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# Dimeric procyanidins as modulators of airway inflammation in the context of allergic asthma

A thesis presented in partial fulfilment of  
the requirements for the degree of

Doctor of Philosophy (PhD)

in

Human Physiology

at

Massey University

Manawatū, Palmerston North, New Zealand

Sara L Coleman, MS

2017



# Declaration

---

It is hereby declared that this thesis has been composed by the undersigned Sara L. Coleman for the degree of Doctor of Philosophy (PhD) at Massey University. This work has not been presented in any previous application for a degree. All work was performed by the undersigned unless otherwise stated in the text. All sources of information have been specifically acknowledged in the text.

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A handwritten signature in black ink, appearing to read 'Sara L. Coleman', is written over a light blue horizontal line. The signature is fluid and cursive.

Sara L. Coleman

August 2017

# Abstract

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Dimeric procyanidins as modulators of airway inflammation in the context of allergic asthma

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Procyanidins are polyphenolic compounds that have come to be known as biologically active in the context of promoting human health. Epidemiological evidence suggests that populations that consume diets rich in procyanidins are less susceptible to inflammatory diseases. Allergic asthma is an inflammatory lung disease with an estimated 100 million affected individuals worldwide, with New Zealand having the world's second highest rate. Inflammation at the airway epithelium and infiltration of immune cells, specifically eosinophils, into the lung tissue are two central characteristics of allergic asthma. Thymic stromal lymphopoietin (TSLP) and eotaxin isoforms, eotaxin-1 (CCL11) and eotaxin-3 (CCL26), are three biomarkers of airway inflammation produced by the epithelium. Cell culture models were successfully optimized for CCL11 and CCL26 production in A549 cells. Investigation of procyanidins effect on epithelial TSLP production was not possible because TSLP production in A549 cells was undetectable. Data suggests that dimeric A-type linked procyanidin A2, but not B-type linked procyanidin B1 or B2, is capable of inhibiting IL-4-induced CCL11 production when incubated on A549 cells prior to an inflammatory insult. Co-incubation of A549 cells with procyanidin A2 and procyanidin B2 demonstrated no evidence of a synergistic relationship for inhibiting cytokine-

induced CCL11 production. Similarly, A549 cells exposed to procyanidin A2, and to a lesser extent procyanidin B2, had reduced production of cytokine-induced CCL26 production. An inhibition time course demonstrated procyanidin A2 had greatest inhibition efficacy on cytokine-induced CCL26 production when incubated for 2 h prior to an inflammatory insult. Comparison of procyanidin A2 inhibition to the known CCL26 inhibitor, IFN $\gamma$ , demonstrated that procyanidin A2 and IFN $\gamma$  did not share the same temporal inhibition patterns. Furthermore, experiments investigating concomitant incubation of procyanidin A2 and IFN $\gamma$  demonstrated that procyanidin A2 could interfere with IFN $\gamma$ -mediated CCL26 inhibition. Two possible mechanisms responsible for the procyanidin A-mediated inhibition of cytokine-induced CCL11 and CCL26 were investigated: the modulation of cytokine receptor expression, and modulation of plasma membrane fluidity. However, there was no evidence to support either of these modes of action. The data presented in this thesis collectively demonstrate the ability of procyanidin A2 to inhibit cytokine-induced eotaxin production from the lung epithelium *in vitro* and support further investigation of procyanidin A2 as a preventative approach for managing airway inflammation.

