966'. *Biochimica et Biophysica Acta - Bioenergetics* [online] 1807 (12), 1507-38. available from <https://www.sciencedirect.com/science/article/pii/S0005272811002283?via%3Dihub> [8 June 2019]

Miyazaki, I. and Asanuma, M. (2008) 'Dopaminergic Neuron-Specific Oxidative Stress Caused by Dopamine Itself Dopaminergic Neuron-Specific Oxidative Stress Caused by Dopamine Itself'. *Acta Medica Okayama* [online] 62 (3). available from <http://ousar.lib.okayama-u.ac.jp/files/public/3/30980/20160528022138672578/fulltext.pdf> [19 December 2017]

Moazen, M., Mazloom, Z., Ahmadi, A., Dabbaghmanesh, M.H., and Roosta, S. (2015) 'Effect of coenzyme Q10 on glycaemic control, oxidative stress and adiponectin in type 2 diabetes'. *Journal of the Pakistan Medical Association* [online] 65 (4), 404-8. available from <https://jpma.org.pk/article-details/7322> [21 June 2019]

Modi, K., Santani, D.D., Goyal, R.K., and Bhatt, P.A. (2006) 'Effect of coenzyme Q10 on catalase activity and other antioxidnat parameters in streptozotocin-induced diabetic rats'. *Biological Trace Element Research* [online] 109 (1), 25-34. available from <https://link.springer.com/article/10.1385%2FBTER%3A109%3A1%3A025> [21 June 2019]

Monticone, M., Taherian, R., Stigliani, S., Carra, E., Monteghirfo, S., Longo, L., Daga, A., Dono, M., Zupo, S., Giaretti, W., and Castagnola, P. (2014) 'NAC, Tiron and Trolox Impair Survival of Cell Cultures Containing Glioblastoma Tumorigenic Initiating Cells by Inhibition of Cell Cycle Progression'. *PLOS ONE* [online] 9 (2), e90085. available from <http://dx.plos.org/10.1371/journal.pone.0090085> [5 April 2017]

Monzo-Beltran, L., Vazquez-Tarragón, A., Cerdà, C., Garcia-Perez, P., Iradi, A., Sánchez, C., Climent, B., Tormos, C., Vázquez-Prado, A., Girbés, J., Estáñ, N., Blesa, S., Cortés, R., Chaves, F.J., and Sáez, G.T. (2017) 'One-Year Follow-up of Clinical, Metabolic and Oxidative Stress Profile of Morbid Obese Patients after Laparoscopic Sleeve Gastrectomy. 8-Oxo-DG as a Clinical Marker'. *Redox Biology* [online] 12, 289-402.

Mookherjee, P., Green, P.S., Watson, G.S., Marques, M.A., Tanaka, K., Meeker, K.D., Meabon, J.S., Li, N., Zhu, P., Olson, V.G., and Cook, D.G. (2011) 'GLT-1 loss accelerates cognitive deficit onset in an Alzheimer's disease animal model'. *Journal of Alzheimer's Disease* [online] 26 (3), 447-55. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3256092/> [18 June 2019]

Moon, G.J., Kim, S.J., Cho, Y.H., Ryoo, S. and Bang, O.Y. (2014) 'Antioxidant Effects of Statins in Patients with Atherosclerotic Cerebrovascular Disease' *Journal of Clinical Neurology* [online] 10 (2), 140-147. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4017017/> [15 June 2019]

Moreno-Macias, H. and Romieu, I. (2014) 'Effects of Antioxidant Supplements and Nutrients on Patients with Asthma and Allergies.' *The Journal of Allergy and Clinical Immunology* [online] 133 (5), 1237–44; quiz 1245. available from <http://www.ncbi.nlm.nih.gov/pubmed/24766873> [26 March 2018]

Morgan, A., Galal, M.K., Ogaly, H.A., Ibrahim, M.A., Abd-Elsalam, R.M., and Noshy, P. (2017) 'Tiron Ameliorates Oxidative Stress and Inflammation in Titanium Dioxide Nanoparticles Induced Nephrotoxicity of Male Rats'. *Biomedicine & Pharmacotherapy* [online] 93, 779–787. available from <http://linkinghub.elsevier.com/retrieve/pii/S0753332217321832> [24 August 2017]

Morgan, A., Ibrahim, M.A., Galal, M.K., Ogaly, H.A., and Abd-Elsalam, R.M. (2018) 'Innovative Perception on Using Tiron to Modulate the Hepatotoxicity Induced by Titanium Dioxide Nanoparticles in Male Rats'. *Biomedicine & Pharmacotherapy* [online] 103, 553–561. available from <https://www.sciencedirect.com/science/article/pii/S0753332217371263> [13 October 2018]

Morris, M.C., Evans, D.A., Bienias, J.L., Tangney, C.C., Bennett, D.A., Aggarwal, N., Wilson, R.S., and Scherr, P.A. (2002) 'Dietary Intake of Antioxidant Nutrients and the Risk of Incident Alzheimer Disease in a Biracial Community Study'. *JAMA* [online] 287 (24), 3230. available from <http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.287.24.3230> [14 August 2017]

Moussa, C., Hebron, M., Huang, X., Ahn, J., Rissman, R.A., Aisen, P.S., and Turner, R.S. (2017) 'Resveratrol regulates neuro-inflammation and induces adaptive immunity in Alzheimer's disease'. *Journal of Neuroinflammation* [online] 14 (1). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5234138/> [29 June 2019]

Mukherjee, M., Gupta, R., Farr, A., Heaven, M., Stoddart, A., Nwaru, B.I., Fitzsimmons, D., Chamberlain, G., Bandyopadhyay, A., Fischbacher, C., Dibben, C., Shields, M., Phillips, C., Strachan, D., Davies, G., McKinstry, B., Sheikh, A., McClelland, K., Hamilton, K., Burgess, K., Stewart, R., McVeigh, S., Murray, P., Gingles, J., Maguire, J., Kennedy, C., Myers, J., Doole, C., Rosato, M., Brunton, C., Walsh, C., McKeown, N., Fitzpatrick, S., McLoughlin, R., Vitty, A., Copyright, C., Batchelor, J., McShane, P., Fleming, S., Parslow, R., Robinson, E., Hickford, D., Dingle, H., Lloyd-Scott, L., Harrison, D., Martin, P., Hussey, L., Agius, R., Price, D., Von Ziegenweidt, J., Carter, V., Skinner, D., Hutton, C., Bathie, E., Chisolm, A., Been, J., Kotz, D., Payne, R., Julious, S., Goodacre, S., Devereux, G., Steiner, M., Weir, C., Parker, R., Feng, Z., McAllister, D., Carter, R., May, L., Limb, E., Vidal-Diez, A., Carey, I., Dickie, C., Malloy, A., Milligan, D., Barclay, K., Ho, D., Alexander, M., Burns, G., McAnaw, J., Hourcastagné, P., Ralph, L., Smith, A., Walker, C., Mikolajczak, D., Briggs, R., Hopkins, L., Wilson, L., Bell, M., Quirk, S., Morris, C., Simpson, M., Sisk, R., Haddow, C., Nowell, S., Hair, D., Duffy, A., Robertson, I., Pritchard, A., Peacock, J., Fleming, M., Kirby, B., Clark, D., Caldwell, J., Young, C., Bailey, A., Melrose, C., Morrice, L., Douglas, A., Bromley, C., Campbell, R., and Wierzoch, A. (2014) 'Estimating the Incidence, Prevalence and True Cost of Asthma in the UK: Secondary Analysis of National Stand-Alone and Linked Databases in England, Northern Ireland, Scotland and Wales-A Study Protocol'. *BMJ Open* [online] 4 (11). available from <http://dx.doi.org/> [17 May 2018]

Mueller, E.E., Eder, W., Ebner, S., Schqaifer,E., Santic, D., Kreindl, T., Stanger, O., Paulweber, B., Iglseder, B., Oberkofler, H., Maier, R., Mayr, J.A., Krempler, F., Weitgasser, R., Patsch, W., Sperl, W., and Kofler, B. (2011) 'The Mitochondrial T16189C Polymorphism Is Associated with Coronary Artery Disease in Middle European Populations'. *PLoS One* [online] 6 (1). available from <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0016455> [15 June 2019]

Mulder, S.D., Veerhuis, R., Blankenstein, M.A., and Nielsen, H.M. (2012) 'The effect of amyloid associated proteins on the expression of genes involved in amyloid-β clearance by adult human astrocytes'. *Experimental Neurology* [online] 233 (1), 373-379. available from <https://www.sciencedirect.com/science/article/pii/S0014488611004055> [22 June 2019]

Murphy, M.P. (2009) 'How Mitochondria Produce Reactive Oxygen Species.' *The Biochemical Journal* [online] 417 (1), 1–13. available from <http://www.biochemj.org/content/417/1/1.abstract> [9 July 2014]

Nadeem, A., Siddiqui, N., Alharbi, N.O., and Alharbi, M.M. (2014) ‘Airway and Systemic Oxidant-Antioxidant Dysregulation in Asthma: A Possible Scenario of Oxidants Spill over from Lung into Blood’. *Pulmonary Pharmacology & Therapeutics* [online] 29 (1), 31–40. available from <http://linkinghub.elsevier.com/retrieve/pii/S1094553914000674> [10 August 2017]

Nakamoto, K., Watanabe, M., Sada, M., Inui, T., Nakamura, M., Honda, K., Wada, H., Mikami, Y., Matsuzaki, H., Horie, M., Noguchi, S., Yamauchi, Y., Koyama, H., Kogane, T., Kohyama, T., and Takizawa, H. (2016) ‘Serum Reactive Oxygen Metabolite Levels Predict Severe Exacerbations of Asthma.’ *PLOS ONE* [online] 11 (10), e0164948. available from <http://www.ncbi.nlm.nih.gov/pubmed/27776186> [21 May 2018]

Namboori, S.C., and Graham, D.E. (2008) 'Enzymatic analysis of UCDP-N-acetylglucosamine'. *Analytical Biochemistry* [online] 381 (1), 94-100. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2597562/> [28 June 2019]

Nasri, H., Behradmanesh, S., Ahmadi, A., and Rafieian-Kopaei, M. (2014) ‘Impact of Oral Vitamin D (Cholecalciferol) Replacement Therapy on Blood Pressure in Type 2 Diabetes Patients; a Randomized, Double-Blind, Placebo Controlled Clinical Trial.’ *Journal of nephropathology* [online] 3 (1), 29–33. available from <http://www.ncbi.nlm.nih.gov/pubmed/24644541> [30 November 2018]

Nass, S. and Nass, M.M. (1963) ‘Intramitochondrial Fibers with DNA Characteristics’. *The Journal of Cell Biology* [online] 19 (3), 613–29. available from <http://www.ncbi.nlm.nih.gov/pubmed/14086139> [23 January 2017]

Nathan, D.M., Cleary, P.A., Backlund, J.-Y.C., Genuth, S.M., Lachin, J.M., Orchard, T.J., Raskin, P., Zinman, B., and Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group (2005) ‘Intensive Diabetes Treatment and Cardiovascular Disease in Patients with Type 1 Diabetes.’ *The New England Journal of Medicine* [online] 353 (25), 2643–53. available from <http://www.ncbi.nlm.nih.gov/pubmed/16371630> [7 January 2018]

Nayernia, Z., Jaquet, V., and Krause, K.-H. (2014) ‘New Insights on NOX Enzymes in the Central Nervous System.’ *Antioxidants & Redox Signaling* [online] 20 (17), 2815–37. available from <http://www.ncbi.nlm.nih.gov/pubmed/24206089> [17 July 2016]

Nemmar, A., Karaca, T., Beegam, S., Yuvaraju, P., Yasin, J., and Ali, B.H. (2017) 'Lung Oxidative Stress, DNA Damage, Apoptosis, and Fibrosis in Adenine-Induced Chronic Kidney Disease in Mice.' *Frontiers in Physiology* [online] 8, 896. available from <https://www.frontiersin.org/articles/10.3389/fphys.2017.00896/full> [11 March 2019]

Niedernhofer, L.J., Daniels, J.S., Rouzer, C.A., Greene, R.E., and Marnett, L.J. (2003) ‘Malondialdehyde, a Product of Lipid Peroxidation, Is Mutagenic in Human Cells.’ *The Journal of biological chemistry* [online] 278 (33), 31426–33. available from <http://www.ncbi.nlm.nih.gov/pubmed/12775726> [25 April 2018]

Nielsen, H.M., Mulder, S.D., Belien, J.A.M., Musters, R.J.P., Eikelenboom, P., and Veerhuis, R. (2010) ‘Astrocytic Aβ1-42 uptake is determined by Aβ-aggregation state and the presence of amyloid-associated proteins’. *Glia* [online] 58 (10). available from <https://onlinelibrary.wiley.com/doi/abs/10.1002/glia.21004> [18 June 2019]

Nijland, P.G., Witte, M.E., van het Hof, B., van der Pol, S., Bauer, J., Lassmann, H., van der Valk, P., de Vries, H.E., and van Horssen, J. (2014) ‘Astroglial PGC-1alpha increases mitochondrial antioxidant capacity and suppresses inflammation: implications for multiple sclerosis’. *Acta Neuropathologica Communications* [online] 2 (10). available from <https://actaneurocomms.biomedcentral.com/track/pdf/10.1186/s40478-014-0170-2> [21 June 2019]

Niki, E. (2014) ‘Role of Vitamin E as a Lipid-Soluble Peroxyl Radical Scavenger: In Vitro and in Vivo Evidence’. *Free Radical Biology and Medicine* [online] 66, 3–12. available from <http://www.sciencedirect.com/science/article/pii/S0891584913001275> [5 May 2017]

NINDS NET-PD Investigators, T.N.N.-P. (2007) ‘A Randomized Clinical Trial of Coenzyme Q10 and GPI-1485 in Early Parkinson Disease.’ *Neurology* [online] 68 (1), 20–8. available from <http://www.ncbi.nlm.nih.gov/pubmed/17200487> [28 March 2018]

Niranjan, R., Rajasekar, N., Nath, C., and Shukla, R. (2012) ‘The effect of guggulipid and nimesulide on MPTP-induced mediators of neuroinflammation in rat astrocytoma cells, C6’. *Chemico-biological Interactions* [online] 200 (2-3), 73-83. available from <http://europepmc.org/abstract/med/22940226> [22 June 2019]

Nixon, R.A., and Yang, D-S. (2012) 'Autophagy and Neuronal Cell Death in Neurological Disorders.' *Cold Spring Harbor Prespectives in Biology* [online] 4 (10). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3475163/> [24 November 2014]

Noeman, S.A., Hamooda, H.E., and Baalash, A.A. (2011) ‘Biochemical Study of Oxidative Stress Markers in the Liver, Kidney and Heart of High Fat Diet Induced Obesity in Rats.’ *Diabetology & Metabolic Syndrome* [online] 3 (1), 17. available from <http://www.ncbi.nlm.nih.gov/pubmed/21812977> [8 February 2018]

Nomiyama, T., Tanaka, Y., Piao, L., Hattori, N., Uchino, H., Watada, H., Kawamori, R., and Ohta, S. (2006) ‘Accumulation of Somatic Mutation in Mitochondrial DNA and Atherosclerosis in Diabetic Patients’. *Annals of the New York Academy of Sciences [*online] 1011 (1). available from <https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1196/annals.1293.020> [15 June 2019]

Nowicka, B., Żądło, A., Pluciński, B., Kruk, J., and Kuczyńska, P. (2017) ‘The Oxidative Stress in Allelopathy: Participation of Prenyllipid Antioxidants in the Response to Juglone in Chlamydomonas Reinhardtii’. *Phytochemistry* [online] 144, 171–179. available from <https://www.sciencedirect.com/science/article/pii/S0031942217303175> [25 August 2018]

Oberg, B.P., McMenamin, E., Lucas, F.L., McMonagle, E., Morrow, J., Ikizler, T.A., and Himmelfarb, J. (2004) ‘Increased Prevalence of Oxidant Stress and Inflammation in Patients with Moderate to Severe Chronic Kidney Disease’. *Kidney International* 65 (3), 1009–1016

Obrosova, I.G. (2005) ‘Increased Sorbitol Pathway Activity Generates Oxidative Stress in Tissue Sites for Diabetic Complications’. *Antioxidants & Redox Signaling* [online] 7 (11–12), 1543–1552. available from <http://www.liebertonline.com/doi/abs/10.1089/ars.2005.7.1543> [14 June 2018]

Ochs-Balcom, H., Grant, B., Muti, P., Sempos, C., Freudenheim, J., Browne, R., Mccann, S., Trevisan, M., Cassano, P., Iacoviello, L., and Schünemann, H. (2006) ‘Antioxidants, Oxidative Stress, and Pulmonary Function in Individuals Diagnosed with Asthma or COPD’. *European Journal of Clinical Nutrition* [online] 60 (60), 991–999. available from <www.nature.com/ejcn> [26 March 2018]

Officioso, A., Panzella, L., Tortora, F., Alfieri, M.L., Napolitano, A., and Manna, C. (2018) ‘Comparative Analysis of the Effects of Olive Oil Hydroxytyrosol and Its 5-S-Lipoyl Conjugate in Protecting Human Erythrocytes from Mercury Toxicity‘. *Oxidative Medicine and Cellular Longevity* [online] 2018. available from <https://www.hindawi.com/journals/omcl/2018/9042192/> [29 June 2019]

