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**Phytochemical metabolites and their effects on  
*in vitro* and *in vivo* measures of oxidative stress**

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A thesis submitted in partial fulfilment of the requirements for the degree Doctor of  
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# Abstract

Phytochemicals, plant based non-nutrient compounds, are important components of the human diet and have been associated with many of the beneficial effects of fruit and vegetable consumption. Their potential ability to function as antioxidants and also to regulate cell signalling pathways and induce endogenous protective mechanisms is both an exciting and a complicated area of research. Currently it is known that phytochemicals can act to enhance cell survival in cells that are challenged with a cytotoxic stimulus, and it has recently been shown that the combination of different fruit extracts can have a synergistic enhancement on this cell survival.

There have been numerous studies into the mechanisms behind this protection, indicating that some phytochemicals may have a direct binding effect on cell signalling molecules, some may act as chemical antioxidants that directly scavenge free radicals and some may induce an adaptive response by upregulation of the body's endogenous defence mechanisms. However, the majority of these studies have been conducted using dietary phytochemicals and have not examined the effects of the phytochemical's blood metabolites.

In this thesis the effects of phytochemical blood metabolites on factors that regulate oxidative stress within the body are examined. It is shown that 3,4-dihydroxybenzoic acid (3,4-DHBA) pretreatment can induce an adaptive response by the upregulation of endogenous antioxidant enzymes and that this effects occurs in four different cell lines that originate from four distinct tissue types. Additionally, an animal experiment was conducted to determine if the results seen *in vitro* could be related to *in vivo* effects. The feeding of 3,4-DHBA to Sprague Dawley rats for two weeks at the non-physiological dose of 100 mg per kg induced endogenous antioxidant expression in both intestine and liver of healthy young adult rats. While this effect is not directly applicable to levels of 3,4-DHBA seen in normal diets, it does highlight the potential for a high 3,4-DHBA or 3,4-DHBA precursor functional food and suggest that long term consumption of dietary levels of these compounds may induce a similar response.



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1,3,7-trimethyl-1*H*-purine-2,6(3*H*,7*H*)-dione.

Dedicated to the memory of Gabbeh, my friend and companion of 15 years, you will be missed.



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## Abbreviations:

**3-AT** 3-amino-1,2,4-triazole  
**8-epi-PGF2** 8-Epi-prostaglandin F2  
**AAPH** 2,2'-azobis-2-methyl-propanimidamide,dihydrochloride  
**AIF** Apoptosis inducing factor  
**ALPS** Autoimmune Lymphoproliferative Syndrome  
**ANT** Adenine nucleotide translocator  
**AP-1** Activator protein 1  
**APAF-1** Apoptotic protease activating factor 1  
**ARE** Antioxidant response element  
**ASK-1** Apoptosis signal-regulating kinase 1  
**ATCC** American Type Culture Collection  
**ATP** Adenosine-5'-triphosphate  
**Bad** Bcl-2-associated death promoter  
**Bak** Bcl-2 homologous antagonist/killer  
**Bax** Bcl-2-associated X protein  
**BBB** Blood brain barrier  
**Bcl-2** B-cell lymphoma 2  
**Bcl-W** Bcl-2-like protein 2  
**Bcl-xL** B-cell lymphoma-extra large  
**BCRP** Breast cancer resistance protein  
**BH** B-cell lymphoma 2 homology  
**Bid** BH3 interacting domain death agonist  
**Bim** Bcl-2-like protein 11  
**BITC** Benzyl-isothiocyanate  
**BTB** Blood testis barrier  
**CAD** Caspase-activated deoxyribonuclease  
**CAPE** Caffeic acid phenyl ester  
**CARD** Caspase recruitment domain  
**CARET** The beta-Carotene and Retinol Efficacy Trial  
**CDA** Cyanidin derived anthocyanin  
**cFLIP** Fas-associated protein with Death Domain -like IL-1beta-converting enzyme-like inhibitory protein  
**cMAX** Maximum concentration  
**CVD** Cardiovascular disease  
**CYP1A1** Cytochrome P450 A1  
**CYP1B1** Cytochrome P450 B1  
**DCFDA** 2',7'-dichlorodihydrofluorescein  
**DED** Death effector domain  
**DHBA** Dihydroxybenzoic acid  
**DHEA** Dehydroepiandrosterone  
**DMSO** Dimethyl sulfoxide



