

ABSTRACT

Erythema is a hallmark skin response to excessive ultraviolet radiation (UVR) and is associated with cutaneous inflammation. Both are mediated by inflammatory mediators including nitric oxide (NO), prostaglandin E₂ (PGE₂) and chemoattractants such as 12-hydroxyeicosatetraenoic acid (12-HETE) leading to vasodilation and increased leukocyte infiltration. The erythematous response is more pronounced in individuals with low basal melanin levels or who fail to respond to UVR with a robust up-regulation of melanogenesis. While melanin production is a key function of melanocytes, these cells can also produce NO and PGE₂, and are located in close proximity to the dermal vasculature. It has been hypothesized that melanocytes with poor melanogenic capacity may participate in the inflammatory response to UVR.

The aim of this project was to investigate the inflammatory response in the skin of individuals with either skin phototype (SPT) 1 or 4 to UVR. Sixteen normal healthy individuals were selected for study (8 SPT-1 & 8 SPT-4). Buttock skin was investigated by immunohistochemistry for leukocyte subtypes, eicosanoid producing enzymes and NO synthases under basal and UVR-stimulated conditions. In addition primary cultures of epidermal melanocytes (EM) were established from 16 individuals (8 SPT-1 & 8 SPT-4) and assessed for the presence of eicosanoid-producing enzymes, melanogenic enzymes and NO synthases, by immunocytochemistry, Polymerase Chain Reaction and Western Blotting and for the production of the main pro-inflammatory eicosanoid PGE₂ by ELISA and Mass Spectrometry. Moreover, the fatty acid composition of cultured melanocytes was assessed by Gas Chromatography.

Results showed that individuals with SPT-1 had significantly greater neutrophil infiltration into the epidermis than those with SPT-4 at 24 hrs post-UVR. Moreover,

CD3⁺ lymphocyte infiltration into the dermis was significantly greater in individuals with SPT-4 than those with SPT-1 at 24 and 72 hrs post-UVR. NOS-1, NOS-3, 12-LOX and COX-2 expression were significantly increased in SPT-1 skin, while NOS-2 and 15-LOX were significantly increased in SPT-4 skin. As 12-LOX and COX-2 products are chemoattractive (for neutrophils) and pro-inflammatory respectively these data could explain the greater observed neutrophil infiltration in SPT-1. The 15-LOX product (15-HETE) is anti-inflammatory and may suggest that 15-LOX up-regulation in SPT-4 skin may aid resolution of the sunburn response, which in part may be mediated by CD3⁺ lymphocytes and a class-switch in eicosanoid production from COX to LOX products.

Melanocyte primary cultures surprisingly showed that SPT was not correlated with melanin content or melanogenic enzyme expression/activity suggesting that all melanocytes *in vitro* contained the necessary cellular machinery to produce melanin. This finding may reflect also their equal treatment under these enriched culture conditions, which may or may not be available to these cells *in situ*. Moreover, all melanocytes expressed the necessary machinery (PLA₂, COX-1, cPGES) to produce PGE₂. However, only some cultures did so at baseline and in response to UVR, and this was not correlated with SPT. A positive correlation was found however between expression level of dopachrome tautomerase (DCT) and protection against PGE₂ production in response to UVR, which may suggest a novel role for DCT unrelated to melanogenesis.

In summary this research project has generated data that highlights differences between the skin of individuals with SPT-1 and those with SPT-4, and may provide evidence that the keratinocyte partner contributes significantly to the SPT-associated response. This research may also suggest DCT as a novel therapeutic target to protect EM from participation in the UVR-associated inflammatory response in skin.

ACKNOWLEDGEMENTS

I would like to thank Prof. D. J. Tobin for providing me with guidance and encouragement for what at times felt an almost impossible task. I would also like to thank him for always making time to see me and discussing any problems that I may have been encountering. Also just as importantly I would like to thank him for stimulating me to come up with solutions for some of these problems.