Ogawa, K., Suzuki, K., Okutsu, M., Yamazaki, K., Shinkai, S., Khuseyinova, N., Koenig, W., Koenig, W., Khuseyinova, N., Baumert, J., Thorand, B., Loewel, H., Chambless, L., Meisinger, C., Schneider, A., Martin, S., Kolb, H., Herder, C., Kalantar-Zadeh, K., Kopple, J., Humphreys, M., Block, G., Ridker, P., Rifai, N., Stampfer, M., Hennekens, C., Harris, T., Ferrucci, L., Tracy, R., Corti, M., Wacholder, S., Ettinger, W., Heimovitz, H., Cohen, H., Wallace, R., Ridker, P., Rifai, N., Pfeffer, M., Sacks, F., Lepage, S., Braunwald, E., Ridker, P., Hennekens, C., Buring, J., Rifai, N., Petersen, A., Pedersen, B., Koenig, W., Khuseyinova, N., Nagano, M., Nakamura, M., Sato, K., Tanaka, F., Segawa, T., Hiramori, K., Nakamura, M., Onoda, T., Itai, K., Ohsawa, M., Satou, K., Sakai, T., Segawa, T., Sasaki, J., Tonari, Y., Hiramori, K., Okayama, A., Libby, P., Suzuki, K., Sato, H., Kikuchi, T., Abe, T., Nakaji, S., Sugawara, K., Totsuka, M., Sato, K., Yamaya, K., Lakshman, R., Bruce, S., Spencer, D., Crawford, D., Galloway, A., Cooper, P., Barge, D., Roos, D., Flood, T., Abinun, M., Harman, D., Sastre, J., Pallardó, F., Asunción, J.G. de la, Viña, J., Sela, S., Shurtz-Swirski, R., Cohen-Mazor, M., Mazor, R., Chezar, J., Shapiro, G., Hassan, K., Shkolnik, G., Geron, R., Kristal, B., Oostrom, A. van, Sijmonsma, T., Verseyden, C., Jansen, E., Koning, E. de, Rabelink, T., Cabezas, M.C., Fialkow, L., Wang, Y., Downey, G., Walrand, S., Moreau, K., Caldefie, F., Tridon, A., Chassagne, J., Portefaix, G., Cynober, L., Beaufrère, B., Vasson, M., Boirie, Y., Plackett, T., Boehmer, E., Faunce, D., Kovacs, E., Haumer, M., Amighi, J., Exner, M., Mlekusch, W., Sabeti, S., Schlager, O., Schwarzinger, I., Wagner, O., Minar, E., Schillinger, M., Madjid, M., Awan, I., Willerson, J., Casscells, S., Lindquist, S., Craig, E., Njemini, R., Abeele, M., Demanet, C., Lambert, M., Vandebosch, S., Mets, T., Njemini, R., Bautmans, I., Lambert, M., Demanet, C., Mets, T., Rothe, G., Valet, G., Hasegawa, H., Suzuki, K., Nakaji, S., Sugawara, K., Shinkai, S., Shore, S., Shek, P., and Shephard, R. (2008) ‘The Association of Elevated Reactive Oxygen Species Levels from Neutrophils with Low-Grade Inflammation in the Elderly’. *Immunity & Ageing* [online] 5 (1), 13. available from <http://immunityageing.biomedcentral.com/articles/10.1186/1742-4933-5-13> [17 October 2016]

Oh, J.Y., Choi, G.E., Lee, H.J., Jung, Y.H., Chae, C.W., Kim, J.S., Lee, C.. and Han, H.J. (2019) '17β-Estradiol protects mesenchymal stem cells against high glucose-induced mitochondrial oxidants production via Nrf2/Sirt3/MnSOD signaling'. *Free Radical Biology and Medicine* [online] 130, 328-42. available from <https://www.sciencedirect.com/science/article/pii/S0891584918313601?via%3Dihub> [22 June 2019]

Oh, M. and Nör, J.E. (2015) ‘The Perivascular Niche and Self-Renewal of Stem Cells.’ *Frontiers in Physiology* [online] 6, 367. available from <http://www.ncbi.nlm.nih.gov/pubmed/26696901> [4 December 2018]

Olea, E., Ferrer, E., Prieto-Lloret, J., Gonzalez-Martin, C., Vega-Agapito, V., Gonzalez-Obeso, E., Agapito, T., Peinado, V., Obeso, A., Barbera, J.A., and Gonzalez, C. (2011) ‘Effects of Cigarette Smoke and Chronic Hypoxia on Airways Remodeling and Resistance. Clinical Significance’. *Respiratory Physiology & Neurobiology* [online] 179 (2–3), 305–313. available from <https://www.sciencedirect.com/science/article/pii/S1569904811003478> [27 August 2018]

Olsen, M., Aguilar, X., Sehlin, D., Fang, X.T., Antoni,G., Erlandsson, A., Syvanen, S. (2018) ‘Astroglial Responses to Amyloid-Beta Progression in a Mouse Model of Alzheimer’s Disease ‘. *Molecular Imaging and Biology* [online] 20 (4), 605-14. available from <https://link.springer.com/article/10.1007/s11307-017-1153-z> [18 June 2019]

Olson, N., Hristova, M., Heintz, N.H., Lounsbury, K.M., and van der Vliet, A. (2011) ‘Activation of Hypoxia-Inducible Factor-1 Protects Airway Epithelium against Oxidant-Induced Barrier Dysfunction.’ *American Journal of Physiology. Lung Cellular and Molecular Physiology* [online] 301 (6), L993–L1002. available from <http://www.ncbi.nlm.nih.gov/pubmed/21926263> [22 January 2019]

Ortiz, G.G., Pacheco-Moisés, F., El Hafidi, M., Jiménez-Delgado, A., Macías-Islas, M.A., Rosales Corral, S.A., Célis de la Rosa, A., Sánchez-González, V.J., Arias-Merino, E.D., and Velázquez-Brizuela, I.E. (2008) 'Detection of Membrane Fluidity in Submitochondrial Particles of Platelets and Erythrocyte Membranes from Mexican Patients with Alzheimer Disease by Intramolecular Excimer Formation of 1,3 Dipyrenylpropane.' *Disease Markers* [online] 24 (3), 151-6. availble from <https://www.hindawi.com/journals/dm/2008/642120/abs/> [12 March 2019]

Otteneder, M.B., Knutson, C.G., Daniels, J.S., Hashim, M., Crews, B.C., Remmel, R.P., Wang, H., Rizzo, C., and Marnett, L.J. (2006) 'In Vivo Oxidative Metabolism of a Major Peroxidation-Derived DNA Adduct, M1dG.' *Proceedings of the National Academy of Sciences of the United States of America* [online] 103 (17), 6665–9. available from <http://www.ncbi.nlm.nih.gov/pubmed/16614064> [14 November 2018]

Owusu-Ansah, E. and Banerjee, U. (2009) 'Reactive Oxygen Species Prime Drosophila Haematopoietic Progenitors for Differentiation'. *Nature* [online] 461 (7263), 537–541. available from <http://www.nature.com/articles/nature08313> [19 December 2018]

Oyewole, A.O. and Birch-Machin, M.A. (2015) 'Mitochondria-Targeted Antioxidants.' *FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology* [online] 29 (12), 4766–71. available from <http://www.ncbi.nlm.nih.gov/pubmed/26253366> [24 August 2017]

Oyewole, A.O., Wilmot, M.C., Fowler, M., and Birch-Machin, M.A. (2014) 'Comparing the Effects of Mitochondrial Targeted and Localized Antioxidants with Cellular Antioxidants in Human Skin Cells Exposed to UVA and Hydrogen Peroxide'. *FASEB Journal* 28 (1), 485–494

Ozaki, E., Campbell, M., and Doyle, S.L. (2015) 'Targeting the NLRP3 Inflammasome in Chronic Inflammatory Diseases: Current Perspectives.' *Journal of Inflammation Research* [online] 8, 15–27. available from <http://www.ncbi.nlm.nih.gov/pubmed/25653548> [13 October 2016]

Ozcankaya, R. and Delibas, N. (2002) 'Malondialdehyde, Superoxide Dismutase, Melatonin, Iron, Copper, and Zinc Blood Concentrations in Patients with Alzheimer Disease: Cross-Sectional Study'. *Clinical Sciences* [online] 43 (1), 28–32. available from <https://pdfs.semanticscholar.org/face/f495317e923f411ae345931b617e1e554aa6.pdf> [23 August 2017]

Öztürk, E., Arslan, A.K.K., Yerer, M.B., and Bishayee, A. (2017) 'Resveratrol and Diabetes: A Critical Review of Clinical Studies'. *Biomedicine & Pharmacotherapy* [online] 95, 230–234. available from <https://www.sciencedirect.com/science/article/pii/S0753332217327683?via%3Dihub> [25 March 2018]

de Pablos, R.M., Esponosa-Oliva, A.M., Hornedo-Ortega, R., Cano, M., and Arguelles, S. (2019) 'Hydroxytyrosol protects from aging process via AMPK and autophagy; a review of its effects on cancer, metabolic syndrome, osteoporosis, immune-mediated and neurodegenerative diseases'. *Pharmacological Research* [online] 143, 58-72. available from <https://www.sciencedirect.com/science/article/abs/pii/S1043661819301045> [29 June 2019]

Padgett, L.E., Broniowska, K.A., Hansen, P.A., Corbett, J.A., and Tse, H.M. (2013) 'The Role of Reactive Oxygen Species and Proinflammatory Cytokines in Type 1 Diabetes Pathogenesis.' *Annals of the New York Academy of Sciences* [online] 1281 (1), 16–35. available from <http://www.ncbi.nlm.nih.gov/pubmed/23323860> [24 September 2017]

Palacios-Callender, M., Quintero, M., Hollis, V.S., Springett, R.J., and Moncada, S. (2004) 'Endogenous NO Regulates Superoxide Production at Low Oxygen Concentrations by Modifying the Redox State of Cytochrome c Oxidase.' *Proceedings of the National Academy of Sciences of the United States of America* [online] 101 (20), 7630–5. available from <http://www.ncbi.nlm.nih.gov/pubmed/15136725> [21 April 2018]

Pálmai-Pallag, T. and Bachrati, C.Z. (2014) 'Inflammation-Induced DNA Damage and Damage-Induced Inflammation: A Vicious Cycle.' *Microbes and infection / Institut Pasteur* [online] 16 (10), 822–32. available from <http://www.ncbi.nlm.nih.gov/pubmed/25449753> [13 October 2016]

Palsamy, P. and Subramanian, S. (2011) 'Resveratrol Protects Diabetic Kidney by Attenuating Hyperglycemia-Mediated Oxidative Stress and Renal Inflammatory Cytokines via Nrf2–Keap1 Signaling'. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* [online] 1812 (7), 719–731. available from <https://www.sciencedirect.com/science/article/pii/S0925443911000639> [4 May 2018]

Pan, Y., Mansfield, K.D., Bertozzi, C.C., Rudenko, V., Chan, D.A., Giaccia, A.J., and Simon, M.C. (2007) 'Multiple Factors Affecting Cellular Redox Status and Energy Metabolism Modulate Hypoxia-Inducible Factor Prolyl Hydroxylase Activity In Vivo and In Vitro'. *Molecular and Cellular Biology* [online] 27 (3), 912-925. available from < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1800695/> [16 June 2019]

Panat, N.A., Maurya, D.K., Ghaskadbi, S.S., and Sandur, S.K. (2016) ‘Troxerutin, a Plant Flavonoid, Protects Cells against Oxidative Stress-Induced Cell Death through Radical Scavenging Mechanism’. *Food Chemistry* 194, 32–45

Pandey, A.K., Bhattacharya, P., Shukla, S.C., Paul, S., and Patnaik, R. (2015) ' Resveratrol inhibits matrix metalloproteinases to attenuate neuronal damage in cerebral ischemia: a molecular docking study exploring possible neuroprotection'. *Neural Regeneration Researc*h [online] 10 (4), 568-75. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4424748/>

Paracha, U.Z., Fatima, K., Alqahtani, M., Chaudhary, A., Abuzenadah, A., Gamanhouri, G., and Qadri, I. (2013) 'Oxidative stress and hepatitis C virus'. *Virology Journa*l [online] 10 (251). available from < https://link.springer.com/article/10.1007/s13277-016-4873-9> [9 March 2019]

Parfenova, H., Leffler, C.W., Basuroy, S., Liu, J., and Fedinec, A.L. (2012) ‘Antioxidant Roles of Heme Oxygenase, Carbon Monoxide, and Bilirubin in Cerebral Circulation during Seizures.’ *Journal of Cerebral Blood Flow and Metabolism: Official Journal of the International Society of Cerebral Blood Flow and Metabolism* [online] 32 (6), 1024–34. available from <http://www.ncbi.nlm.nih.gov/pubmed/22354150> [2 March 2018]

Park, E.-J. and Park, K. (2007) ‘Induction of Reactive Oxygen Species and Apoptosis in BEAS-2B Cells by Mercuric Chloride’. *Toxicology in Vitro* 21 (5), 789–794

Park, E.-J., Yi, J., Chung, K.-H., Ryu, D.-Y., Choi, J., and Park, K. (2008) ‘Oxidative Stress and Apoptosis Induced by Titanium Dioxide Nanoparticles in Cultured BEAS-2B Cells’. *Toxicology Letters* [online] 180 (3), 222–229. available from <https://www.sciencedirect.com/science/article/pii/S0378427408010552> [19 March 2018]

Park, H.S., Kim, S.R., and Lee, Y.C. (2009) 'Impact of Oxidative Stress on Lung Diseases'. *Respirology* [online]14, 27-38 available from <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1440-1843.2008.01447.x> [5 September 2018]

Park, S.J., Lee, J.H., Kim, H.Y., Choi, Y.H., Park, J.S., Suh, Y.H., Park, S.M., Joe, E., and Jou, I. (2012) ‘Astrocytes, but Not Microglia, Rapidly Sense H_2O_2via STAT6 Phosphorylation, Resulting in Cyclooxygenase-2 Expression and Prostaglandin Release.’ *Journal of Immunology* [online] 188 (10), 5132–41. available from <http://www.ncbi.nlm.nih.gov/pubmed/22504638> [22 January 2018]

Passos, J.F., Saretzki, G., Ahmed, S., Nelson, G., Richter, T., Peters, H., Wappler, I., Birket, M.J., Harold, G., Schaeuble, K., Birch-Machin, M.A., Kirkwood, T.B.L., and von Zglinicki, T. (2007) ‘Mitochondrial Dysfunction Accounts for the Stochastic Heterogeneity in Telomere-Dependent Senescence.’ *PLOS Biology* [online] 5 (5), e110. available from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1858712&tool=pmcentrez&rendertype=abstract> [5 March 2016]

Pastukh, V.M., Zhang, L., Ruchko, M. V, Gorodnya, O., Bardwell, G.C., Tuder, R.M., and Gillespie, M.N. (2011) ‘Oxidative DNA Damage in Lung Tissue from Patients with COPD Is Clustered in Functionally Significant Sequences.’ *International Journal of Chronic Obstructive Pulmonary Disease* [online] 6, 209–17. available from <http://www.ncbi.nlm.nih.gov/pubmed/21660298> [19 November 2017]

Patel, V., Kanani, D., Chauhan, K., Haridas, N., Makadia, M., and Patel, V. (2015) ‘Assessment of Oxidative Stress in Type 2 Diabetes Mellitus by Measurement of Plasma Lipid Hydroperoxides and Total Antioxidant Capacity’. *International Journal of Advanced Research* [online] 3 (5), 1078–1084. available from <http://www.journalijar.com/uploads/567_IJAR-5849.pdf> [6 May 2018]

Pearson, J., Pullar, J., Wilson, R., Spittlehouse, J., Vissers, M., Skidmore, P., Willis, J., Cameron, V., Carr, A., Pearson, J.F., Pullar, J.M., Wilson, R., Spittlehouse, J.K., Vissers, M.C.M., Skidmore, P.M.L., Willis, J., Cameron, V.A., and Carr, A.C. (2017) ‘Vitamin C Status Correlates with Markers of Metabolic and Cognitive Health in 50-Year-Olds: Findings of the CHALICE Cohort Study’. *Nutrients* [online] 9 (8), 831. available from <http://www.mdpi.com/2072-6643/9/8/831> [6 September 2018]

Peng, S., Zhang, B., Yao, J., Duan, D., and Fang, J. (2015) ‘Dual protection of hydroxytyrosol, an olive oil polyphenol, against oxidative damage in PC12 cells‘. *Food & Function* [online] 6 (6). 2091-100. available from <https://pubs.rsc.org/en/content/articlelanding/2015/FO/C5FO00097A#!divAbstract> [29 June 2019]

Pereira, C., Coelho, R., Gracio, D., Dias, C., Silva, M., Peixoto, A., Lopes, P., Costa, C., Teixeira, J.P., Macedo, F., and Magro, F. (2016) 'DNA Damage and OxidativeDNA Damage in Inflammatory Bowel Disease.' *Journal of Crohn's & Colitis* [online] 10 (11), 1316-23. available from <https://academic.oup.com/ecco-jcc/article/10/11/1316/2479998> [9 March 2019]

