Development of Assays for Biomarkers of Oxidative Damage to Assess the Efficacy of Fruit-derived Antioxidants

A thesis presented to in partial fulfilment of the requirements for the degree of Master of Science in Biochemistry at Massey University

Laura Evelyn Barnett

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Acknowledgements

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Abstract

The diet is a very important part of maintaining a healthy lifestyle. Increased consumption of fruits and vegetables is one practice postulated to decrease the incidence of diseases such as cancer, cardiovascular disease and other disorders. Although there are a number of possible beneficial compounds in fruit, it is believed that the antioxidant components found in these foods may decrease the oxidative damage that could lead to such diseases. Oxidative damage to cellular proteins, lipids and DNA is considered to result from an increase in the production of free radicals, which overwhelm the body's defence system.

This research investigated fruit-derived antioxidants, and developed biomarker assays to measure the potential health benefits they may offer. To determine the in vivo antioxidant efficacy of berry fruit anthocyanins, oxidative damage to proteins, lipids and DNA was measured in rats fed several combinations of natural and synthetic diets. Mild oxidative damage was induced by the inclusion of fish oil in these diets.

DNA oxidation was determined by measuring urinary 8-hydroxy-2'-deoxyguanosine using reversed-phase high performance liquid chromatography with electrochemical detection. ELISA and colorimetric techniques were used to measure protein carbonyl content of plasma as a reflection of protein oxidation. Oxidation to lipids was assessed by measuring malondialdehyde, which results from lipid peroxidation.

Supplementation with fish oil induced a mild form of dietary oxidative damage, as shown by an increase in lipid and protein oxidation. In most cases the berry fruit extracts had little effect on the level of fish oil-induced oxidative damage, however, boysenberry anthocyanin extract significantly reduced protein oxidation when used in combination with the natural diet. Taken together the results suggest that oxidative damage to biomacromolecules may occur by different pathways of oxidative stress, which selectively target either DNA, protein or lipids at varying levels, and the antioxidant is effective only with selected mechanisms of oxidative damage.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>8OHdG</td>
<td>8-hydroxy-2’-deoxyguanosine</td>
</tr>
<tr>
<td>AAPH</td>
<td>2,2’-azobis(2-amidinopropane) dihydrochloride</td>
</tr>
<tr>
<td>ABTS</td>
<td>2,2’-azinobis-(3-ethyl-benzothiazoline-6-sulphonic acid)</td>
</tr>
<tr>
<td>AUC</td>
<td>area under curve</td>
</tr>
<tr>
<td>BHT</td>
<td>butylated hydroxytoluene</td>
</tr>
<tr>
<td>BSA</td>
<td>bovine serum albumin</td>
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<tr>
<td>CHCl₃</td>
<td>carbon tetrachloride</td>
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<tr>
<td>CoA</td>
<td>coenzyme A</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNPH</td>
<td>dinitrophenylhydrazine</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylene diamine tetra-acetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunoabsorbent assay</td>
</tr>
<tr>
<td>FPG</td>
<td>formamidopyrimidine DNA N-glycosylase</td>
</tr>
<tr>
<td>FO</td>
<td>fish oil</td>
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<tr>
<td>FRAP</td>
<td>free radical antioxidant power</td>
</tr>
<tr>
<td>GC-MS</td>
<td>gas chromatography – mass spectrometry</td>
</tr>
<tr>
<td>HCl</td>
<td>hydrochloric acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>high-performance liquid chromatography</td>
</tr>
<tr>
<td>IPA</td>
<td>isopropyl alcohol</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography with mass spectrometry</td>
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<tr>
<td>LDL</td>
<td>low density lipoprotein</td>
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<tr>
<td>MDA</td>
<td>malondialdehyde</td>
</tr>
<tr>
<td>MeOH</td>
<td>methanol</td>
</tr>
<tr>
<td>NaOAc</td>
<td>sodium acetate</td>
</tr>
<tr>
<td>ORAC</td>
<td>oxygen radical absorbance capacity</td>
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<tr>
<td>ORAC&lt;sub&gt;FL&lt;/sub&gt;</td>
<td>oxygen radical absorbance capacity assay using fluorescein</td>
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<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acid</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>reversed-phase high-performance liquid chromatography</td>
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<tr>
<td>SBO</td>
<td>soybean oil</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>SPE</td>
<td>solid phase extraction</td>
</tr>
<tr>
<td>TBA</td>
<td>thiobarbituric acid</td>
</tr>
<tr>
<td>TCA</td>
<td>trichloroacetic acid</td>
</tr>
<tr>
<td>TE</td>
<td>trolox equivalent</td>
</tr>
<tr>
<td>TEAC</td>
<td>trolox equivalent antioxidant capacity</td>
</tr>
<tr>
<td>Trolox</td>
<td>6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid</td>
</tr>
</tbody>
</table>
# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acknowledgements</td>
<td>ii</td>
</tr>
<tr>
<td>Abstract</td>
<td>iv</td>
</tr>
<tr>
<td>Abbreviations</td>
<td>v</td>
</tr>
<tr>
<td>List of Figures</td>
<td>xvi</td>
</tr>
<tr>
<td>List of Tables</td>
<td>xix</td>
</tr>
</tbody>
</table>