I would like to thank Prof. A. Nicolaou for acting as my second supervisor. Her knowledge of lipid chemistry was invaluable. I would like to thank Prof. L. E. Rhodes, M. Brownrigg and A. Haylet for providing me with the skin specimens, without which this project would not have been possible and Prof. A. J. Thody and aforementioned for the opportunity for discussion of my data. I would like to thank Dr. Mojgan Masoodi for her help with Mass Spectrometry and Dr. Karen Massey and Juliano Miyake for their help with Gas Chromatography.

I would like to thank my Tobin lab colleagues Dr. Suman K. Singh, Dr. Judy (Man Ching) Leung, Dr. Aisha N. Meskiri, Dr. Sobia Kauser and Dr. Nik Papageorgiou for their help in and out of the lab.

I would like to thank my girlfriend Victoria Hutchinson, my parents Martin and Dee Gledhill and my sister Joanne Gledhill for their love and support.

Finally, I would like to thank the School of Life Sciences at the University of Bradford and the Wellcome Trust for funding this research project.

CONTENTS

ABSTRACT.....	i
ACKNOWLEDGEMENTS.....	iii
CONTENTS.....	iv
LIST OF FIGURES	x
LIST OF TABLES	xvi
ABBREVIATIONS	xvii
REAGENTS.....	xxii
EQUIPMENT AND CONSUMABLES	xxv
PRIMARY ANTIBODIES	xxvii
SECONDARY ANTIBODIES	xxviii
BLOCKING PEPTIDES.....	xxviii
PRIMERS.....	xxix
BIOLOGICAL MATERIAL.....	xxx
1 INTRODUCTION	1
1.1 Structure and Function of the Skin	2
1.1.1 Epidermis	2
1.1.1.1 Keratinocytes.....	3
1.1.1.2 Melanocytes	7
1.1.1.3 Langerhans Cells.....	8
1.1.1.4 Merkel Cells.....	8
1.1.2 Basement Membrane Zone	9
1.1.3 Dermis.....	9
1.1.3.1 Fibroblasts.....	10
1.1.3.2 Mast Cells	10
1.1.3.3 Appendages of the Skin	11
1.1.4 Subcutaneous Layer	11
1.2 Response of the Skin to Ultraviolet Radiation.....	12
1.3 Melanocyte Biology.....	13
1.3.1 Melanocyte Function.....	13
1.3.2 Melanogenesis.....	14
1.3.3 Melanin Structure.....	17
1.3.3 Regulation of Melanogenesis.....	18
1.4 Variation in Skin Pigmentation and Inflammation in Response to UVR	21
1.5 Skin Phototypes.....	23
1.6 Eicosanoids	24
1.6.1 Origins.....	24
1.6.2 Phospholipase-A ₂	25
1.6.3 Cyclooxygenase Pathway.....	27
1.6.3.1 Prostaglandin I ₂ (PGI ₂).....	30
1.6.3.2 Prostaglandin E ₂ (PGE ₂)	31
1.6.3.3 Prostaglandin F _{2α} (PGF _{2α})	31
1.6.3.4 Prostaglandin D ₂ (PGD ₂)	32
1.6.4 Lipoxygenase Pathway	32
1.7 Photobiology of the Skin.....	33
1.7.1 Ultraviolet Radiation.....	33