Pérez-Rodríguez, L., Romero-Haro, A.A., Sternalski, A., Muriel, J., Mougeot, F., Gil, D., and Alonso-Alvarez, C. (2015) 'Measuring Oxidative Stress: The Confounding Effect of Lipid Concentration in Measures of Lipid Peroxidation'. *Physiological and Biochemical Zoology* [online] 88 (3), 345–351. available from <http://www.journals.uchicago.edu/doi/10.1086/680688>

Petrillo, L.A., Gan, S., Jing, B., Lang-Brown, S., Boscardin, W.J., and Lee, S.J. (2018) 'Hypoglycemia in Hospice Patients With Type 2 Diabetes in a National Sample of Nursing Homes'. *JAMA Internal Medicine* [online] 178 (5), 713. available from <http://archinte.jamanetwork.com/article.aspx?doi=10.1001/jamainternmed.2017.7744> [13 July 2018]

Pham-Huy, L.A., He, H., and Pham-Huy, C. (2008) 'Free Radicals, Antioxidants in Disease and Health'. *International Journal of Biomedical Science* [online] 4 (2), 89–96. available from <https://pdfs.semanticscholar.org/162a/2b26ae37c18cb0d8ef875f581d57538c6108.pdf>

Phull, A.-R., Nasir, B., Haq, I. ul, and Kim, S.J. (2018) 'Oxidative Stress, Consequences and ROS Mediated Cellular Signaling in Rheumatoid Arthritis'. *Chemico-Biological Interactions* [online] 281, 121–136. available from <https://www.sciencedirect.com/science/article/pii/S0009279717305239> [16 February 2019]

Pi, J., Qu, W., Reece, J.M., Kumagai, Y., and Waalkes, M.P. (2003) 'Transcription Factor Nrf2 Activation by Inorganic Arsenic in Cultured Keratinocytes: Involvement of Hydrogen Peroxide'. *Experimental Cell Research* [online] 290 (2), 234–245. available from <http://linkinghub.elsevier.com/retrieve/pii/S0014482703003410> [25 August 2017]

Piao, M.J., Kang, K.A., Zhang, R., Ko, D.O., Wang, Z.H., You, H.J., Kim, H.S., Kim, J.S., Kang, S.S., and Hyun, J.W. (2008) 'Hyperoside Prevents Oxidative Damage Induced by Hydrogen Peroxide in Lung Fibroblast Cells via an Antioxidant Effect'. *Biochimica et Biophysica Acta (BBA) - General Subjects* [online] 1780 (12), 1448–1457. available from <https://linkinghub.elsevier.com/retrieve/pii/S0304416508001633> [21 January 2019]

Pierrot, N., Santos, S.F., Feyt, C., Morel, M., Brion, J., and Octave, J. (2006) 'Calcium-mediated Transient Phosphorylation of Tau and Amyloid Precursor Protein Followed by Intraneuronal Amyloid-β Accumulation'. *Journal of Biological Chemistry* [online] 281, 39907-14. available from <http://www.jbc.org/content/281/52/39907.long>

Pisoschi, A.M. and Pop, A. (2015) 'The Role of Antioxidants in the Chemistry of Oxidative Stress: A Review'. *European Journal of Medicinal Chemistry* 97, 55–74

Plotnikov, E.Y., Morosanova, M. a, Pevzner, I.B., Zorova, L.D., Manskikh, V.N., Pulkova, N. V, Galkina, S.I., Skulachev, V.P., and Zorov, D.B. (2013) 'Protective Effect of Mitochondria-Targeted Antioxidants in an Acute Bacterial Infection.' *Proceedings of the National Academy of Sciences of the United States of America* [online] 110 (33), E3100-8. available from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3746855&tool=pmcentrez&rendertype=abstract>

Poljsak, B. and Milisav, I. (2012) 'The Neglected Significance of "Antioxidative Stress"'. *Oxidative Medicine and Cellular Longevity* [online] 2012. available from <file:///C:/Users/Student/Downloads/480895 (1).pdf> [7 May 2018]

Popa-Wagner, A., Mitran, S., Sivanesan, S., Chang, E., and Buga, A. (2013) 'ROS and Brain Diseases : The Good , the Bad , and the Ugly'. *Oxidative Medicine and Cellular Longevity* 2013

Porter, K.M., Kang, B.-Y., Adesina, S.E., Murphy, T.C., Hart, C.M., and Sutliff, R.L. (2014) 'Chronic Hypoxia Promotes Pulmonary Artery Endothelial Cell Proliferation through H2O2-Induced 5-Lipoxygenase'. *PLOS ONE* [online] 9 (6), e98532. available from <http://dx.plos.org/10.1371/journal.pone.0098532> [24 August 2018]

Prakash, S., Sunitha, J., and Hans, M. (2010) 'Role of Coenzyme Q(10) as an Antioxidant and Bioenergizer in Periodontal Diseases.' *Indian Journal of Pharmacology* [online] 42 (6), 334–7. available from <http://www.ncbi.nlm.nih.gov/pubmed/21189900> [2 May 2017]

Prasad, K. and Dhar, I. (2014) 'Oxidative Stress as a Mechanism of Added Sugar-Induced Cardiovascular Disease.' *The International Journal of Angiology: Official Publication of the International College of Angiology, Inc* [online] 23 (4), 217–26. available from <http://www.ncbi.nlm.nih.gov/pubmed/25484552> [21 April 2018]

Praticò, D., Uryu, K., Leight, S., Trojanoswki, J.Q., and Lee, V.M. (2001) 'Increased Lipid Peroxidation Precedes Amyloid Plaque Formation in an Animal Model of Alzheimer Amyloidosis.' *The Journal of Neuroscience: the Official Journal of the Society for Neuroscience* [online] 21 (12), 4183–7. available from <http://www.ncbi.nlm.nih.gov/pubmed/11404403> [19 August 2018]

Pryde, K.R. and Hirst, J. (2011) 'Superoxide Is Produced by the Reduced Flavin in Mitochondrial Complex I: A Single, Unified Mechanism That Applies during Both Forward and Reverse Electron Transfer.' *The Journal of Biological Chemistry* [online] 286 (20), 18056–65. available from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3093879&tool=pmcentrez&rendertype=abstract> [19 January 2016]

Puente-Maestu, L., Perez-Parra, J., Godoy, R., Moreno, N., Tejedor, A., Gonzalez-Aragoneses, F., Bravo, J.-L., Villar Alvarez, F., Camano, S., and Agusti, A. (2009) 'Abnormal Mitochondrial Function in Locomotor and Respiratory Muscles of COPD Patients'. *European Respiratory Journal* [online] 33 (5), 1045–1052. available from <http://www.ncbi.nlm.nih.gov/pubmed/19129279> [22 September 2018]

Purushothaman, J., Suryakumar, G., Shukla, D., Jayamurthy, H., Kasiganesan, H., Kumar, R., and Sawhney, R.C. (2011) 'Modulation of Hypoxia-Induced Pulmonary Vascular Leakage in Rats by Seabuckthorn (Hippophae Rhamnoides L.).' *Evidence-based Complementary and Alternative Medicine : eCAM* [online] 2011, 574524. available from <http://www.ncbi.nlm.nih.gov/pubmed/19996155> [22 January 2019]

Puspita, L., Chung, S.Y., and Shim, J.-W. (2017) 'Oxidative Stress and Cellular Pathologies in Parkinson's Disease.' *Molecular Brain* [online] 10 (1), 53. available from <http://www.ncbi.nlm.nih.gov/pubmed/29183391> [16 February 2019]

Qi, X., Sun, L., Hauswirth, W.W., Lewin, A.S., and Guy, J. (2007) 'Use of mitochondrial antioxidant defenses for rescue of cells with a Leber hereditary optic neuropathy-causing mutation'. Archives of Opthalmology [online] 125 (2), 268-72. available from <https://jamanetwork.com/journals/jamaophthalmology/fullarticle/817094> [15 June 2019]

Qin, Z., Hu, D., Han, S., Reaney, S.H., Di Monte, D.A., and Fink, A.L. (2007) 'Effect of 4-hydroxy-2-nonenal modification on alpha-synuclein aggregation'. *Journal of Biological Chemistry* [online] 282 (8), 5862-70. available from <http://www.jbc.org/content/282/8/5862.long> [21 June 2019]

Qiu, C., Hevner, K., Abetew, D., Sednesky, M., Morgan, P., Enquobahrie, D.A., and Williams, M.A. (2013) 'Mitochondrial DNA copy number and oxidative DNA damage in placental tissues from gestational diabetes and control pregnancies: a pilot study'. *Clinical Laboratory* [online] 59 (5-6), 655-60. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4143244/> [27 June 2019]

Qu, J., Li, Y., Zhong, W., Gao, P., and Hu, C. (2017) 'Recent Developments in the Role of Reactive Oxygen Species in Allergic Asthma.' *Journal of thoracic disease* [online] 9 (1), E32–E43. available from <http://www.ncbi.nlm.nih.gov/pubmed/28203435> [22 May 2018]

Quincozes-Santos, A., Bobermin, L.D., Latini, A., Wajner, M., Souza, D.O., Gonçalves, C.-A., and Gottfried, C. (2013) 'Resveratrol Protects C6 Astrocyte Cell Line against Hydrogen Peroxide-Induced Oxidative Stress through Heme Oxygenase 1'. *PLOS ONE* [online] 8 (5), e64372. available from <http://dx.plos.org/10.1371/journal.pone.0064372> [28 June 2018]

Quindry, J., Dumke, C., Slivka, D., and Ruby, B (2015) 'Impact of extreme exercise at high altitude on oxidative stress in humans.' *Journal of Physiology* [online] 594 (18), 5093-104. available from <https://physoc.onlinelibrary.wiley.com/doi/pdf/10.1113/JP270651> [23 March 2018]

Quintero, M., Brennan, P.A., Thomas, G.J., and Moncada, S. (2006) ‘Nitric Oxide Is a Factor in the Stabilization of Hypoxia-Inducible Factor-1α in Cancer: Role of Free Radical Formation‘. *Cancer Research* [online] 66 (2). available from <http://cancerres.aacrjournals.org/content/66/2/770> [16 June 2019]

Racusen, L.C., Monteil, C., Sgrignoli, A., Lucskay, M., Marouillat, S., Rhim, J.G.S., and Morin, J. (1997) ‘Cell Lines with Extended in Vitro Growth Potential from Human Renal Proximal Tubule: Characterization, Response to Inducers, and Comparison with Established Cell Lines’. *Journal of Laboratory and Clinical Medicine* [online] 129 (3), 318–329. available from <http://linkinghub.elsevier.com/retrieve/pii/S0022214397901803> [16 March 2018]

Rahman, I. (2008) ‘Antioxidant Therapeutic Advances in COPD.’ *Therapeutic Advances in Respiratory Disease* [online] 2 (6), 351–74. available from <http://www.ncbi.nlm.nih.gov/pubmed/19124382> [19 November 2017]

Rahman, I. (2012) ‘Pharmacological Antioxidant Strategies as Therapeutic Interventions for COPD’. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* [online] 1822 (5), 714–728. available from <http://www.ncbi.nlm.nih.gov/pubmed/22101076> [17 January 2017]

Rahmoune, H., Thompson, P.W., Ward, J.M., Smith, C.D., Hong, G., and Brown, J. (2005) *Glucose Transporters in Human Renal Proximal Tubular Cells Isolated From the Urine of Patients With Non-Insulin-Dependent Diabetes*. [online] available from <http://diabetes.diabetesjournals.org/content/diabetes/54/12/3427.full.pdf> [13 July 2018]

Rains, J.L. and Jain, S.K. (2011) ‘Oxidative Stress, Insulin Signaling, and Diabetes.’ *Free Radical Biology and Medicine* [online] 50 (5), 567–75. available from <http://www.ncbi.nlm.nih.gov/pubmed/21163346> [7 January 2018]

Rajendran, P., Nandakumar, N., Rengarajan, T., Palaniswami, R., Gnanadhas, E.N., Lakshminarasaiah, U., Gopas, J., and Nishigaki, I. (2014) ‘Antioxidants and Human Diseases’. *Clinica Chimica Acta* [online] 436, 332–347. available from <http://dx.doi.org/10.1016/j.cca.2014.06.004> [12 May 2015]

Ramana, K.V., Friedrich, B., Tammali, R., West, M.B., Bhatnagar, A., and Srivastava, S.K. (2005) 'Requirement of Aldose Reductase for the Hyperglycemic Activation of Protein Kinase C and Formation of Diacylglycerol in Vascular Smooth Muscle Cells'. *Diabetes* [online] 54 (3), 818-29. available from <https://diabetes.diabetesjournals.org/content/54/3/818>

Ramos-Espinosa, P., Rojas, E., and Valverde, M. (2012) ‘Differential DNA Damage Response to UV and Hydrogen Peroxide Depending of Differentiation Stage in a Neuroblastoma Model’. *NeuroToxicology* [online] 33 (5), 1086–1095. available from <http://dx.doi.org/10.1016/j.neuro.2012.05.017> [10 May 2016]

Rani, P., Krishnan, S., and Cathrine, C.R. (2017) ‘Study on Analysis of Peripheral Biomarker for Alzheimer’s Disease Diagnosis’. *Frontiers in Neurology* [online] 8 (328). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5509803/> [21 June 2019]

Rashidipour, M., Hajializadeh, Z., Esmaeili-Mahani, S., and Kaeidi, A. (2017) 'The Effect of Rosmarinus officinalis LExtract on the Inhibition of High Glucose-Induced Neurotoxicity in PC12Cells: an In Vitro Model of Diabetic Neuropathy' *Herbal Medicines Journal* [online] 2 (3), 114-21. available from <https://www.researchgate.net/publication/324167773_The_Effect_of_Rosmarinus_officinalis_L_Extract_on_the_Inhibition_of_High_Glucose-Induced_Neurotoxicity_in_PC12_Cells_an_In_Vitro_Model_of_Diabetic_Neuropathy> [21 June 2019]

Rawshani, A., Rawshani, A., Franzén, S., Eliasson, B., Svensson, A.-M., Miftaraj, M., McGuire, D.K., Sattar, N., Rosengren, A., and Gudbjörnsdottir, S. (2017) ‘Mortality and Cardiovascular Disease in Type 1 and Type 2 Diabetes’. *New England Journal of Medicine* [online] 376 (15), 1407–1418. available from <http://www.nejm.org/doi/10.1056/NEJMoa1608664> [16 December 2018]

Raza, H., Prabu, S.K., John, A., and Avadhani, N.G. (2011) 'Impaired Mitochondrial Respiratory Functions and Oxidative Stress in Streptozotocin-Induced Diabetic Rats'. *International Journal of Molecular Sciences* [online] 12 (5), 3133-47. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3116180/> [26 June 2019]

Reddel, R.R., De Silva, R., Duncan, E.L., Rogan, E.M., Whitaker, N.J., Zahra, D.G., Ke, Y., McMenamin, M.G., Gerwin, B.I., and Harris, C.C. (1995) 'SV40-Induced Immortalization and Ras-Transformation of Human Bronchial Epithelial Cells.' *International Journal of Cancer* [online] 61 (2), 199–205. available from <http://www.ncbi.nlm.nih.gov/pubmed/7705948> [8 April 2018]

Reddy, P.H. (2011) 'Mitochondrial Dysfunction and Oxidative Stress in Asthma: Implications for Mitochondria-Targeted Antioxidant Therapeutics.' *Pharmaceuticals* [online] 4 (3), 429–456. available from <http://www.ncbi.nlm.nih.gov/pubmed/21461182> [17 January 2017]

Reinhardt, H.C., and Schumacher, N. (2012) ' The p53 network: Cellular and systemic DNA damage responses in aging and cancer.' *Trends in Genetics* [online] 28 (3), 128-36. available from < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4120491/> [11 October 2014]

Reis, G.S., Augusto, V.S., Silveira, A.P.C., Jordão, A.A., Baddini-Martinez, J., Poli Neto, O., Rodrigues, A.J., Evora, P.R.B., and Evora, P.R.B. (2013) 'Oxidative-Stress Biomarkers in Patients with Pulmonary Hypertension.' *Pulmonary Circulation* [online] 3 (4), 856–61. available from <http://www.ncbi.nlm.nih.gov/pubmed/25006401> [3 November 2018]

Reichenbach, N., Delekate, A., Plescher, M., Schmitt, F., Krauss, S., Blank, N., Halle, A., and Petzold, G.C. (2019) 'Inhibition of Stat3-mediated astrogliosis ameliorates pathology in an Alzheimer's disease model'. *EMBO Molecular Medicine* [online] 11 (6). available from <http://embomolmed.embopress.org/content/early/2019/01/07/emmm.201809665#sec-10> [18 June 2019]

Ren, B., Deng, L., Wang, J., Zhu, Y., Wei, L., and Zhou, Q. (2008) 'Hypoxia regulation of facilitated glucose transporter-1 and glucose transporter-3 in mouse chondrocytes mediated by HIF-1α'. *Joint Bone Spine* [online] 75 (2), 176-181. available from <https://www.sciencedirect.com/science/article/pii/S1297319X07002783> [16 June 2019]