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## Abbreviations

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|                     |   |
|---------------------|---|
| 16HBE14o-           | human airway epithelial cell-line           |
| A549                | human alveolar epithelial cell-line         |
| AD                  | Alzheimer's disease                         |
| AP-1                | activator protein-1                         |
| APC                 | allophycocyanin fluorophore                 |
| ARE                 | antioxidant response element                |
| B2G2                | procyanidin B2 3,3''-di-O-gallate           |
| BALF                | bronchoalveolar lavage fluid                |
| BD                  | Becton Dickinson                            |
| BEAS-2B             | human bronchial epithelial cell-line        |
| BSA                 | bovine serum albumin                        |
| CA                  | catechin                                    |
| Caco2               | human colon epithelial cell-line            |
| Calu-3              | human lung epithelial cell-line             |
| Calu-6              | human lung epithelial cell-line             |
| cAMP                | cyclic adenosine monophosphate              |
| CCL11               | eotaxin-1                                   |
| CCL24               | eotaxin-2                                   |
| CCL26               | eotaxin-3                                   |
| CCL3                | macrophage inflammatory protein 1-alpha     |
| CCR3                | C-C chemokine receptor type 3               |
| CD4+                | cluster of differentiation 4 positive       |
| CgC                 | common gamma chain                          |
| CO <sub>2</sub>     | carbon dioxide                              |
| COPD                | chronic obstructive pulmonary disease       |
| COX-2               | cyclooxygenase-2                            |
| CSB                 | cell staining buffer                        |
| -Cy <sup>TM</sup> 7 | cyanine 7 fluorophore                       |
| DC                  | dendritic cell                              |
| DiO                 | 3,3'-dioctadecyloxycarbocyanine perchlorate |
| DMEM                | Dulbecco's modified eagle medium            |
| DMEM/F-12           | DMEM with Nutrient Mixture F-12             |
| DMSO                | dimethyl sulfoxide                          |
| DP                  | degree of polymerization                    |
| DPH                 | 1,6-diphenyl-1,3,5-hexatriene               |
| EC                  | epicatechin                                 |
| EDN                 | eosinophil-derived neurotoxin               |
| EDTA                | ethylenediaminetetraacetic acid             |
| ELISA               | enzyme-linked immunosorbent assay           |
| em                  | emission                                    |



|                               |   |
|-------------------------------|---|
| EPA                           | eicosapentaenoic acid   |
| ERK1/2                        | extracellular-regulated kinase 1 and 2  |
| ex                            | excitation  |
| F1                            | first filial generation   |
| FBS                           | foetal bovine serum   |
| FcεRI                         | high-affinity IgE receptor  |
| FITC                          | fluorescein   |
| FLVR                          | <i>Faecalibacterium, Lachnospira, Veillonella, and Rothia</i>                     |
| FSC                           | forward scatter   |
| GM-CSF                        | granulocyte-macrophage colony-stimulating factor                                  |
| H <sub>2</sub> O <sub>2</sub> | hydrogen peroxide   |
| HeLa                          | human cervix epithelial cell-line   |
| HepG2                         | human liver epithelial cell-line  |
| HRP                           | horseradish peroxidase  |
| IBD                           | inflammatory bowel disease  |
| IFNγ                          | interferon gamma  |
| IgE                           | immunoglobulin E  |
| IgG1                          | immunoglobulin G1   |
| IL                            | interleukin   |
| IL-13Rα1                      | interleukin 13 receptor alpha one   |
| IL-4Rα                        | interleukin 4 receptor alpha  |
| ILC                           | innate lymphoid cell  |
| ILC2                          | ILC type 2  |
| IM9                           | human peripheral blood B lymphoblast cell-line                                    |
| JAK                           | janus kinase  |
| JNK1/2                        | C-Jun N-terminal kinase 1 and 2   |
| kDa                           | kilo Dalton   |
| LDH                           | lactate dehydrogenase   |
| LPS                           | lipopolysaccharide  |
| MALDI-TOF MS                  | matrix-assisted laser desorption/ionization with time-of-flight mass spectrometer |
| MAPK                          | mitogen-activated protein kinase  |
| mRNA                          | messenger RNA   |
| NAD <sup>+</sup>              | nicotinamide adenine dinucleotide (oxidized)                                      |
| NADH                          | nicotinamide adenine dinucleotide (reduced)                                       |
| NFAT                          | nuclear factor of activated T cells   |
| NF-κB                         | nuclear factor kappa-light-chain-enhancer of activated B cells                    |
| NO                            | nitric oxide  |
| Nrf2                          | nuclear factor E2-related factor 2  |
| NS                            | not significant   |
| NZ                            | New Zealand   |
| OVA                           | ovalbumin   |
| Ox40L                         | Ox40 ligand   |