1.7.3 Formation of Reactive Oxygen Species	35
1.7.2 Effect of Ultraviolet Radiation on Biological Molecules	37
1.8 Defense Mechanisms of Cell to Combat Oxidative Stress	42
2 AIMS OF THE PROJECT	45
3 MATERIALS AND METHODS	46
3.1 Isolation of Epidermal Melanocytes from Human Skin Blister Roofs	47
3.2 Cell Culture	48
3.2.1 Feeding of Cell Cultures	48
3.2.2 Passaging of Cell Cultures	48
3.2.3 Freezing and Thawing of Cell Cultures	49
3.2.4 Cell Counting	49
3.3 Cell Stimulations	50
3.3.1 Ultraviolet Radiation	50
3.3.2 Arachidonic Acid	51
3.3.3 Luzindole.....	51
3.3.4 Melatonin	51
3.4 Tissues.....	51
3.5 Immunocytochemistry.....	52
3.5.1 Preparation of Skin Sections for Immunohistochemistry	52
3.5.2 Immunohistochemical Staining.....	53
3.5.3 Quantification of Immunohistochemical Analysis.....	54
3.5.4 Preparation of Cells for Immunocytochemistry	55
3.5.5 Immunocytochemical Staining.....	55
3.5.6 Single Immunofluorescence.....	56
3.5.7 Double Immunofluorescence	57
3.6 Polymerase Chain Reaction	58
3.6.1 RNA Extraction.....	59
3.6.2 RNA Quantification	60
3.6.3 cDNA Synthesis	60
3.6.4 Polymerase Chain Reaction	61
3.6.5 Visualization of cDNA.....	61
3.7 Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis and Western Blotting.....	61
3.7.1 Protein Extraction.....	62
3.7.2 Quantification of Protein in Cell Extract (Bradford Assay)	63
3.7.3 SDS-PAGE of Proteins	63
3.7.4 Transblotting of SDS-PAGE Separated Proteins.....	64
3.7.5 Western Immunoblotting of Proteins	64
3.7.6 Visualization of Antibody-Reactive Proteins	65
3.7.7 Densitometric analysis of Results Produced by Western Blotting.....	66
3.8 Prostaglandin E ₂ and D ₂ -MOX Enzyme Immunoassay	66
3.8.1 Pre-Assay Preparation.....	67
3.8.2 Preparation of Assay Specific Reagents	67
3.8.3 Immunoassay Plate Set-up	68
3.8.4 Performing the Assay	68
3.8.5 Analysis of Assay Results.....	69
3.9 Liquid Chromatography/Electrospray Tandem Mass Spectrometry.....	70

3.9.1 Sample preparation.....	70
3.9.2 LC/ESI-MS/MS Analysis	71
3.10 Gas Chromatography	72
3.10.1 Lipid Extraction from Cell Pellets	72
3.10.2 Preparation of Fatty Acid Methyl Esters.....	74
3.10.3 Gas Chromatography Analysis	75
3.11 Melanin Assay.....	75
3.12 Dopa-Oxidase (Tyrosinase Activity) Assay.....	76
3.13 Statistical Analysis.....	77
4 RESULTS	78
4.1 Leukocyte Infiltration into Human Skin in Response to UVR	79
4.1.1 UVR Increases Neutrophil Infiltration into Human Skin (Phototypes 1 and 4)	79
4.1.1.1 UVR Increases Neutrophil Infiltration into Human Skin (Phototype 1)..	80
4.1.1.2 UVR Increases Neutrophil Infiltration into Human Skin (Phototype 4)..	81
4.1.2 UVR Increases CD3 ⁺ Lymphocyte Infiltration Into Human Skin (Phototypes 1 and 4).....	84
4.1.2.1 UVR has no Effect on CD3 ⁺ Lymphocyte Infiltration into Human Skin (Phototype 1).....	85
4.1.2.2 UVR Increases CD3 ⁺ Lymphocyte Infiltration into Human Skin (Phototype 4).....	87
4.1.3 Infiltration of CD4 ⁺ T-Helper Cells, but not CD8 ⁺ T-Cytotoxic Cells, Occurs in the Sunburn Response in Human Skin.....	89
4.2 Effect of UVR on Nitric Oxide Synthase Expression in Human Skin.....	92
4.2.1 UVR Increases NOS-1 Expression in Human Skin (Phototypes 1 and 4).....	92
4.2.1.1 UVR Increases NOS-1 Expression in Human Skin (Phototype 1)	94
4.2.1.2 UVR has no Effect on NOS-1 Expression in Human Skin (Phototype 4)	97
4.2.2 UVR Increases NOS-2 Expression in Human Skin (Phototypes 1 and 4)....	100
4.2.2.1 UVR has no Effect on NOS-2 Expression in Human Skin (Phototype 1)	101
4.2.2.2 UVR Increases NOS-2 Expression in Human Skin (Phototype 4)	103
4.2.3 UVR Increases NOS-3 Expression in Human Skin (Phototypes 1 and 4)....	106
4.2.3.1 UVR Increases NOS-3 Expression in Human Skin (Phototype 1)	107
4.2.3.2 UVR has no Significant Effect on NOS-3 Expression in Human Skin (Phototype 4).....	110
4.2.4 Expression of Nitric Oxide Synthases in Epidermal Melanocytes <i>In situ</i> and <i>In vitro</i>	113
4.2.4.1 Epidermal Melanocytes Express NOS-1 <i>In situ</i> and <i>In vitro</i>	113
4.2.4.2 Epidermal Melanocytes Express NOS-2 <i>In situ</i> and <i>In vitro</i>	115
4.2.4.3 Epidermal Melanocytes Express NOS-3 <i>In situ</i> and <i>In vitro</i>	116
4.3 Effect of UVR on Lipoxygenase Expression in Human Skin.....	117
4.3.1 UVR Increases 12-LOX Expression in Human Skin (Phototypes 1 and 4)..	118
4.3.1.1 UVR Increases 12-LOX Expression in Human Skin (Phototype 1).....	119
4.3.1.2 UVR had no Effect on 12-LOX Expression in Human Skin (Phototype 4)	122
4.3.2 Expression of 12-LOX in Epidermal Melanocytes <i>In vitro</i>	124
4.3.3 UVR Increases 15-LOX-2 Expression in Human Skin (Phototype 1 and 4)	125