Reuter, S., Gupta, S.C., Chaturvedi, M.M., and Aggarwal, B.B. (2010) 'Oxidative Stress, Inflammation, and Cancer: How Are They Linked?' *Free Radical Biology and Medicine* [online] 49 (11), 1603–16. available from <http://www.ncbi.nlm.nih.gov/pubmed/20840865> [13 October 2016]

Reynolds, J.P., Zheng, K., and Rusakov, D.A. (2019) 'Multiplexed calcium imaging of single-synapse activity and astroglial responses in the intact brain'. *Neuroscience Letters* [online] 689, 26-32. available from <https://www.sciencedirect.com/science/article/pii/S0304394018304270> [18 June 2019]

Rhee, S.Y., and Kim, Y.S. (2018) 'The Role of Advanced Glycation End Products in Diabetic Vascular Complications'. *Diabetes & Metabolism Journal* [online] 42 (3), 188-195. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6015964/> [18 January 2019]

Ricci, C., Pastukh, V., Leonard, J., Turrens, J., Wilson, G., Schaffer, D., and Schaffer, S.W. (2008) 'Mitochondrial DNA Damage Triggers Mitochondrial-Superoxide Generation and Apoptosis'. *American Journal of Physiology - Cell Physiology* 294 (2)

Richardson, B.G., Jain, A.D., Speltz, T.E., and Moore, T.W. (2015) 'Non-Electrophilic Modulators of the Canonical Keap1/Nrf2 Pathway'. *Bioorganic & Medicinal Chemistry Letters* 25 (11), 2261–2268

Richter, K., Konzack, A., Pihlajaniemi, T., Heljasvaara, R., and Kietzmann, T. (2015) 'Redox-Fibrosis: Impact of TGFβ1 on ROS Generators, Mediators and Functional Consequences'. *Redox Biology* 6, 344–352

Riss, T.L., Moravec, R.A., Niles, A.L., Duellman, S., Benink, H.A., Worzella, T.J., and Minor, L. (2013) 'Cell Viability Assays'. *Assay Guidance Manual* [online] available from <https://www.ncbi.nlm.nih.gov/books/NBK144065/>

Rodacka, A., Strumillo, J., Serafin, E., and Puchala, M. (2014) 'Effect of Resveratrol and Tiron on the Inactivation of Glyceraldehyde-3- Phosphate Dehydrogenase Induced by Superoxide Anion Radical.' *Current Medicinal Chemistry* [online] 21 (8), 1061–9. available from <http://www.ncbi.nlm.nih.gov/pubmed/24059224> [7 April 2018]

Rodríguez-Carrizalez, A.D., Castellanos-González, J.A., Martínez-Romero, E.C., Miller-Arrevillaga, G., Pacheco-Moisés, F.P., Román-Pintos, L.M., and Miranda-Díaz, A.G. (2016) 'The Effect of Ubiquinone and Combined Antioxidant Therapy on Oxidative Stress Markers in Non-Proliferative Diabetic Retinopathy: A Phase IIa, Randomized, Double-Blind, and Placebo-Controlled Study'. *Redox Report* [online] 21 (4), 155–163. available from <http://www.tandfonline.com/doi/full/10.1179/1351000215Y.0000000040> [25 August 2018]

Rose, S., Melnyk, S., Trusty, T.A., Pavliv, O., Seidel, L., Li, J., Nick, T., and James, S.J. (2012) 'Intracellular and Extracellular Redox Status and Free Radical Generation in Primary Immune Cells from Children with Autism'. *Autism Research and Treatment* [online] 2012, 1–10. available from <http://www.ncbi.nlm.nih.gov/pubmed/22928106> [16 February 2019]

Rosenfeld, W.N., Davis, J.M., Parton, L., Richter, S.E., Price, A., Flaster, E., and Kassem, N. (1996) 'Safety and Pharmacokinetics of Recombinant Human Superoxide Dismutase Administered Intratracheally to Premature Neonates with Respiratory Distress Syndrome.' *Pediatrics* [online] 97 (6 Pt 1), 811–7. available from <http://www.ncbi.nlm.nih.gov/pubmed/8657519> [26 April 2018]

Rothfuss, O., Gasser, T., and Patenge, N. (2010) 'Analysis of Differential DNA Damage in the Mitochondrial Genome Employing a Semi-Long Run Real-Time PCR Approach.' *Nucleic acids research* [online] 38 (4), e24. available from <http://www.ncbi.nlm.nih.gov/pubmed/19966269> [29 June 2018]

Rusciani, L., Proietti, I., Paradisi, A., Rusciani, A., Guerriero, G., Mammone, A., De Gaetano, A., and Lippa, S. (2007) 'Recombinant Interferon ??-2b and Coenzyme Q10 as a Postsurgical Adjuvant Therapy for Melanoma: A 3-Year Trial with Recombinant Interferon-?? And 5-Year Follow-Up'. *Melanoma Research* [online] 17 (3), 177–183. available from <http://www.ncbi.nlm.nih.gov/pubmed/17505263> [16 February 2019]

Sackesen, C., Ercan, H., Dizdar, E., Soyer, O., Gumus, P., Tosun, B.N., Büyüktuncer, Z., Karabulut, E., Besler, T., and Kalayci, O. (2008) 'A Comprehensive Evaluation of the Enzymatic and Nonenzymatic Antioxidant Systems in Childhood Asthma.' *The Journal of Allergy and Clinical Immunology* [online] 122 (1), 78–85. available from <http://www.ncbi.nlm.nih.gov/pubmed/18485467> [21 May 2018]

Sadek, A., Berk, L.S., Mainess, K., and Daher, N.S. (2018) 'Antioxidants and Autism: Teachers' Perceptions of Behavioral Changes.' *Advances in Mind-Body Medicine* [online] available from <http://www.ncbi.nlm.nih.gov/pubmed/29870399> [16 February 2019]

Saeidnia, S. and Abdollahi, M. (2013) 'Toxicological and Pharmacological Concerns on Oxidative Stress and Related Diseases'. *Toxicology and Applied Pharmacology* [online] 273 (3), 442–455. available from <http://dx.doi.org/10.1016/j.taap.2013.09.031>

Sage, A.T., Walter, L.A., Shi, Y., Khan, M.I., Kaneto, H., Capretta, A., and Werstuck, G.H. (2010) 'Hexosamine biosynthesis pathway flux promotes endoplasmic reticulum stress, lipid accumulation, and inflammatory gene expression in hepatic cells.' *American Journal of Physiology Endocrinology and Metabolism* [online] 298 (3), 499-511. available from <https://www.physiology.org/doi/full/10.1152/ajpendo.00507.2009> [15 June 2019]

Sahiner, U.M., Birben, E., Erzurum, S., Sackesen, C., and Kalayci, O. (2011) 'Oxidative Stress in Asthma.' *The World Allergy Organization Journal* [online] 4 (10), 151–8. available from <http://www.ncbi.nlm.nih.gov/pubmed/23268432> [17 January 2017]

Saio, V., Syiem, D., and Sharma, R. (2012) 'Effect of Potentilla Fulgens on Lipid Peroxidation and Antioxidant Status in Alloxan-Induced Diabetic Mice'. *Journal of Basic and Clinical Pharmacy* [online] 3 (2). available from <https://www.jbclinpharm.org/articles/effect-of-potentilla-fulgens-on-lipid-peroxidation-and-antioxidant-status-in-alloxaninduced-diabetic-mice.html> [17 August 2018]

Salim, S. (2014) 'Oxidative Stress and Psychological Disorders.' *Current Neuropharmacology* [online] 12 (2), 140–7. available from <http://www.ncbi.nlm.nih.gov/pubmed/24669208> [3 March 2017]

Salucci, S., Ambrogini, P., Lattazni, D., Betti, M., Gobbi, P., Galati, C., Galli, F., Cuppini, R., and Minelli, A. (2014) 'Maternal Dietary Loads of Alpha-Tocopherol Increase Synapse Density and Glial Synaptic Coverage in the Hippocampus of Adult Offspring'. *European Journal of Histochemistry* [online] 58 (2). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4083323/> [29 June 2019]

Sanders, L.H., McCoy, J., Hu, X., Mastroberardino, P.G., Dickinson, B.C., Chang, C.J., Chu, C.T., Van Houten, B., and Greenamyre, J.T. (2014) 'Mitochondrial DNA Damage: Molecular Marker of Vulnerable Nigral Neurons in Parkinson's Disease'. *Neurobiology of Disease* [online] 70, 214–223. available from <http://linkinghub.elsevier.com/retrieve/pii/S0969996114001788> [20 August 2017]

Sango, K., Suzuki, T., Yanagisawa, H., Takaku, S., Hirooka, H., Tamura, M., and Watabe, K. (2006) 'High Glucose-Induced Activation of the Polyol Pathway and Changes of Gene Expression Profiles in Immortalized Adult Mouse Schwann Cells IMS32'. *Journal of Neurochemistry* [online] 98 (2), 446–458. available from <http://www.ncbi.nlm.nih.gov/pubmed/16805838> [20 June 2018]

Sangomla, S., Saifi, M.A., Khurana, A., and Godugu, C. (2018) 'Nanoceria Ameliorates Doxorubicin Induced Cardiotoxicity: Possible Mitigation via Reduction of Oxidative Stress and Inflammation'. *Journal of Trace Elements in Medicine and Biology* [online] 47, 53–62. available from <https://www.sciencedirect.com/science/article/pii/S0946672X1730771X> [25 August 2018]

Sano, M., Ernesto, C., Thomas, R.G., Klauber, M.R., Schafer, K., Grundman, M., Woodbury, P., Growdon, J., Cotman, C.W., Pfeiffer, E., Schneider, L.S., and Thal, L.J. (1997) 'A Controlled Trial of Selegiline, Alpha-Tocopherol, or Both as Treatment for Alzheimer's Disease. The Alzheimer's Disease Cooperative Study.' *The New England Journal of Medicine* [online] 336 (17), 1216–22. available from <http://www.ncbi.nlm.nih.gov/pubmed/9110909> [23 July 2016]

Santos, J.H., Meyer, J.N., Mandavilli, B.S., and Houten, B. (2006) 'Quantitative PCR-Based Measurement of Nuclear and Mitochondrial DNA Damage and Repair in Mammalian Cells'. *DNA Repair Protocols* [online], 314, 183–199. available from <http://link.springer.com/10.1385/1-59259-973-7:183> [11 May 2018]

Sanyal, J., Bandyopadhyay, S.K., Banerjee, T.K., Mukherjee, S.C., Chakraborty, D.P., Ray, B.C., and Rao, V.R. (2009) 'Plasma Levels of Lipid Peroxides in Patients with Parkinson's Disease'. *European Review of Medical and Pharmacological Sciences* [online] 13, 129–132. available from < http://www.europeanreview.org/wp/wp-content/uploads/608.pdf> [23 August 2017]

Sarrafchi, A., Bahmani, M., Shirzad, H., and Rafieian-Kopaei, M. (2016) 'Oxidative Stress and Parkinson's Disease: New Hopes in Treatment with Herbal Antioxidants'. *Current Pharmaceutical Design* [online] 22. available from <http://eprints.umsu.ac.ir/466/1/Parkinson_s-Rafieian-Kopaei-2-MS.pdf> [25 February 2018]

Sasaki, M., Shibata, E., Tohyama, K., Takahashi, J., Otsuka, K., Tsuchiya, K., Takahashi, S., Ehara, S., Terayama, Y., and Sakai, A. (2006) 'Neuromelanin Magnetic Resonance Imaging of Locus Ceruleus and Substantia Nigra in Parkinson's Disease'. *NeuroReport* [online] 17 (11), 1215–1218. available from <https://insights.ovid.com/pubmed?pmid=16837857> [25 February 2018]

Scapagnini, G., Sonya, V., Nader, A.G., Calogero, C., Zella, D., and Fabio, G. (2011) 'Modulation of Nrf2/ARE Pathway by Food Polyphenols: A Nutritional Neuroprotective Strategy for Cognitive and Neurodegenerative Disorders'. *Molecular Neurobiology* [online] 44 (2), 192–201. available from <http://link.springer.com/10.1007/s12035-011-8181-5> [4 May 2017]

Schaefer, A.M., McFarland, R., Blakely, E.L., He, L., Whittaker, R.G., Taylor, R.W., Chinnery, P.F., and Turnbull, D.M. (2007) 'Prevalence of mitochondrial DNA disease in adults'. *Annals of Neurology* [online] 63 (1). available from <https://onlinelibrary.wiley.com/doi/full/10.1002/ana.21217> [11 June 2019]

Schieber, M. and Chandel, N.S. (2014) 'ROS Function in Redox Signaling and Oxidative Stress'. *Current Biology* 24 (10), 453–462

Schipper, H.M., Bennett, D.A., Liberman, A., Bienias, J.L., Schneider, J.A., Kelly, J., and Arvanitakis, Z. (2006) 'Glial Heme Oxygenase-1 Expression in Alzheimer Disease and Mild Cognitive Impairment'. *Neurobiology of Aging* [online] 27 (2), 252–261. available from <https://www.sciencedirect.com/science/article/pii/S0197458005000679> [24 February 2018]

Schmatz, R., Perreira, L.B., Stefanello, N., Mazzanti, C., Spanevello, R., Gutierres, J., Bagatini, M., Martins, C.C., Abdalla, F.H., Daci da Silva Serres, J., Zanini, D., Vieira, J.M., Cardoso, A.M., Schetinger, M.R., and Morsch, V.M. (2012) 'Effects of Resveratrol on Biomarkers of Oxidative Stress and on the Activity of Delta Aminolevulinic Acid Dehydratase in Liver and Kidney of Streptozotocin-Induced Diabetic Rats'. *Biochimie* [online] 94 (2), 374–383. available from <https://www.sciencedirect.com/science/article/pii/S0300908411003105> [4 May 2018]

Schriner, S.E., Linford, N.J., Martin, G.M., Treuting, P., Ogburn, C.E., Emond, M., Coskun, P.E., Ladiges, W., Wolf, N., Van Remmen, H., Wallace, D.C., and Rabinovitch, P.S. (2005) 'Extension of Murine Life Span by Overexpression of Catalase Targeted to Mitochondria.' *Science* [online] 308 (5730), 1909–11. available from <http://www.ncbi.nlm.nih.gov/pubmed/15879174> [13 August 2015]

Selvakumar, G.P., Janakiraman, U., Essa, M.M., Justin Thenmozhi, A., and Manivasagam, T. (2014) 'Escin Attenuates Behavioral Impairments, Oxidative Stress and Inflammation in a Chronic MPTP/Probenecid Mouse Model of Parkinson's Disease'. *Brain Research* [online] 1585, 23–36. available from <https://www.sciencedirect.com/science/article/pii/S0006899314003588?via%3Dihub> [1 February 2018]

Seyyedebrahimi, S., Khodabandehloo, H., Nasli Esfahani, E., and Meshkani, R. (2018) 'The effects of resveratrol on markers of oxidative stress in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled clinical trial'. *Acta Diabetologica* [online] 55 (4), 341-53. available from <https://link.springer.com/article/10.1007%2Fs00592-017-1098-3> [29 June 2019]

Sharma, P., Ahmad Shah, Z., Kumar, A., Islam, F., and Mishra, K.P. (2007) 'Role of Combined Administration of Tiron and Glutathione against Aluminum-Induced Oxidative Stress in Rat Brain'. *Journal of Trace Elements in Medicine and Biology* [online] 21 (1), 63–70. available from <http://linkinghub.elsevier.com/retrieve/pii/S0946672X06001635> [21 March 2017]

Sharma, S.R., and Sharma, N. (2008) 'Epalrestat, an aldose reductase inhibitor, in diabetic neuropathy: An Indian Perspective'. Annals of Indian Academy of Neurology [online] 11 (4), 231-235. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2771994/> [17 June 2019]

Shi, T.S., Zhang, M., Zeberg, H., Nilsson, J., Grunler, J., Liu, S., Xiang, W., Persson, J., Fried, K.J., Catrina, S.B., Watanabe, M., Arhem, P., Brismar, K., and Hokfelt, T.G.M. (2013) 'Coenzyme Q10 prevents peripheral neuropathy and attenuates neuron loss in the db−/db− mouse, a type 2 diabetes model' *PNAS* [online] 110 (2), 690-695. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3545822/> [15 June 2019]

Shichiri, M. (2014) 'The Role of Lipid Peroxidation in Neurological Disorders.' *Journal of Clinical Biochemistry and Nutrition* [online] 54 (3), 151–60. available from <http://www.ncbi.nlm.nih.gov/pubmed/24895477> [17 August 2018]

Shintani, T., and Klionsky, D.J. (2004) 'Autophagy in Health and Disease: A Double-Edged Sword.' *Science* [online] 306 (5698), 990-5. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1705980/> [11 November 2014]

Shoeib, A.M., Said, E., Nader, M.A., Salem, H.A.-E., and Ammar, E.M. (2016) 'Tiron Mitigates Thioacetamide-Induced Acute Liver Injury'. *Journal of Food and Pharmaceutical Sciences* [online] 3 (3). available from <https://journal.ugm.ac.id/jfps/article/view/8774> [4 May 2017]