## Chapter One – Introduction

1.1 Introduction

1.2 Oxidative Damage

1.2.1 Free Radicals

1.2.2 The Biological Consequences of Oxidative Damage

1.2.2.1 Cellular Consequences

1.2.2.2 Disease Pathogenesis

1.3 Antioxidants

1.3.1 Non-Enzymatic Antioxidant Systems

1.3.1.1 Endogenous Antioxidants

1.3.1.2 Dietary Antioxidants
1.3.2 Enzymatic Antioxidant Systems

1.3.2.1 Redox Cycling Enzymes (Phase 2 Enzymes)
1.3.2.2 Repair Enzymes

1.4 Methods for Measuring Oxidative Damage

1.4.1 The Concept of Biomarkers

1.4.2 Oxidative Damage to DNA

1.4.2.1 8OHdG Keto-enol Tautomerism
1.4.2.2 Non-invasive Urinary Analysis
1.4.2.3 Analysis of Cells for Oxidative DNA Damage

1.4.3 Oxidative Damage to Proteins

1.4.3.1 Methodology

1.4.4 Oxidative Damage to Lipids

1.4.4.1 Malondialdehyde Analysis
1.4.4.2 Isoprostane Analysis
1.4.4.3 Volatile Hydrocarbon Analysis

1.4.5 Overall Antioxidant Capacity

1.4.5.1 Oxygen Radical Absorbance Capacity Analysis
1.4.5.2 Free Radical Antioxidant Power Analysis
1.4.5.3 Trolox Equivalent Antioxidant Capacity Analysis
1.4.5.4 Comparison of FRAP, ORAC and TEAC

1.4.6 Oxidative Damage in Mammalian Systems

1.4.6.1 Inducing Oxidative Damage
1.4.6.2 Cell-Based Studies
1.4.6.3 Mammalian Studies
1.5 Hypothesis and Aims

1.5.1 Hypothesis

1.5.2 Aims

Chapter Two - Method Development

2.1 8OHdG Assay to Measure Oxidative Damage to DNA

2.1.1 Standard Preparation

2.1.2 Sample Preparation by Solid Phase Extraction

2.1.2.1 Preliminary Method Development: Is Solid Phase Extraction Necessary? 26

2.1.2.2 Solid Phase Extraction Protocol 28

2.1.2.3 pH control of 8OHdG to Optimise Recovery 29

2.1.2.4 Intra-sample Variation 32

2.1.2.5 Implementation of Quality Controls for Sample Recovery Analysis – Inter-batch Variation 32

2.1.3 High Performance Liquid Chromatography Analysis

2.1.3.1 HPLC Columns and Solvent Programmes 32

2.1.3.2 Detection of 8OHdG 35

2.1.3.3 Inter- and Intra-assay Variation Associated with HPLC 36

2.1.4 Summary 36

2.2 Protein Carbonyl Assay to Measure Oxidative Damage to Proteins 37
2.2.1 Colorimetric Determination of Carbonyl Content

2.2.1.1 Initial Methodology Used

2.2.1.2 Final Methodology Used

2.2.2 Determination of Carbonyl Content Using an Enzyme-linked Immuno-adsorbent Assay (ELISA)

2.2.3 Comparison of ELISA and Colorimetric Methods for Determination of Protein Carbonyl Content of Plasma

Chapter Three - Materials and Methods

3.1 Rat Feeding Experiments

3.1.1 General Protocol

3.1.1.1 Animals

3.1.1.2 Diets

3.1.1.3 Sample Collection

3.2 Sample Preparation and Analysis

3.2.1 8OHdG Assay to Measure Oxidative Damage to DNA

3.2.1.1 Sample Preparation: Solid Phase Extraction to Clean-up Samples and Concentrate 8OHdG

3.2.1.2 Reversed-Phase High Performance Liquid Chromatography

3.2.1.3 Quantification of 8OHdG in Urine

3.2.2 Protein Carbonyl Assay to Measure Oxidative Damage to Proteins
3.2.2.1 Colorimetric Method
3.2.2.2 ELISA Method

3.2.3 Malondialdehyde Assay to Measure Oxidative Damage to Lipids
3.2.3.1 Standard Preparation
3.2.3.2 Sample Preparation
3.2.3.3 Determination of MDA Concentration