4.3.3.1 UVR Increases 15-LOX-2 Expression in Human Skin (Phototype 1)...	126
4.3.3.2 UVR Increases 15-LOX-2 Expression in Human Skin (Phototype 4)...	128
4.3.4 Expression of 15-LOX-2 in Epidermal Melanocytes <i>In vitro</i>	130
4.4 Effect of UVR on Cyclooxygenase Expression in Human Skin.....	132
4.4.1 UVR Increases COX-1 Expression in Human Skin (Phototypes 1 and 4) ...	132
4.4.1.1 UVR has no Significant Effect on COX-1 Expression in Human Skin (Phototype 1).....	134
4.4.1.2 UVR Increases COX-1 Expression in Human Skin (Phototype 4).....	136
4.4.2 Expression of COX-1 in Epidermal Melanocytes <i>In vitro</i>	138
4.4.3 UVR Increases COX-2 Expression in Human Skin (Phototypes 1 and 4) ...	140
4.4.3.1 UVR Increases COX-2 Expression in Human Skin (Phototype 1).....	141
4.4.3.2 UVR has no Effect on COX-2 Expression in Human Skin (Phototype 4)	144
4.4.4 Expression of COX-2 in Epidermal Melanocytes <i>In vitro</i>	147
4.5 Summary of Findings: Effect of UVR on Human Skin (Phototypes 1 and 4).....	149
4.6 Summary of Findings: Differences in Response to UVR Between Skin Phototype- 1 and -4.....	150
4.7 Melanogenic Capability of Primary Epidermal Melanocytes Derived from Individuals with SPT-1 or SPT-4.....	151
4.7.1 Donor Skin Phototype does not Correlate with Melanogenic Capability in Epidermal Melanocytes <i>In vitro</i>	151
4.7.1.1 Dendricity and Melanin Content.....	151
4.7.1.2 Expression/Activity of Melanogenic Enzymes.....	156
4.8 Summary of Findings: Dendricity and Melanogenic Capability of Epidermal Melanocytes Derived From Phototype-1 or -4 Skin	159
4.9 Eicosanoid Production by Epidermal Melanocytes	160
4.9.1 Epidermal Melanocytes Produce Prostanoids	160
4.9.1.1 Mass Spectrometric Analysis.....	160
4.9.1.2 ELISA Analysis	161
4.9.2 Prostaglandin E ₂ Production by Epidermal Melanocytes: Effect of Skin Phototype	163
4.9.2.1 ELISA Analysis	163
4.9.3 Expression of Eicosanoid-Producing Enzymes	165
4.9.3.1 Cytoplasmic Phospholipase A ₂	166
4.9.3.2 Cyclooxygenase-1	168
4.9.3.3 Cytoplasmic Prostaglandin E Synthase.....	172
4.10 Summary of Findings: Eicosanoid Production by Epidermal Melanocytes Under Baseline and Arachidonic Acid-Stimulated Conditions	174
4.11 Effect of UVR on Prostaglandin E ₂ Production by Epidermal Melanocytes.....	175
4.11.1 Stimulation of Epidermal Melanocytes with UVR	175
4.11.2 UVR Alters PGE ₂ Production by Epidermal Melanocytes	176
4.11.3 UVR did not Affect Malondialdehyde Formation in Epidermal Melanocytes	182
4.11.4 H ₂ O ₂ Increased PGE ₂ Production by Epidermal Melanocytes	183
4.11.5 H ₂ O ₂ Decreased COX-1 Expression in Epidermal Melanocytes	184
4.12 Summary of Findings: Effect of UVR on Prostaglandin E ₂ Production by Epidermal Melanocytes.....	186
4.13 Effect of Melatonin and Luzindole on Prostaglandin E ₂ Production by Epidermal Melanocytes	187