Shokolenko, I., Venediktova, N., Bochkareva, A., Wilson, G.L., and Alexeyev, M.F. (2009) 'Oxidative Stress Induces Degradation of Mitochondrial DNA.' *Nucleic Acids Research* [online] 37 (8), 2539–48. available from <http://www.ncbi.nlm.nih.gov/pubmed/19264794> [25 September 2018]

Shokrzadeh, M., Abdi, H., Asadollah-Pour, A., and Shaki, F. (2016) 'Nanoceria Attenuated High Glucose-Induced Oxidative Damage in HepG2 Cells.' *Cell journal* [online] 18 (1), 97–102. available from <http://www.ncbi.nlm.nih.gov/pubmed/27054124> [2 May 2017]

Shrivastava, S., Jadon, A., and Shukla, S. (2007) 'Effect of Tiron and Its Combination with Nutritional Supplements against Vanadium Intoxication in Female Albino Rats.' *The Journal of Toxicological Sciences* [online] 32 (2), 185–92. available from <http://www.ncbi.nlm.nih.gov/pubmed/17538242> [21 March 2017]

Shukla, K., Pal, P.B., Sonowal, H., Srivastava, S.K., and Ramana, K. V. (2017) 'Aldose Reductase Inhibitor Protects against Hyperglycemic Stress by Activating Nrf2-Dependent Antioxidant Proteins'. *Journal of Diabetes Research* [online] 2017, 1–9. available from <https://www.hindawi.com/journals/jdr/2017/6785852/> [16 December 2018]

Sidoryk-Wegrzynowicz, M., Wegrzynowicz, M., Lee, E., Bowman, A.B., and Aschner, M. (2011) 'Role of Astrocytes in Brain Function and Disease.' *Toxicologic Pathology* [online] 39 (1), 115–23. available from <http://www.ncbi.nlm.nih.gov/pubmed/21075920> [11 November 2015]

Sigma-Aldrich (n.d) *Tiron* [online] available from <https://www.sigmaaldrich.com/catalog/product/sigma/d7389?lang=en&region=GB> [01 October 2014]

da Silva, J. (2016) 'DNA Damage Induced by Occupational and Environmental Exposure to Miscellaneous Chemicals'. *Mutation Research/Reviews in Mutation Research* [online] available from <http://www.sciencedirect.com/science/article/pii/S1383574216300096> [10 March 2016]

Silveira, L.R., Pereira-Da-Silva, L., Juel, C., and Hellsten, Y. (2003) 'Formation of Hydrogen Peroxide and Nitric Oxide in Rat Skeletal Muscle Cells during Contractions'. *Free Radical Biology and Medicine* [online] 35 (5), 455–464. available from <http://linkinghub.elsevier.com/retrieve/pii/S0891584903002715> [24 August 2017]

Sima, C., Aboodi, G.M., Lakschevitz, F.S., Sun, C., Goldberg, M.B., and Glogauer, M. (2016) ' Nuclear Factor Erythroid 2-Related Factor 2 Down-Regulation in Oral Neutrophils Is Associated with Periodontal Oxidative Damage and Severe Chronic Periodontitis'. The American Journal of Pathology [online] 186 (6), 1417-26. available from <https://ajp.amjpathol.org/article/S0002-9440(16)30004-9/fulltext> [22 June 2019]

Singh, C.K., Ndiaye, M.A., and Ahmad, N. (2015) 'Resveratrol and Cancer: Challenges for Clinical Translation'. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* [online] 1852 (6), 1178–1185. available from <http://linkinghub.elsevier.com/retrieve/pii/S0925443914003342> [3 July 2017]

Singh, M., Sharma, H., and Singh, N. (2007) 'Hydrogen Peroxide Induces Apoptosis in HeLa Cells through Mitochondrial Pathway'. *Mitochondrion* 7 (6), 367–373

Singh, U.N., Kumar, S., and Dhakal, S. (2017) 'Study of Oxidative Stress in Hypercholesterolemia'. *International Journal of Contemporary Medical Research ICV ISSN* [online] 483 (5), 77–2393. available from <www.ijcmr.com> [5 August 2017]

Sinha, K., Das, J., Pal, P.B., and Sil, P.C. (2013) 'Oxidative Stress: The Mitochondria-Dependent and Mitochondria-Independent Pathways of Apoptosis'. *Archives of Toxicology* [online] 87 (7), 1157–1180. available from <http://link.springer.com/10.1007/s00204-013-1034-4> [9 January 2017]

Sireesh, D., Dhamodhara, U., Ezhilarasi, K., Vijay, V., and Ramkumar, K.M. (2018) ' Association of NF-E2 Related Factor 2 (Nrf2) and inflammatory cytokines in recent onset Type 2 Diabetes Mellitus'. Scientific Reports [online] 8 (5126). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5865120/> [22 June 2019]

Smeriglio, A., Denaro, M., Mastracci, L., Grillo, F., Cornara, L., Shirooie, S., Nabavi, S.N., and Trombetta, D. (2019) 'Safety and efficacy of hydroxytyrosol-based formulation on skin inflammation: in vitro evaluation on reconstructed human epidermis model'. *DARU Journal of Pharmaceutical Sciences* [online] 27 (1), 283-93. available from <https://link.springer.com/article/10.1007/s40199-019-00274-3> [29 June 2019]

Smith, F., Smith, G.L., and Loo, A.F. (2012) 'Upregulation of Heme Oxygenase-1 by Zinc in HCT-116 Cells'. *Free Radical Research* [online] 46 (9), 1099–1107. available from <http://www.dx.doi.org/10.3109/10715762.2012.690872> [18 February 2019]

Smith, I.F., Boyle, J.P., Green, K.N., Pearson, H.A., and Peers, C. (2003) 'Hypoxic Remodelling of Ca2+ Mobilization in Type I Cortical Astrocytes: Involvement of ROS and pro-Amyloidogenic APP Processing'. *Journal of Neurochemistry* [online] 88 (4), 869–877. available from <http://doi.wiley.com/10.1046/j.1471-4159.2003.02212.x> [22 August 2017]

Smith, M.A., Harris, P.L., Sayre, L.M., and Perry, G. (1997) 'Iron Accumulation in Alzheimer Disease Is a Source of Redox-Generated Free Radicals.' *Proceedings of the National Academy of Sciences of the United States of America* [online] 94 (18), 9866–8. available from <http://www.ncbi.nlm.nih.gov/pubmed/9275217> [23 July 2016]

Smith, P.S., Zhao, W., Spitz, D.R., and Robbins, M.E. (2007) 'Inhibiting Catalase Activity Sensitizes 36B10 Rat Glioma Cells to Oxidative Stress'. *Free Radical Biology and Medicine* [online] 42 (6), 787–797. available from <https://www.sciencedirect.com/science/article/pii/S0891584906007702>

Smriti, K., Pai, K.M., Ravindranath, V., and Pentapati, K.C. (2016) 'Role of Salivary Malondialdehyde in Assessment of Oxidative Stress among Diabetics.' *Journal of Oral Biology and Craniofacial Research* [online] 6 (1), 41–4. available from <http://www.ncbi.nlm.nih.gov/pubmed/26937368> [10 February 2018]

Snedeker, J.G. and Gautieri, A. (2014) 'The Role of Collagen Crosslinks in Ageing and Diabetes - the Good, the Bad, and the Ugly.' *Muscles, Ligaments and Tendons Journal* [online] 4 (3), 303–8. available from <http://www.ncbi.nlm.nih.gov/pubmed/25489547> [17 July 2018]

Sommer, A. (2008) 'Vitamin a Deficiency and Clinical Disease: An Historical Overview.' *The Journal of Nutrition* [online] 138 (10), 1835–9. available from <http://www.ncbi.nlm.nih.gov/pubmed/18806089> [5 May 2017]

Son, N.H., Ananthakrishnan, R., Yu, S., Khan, R.S., Jiang, H., Ji, R., Akashi, H., Li, Q., O'Shea, K., Homma, S., Goldberg, I.J., and Ramasamy, R. (2012) 'Cardiomyocyte aldose reductase causes heart failure and impairs recovery from ischemia'. PLoS One [online] 7 (9). available from <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0046549> [24 June 2019]

Song, Y.J.C., Halliday, G.M., Holton, J.L., Lashley, T., O'Sullivan, S.S., McCann, H., Lees, A.J., Ozawa, T., Williams, D.R., Lockhart, P.J., Revesz, T.R., (2009) 'Degeneration in Different Parkinsonian Syndromes Relates to Astrocyte Type and Astrocyte Protein Expression'. *Journal of Neuropathology & Experimental Neurology* [online] 68 (10), 1073–1083. available from <https://academic.oup.com/jnen/article/68/10/1073/2916983> [22 June 2019]

Sook Cho, Y., Lee, J., Lee, T.-H., Young Lee, E., Lee, K.-U., Yeol Park, J., and Moon, H.-B. (2004) 'A-Lipoic Acid Inhibits Airway Inflammation and Hyperresponsiveness in a Mouse Model of Asthma'. *Journal of Allergy and Clinical Immunology* [online] 114, 429–464. available from <https://www.jacionline.org/article/S0091-6749(04)01309-0/pdf> [8 November 2018]

Sourris, K.C., Harcourt, B.E., Tang, P.H., Morley, A.L., Huynh, K., Penfold, S.A., Coughlan, M.T., Cooper, M.E., Nguyen, T.-V., Ritchie, R.H., and Forbes, J.M. (2012) 'Ubiquinone (Coenzyme Q10) Prevents Renal Mitochondrial Dysfunction in an Experimental Model of Type 2 Diabetes'. *Free Radical Biology and Medicine* [online] 52 (3), 716–723. available from <http://linkinghub.elsevier.com/retrieve/pii/S0891584911012044> [5 September 2017]

de Souza Bastos, A., Graves, D.T., de Melo Loureiro, A.P., Rossa, C., Corbi, S.C.T., Frizzera,F., Scarel-Caminaga, R.M., Camara, N.O., Andriankaja, O.M., Hiyane, M.I., and Orrico, S.P. (2016) ' Diabetes and increased lipid peroxidation are associated with systemic inflammation even in well-controlled patients.' *Journal of Diabetic Complications* [online] 30 (8). 1593-99. available from < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5120401/> [12 August 2018]

Spickett, C.M., Wiswedel, I., Siems, W., Zarkovic, K., and Zarkovic, N. (2010) 'Advances in Methods for the Determination of Biologically Relevant Lipid Peroxidation Products'. *Free Radical Research* [online] 44 (10), 1172–1202. available from <http://www.tandfonline.com/doi/full/10.3109/10715762.2010.498476> [13 June 2017]

Stefanska, J., Sarniak, A., Wlodarczyk, A., Sokolowska, M., Pniewska, E., Doniec, Z., Nowak, D., and Pawliczak, R. (2012) 'Apocynin Reduces Reactive Oxygen Species Concentrations in Exhaled Breath Condensate in Asthmatics'. *Experimental Lung Research* [online] 38 (2), 90–99. available from <http://www.tandfonline.com/doi/full/10.3109/01902148.2011.649823> [8 November 2018]

Stewart, J.B. and Chinnery, P.F. (2015) 'The Dynamics of Mitochondrial DNA Heteroplasmy: Implications for Human Health and Disease'. *Nature Reviews Genetics* [online] 16 (9), 530–542. available from <http://www.nature.com/articles/nrg3966> [7 May 2018]

Suhail, N., Bilal, N., Khan, H.Y., Hasan, S., Sharma, S., Khan, F., Mansoor, T., and Banu, N. (2012) 'Effect of Vitamins C and E on Antioxidant Status of Breast-Cancer Patients Undergoing Chemotherapy'. *Journal of Clinical Pharmacy and Therapeutics* [online] 37 (1), 22–26. available from <http://www.ncbi.nlm.nih.gov/pubmed/21204889> [16 February 2019]

Sultana, R., Perluigi, M., and Allan Butterfield, D. (2013) 'Lipid Peroxidation Triggers Neurodegeneration: A Redox Proteomics View into the Alzheimer Disease Brain.' *Free Radical Biology and Medicine* [online] 62, 157–69. available from <http://www.ncbi.nlm.nih.gov/pubmed/23044265> [23 August 2017]

Sun, X., He, G., Qing, H., Zhou, W., Dobie, F., Cai, F., Staufenbiel, M., Huang, L.E., and Song, W. (2006) 'Hypoxia Facilitates Alzheimer's Disease Pathogenesis by up-Regulating BACE1 Gene Expression.' *Proceedings of the National Academy of Sciences of the United States of America* [online] 103 (49), 18727–32. available from <http://www.ncbi.nlm.nih.gov/pubmed/17121991> [22 August 2017]

Sun, Z.-J., Yang, Y.-C., Wu, J.-S., Wang, M.-C., Chang, C.-J., and Lu, F.-H. (2016) 'Increased Risk of Glomerular Hyperfiltration in Subjects with Impaired Glucose Tolerance and Newly Diagnosed Diabetes'. *Nephrology Dialysis Transplantation* [online] 31 (8), 1295–1301. available from <https://academic.oup.com/ndt/article-lookup/doi/10.1093/ndt/gfv385> [13 July 2018]

Sung, C.-C., Hsu, Y.-C., Chen, C.-C., Lin, Y.-F., and Wu, C.-C. (2013) 'Oxidative Stress and Nucleic Acid Oxidation in Patients with Chronic Kidney Disease'. *Oxidative Medicine and Cellular Longevity* [online] 2013, 1–15. available from <http://www.hindawi.com/journals/omcl/2013/301982/> [14 March 2016]

Supinski, G., Nethery, D., Stofan, D., and Dimarco, A. (1999) 'Extracellular Calcium Modulates Generation of Reactive Oxygen Species by the Contracting Diaphragm'. *Journal of Applied Physiology* [online] 87 (6), 2177–2185. available from <http://jap.physiology.org/content/jap/87/6/2177.full.pdf> [24 August 2017]

Suwalsky, M., Vileena, F., and Gallardo, M.J. (2015) 'In vitro protective effects of resveratrol against oxidative damage in human erythrocytes'. *Biochimica et Biophysica Acta* [online] 1848 (1), 76-82. available from <https://www.sciencedirect.com/science/article/pii/S0005273614003241?via%3Dihub> [29 June 2019]

Suzuki, T., Motohashi, H., and Yamamoto, M. (2013) 'Toward Clinical Application of the Keap1–Nrf2 Pathway'. *Trends in Pharmacological Sciences* 34 (6), 340–346

Suzuki, T., Sekido, H., Kato, N., Nakayama, Y., and Yabe-Nishimura, C. (2004) 'Neurotrophin-3-Induced Production of Nerve Growth Factor Is Suppressed in Schwann Cells Exposed to High Glucose: Involvement of the Polyol Pathway'. *Journal of Neurochemistry* [online] 91 (6), 1430–1438. available from <http://www.ncbi.nlm.nih.gov/pubmed/15584919> [20 June 2018]

Szeto, Y.T., Tomlinson, B., and Benzie, I.F.F. (2002) 'Total Antioxidant and Ascorbic Acid Content of Fresh Fruits and Vegetables: Implications for Dietary Planning and Food Preservation'. *British Journal of Nutrition* [online] 87 (01), 55. available from <http://www.journals.cambridge.org/abstract_S0007114502000089> [25 April 2018]

Taghizadeh, M., Tamtaji, O.R., Dadgostar, E., Daneshvar Kakhaki, R., Bahmani, F., Abolhassani, J., Aarabi, M.H., Kouchaki, E., Memarzadeh, M.R., and Asemi, Z. (2017) 'The Effects of Omega-3 Fatty Acids and Vitamin E Co-Supplementation on Clinical and Metabolic Status in Patients with Parkinson's Disease: A Randomized, Double-Blind, Placebo-Controlled Trial'. *Neurochemistry International* [online] 108, 183–189. available from <http://linkinghub.elsevier.com/retrieve/pii/S0197018617300736> [14 August 2017]

Taguchi, K., Motohashi, H., and Yamamoto, M. (2011) 'Molecular Mechanisms of the Keap1-Nrf2 Pathway in Stress Response and Cancer Evolution'. *Genes to Cells* [online] 16 (2), 123–140. available from <http://doi.wiley.com/10.1111/j.1365-2443.2010.01473.x> [25 July 2016]

Taha, R., Blaise, G., and Blaise, G.A. (2014) 'Nrf2 Activation as a Future Target of Therapy for Chronic Diseases'. *Functional Foods in Health and Disease* 4 (11), 510–523

Taiwo, F.A. (2008) 'Mechanism of Tiron as Scavenger of Superoxide Ions and Free Electrons'. *Journal of Spectroscopy* 22 (6), 491–498

Talarowska, M., Galecki, P., Maes, M., Gardner, A., Chamielec, M., Orzechowska, A., Bobinska, K., and Kowalczyk, E. (2012) 'Malondialdehyde plasma concentration correlates with declarative and working memory in patients with recurrent depressive disorder'. *Molecular Biology Reports* [online] 39 (5), 5359-66. available from <https://link.springer.com/article/10.1007%2Fs11033-011-1335-8> [21 June 2019]