|              |   |
|--------------|---|
| PBS          | phosphate buffered saline                           |
| PD-L1        | programmed death ligand 1                           |
| PE           | phycoerythrin fluorophore                           |
| PerCP        | peridinin chlorophyll protein fluorophore           |
| PFR          | Plant & Food Research                               |
| PMT          | photomultiplier tube                                |
| Procy        | procyanidin   |
| PSN          | penicillin streptomycin neomycin antibiotic mixture |
| RPMI         | Roswell Park Memorial Institute                     |
| SCFA         | short chain fatty acid                              |
| SEM          | standard error mean                                 |
| SPTS         | Science Publication Tracking System                 |
| SSC          | side scatter  |
| STAT         | signal transducers and activation of transcription  |
| T reg        | T regulatory cell                                   |
| TEER         | transepithelial electrical resistance               |
| Th1          | T-helper 1  |
| Th2          | T-helper 2  |
| THLE-2       | human liver epithelial cell-line                    |
| TMB          | 3,3',5,5'-tetramethylbenzidine                      |
| TNF R1       | tumour necrosis factor receptor one                 |
| TNF $\alpha$ | tumour necrosis factor alpha                        |
| TSLP         | thymic stromal lymphopoietin                        |
| UK           | United Kingdom                                      |
| US           | United States                                       |
| USDA         | United States Department of Agriculture             |
| UV           | ultraviolet   |
| WST-1        | water soluble tetrazolium-1                         |

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## External Contributions

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Portions of this thesis have been published elsewhere in the form of a book chapter, research articles, and a review article. Original drafts of these publications were solely written by Sara Coleman and then subsequently edited by co-authors of each publication. Manuscripts were submitted to Science Publication Tracking system (SPTS) at PFR, a compulsory internal process which includes scientific peer review and professional editing. All portions of this thesis not published elsewhere, are the product of Sara L Coleman alone.

A declaration of contribution detailing the specifics is included at the beginning of each chapter.

# Outputs

---

## Publications

Coleman, S.L.; Hurst, R.D.; Sawyer, G.M.; Kruger, M.C. Fruit Procyanidins: Modulating Inflammation to Promote Health. In: Sullivan I, editor. Proanthocyanidins: Food Sources, Antioxidant Properties, and Health Benefits. New York: Nova Science Publishers, Inc.; 2015. p. 73-97.

Coleman, S.L.; Hurst, R.D.; Sawyer, G.M.; Kruger, M.C.; The in vitro evaluation of isolated procyanidins as modulators of cytokine-induced eotaxin production in human alveolar epithelial cells. *J. Berry Res.* 2016; 5: 115-124; DOI: 10.3233/JBR-160121

Coleman, S.L.; Kruger, M.C.; Sawyer, G.M.; Hurst, R.D. Procyanidin A2 Modulates IL-4-Induced CCL26 Production in Human Alveolar Epithelial Cells. *Int. J. Mol. Sci.* 2016, 17, 1888; DOI: 10.3390/ijms17111888

Coleman, S.L.; Shaw, O.M. Progress in our understanding of allergic asthma pathology supports the potential of fruit proanthocyanidins as modulators of airway inflammation. 2017 (Under Review)

Abstracts for Publications can be seen in Appendix I

# Presentations

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## **Presentations:**

- Dec 2014 Confirmation Presentation at Massey University, Palmerston North. New Zealand
- July 2015 Oral Presentation at NZASI Conference Auckland, New Zealand
- October 2015 Poster Presentation at Berry Health Benefits Symposium Conference, Madison, Wisconsin, USA
- June 2016 Oral Presentation at Joint Graduate School of Horticulture and Food Enterprise, Massey University, Palmerston North, New Zealand

## **Additional Scientific Education:**

- July 2014 Attendance NZASI Conference Palmerston North, NZ
- October 2015 US:NZ Science Workshop- Building the Health Claims Dossier for Berry Fruits
- April 2016 Polychromatic Flow Cytometry Roadshow, Wellington, NZ