**DNA** Deoxyribonucleic acid  
**DNA-PK** DNA-dependent protein kinase, catalytic subunit  
**DTNB** 5,5'-dithiobis-(2-nitrobenzoic acid)  
**DTT** Dithiothreitol  
**EGCG** Epigallocatechin gallate  
**EGTA** Ethylene glycol tetraacetic acid  
**eNOS** Endothelial nitricoxide synthase  
**ER** Endoplasmic reticulum  
**ERK** Extracellular signal-regulated kinases  
**ET** Electron transfer  
**ETDA** Ethylenediaminetetraacetic acid  
**FACS** Fluorescence-activated cell sorting  
**FADD** Fas-Associated protein with Death Domain  
**FBS** Fetal bovine serum  
**FDA** The American food and drug administration  
**FITC** Fluorescein isothiocyanate  
**FRAP** Ferric reducing ability of plasma  
**FTO** Fat mass and obesity associated gene  
**GAB-1** GRB2-associated-binding protein 1  
**GAS2** Growth arrest-specific protein 2  
**GI** Gastrointestinal  
**GPx** Glutathione peroxidase  
**GSH** Reduced glutathione  
**GST $\pi$**  Glutathione s-transferase Pi  
**H<sub>2</sub>O<sub>2</sub>** Hydrogen peroxide  
**HAT** Hydrogen atom transfer  
**HBA** Hydroxybenzoic acid  
**HED** Human equivalent dose  
**HEK** Transformed embryonic kidney  
**HIV** Human immunodeficiency virus  
**HPCL** High-performance liquid chromatography  
**HSP** Heat shock proteins  
**ICAD** Inhibitor of aspartate activated deoxyribonuclease  
**ICAM-1** Inter-cellular adhesion molecule 1  
**IFN- $\gamma$**  Interferon-gamma  
**iNOS** Inducible nitricoxide synthase  
**JNK1** C-Jun N-terminal kinase 1  
**KEAP-1** Kelch-like ECH-associated protein 1  
**LPS** Lipopolysaccharide  
**MCF7** Michigan cancer foundation-7  
**NAC** N-acetyl-L-cysteine  
**NADPH** Reduced nicotinamide adenine dinucleotide phosphate  
**NF- $\kappa$ B** Nuclear factor kappa-light-chain-enhancer of activated B cells  
**nNOS** Neuronal nitricoxide synthase

**NQO1** NAD(P)H:quinone oxidoreductase 1  
**NQO2** NAD(P)H quinone oxidoreductase 2  
**Nrf2** NF-E2-related factor-2  
**NTB** 2-nitro-5-thiobenzoate  
**ORAC** Oxygen radical absorbance capacity  
**P53** Protein 53  
**PARP** Poly ADP ribose polymerase  
**PB** Buffer solution  
**PBS** Phosphate Buffered Saline  
**PC-3** human prostate cancer cell line  
**P-gp** P-glycoprotein  
**PI** Propidium iodide  
**PLA2** Phospholipase A2  
**PON1** Paraoxonase 1  
**RIP** Receptor interacting protein  
**RNS** Reactive nitrogen species  
**ROS** Reactive oxygen species  
**RPMI** Roswell Park Memorial Institute  
**SEM** Standard error of the mean  
**SOD** Super oxide dismutase  
**TNB** 5-thio-2-nitrobenzoic acid  
**TNF-R1** Tumour necrosis factor receptor 1  
**TNF-R2** Tumour necrosis factor receptor 2  
**TPTZ** Tro-triarydimethyltriazine  
**TRADD** Tumor necrosis factor receptor type 1-associated death domain protein  
**TRAF2** Tumor necrosis factor receptor-associated factor 2  
**TRAIL** Tumor necrosis factor-related apoptosis-inducing ligand  
**Trx** Thioredoxin  
**TrxR** Thioredoxin reductase  
**UV** Ultraviolet  
**UVB** Ultraviolet radiation B  
**VCAM-1** Vascular cell adhesion protein 1  
**VDAC** voltage-dependent anion channel  
**Z-DEVD-FMK** Z-DEVD-Fluor methyl ketone