Tan, W.S.D., Liao, W., Peh, H.Y., Vila, M., Dong, J., Shen, H.-M., and Wong, W.S.F. (2018) 'Andrographolide Simultaneously Augments Nrf2 Antioxidant Defense and Facilitates Autophagic Flux Blockade in Cigarette Smoke-Exposed Human Bronchial Epithelial Cells'. *Toxicology and Applied Pharmacology* [online] 360, 120–130. available from <https://www.sciencedirect.com/science/article/pii/S0041008X18304630> [20 October 2018]

Tang, W.H., Martin, K.A., and Hwa, J. (2012) 'Aldose reductase, oxidative stress, and diabetic mellitus.' *Frontiers in Pharmacology* [online] 9 (3), 87. available from <https://www.ncbi.nlm.nih.gov/pubmed/22582044> [22 December 2018]

Tangvarasittichai, S., Poonsub, P., Tangvarasittichai, O., and Sirigulsatien, V. (2009) 'Serum Levels of Malondialdehyde in Type 2 Diabetes Mellitus Thai Subjects'. *Siriraj Medical Journal* 61 (1), 20–23

Tarozzi, A., Angeloni, C., Malaguti, M., Morroni, F., Hrelia, S., and Hrelia, P. (2013) 'Sulforaphane as a Potential Protective Phytochemical against Neurodegenerative Diseases.' *Oxidative Medicine and Cellular Longevity* [online] 2013, 415078. available from <http://www.ncbi.nlm.nih.gov/pubmed/23983898> [13 November 2018]

Tatsch, E., Bochi, G. V., Piva, S.J., De Carvalho, J.A.M., Kober, H., Torbitz, V.D., Duarte, T., Signor, C., Coelho, A.C., Duarte, M.M.M.F., Montagner, G.F.F.S., Da Cruz, I.B.M., and Moresco, R.N. (2012) 'Association between DNA Strand Breakage and Oxidative, Inflammatory and Endothelial Biomarkers in Type 2 Diabetes'. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* [online] 732 (1), 16–20. available from <http://www.sciencedirect.com/science/article/pii/S002751071200022X> [5 September 2017]

Tatsch, E., Carvalho, J.A.M.D., Hausen, B.S., Bollick, Y.S., Torbitz, V.D., Duarte, T., Scolari, R., Duarte, M.M.M.F., Londero, S.W.K., Vaucher, R.A., Premaor, M.O., Comim, F. V., and Moresco, R.N. (2015) 'Oxidative DNA Damage Is Associated with Inflammatory Response, Insulin Resistance and Microvascular Complications in Type 2 Diabetes'. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* [online] 782, 17–22. available from <http://www.sciencedirect.com/science/article/pii/S0027510715300488> [5 September 2017]

Tatsuta, T., Scharwey, M., and Langer, T. (2013) 'Mitochondrial lipid trafficking' *Trends in Cell Biology* [online] 24 (1), 44-52. available from <https://www.cell.com/trends/cell-biology/fulltext/S0962-8924(13)00132-3> [13 June 2019]

Ten, V.S. and Starkov, A. (2012) 'Hypoxic-Ischemic Injury in the Developing Brain: The Role of Reactive Oxygen Species Originating in Mitochondria'. *Neurology Research International* [online] 2012, 1–10. available from <http://www.hindawi.com/journals/nri/2012/542976/> [16 January 2017]

Terraneo, L., Paroni, R., Bianciardi, P., Giallongo, T., Carelli, S., Gorio, A., and Samaja, M. (2017) 'Brain Adaptation to Hypoxia and Hyperoxia in Mice'. *Redox Biology* [online] 11, 12–20. available from <https://www.sciencedirect.com/science/article/pii/S2213231716302075> [23 January 2018]

Thomas, C., Mackey, M.M., Diaz, A.A., and Cox, D.P. (2009) 'Hydroxyl radical is produced via the Fenton reaction in submitochondrial particles under oxidative stress: implications for diseases associated with iron accumulation'. *Redox Report* [online] 14 (3), 102-108. available from <https://www.tandfonline.com/doi/abs/10.1179/135100009X392566> [7 June 2019]

Thummasorn, S., Shinlapawittayatorn, K., Khamseekaew, J., Jaiwongkam, T., Chattipakorn, S.C., and Chattipakorn, N. (2018) 'Humanin Directly Protects Cardiac Mitochondria against Dysfunction Initiated by Oxidative Stress by Decreasing Complex I Activity'. *Mitochondrion* [online] 38, 31–40. available from <https://www.sciencedirect.com/science/article/pii/S1567724917300739> [14 June 2018]

Thyagarajan, A. and Sahu, R.P. (2018) 'Potential Contributions of Antioxidants to Cancer Therapy: Immunomodulation and Radiosensitization.' *Integrative Cancer Therapies* [online] 17 (2), 210–216. available from <http://www.ncbi.nlm.nih.gov/pubmed/28627256> [16 February 2019]

Tiganis, T. (2011) ‘Reactive Oxygen Species and Insulin Resistance: The Good, the Bad and the Ugly’. *Trends in Pharmacological Sciences* [online] 32 (2), 82–89. available from <https://www.sciencedirect.com/science/article/pii/S0165614710002075> [14 March 2018]

To, M., Yamamura, S., Akashi, K., Charron, C.E., Haruki, K., Barnes, P.J., and Ito, K. (2012) ‘Defect of Adaptation to Hypoxia in Patients with COPD Due to Reduction of Histone Deacetylase 7.’ *Chest* [online] 141 (5), 1233–1242. available from <http://www.ncbi.nlm.nih.gov/pubmed/22172637> [22 January 2019]

Tomé-Carneiro, J., Larrosa, M., González-Sarrías, A., Tomás-Barberán, F.A., García-Conesa, M.T., and Espín, J.C. (2013) ‘Resveratrol and Clinical Trials: The Crossroad from in Vitro Studies to Human Evidence.’ *Current Pharmaceutical Design* [online] 19 (34), 6064–93. available from <http://www.ncbi.nlm.nih.gov/pubmed/23448440> [30 January 2017]

Tong, J., Ang, L-C., Williams, B., Furukawa, Y., Fitzmaurice, P., Guttman, M., Boileau, I., Hornykiewicz, O., and Kish, S.J. (2015) ‘Low Levels of Astroglial Markers in Parkinson's Disease: Relationship to α-Synuclein Accumulation’. *Neurobiological Disorders* [online] 82, 243-53. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4641013/> [22 June 2019]

Torres, L.L., Barbosa Quaglio, N., Tavares De Souza, G., Garcia, R.T., Mendonça, L., Dati, M., Moreira, W.L., Paula De Melo Loureiro, A., Nery De Souza-Talarico, J., Smid, J., Porto, C.S., Machado De Campos Bottino, C., Nitrini, R., Berlanga De Moraes Barros, S., Camarini, R., and Marcourakis, T. (2011) ‘Peripheral Oxidative Stress Biomarkers in Mild Cognitive Impairment and Alzheimer’s Disease’. *Journal of Alzheimer’s Disease* [online] 26, 59–68. available from < https://content.iospress.com/articles/journal-of-alzheimers-disease/jad110284 > [23 August 2017]

Toth, R.K. and Warfel, N.A. (2017) ‘Strange Bedfellows: Nuclear Factor, Erythroid 2-Like 2 (Nrf2) and Hypoxia-Inducible Factor 1 (HIF-1) in Tumor Hypoxia.’ *Antioxidants* [online] 6 (2). available from <http://www.ncbi.nlm.nih.gov/pubmed/28383481> [9 October 2018]

Toyota,N., Ogawa, D., Ishii, K., Hirata, K., Wada, J., Shikata, K., Makino, H. (2011) ‘Emphysematous Cystitis in a Patient with Type 2 Diabetes Mellitus’. *Acta Medica Okayama* [online] 65 (2), 129–133. available from <http://ousar.lib.okayama-u.ac.jp/files/public/4/45272/20160528073930820454/65_2_129.pdf> [13 July 2018]

Tranah, G., Katzman, S.M., Lauterjung, K., Yaffe, K., Manini, T.M., Kritchevsky, S., Newman, A.B., Harris, T.B., and Cummings, S.R. (2018) ‘Mitochondrial DNA m.3243A > G heteroplasmy affects multiple aging phenotypes and risk of mortality’. *Scientific Reports* [online] 8. available from <https://www.nature.com/articles/s41598-018-30255-6> [15 June 2019]

Tsao, S. and Yin, M. (2017) 'Antioxidative and Antiinflammatory Activities of Asiatic Acid, Glycyrrhizic Acid, and Oleanolic Acid in Human Bronchial Epithelial Cells'. *Journal of Agricultural and Food Chemistry* [online] 65. available from <https://pubs.acs.org/doi/pdfplus/10.1021/acs.jafc.5b00102?src=recsys>

Tsiligianni, I.G. and van der Molen, T. (2010) ‘A Systematic Review of the Role of Vitamin Insufficiencies and Supplementation in COPD’. *Respiratory Research* [online] 11 (1), 171. available from <http://respiratory-research.biomedcentral.com/articles/10.1186/1465-9921-11-171> [26 March 2018]

Tsuneki, H., Sekizaki, N., Suzuki, T., Kobayashi, S., Wada, T., Okamoto, T., Kimura, I., and Sasoaka, T. (2007) ‘Coenzyme Q10 prevents high glucose-induced oxidative stress in human umbilical vein endothelial cells’. *European Journal of Pharmacology* [online] 566 (1-3), 1-10. available from <https://www.sciencedirect.com/science/article/pii/S0014299907003354?via%3Dihub> [21 June 2019]

Tug, T., Karatas, F., and Terzi, S.M. (2004) ‘Antioxidant Vitamins (A, C and E) and Malondialdehyde Levels in Acute Exacerbation and Stable Periods of Patients with Chronic Obstructive Pulmonary Disease.’ *Clinical and Investigative Medicine* [online] 27 (3), 123–8. available from <http://www.ncbi.nlm.nih.gov/pubmed/15305803> [20 January 2019]

Turrens, J.F. (2003) ‘Mitochondrial Formation of Reactive Oxygen Species.’ *The Journal of Physiology* 552 (Pt 2), 335–344

Turunc Bayrakdar, E., Uyanikgil, Y., Kanit, L., Koylu, E., and Yalcin, A. (2014) ' Nicotinamide treatment reduces the levels of oxidative stress, apoptosis, and PARP-1 activity in Aβ(1–42)-induced rat model of Alzheimer's disease.' *Free Radical Research* [online] 48(2), 146-158. available from <https://www.tandfonline.com/doi/abs/10.3109/10715762.2013.857018?src=recsys&journalCode=ifra20> [21 April 2018]

Ujita, S., Sasaki, T., Asada, A., Funayama, K., Gao, M., Mikoshiba, K., Matsuki, N., and Ikegaya, Y. (2017) 'cAMP-Dependent Calcium Oscillations of Astrocytes: An Implication for Pathology'. *Cerebral Cortex* [online] 27 (2), 1602-14. available from <https://academic.oup.com/cercor/article/27/2/1602/3056218> [18 June 2019]

Umanath, K. and Lewis, J.B. (2018) 'Update on Diabetic Nephropathy: Core Curriculum 2018.' *American Journal of Kidney Diseases : the official journal of the National Kidney Foundation* [online] 71 (6), 884–895. available from <http://www.ncbi.nlm.nih.gov/pubmed/29398179> [14 October 2018]

Umbrello, M., Dyson, A., Feelisch, M., and Singer, M. (2013) 'The Key Role of Nitric Oxide in Hypoxia: Hypoxic Vasodilation and Energy Supply–Demand Matching'. *Antioxidants & Redox Signaling* [online] 19 (14), 1690–1710. available from <http://www.ncbi.nlm.nih.gov/pubmed/23311950> [21 April 2018]

Umeno, A., Biju, V., and Yoshida, Y. (2017) '*In Vivo* ROS Production and Use of Oxidative Stress-Derived Biomarkers to Detect the Onset of Diseases Such as Alzheimer's Disease, Parkinson's Disease, and Diabetes'. *Free Radical Research* [online] 51 (4), 413–427. available from <https://www.tandfonline.com/doi/full/10.1080/10715762.2017.1315114> [28 March 2018]

Uttara, B., Singh, A. V, Zamboni, P., and Mahajan, R.T. (2009) 'Oxidative Stress and Neurodegenerative Diseases: A Review of Upstream and Downstream Antioxidant Therapeutic Options.' *Current Neuropharmacology* [online] 7 (1), 65–74. available from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2724665&tool=pmcentrez&rendertype=abstract> [6 February 2016]

Valavanidis, A., Vlachogianni, T., Fiotakis, K., and Loridas, S. (2013) 'Pulmonary Oxidative Stress, Inflammation and Cancer: Respirable Particulate Matter, Fibrous Dusts and Ozone as Major Causes of Lung Carcinogenesis through Reactive Oxygen Species Mechanisms'. *International Journal of Environmental Research and Public Health* [online] 10 (9), 3886–3907. available from <http://www.mdpi.com/1660-4601/10/9/3886/> [13 October 2016]

Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., and Telser, J. (2007) 'Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease.' *The International Journal of Biochemistry & Cell Biology* 39 (1), 44–84

Vallon, V. (2011) 'The Proximal Tubule in the Pathophysiology of the Diabetic Kidney.' *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology* [online] 300 (5), R1009-22. available from <http://www.ncbi.nlm.nih.gov/pubmed/21228342> [1 February 2017]

Vallon, V., Platt, K.A., Cunard, R., Schroth, J., Whaley, J., Thomson, S.C., Koepsell, H., and Rieg, T. (2011) 'SGLT2 Mediates Glucose Reabsorption in the Early Proximal Tubule.' *Journal of the American Society of Nephrology : JASN* [online] 22 (1), 104–12. available from <http://www.ncbi.nlm.nih.gov/pubmed/20616166> [14 October 2018]

Van Vré, E.A., Ait-Oufella, H., Tedgui, A., and Mallat, Z. (2012) 'Apoptotic Cell Death and Efferocytosis in Atherosclerosis' *Arteriosclerosis, Thrombosis, and Vascular Biology* [online] 32 (4), 887-93. available from <https://www.ahajournals.org/doi/10.1161/atvbaha.111.224873> [11 March 2019]

Van Welden, S., Selfridge, A.C., and Hindryckx, P. (2017) 'Intestinal hypoxia and hypoxia-induced signalling as therapeutic targets for IBD'. *Nature Reviews Gastroenterology & Hepatology* [online] 14, 596-611. available from <https://www.nature.com/articles/nrgastro.2017.101> [16 June 2019]

Vaquero, E.C., Edderkaoui, M., Pandol, S.J., Gukovsky, I., and Gukovskaya, A.S. (2004) 'Reactive Oxygen Species Produced by NAD(P)H Oxidase Inhibit Apoptosis in Pancreatic Cancer Cells.' *The Journal of Biological Chemistry* [online] 279 (33), 34643–54. available from <http://www.ncbi.nlm.nih.gov/pubmed/15155719> [7 April 2018]

Vendelbo, M.H. and Nair, K.S. (2011) ‘Mitochondrial Longevity Pathways’. *Biochimica et Biophysica Acta - Molecular Cell Research* [online] 1813 (4), 634–644. available from <http://dx.doi.org/10.1016/j.bbamcr.2011.01.029>

Venkateshappa, C., Harish, G., Mythri, R. B., Mahadevan, A., Srinivas Bharath, M. M., and Shankar, S. K. (2011) ‘Increased Oxidative Damage and Decreased Antioxidant Function in Aging Human Substantia Nigra Compared to Striatum: Implications for Parkinson’s Disease’. *Neurochemical Research* [online] 37(2), 358–369. available from <doi:10.1007/s11064-011-0619-7> [22 June 2019]

Verkhratsky, A., Steardo, L., Parpura, V., and Montana, V. (2015) ‘Translational Potential of Astrocytes in Brain Disorders.’ *Progress in Neurobiology* [online] available from <http://www.sciencedirect.com/science/article/pii/S0301008215300265> [3 May 2016]

Verzola, D., Bertolotto, M.B., Villaggio, B., Ottonello, L., Dallegri, F., Salvatore, F., Berruti, V., Gandolfo, M.T., Garibotto, G., and Deferrari, G. (2004) ' Oxidative stress mediates apoptotic changes induced by hyperglycemia in human tubular kidney cells.' *Journal of the American Society of Nephrology: JASN* [online] 15 (1), 85-7. available from <https://www.ncbi.nlm.nih.gov/pubmed/14684680/> [12 December 2016]

Verzola, D., Gandolfo, M.T., Ferrario, F., Rastaldi, M.P., Villaggio, B., Gianiorio, F., Giannoni, M., Rimoldi,L., Lauria, F., Miji, M., Defarrari, G., and Garibotto, G. (2007) ' Apoptosis in the kidneys of patients with type II diabetic nephropathy.' *Kidney International* [online] 72 (10), 1262-72. available from < https://www.sciencedirect.com/science/article/pii/S0085253815525362> [12 December 2016]