3.2.4 ORAC<sub>FL</sub> Assay to Measure the Overall Antioxidant Capacity
3.2.4.1 Sample Preparation
3.2.4.2 Procedure
3.2.4.3 Determination of Trolox Equivalent ORAC Value

3.2.5 α-tocopherol Assay
3.2.5.1 Sample Preparation
3.2.5.2 RP-HPLC Analysis
3.2.5.3 Determination of α-tocopherol Concentration

3.2.6 Statistical Analysis

Chapter Four – Fish Oil Feeding Trial Results and Discussion

4.1 Overall Observations

4.1.1 Rat Weight Gain

4.2 Biomarkers of Oxidative Damage

4.2.1 Oxidised DNA
4.2.2 Oxidised Protein

4.2.3 Oxidised Lipid

4.2.4 Plasma Antioxidant Status

4.3 Discussion

4.3.1 Increasing Oxidative Damage with Increasing Fish Oil

Chapter Five – Fruit-derived Antioxidant Feeding Trials
#1 and #2 Results and Discussion

5.1 Overall Observations

5.1.1 Rat Weight Gain

5.1.2 Dietary Consumption

5.2 Biomarkers of Oxidative Damage

5.2.1 Oxidised DNA

5.2.2 Oxidised Protein

5.2.3 Oxidised Lipid

5.2.4 Plasma Antioxidant Status
Chapter Six – Fruit-derived Antioxidant Feeding Trial #3

Results and Discussion

6.1 Overall Observations

6.1.1 Rat Weight Gain

6.1.2 Dietary Consumption

6.2 Biomarkers of Oxidative Damage

6.2.1 Oxidised DNA

6.2.2 Oxidised Protein

6.2.3 Oxidised Lipid

6.2.4 Plasma Antioxidant Status

6.3 Discussion

6.3.1 Fish Oil-Induced Oxidative Damage
6.3.2 Reduction of Oxidative Damage by Berry fruit Anthocyanins

Chapter Seven - Summary and Future Direction

7.1 Overall Summary

7.2 Future Plans

7.2.1 Biomarker Assays

7.2.1.1 Oxidised DNA Assay
7.2.1.2 Additional Biomarker Analyses

7.2.2 Future Feeding Trials

7.2.2.1 Animal Trials
7.2.2.2 Human Trials

7.2.3 Mechanistic Studies

7.2.3.1 Mechanisms of Oxidative Stress
7.2.3.2 Mechanisms of Anthocyanin Antioxidant Action

Appendices

Appendix One - Calibration Curve for 8OHdG Analysis

Appendix Two - Calibration Curve for Determination of Protein Content (Bradford Method)
List of Figures

**Figure 1.1** Generalised structure of an anthocyanin molecule. 8

**Figure 1.2** 8-hydroxy-2’-deoxyguanosine keto-enol tautomerism. 12

**Figure 2.1** Chromatograms for 100 µL injections of: 8OHdG standard (A), urine that has not been through SPE (B), and urine that has been through SPE (C). 27

**Figure 2.2** Percentage recoveries for 1 µg/mL 8OHdG standard through different SPE cartridges to determine which cartridge produces the optimum recovery of 8OHdG. 29

**Figure 2.3** Percentage recoveries for 1 µg/mL 8OHdG standards prepared in either pH 3.5 or pH 7.5 buffer. 30

**Figure 2.4** Percentage recoveries for spiked urine samples where the pH had been changed to 3-4 with 2 mol/L HCl prior to storage compared to urine where the pH had not been altered. 31

**Figure 2.5** Example chromatogram of 1 µg/mL 8OHdG standard where the mobile phase conditions were pH 5.5. 33

**Figure 2.6** Comparison of the ELISA and colorimetric (at 370 nm) methods for determination of protein carbonyl content for fruit-derived antioxidant feeding trials #1 and #2. 39
Figure 3.1  Schematic diagram of switching valve positions used to isolate 8OHdG in the 1 mL loop and apply to column B for analysis by RP-HPLC with CoulArray® detection.

Figure 3.2  Example chromatogram from UV detection (at 260 nm) of a 5 µg/mL 8OHdG standard prior to a sample run.

Figure 4.1  Study design for fish oil feeding trial to determine which level of fish oil is most effective at inducing oxidative stress to a measurable level.