Vestbo, J., Hurd, S.S., Agustí, A.G., Jones, P.W., Vogelmeier, C., Anzueto, A., Barnes, P.J., Fabbri, L.M., Martinez, F.J., Nishimura, M., Stockley, R.A., Sin, D.D., and Rodriguez-Roisin, R. (2013) ‘Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease’. *American Journal of Respiratory and Critical Care Medicine* [online] 187 (4), 347–365. available from <http://www.atsjournals.org/doi/abs/10.1164/rccm.201204-0596PP> [26 March 2018]

Vichai, V. and Kirtikara, K. (2006) 'Sulforhodamine B Colorimetric Assay for Cytotoxicity Screening'. *Nature Protocols* [online] 1, 1112-1116. available from <https://www.nature.com/articles/nprot.2006.179#article-info> [21 February 2016]

Viña, J., Lloret, A., Ortı, R., and Alonso, D. (2004) ‘Molecular Bases of the Treatment of Alzheimer’s Disease with Antioxidants: Prevention of Oxidative Stress’. *Molecular Aspects of Medicine* 25 (1), 117–123

Voigt, W. (2005) ‘Sulforhodamine B Assay and Chemosensitivity’. *Chemosensitivity* [online] 110, 039–048. available from <http://www.ncbi.nlm.nih.gov/pubmed/15901925> [20 May 2017]

Vorobjeva, N. V and Pinegin, B. V (2016) ‘Effects of the Antioxidants Trolox, Tiron and Tempol on Neutrophil Extracellular Trap Formation.’ *Immunobiology* [online] 221 (2), 208–19. available from <http://www.sciencedirect.com/science/article/pii/S0171298515300565> [10 March 2016]

de Vos, L.C., Lefrandt, J.D., Dullaart, R.P.F., Zeebregts, C.J., and Smit, A.J. (2016) ' Advanced glycation end products: An emerging biomarker for adverse outcome in patients with peripheral artery disease'. Artherosclerosis [online] 254, 291-99. available from <https://www.sciencedirect.com/science/article/pii/S0021915016314150> [28 June 2019]

Voskresenska, N., Voicehovska, J., Vojcehovska, A., Orlikovs, G., and Skesters, A. (2015) ‘Malondialdehyde Level in Chronic Obstructive Pulmonary Disease Exacerbations’. *European Respiratory Journal* [online] 46 (59). available from <http://erj.ersjournals.com/lookup/doi/10.1183/13993003.congress-2015.OA2920> [17 July 2018]

Voulgaridou, G.-P., Anestopoulos, I., Franco, R., Panayiotidis, M.I., and Pappa, A. (2011) ‘DNA Damage Induced by Endogenous Aldehydes: Current State of Knowledge’. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis* [online] 711 (1–2), 13–27. available from <https://www.sciencedirect.com/science/article/pii/S002751071100056X?via%3Dihub#bib0365> [19 August 2018]

de Vries, D.K., Kortekaas, K.A., Tsikas, D., Wijermars, L.G.M., van Noorden, C.J.F., Suchy, M.T., Cobbaert, C.M., Klautz, R.J.M., Schaapherder, A.F.M., and Lindeman, J.H.N. (2013) 'Oxidative Damage in Clinical Ischemia/Reperfusion Injury: A Reappraisal.' *Antioxidant and Redox Signalling* [online] 19 (6), 535-45. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3717197/> [29 September 2015]

de Vries, H.E., Witte, M., Hondius, D., Rozemuller, A.J.M., Drukarch, B., Hoozemans, J., and van Horssen, J. (2008) 'Nrf2-Induced Antioxidant Protection: A Promising Target to Counteract ROS-Mediated Damage in Neurodegenerative Disease?' *Free Radical Biology and Medicine* [online] 45 (10), 1375–1383. available from <https://www.sciencedirect.com/science/article/pii/S0891584908005133> [7 April 2018]

Wages, P.A., Silbajoris, R., Speen, A., Brighton, L., Henriquez, A., Tong, H., Bromber, P.A., Simmons, S.O., and Samet, J.M. (2014) 'Role of H_2O_2 in the oxidative effects of zinc exposure in human airway epithelial cells'. *Redox Biology* [online] 3, 47-55. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4297933/> [14 June 2019]

Wallace, D.C. and Chalkia, D. (2013) 'Mitochondrial DNA Genetics and the Heteroplasmy Conundrum in Evolution and Disease.' *Cold Spring Harbor Perspectives in Biology* 5 (11), 1–47

Wang, H., Jiang, T., Li, W., Gao, N., and Zhang, T. (2018) 'Resveratrol attenuates oxidative damage through activating mitophagy in an in vitro model of Alzheimer's disease'. *Toxicology Letters* [online] 282, 100-8. available from <https://www.sciencedirect.com/science/article/pii/S0378427417314443> [29 June 2019]

Wang, J., Ho, L., Zhao, Z., Seror, I., Humala, N., Dickstein, D.L., Thiyagarajan, M., Percival, S.S., Talcott, S.T., and Pasinetti, G.M. (2006) 'Moderate Consumption of Cabernet Sauvignon Attenuates Aβ Neuropathology in a Mouse Model of Alzheimer's Disease'. *The FASEB Journal* [online] 20 (13), 2313–2320. available from <http://www.fasebj.org/doi/10.1096/fj.06-6281com> [27 March 2018]

Wang, J., Markesbery, W.R., and Lovell, M.A. (2006) 'Increase oxidative damage in nuclear and mitochondrial DNA in mild cognitive impairment'. *Journal of Neurochemistry* [online] 96, 825-832. available from <https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1471-4159.2005.03615.x> [21 June 2019]

Wang, M.S., Boddapati, S., Emadi, S., and Sierks, M.R. (2010) 'Curcumin Reduces α-Synuclein Induced Cytotoxicity in Parkinson's Disease Cell Model'. *BMC Neuroscience* [online] 11 (1), 57. available from <http://bmcneurosci.biomedcentral.com/articles/10.1186/1471-2202-11-57> [19 August 2018]

Wang, Y., Bai, C., Li, K., Adler, K.B., and Wang, X. (2008) 'Role of Airway Epithelial Cells in Development of Asthma and Allergic Rhinitis'. *Respiratory Medicine* [online] 102 (7), 949–955. available from <https://www.sciencedirect.com/science/article/pii/S0954611108000504> [8 April 2018]

Wang, Y.C., Lee, W.C., Liao, S.C., Lee, L.C., Su, Y.J., Lee, C.T., and Chen, J.B. (2009) 'Mitochondrial DNA copy number correlates with oxidative stress and predicts mortality in nondiabetic hemodialysis patients'. *Journal of Nephrology* [online] 24 (3), 351-8. available from <https://www.semanticscholar.org/paper/Mitochondrial-DNA-copy-number-correlates-with-and-Wang-Lee/f87704118b6a64ec318c272fc787a3265e899c4e> [27 June 2019]

Waris, G. and Ahsan, H. (2006) 'Reactive Oxygen Species: Role in the Development of Cancer and Various Chronic Conditions.' *Journal of Carcinogenesis* [online] 5, 14. available from <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1479806&tool=pmcentrez&rendertype=abstract> [7 November 2014]

Wauchope, O.R., Mitchener, M.M., Beavers, W.N., Galligan, J.J., Camarillo, J.M., Sanders, W.D., Kingsley, P.J., Shim, H.-N., Blackwell, T., Luong, T., deCaestecker, M., Fessel, J.P., and Marnett, L.J. (2018) 'Oxidative Stress Increases M1dG, a Major Peroxidation-Derived DNA Adduct, in Mitochondrial DNA.' *Nucleic Acids Research* [online] 46 (7), 3458–3467. available from <http://www.ncbi.nlm.nih.gov/pubmed/29438559> [14 November 2018]

Waypa, G.., Smith, K.., and Schumacker, P.. (2016) 'O2 Sensing, Mitchondira and ROS Signaling: The Fog Is Lifting'. *Molecular Aspects of Medicine* 47, 76–89

Wedes, S.H., Khatri, S.B., Zhang, R., Wu, W., Comhair, S.A.A., Wenzel, S., Teague, W.G., Israel, E., Erzurum, S.C., and Hazen, S.L. (2009) 'Noninvasive Markers of Airway Inflammation in Asthma.' *Clinical and Translational Science* [online] 2 (2), 112–7. available from <http://www.ncbi.nlm.nih.gov/pubmed/20234847> [14 January 2018]

Wei, G., and Yu, X. (2016) 'Functions of PARylation in DNA Damage Repair Pathways.' *Genomic, Proteomics & Bioinformatics* [online] 14 (3), 131-9. available from <https://www.sciencedirect.com/science/article/pii/S1672022916300705> [23 January 2018]

Wei, W., Shurui, C., Zipeng, Z., Hongliang, D., Hongyu, W., Yuanlong, L., Kang, Z., Zhaoliang, S., Yue, G., Chang, L., and Mei, X. (2018) 'Aspirin suppresses neuronal apoptosis, reduces tissue inflammation, and restrains astrocyte activation by activating the Nrf2/HO-1 signaling pathway' *NeuroReport* [online] 29 (7), 524-531. available from <https://insights.ovid.com/pubmed?pmid=29381509> [25 June 2019]

Wiegman, C.H., Michaeloudes, C., Haji, G., Narang, P., Clarke, C.J., Russell, K.E., Bao, W., Pavlidis, S., Barnes, P.J., Kanerva, J., Bittner, A., Rao, N., Murphy, M.P., Kirkham, P.A., Chung, K.F., Adcock, I.M., Brightling, C.E., Davies, D.E., Finch, D.K., Fisher, A.J., Gaw, A., Knox, A.J., Mayer, R.J., Polkey, M., Salmon, M., and Singh, D. (2015) 'Oxidative Stress–induced Mitochondrial Dysfunction Drives Inflammation and Airway Smooth Muscle Remodeling in Patients with Chronic Obstructive Pulmonary Disease'. *Journal of Allergy and Clinical Immunology* [online] 136 (3), 769–780. available from <https://www.sciencedirect.com/science/article/pii/S0091674915002651> [22 August 2017]

Wilcox, G. (2005) 'Insulin and Insulin Resistance.' *The Clinical Biochemist. Reviews* [online] 26 (2), 19–39. available from <http://www.ncbi.nlm.nih.gov/pubmed/16278749> [14 March 2018]

Williamson, J. and Ido, Y. (2012) 'Linking Diabetic Complications to Sorbitol Oxidation, Oxidative Stress and Metabolic Suppression'. *Journal of Diabetes & Metabolism* [online] 03 (08). available from <https://www.omicsonline.org/linking-diabetic-complications-to-sorbitol-oxidation-oxidative-stress-and-metabolic-suppression-2155-6156.1000219.php?aid=9670> [14 June 2018]

Won, K.-J., Lin, H.Y., Jung, S., Cho, S.M., Shin, H.-C., Bae, Y.M., Lee, S.H., Kim, H.-J., Jeon, B.H., and Kim, B. (2012) 'Antifungal Miconazole Induces Cardiotoxicity Via Inhibition of APE/Ref-1-Related Pathway in Rat Neonatal Cardiomyocytes'. *Toxicological Sciences* [online] 126 (2), 298–305. available from <https://academic.oup.com/toxsci/article-lookup/doi/10.1093/toxsci/kfr347> [26 June 2017]

Wong, C.-M., Bansal, G., Pavlickova, L., Marcocci, L., and Suzuki, Y.J. (2013) 'Reactive Oxygen Species and Antioxidants in Pulmonary Hypertension.' *Antioxidants & Redox Signaling* [online] 18 (14), 1789–96. available from <http://www.ncbi.nlm.nih.gov/pubmed/22657091> [22 January 2019]

Wong, P.S., Roberts, R.E., and Randall, M.D. (2015) 'Hyperoxic Gassing with Tiron Enhances Bradykinin-Induced Endothelium-Dependent and EDH-Type Relaxation through Generation of Hydrogen Peroxide'. *Pharmacological Research* [online] 91, 29–35. available from <https://www.sciencedirect.com/science/article/pii/S104366181400173X> [20 October 2018]

Wood, L.G., Gibson, P.G., Garg, M.L., and Gibson, P. (2003) 'Biomarkers of Lipid Peroxidation, Airway Inflammation and Asthma'. *European Respiratory Journal* [online] 21, 177–186. available from <https://pdfs.semanticscholar.org/0239/5fdf95b01a4d653d5628d01ef87fff5b5655.pdf> [9 November 2017]

World Health Organisation (2018) *WHO | Projections of Mortality and Causes of Death, 2016 to 2060* [online] available from <https://www.who.int/healthinfo/global_burden_disease/projections/en/> [7 January 2019]

Wyss-Coray, T., Loike, J.D., Brionne, T.C., Lu, E., Anakov, R., Yan,F., Silverstein, S.C., and Husemann, J. (2003) 'Adult mouse astrocytes degrade amyloid-β in vitro and in situ'. *Nature Medicine* [online] 9, 453-7. available from <https://www.nature.com/articles/nm838> [18 June 2019]

Xia, Z., Li, H., and Irwin, M.G. (2016) 'Myocardial ischaemia reperfusion injury: the challenge of translating ischaemic and anaesthetic protection from animal models to humans.' *British Journal of Anaesthesia* [online] 117 (S2), 44-62. available from <https://bjanaesthesia.org/article/S0007-0912(17)30145-9/pdf> [9 March 2019]

Yamada, J., Yoshimura, S., Yamakawa, H., Sawada, M., Nakagawa, M., Hara, S., Kaku, Y., Iwama, T., Naganawa, T., Banno, Y., Nakashima, S., and Sakai, N. (2003) 'Cell Permeable ROS Scavengers, Tiron and Tempol, Rescue PC12 Cell Death Caused by Pyrogallol or Hypoxia/Reoxygenation'. *Neuroscience Research* [online] 45 (1), 1–8. available from <http://linkinghub.elsevier.com/retrieve/pii/S0168010202001967> [4 April 2018]

Yamada, K., Asai, K., Nagayasu, F., Sato, K., Ijiri, N., Yoshii, N., Imahashi, Y., Watanabe, T., Tochino, Y., Kanazawa, H., and Hirata, K. (2016) 'Impaired Nuclear Factor Erythroid 2-Related Factor 2 Expression Increases Apoptosis of Airway Epithelial Cells in Patients with Chronic Obstructive Pulmonary Disease Due to Cigarette Smoking'. *BMC Pulmonary Medicine* [online] 16 (1), 27. available from <http://www.biomedcentral.com/1471-2466/16/27> [20 October 2018]

Yang, B., Yang, Q., Yang, X., Yan, H.-B., and Lu, Q.-P. (2016) 'Hyperoside Protects Human Primary Melanocytes against H2O2-Induced Oxidative Damage'. *Molecular Medicine Reports* [online] 13 (6), 4613–4619. available from <http://www.ncbi.nlm.nih.gov/pubmed/27082158> [21 January 2019]

Yang, J., Su, Y., and Richmond, A. (2007) 'Antioxidants Tiron and N-Acetyl-L-Cysteine Differentially Mediate Apoptosis in Melanoma Cells via a Reactive Oxygen Species-Independent NF-KappaB Pathway.' *Free Radical Biology and Medicine* [online] 42 (9), 1369–80. available from <http://www.ncbi.nlm.nih.gov/pubmed/17395010> [4 August 2018]

Yang, X., Yao, W., Liu, H., Gao, Y., Liu, R., and Xu, L. (2017) 'Tangluoning, a traditional Chinese medicine, attenuates in vivo and in vitro diabetic peripheral neuropathy through modulation of PERK/Nrf2 pathway'. *Scientific Reports* [online] 7 (1). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5430716/> [21 June 2019]

Yang, X., Yao, W., Shi, H., Liu, H., Li, Y., Gao, Y., Liu, R., and Xu, L. (2016) 'Paeoniflorin Protects Schwann Cells against High Glucose Induced Oxidative Injury by Activating Nrf2/ARE Pathway and Inhibiting Apoptosis'. *Journal of Ethnopharmacology* [online] 185, 361–369. available from <https://www.sciencedirect.com/science/article/pii/S0378874116301295> [6 May 2018]

Yang, Y., Gozen, O., Watkins, A., Lorenzini, I., Lepore, A., Gao, Y., Videnskey, S., Brennan, J., Poulsen, D., Park, J.W., Jeon, N.L., Robinson, M.B., and Rothstein, J.D. (2009) 'Presynaptic Regulation of Astroglial Excitatory Neurotransmitter Transporter GLT1'. *Neuron* [online] 61 (6), 880-94. available from <https://www.sciencedirect.com/science/article/pii/S089662730900155X> [18 June 2019]

Yao, H., Arunachalam, G., Hwang, J.-W., Chung, S., Sundar, I.K., Kinnula, V.L., Crapo, J.D., and Rahman, I. (2010) 'Extracellular Superoxide Dismutase Protects against Pulmonary Emphysema by Attenuating Oxidative Fragmentation of ECM.' *Proceedings of the National Academy of Sciences of the United States of America* [online] 107 (35), 15571–6. available from <http://www.ncbi.nlm.nih.gov/pubmed/20713693> [17 January 2017]

Yao, H.,Sundar, I.K., Gorbunova, V., and Rahman, I. (2013) ' P21-PARP-1 Pathway Is Involved in Cigarette Smoke-Induced Lung DNA Damage and Cellular Senescence.' *PLoS One* [online] 8 (11). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3823706/> [25 June 2018]

Yasueda, A., Urushima, H., and Ito, T. (2016) 'Efficacy and Interaction of Antioxidant Supplements as Adjuvant Therapy in Cancer Treatment: A Systematic Review.' *Integrative Cancer Therapies* [online] 15 (1), 17–39. available from <http://www.ncbi.nlm.nih.gov/pubmed/26503419> [16 February 2019]

Yasuo, M., Mizuno, S., Kraskauskas, D., Bogaard, H.J., Natarajan, R., Cool, C.D., Zamora, M., and Voelkel, N.F. (2011) 'Hypoxia Inducible Factor-1α in Human Emphysema Lung Tissue'. *European Respiratory Journal* [online] 37 (4), 775–783. available from <http://www.ncbi.nlm.nih.gov/pubmed/20562128> [22 January 2019]

Yazgan, Ü.C., Taşdemir, E., Bilgin, H.M., Deniz Obay, B., Şermet, A., and Elbey, B. (2015) 'Comparison of the Anti-Diabetic Effects of Resveratrol, Gliclazide and Losartan in Streptozotocin-Induced Experimental Diabetes'. *Archives of Physiology and Biochemistry* [online] 121 (4), 157–161. available from <http://www.ncbi.nlm.nih.gov/pubmed/26161595> [25 March 2018]

Ye, K., Lu, J., Ma,F., Keinan, A., and Gu, Z. (2014) 'Extensive pathogenicity of mitochondrial heteroplasmy in healthy human individuals'. *PNAS* [online] 111 (29), 10654-10659. available from <https://www.pnas.org/content/111/29/10654> [12 June 2019]

Ye, P., Yang, X., Chen, X., and Shi, C. (2017) 'Hyperoside Attenuates OVA-Induced Allergic Airway Inflammation by Activating Nrf2'. *International Immunopharmacology* [online] 44, 168–173. available from <http://www.ncbi.nlm.nih.gov/pubmed/28107754> [21 January 2019]

Yokomizo, A. and Moriwaki, M. (2006) 'Effects of Uptake of Flavonoids on Oxidative Stress Induced by Hydrogen Peroxide in Human Intestinal Caco-2 Cells'. *Bioscience, Biotechnology, and Biochemistry* [online] 70 (6), 1317–1324. available from <http://www.tandfonline.com/doi/full/10.1271/bbb.50604>

Yoshida, Y., Itoh, N., Hayakawa, M., Habuchi, Y., Saito, Y., Tsukamato, Y., Cynshi, O., Jishage, K., Arai, H., and Niki, E. (2010) 'The role of alpha-tocopherol in motor hypofunction with aging in alpha-tocopherol transfer protein knockout mice as assessed by oxidative stress biomarkers'. *Journal of Nutritional Biochemistry* [online] 21 (1), 66-76. available from <https://www.sciencedirect.com/science/article/pii/S0955286308002258?via%3Dihub> [29 June 2019]

Yoshida, Y., Umeno, A., Shichiri, M., Gershman, R., Gilbert, D., Nye, S., Dwyer, P., Fenn, W., Harman, D., Forman, H., Fukuto, J., Miller, T., Zhang, H., Rinna, A., Levy, S., Poli, G., Schaur, R., Siems, W., Leonarduzzi, G., Noguchi, N., Zmijewski, J., Landar, A., Watanabe, N., Dickinson, D., Noguchi, N., Darley-Usmar, V., Porter, N., Caldwell, S., Mills, K., Yamamoto, S., Roberts, L., Fessel, J., Davies, S., Cracowski, J., Diczfalusy, U., Erickson, S., Cooper, A., Matsui, S., Gould, R., Song, W., Pierce, W., Saeki, Y., Redinger, R., Prough, R., Brown, A., Jessup, W., Schiller, J., Süss, R., Arnhold, J., Yoshida, Y., Niki, E., Yoshida, Y., Itoh, N., Hayakawa, M., Yoshida, Y., Hayakawa, M., Niki, E., Yoshida, Y., Itoh, N., Hayakawa, M., Yoshida, Y., Hayakawa, M., Habuchi, Y., Niki, E., Yoshida, Y., Saito, Y., Hayakawa, M., Yoshida, Y., Hayakawa, M., Habuchi, Y., Itoh, N., Niki, E., Yoshida, Y., Kodai, S., Takemura, S., Minamiyama, Y., Niki, E., Yoshida, Y., Hayakawa, M., Cynshi, O., Jishage, K., Niki, E., Yoshida, Y., Hayakawa, M., Niki, E., Yoshida, Y., Yoshikawa, A., Kinumi, T., Liu, W., Yin, H., Ogawa, Y., Yoshida, Y., Niki, E., Porter, N., Lee, C., Huang, S., Jenner, A., Halliwell, B., Terao, K., Niki, E., Yoshida, Y., Hayakawa, M., Cynshi, O., Jishage, K., Niki, E., Shichiri, M., Yoshida, Y., Ishida, N., Bachi, A., Zuccato, E., Baraldi, M., Fanelli, R., Chiabrando, C., Dalle-Donne, I., Rossi, R., Colombo, R., Giustarini, D., Milzani, A., Kitano, S., Yoshida, Y., Kawano, K., Hibi, N., Niki, E., Jira, W., Spiteller, G., Carson, W., Schramm, A., Platicò, D., Iuliano, L., Mauriello, A., Gniwotta, C., Morrow, J., Roberts, L., Kühn, H., Li, L., Duker, J., Yoshida, Y., Hirashima, Y., Doshi, M., Hayashi, N., Montine, T., Neely, M., Quinn, J., Roberts, L., Fessel, J., Davies, S., Su, B., Wang, X., Nunomura, A., Yeum, K., Russell, R., Krinsky, N., Aldini, G., Prior, R., Wu, X., Schaich, K., Niki, E., Omata, Y., Fukuhara, A., Saito, Y., Yoshida, Y., Chen, Z., Saito, Y., Yoshida, Y., Sekine, A., Noguchi, N., Niki, E., Kawamoto, Y., Nakamura, Y., Naito, Y., Levonen, A., Dickinson, D., Moellering, D., Mulcahy, R., Forman, H., Darley-Usmar, V., Musiek, E., Milne, G., McLaughlin, B., Morrow, J., Nagy, L., Tontonoz, P., Alvarez, J., Chen, H., Evans, R., Nishimura, T., Inoue, T., Shibata, N., Poli, G., Leonarduzzi, G., Biasi, F., Chiarpotto, E., Repa, J., Mangelsdorf, D., Brown, H., and Marnett, L. (2013) 'Lipid Peroxidation Biomarkers for Evaluating Oxidative Stress and Assessing Antioxidant Capacity in Vivo'. *Journal of Clinical Biochemistry and Nutrition* [online] 52 (1), 9–16. available from <http://japanlinkcenter.org/DN/JST.JSTAGE/jcbn/12-112?lang=en&from=CrossRef&type=abstract> [10 December 2016]

Youle, R.J. and van der Bliek, A.M. (2012) 'Mitochondrial Fission, Fusion and Stress'. *Science* [online] 337 (6098), 1062–1065. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4762028/>

Zahedi, H., Eghtesaid, S., Saifirad, S., Rezaee, N., Shidfar, F., Heydari, I., Golestan, B., and Jazayeri, S. (2014) 'Effects of CoQ10 Supplementation on Lipid Profiles and Glycemic Control in Patients with Type 2 Diabetes: a randomized, double blind, placebo-controlled trial'. *Journal of Diabetes and Metabolic Disorders* [online] 13 (81). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4583053/> [19 June 2019]

Zakkar, M., Van der Heiden, K., Luong, L.A., Chaudhury, H., Cuhlmann, S., Hamdulay, S.S., Krams, R., Edirisinghe, I., Rahman, R., Carlsen, H., Haskard, D.O., Mason, J.C., and Evans, P.C. (2009) ' Activation of Nrf2 in Endothelial Cells Protects Arteries From Exhibiting a Proinflammatory State'. *Arteriosclerosis, Thrombosis and Vascular Biology* [online] 29, 1851-57. available from < https://www.ahajournals.org/doi/full/10.1161/ATVBAHA.109.193375> [25 February 2019]

Zhai, J. and Wang, Y. (2017) ‘MDI 301, a Synthetic Retinoid, Depressed Levels of Matrix Metalloproteinases and Oxidative Stress in Diabetic Dermal Fibroblasts’. *Oncotarget* [online] 5. available from <http://www.oncotarget.com/abstract/16803> [25 April 2017]

Zhang, F., Zhong, R., Qi, H., Li, S., Cheng, C., Liu, X., Liu, Y., and Le, W. (2018) ‘Impacts of Acute Hypoxia on Alzheimer’s Disease-Like Pathologies in APPswe/PS1dE9 Mice and Their Wild Type Littermates.’ *Frontiers in Neuroscience* [online] 12, 314. available from <http://www.ncbi.nlm.nih.gov/pubmed/29867325> [11 November 2018]

Zhang, F.L., Zhang, W.G., Wei, Y.C., Meng, S., Bai, G.G., Wang, B.Y., Yang, H.Y., Tian, W., Meng, X., Zhang, H., and Chen, S.P. (2010) 'Involvement of Oxidative Stress in the Relapse of Acute Myeloid Leukemia.' *Journal of Biological Chemistry* [online] 285 (20), 15010-15. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2865279/> [10 March 2019]

Zhang, H., Morgan, B., Potter, B.J., Ma, L., Dellsperger, K.C., Ungvari, Z., and Zhang, C. (2010) ‘Resveratrol improves left ventricular diastolic relaxation in type 2 diabetes by inhibiting oxidative/nitrative stress: in vivo demonstration with magnetic resonance imaging’. *Translational Physiology* [online] 299 (4), 985-94. available from <https://www.physiology.org/doi/full/10.1152/ajpheart.00489.2010> [29 June 2019]

Zhang, H.-M. and Zhang, Y. (2014) ‘Melatonin: A Well-Documented Antioxidant with Conditional pro-Oxidant Actions’. *Journal of Pineal Research* [online] 57 (2), 131–146. available from <http://doi.wiley.com/10.1111/jpi.12162> [2 May 2017]

Zhang, J., Zhang, R., Zhan, Z., Li, X., Zhou, F., Xing, A., Jiang, C., Chen, Y., and An, L. (2017) ‘Beneficial Effects of Sulforaphane Treatment in Alzheimer’s Disease May Be Mediated through Reduced HDAC1/3 and Increased P75NTR Expression.’ *Frontiers in Aging Neuroscience* [online] 9, 121. available from <http://www.ncbi.nlm.nih.gov/pubmed/28507518> [26 January 2019]

Zhang, S., Yang, K., Zeng, L., Wu, X., and Huang, H. (2018) ‘Effectiveness of Coenzyme Q10 Supplementation for Type 2 Diabetes Mellitus: A Systematic Review and Meta-Analysis’. *International Journal of Endocrinology* [online] 2018, 1–11. available from <https://www.hindawi.com/journals/ije/2018/6484839/> [30 November 2018]

Zhang, S.M., Hernán, M.A., Chen, H., Spiegelman, D., Willett, W.C., and Ascherio, A. (2002) ‘Intakes of Vitamins E and C, Carotenoids, Vitamin Supplements, and PD Risk.’ *Neurology* [online] 59 (8), 1161–9. available from <http://www.ncbi.nlm.nih.gov/pubmed/12391343> [23 July 2016]

Zhang, X., Wang, R., Chen, G., Dejean, L., and Chen, Q. (2015) ' The Effects of Curcumin-based Compounds on Proliferation and Cell Death in Cervical Cancer Cells'. *Anticancer Research* [online] 35 (10), 5293-98. available from <http://ar.iiarjournals.org/content/35/10/5293.full> [23 June 2019]

Zhang, Y., Kong, J., Deb, D.K., Chang, A., and Li, Y.C. (2010) ‘Vitamin D Receptor Attenuates Renal Fibrosis by Suppressing the Renin-Angiotensin System’. *Journal of the American Society of Nephrology* [online] 21 (6), 966–973. available from <http://www.ncbi.nlm.nih.gov/pubmed/20378820> [30 November 2018]

Zhang, Y., Ma, Y., Bu, D., Liu, H., Xia, C., Zhang, Y., Zhu, S., Pan, H., Pei, P., Zheng, X., Wang, S., Xu, Y., and Qi, Y. (2015) ‘Deletion of a 4977-bp Fragment in the Mitochondrial Genome Is Associated with Mitochondrial Disease Severity’. *PLoS One* [online] 10 (5). available from <https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0128624> [15 June 2019]

Zhang, Z., Yan, J., Chang, Y., ShiDu Yan, S., and Shi, H. (2011) ‘Hypoxia Inducible Factor-1 as a Target for Neurodegenerative Diseases.’ *Current Medicinal Chemistry* [online] 18 (28), 4335–43. available from <http://www.ncbi.nlm.nih.gov/pubmed/21861815> [22 August 2017]

Zheng, Z., Chen, H., Ke, G., Fan, Y., Zou, H., Sun, X., Gu, Q., Xu, X., and Ho, P.C.P. (2009) ‘Protective Effect of Perindopril on Diabetic Retinopathy Is Associated with Decreased Vascular Endothelial Growth Factor-to-Pigment Epithelium-Derived Factor Ratio: Involvement of a Mitochondria-Reactive Oxygen Species Pathway.’ *Diabetes* [online] 58 (4), 954–64. available from <http://www.ncbi.nlm.nih.gov/pubmed/19188429> [16 December 2018]

Zhou, J., Li, J., Rosenbaum, D.M., Zhuang, J., Poon, C., Qin, P., Rivera, K., Lepore, J., Willette, R.N., Hu, E., and Barone, F.C. (2017) 'The prolyl 4-hydroxylase inhibitor GSK360A decreases post-stroke brain injury and sensory, motor, and cognitive behavioural deficits'. *PLoS One* [online] 12 (9). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5589177/> [16 June 2019]

Zhou, L., Xu, D., Sha, W., Shen, L., Lu, G., Yin, X., and Wang, M. (2015) 'High glucose induces renal tubular epithelial injury via Sirt1/NF-kappaB/microR-29/Keap1 signal pathway'. Journal of Translational Medicine [online] 13 (352). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4640239/> [22 June 2019]

Zhou, T., Prather, E.R., Garrison, D.E., and Zuo, L. (2018) 'Interplay between ROS and Antioxidants during Ischaemia-Reperfusion Injuries in Cardiac and Skeletal Muscle.' *International Journal of Molecular Sciences* [online] 19 (2), 417. available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5855639/> [13 September 2018]

Zhou, W., Gao, J., Zhao, H., Meng, X., and Wu, S. (2016) 'The role of quinone cycle in Fe^{2+}- H_2O_2 system in the regeneration of Fe^{2+}.' *Environmental Technology* [online], 38 (5). 1887-1896. available from <https://www.tandfonline.com/doi/abs/10.1080/09593330.2016.1240241?journalCode=tent20> [7 June 2019]

Zhu, D., Tan, K.S., Zhang, X., Sun, A.Y., Sun, G.Y., and Lee, J.C.-M. (2005) 'Hydrogen Peroxide Alters Membrane and Cytoskeleton Properties and Increases Intercellular Connections in Astrocytes.' *Journal of Cell Science* [online] 118 (16), 3695–703. available from <http://www.ncbi.nlm.nih.gov/pubmed/11884517> [20 January 2018]

Zhu, W., Cromie, M.M., Cai, Q., Lv, T., Singh, K., and Gao, W. (2014) 'Curcumin and Vitamin E Protect against Adverse Effects of Benzo[a]Pyrene in Lung Epithelial Cells'. *PLOS ONE* [online] 9 (3), e92992. available from <https://dx.plos.org/10.1371/journal.pone.0092992> [2 November 2018]

Zhu, X., Wu, C., Qiu, S., Yuan, X., and Li, L. (2017) 'Effects of resveratrol on glucose control and insulin sensitivity in subjects with type 2 diabetes: systematic review and meta-analysis'. *Nutrition and Metabolism* [online] 14 (60). available from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5610395/> [29 June 2019]

Zucker, S.N., Fink, E.E., Bagati, A., Mannava, S., Bianchi-Smiraglia, A., Bogner, P.N., Wawrzyniak, J.A., Foley, C., Leonova, K.I., Grimm, M.J., Moparthy, K., Ionov, Y., Wang, J., Liu, S., Sexton, S., Kandel, E.S., Bakin, A. V, Zhang, Y., Kaminski, N., Segal, B.H., and Nikiforov, M.A. (2014) 'Nrf2 Amplifies Oxidative Stress via Induction of Klf9.' *Molecular Cell* [online] 53 (6), 916–928. available from <http://www.ncbi.nlm.nih.gov/pubmed/24613345> [20 October 2018]

Appendix

Certificate of Ethical Approval

Applicant:

Danielle Meyer

Project Title:

The use of antioxidants to inhibit the effects of reactive oxygen species in vitro

This is to certify that the above named applicant has completed the Coventry University Ethical Approval process and their project has been confirmed and approved as Medium Risk

Date of approval:

27 October 2015

Project Reference Number:

P36954