Figure 4.2  Cumulative weight gain of rats fed diets supplemented with the given percentages of fish oil (FO) and soybean oil (SBO).

Figure 4.3  MDA concentration (ng/mL) in plasma samples from rat feeding experiment where the diets were supplemented with the given percentages of fish oil in order to increase oxidative damage.

Figure 5.1  Study design for fruit-derived antioxidants feeding trial #1.

Figure 5.2  Cumulative weight gain in trial #1.

Figure 5.3  Cumulative weight gain in trial #2.

Figure 5.4  A representation of a chromatogram for a typical urine sample, after solid phase extraction and concentration, for 8OHdG analysis by HPLC with electrochemical detection.

Figure 5.5  8OHdG concentration (ng/mL) in urine taken from rats at the end of the trial, for fruit-derived antioxidants feeding trial #1.
Figure 6.1  Study design for fruit-derived antioxidant feeding trial #3. 74

Figure 6.2  Cumulative weight gain for fruit-derived antioxidant feeding trial #3. 76

Figure 6.3  8OHdG concentration for each diet throughout trial #3. 79

Figure 6.4  Comparison of the 8OHdG concentration ng/mL (A) nmol 8OHdG/kg body weight/24 hours (B) and nmol 8OHdG/nmol creatinine (C). 81

Figure 6.5  Protein carbonyl concentration (nmol/mg protein) from ELISA analysis of plasma from trial #3. 83

Figure 6.6  MDA concentration in plasma samples from trial #3. 84

Figure 6.7  Vitamin E content, as measured by plasma α-tocopherol concentration (µg/mL) from samples from trial #3. 85

Figure 7.1  Markers for development of methodology to assess the efficacy of functional food. 93

Figure 7.2  Chromatogram for 5 µg/mL 8OHdG standard from column A showing switching valve times currently used, and proposed switching times. 96
List of Tables

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table 1.1</td>
<td>Active oxygen and related species.</td>
<td>2</td>
</tr>
<tr>
<td>Table 3.1</td>
<td>Constituents of the natural and synthetic diets used in rat feeding trials.</td>
<td>42</td>
</tr>
<tr>
<td>Table 3.2</td>
<td>Composition of boysenberry anthocyanin powder used in trial #3, and blackcurrant powder used in trial #1.</td>
<td>43</td>
</tr>
<tr>
<td>Table 3.3</td>
<td>Solvent gradient used by Jasco pump for column B in the assay for urinary 8OHdG using a dual column, switching valve method with HPLC and CoulArray® detection.</td>
<td>46</td>
</tr>
<tr>
<td>Table 4.1</td>
<td>Mean concentrations of 8OHdG, oxidised protein from colorimetric analysis, MDA, ORAC_{FL} value and vitamin E for samples from the fish oil rat trial.</td>
<td>56</td>
</tr>
<tr>
<td>Table 5.1</td>
<td>Concentration (mg/g extract) of anthocyanin at ( A_{530} ) and total phenolics at ( A_{280} ) of the extracts used in trials #1 and #2.</td>
<td>64</td>
</tr>
<tr>
<td>Table 5.2</td>
<td>Total concentration (mg/feed) of anthocyanin at ( A_{530} ) phenolics at ( A_{280} ) and ORAC value.</td>
<td>65</td>
</tr>
<tr>
<td>Table 5.3</td>
<td>Mean concentrations of 8OHdG, oxidised protein from ELISA analysis, MDA, ORAC_{FL} value and vitamin E for samples from rat trials #1 and #2.</td>
<td>66</td>
</tr>
<tr>
<td>Table 6.1</td>
<td>Concentration (mg/g extract) of anthocyanin at A₅₃₀ and total phenolics at A₂₈₀ of the boysenberry extract used in trial #3.</td>
<td></td>
</tr>
<tr>
<td>Table 6.2</td>
<td>Concentration (mg/g of feed) of anthocyanin at A₅₃₀ and total phenolics at A₂₈₀.</td>
<td></td>
</tr>
<tr>
<td>Table 6.3</td>
<td>Concentration of 8OHdG, oxidised protein from ELISA analysis, MDA and vitamin E for samples from rat trial #3.</td>
<td></td>
</tr>
<tr>
<td>Table 6.4</td>
<td>Mean volume of urine excreted over 24 hours from rats in trial #3.</td>
<td></td>
</tr>
<tr>
<td>Table 7.1</td>
<td>Suggested alterations to solvent gradient used by Jasco pump for column B in the assay for urinary 8OHdG using a dual column, switching valve method of HPLC with CoulArray® detection.</td>
<td></td>
</tr>
</tbody>
</table>