4.13.1 Melatonin did not Protect Against Increased PGE ₂ Production in Response to H ₂ O ₂ or UVR	187
4.13.2 Luzindole had Variable Effects on PGE ₂ Production by Epidermal Melanocytes	188
4.14 Summary of Findings: Effect of Melatonin and Luzindole on Prostaglandin E ₂ production in Epidermal Melanocytes	193
4.15 Effect of UVR, Melatonin and Luzindole on Fatty Acid Composition of Epidermal Melanocytes.....	194
4.15.1 UVR had Variable Effects on Arachidonic Acid Levels in Epidermal Melanocytes	194
4.15.2 UVR had Variable Effects on Fatty Acid Composition in Epidermal Melanocytes	195
4.16 Summary of Findings: Effect of UVR, Melatonin and Luzindole on Fatty Acid Composition of Epidermal Melanocytes.....	200
5 DISCUSSION	201
Aim of this Project	202
5.1 Summary of Results Obtained from Skin Sections.....	202
5.1.1 Neutrophil Infiltration into the Skin is Increased Post-UVR Exposure.....	203
5.1.2 Lymphocyte Infiltration into the Skin is Increased Post-UVR Exposure.....	209
5.2 Primary Epidermal Melanocyte Cultures - Melanin Levels and Melanogenic Enzyme Expression/Activity.....	213
5.2.1 Donor Skin Phototype does not Correlate with Melanin Content or Melanogenic Enzyme Expression/Activity in Epidermal Melanocytes <i>In vitro</i>	214
5.3 Primary Epidermal Melanocyte Cultures – Production of PGD ₂ and PGE ₂ <i>in vitro</i>	217
5.3.1 Epidermal Melanocytes Produce PGD ₂ and PGE ₂ <i>In vitro</i>	218
5.4 Primary Epidermal Melanocyte Cultures – PGE ₂ Production in Epidermal Melanocytes in Response to UVR	221
5.4.1 PGE ₂ Production in Response to UVR does not Correlate with Melanin Levels in Epidermal Melanocytes <i>In vitro</i>	222
5.5 Primary Epidermal Melanocyte Cultures – The Effect of Melatonin on PGE ₂ Production in Epidermal Melanocytes in Response to UVR	225
5.5.1 Melatonin Decreases Melanogenesis but does not Protect Epidermal Melanocytes from Increased PGE ₂ Production in Response to a Pro-oxidant Stimuli	226
5.6 Primary Epidermal Melanocyte Cultures – The Effect of UVR and Melatonin on Fatty Acid Levels in Epidermal Melanocytes	228
5.6.1 Melatonin has Variable Effects on Fatty Acid Composition of Epidermal Melanocytes	228
6 CONCLUSION	230
7 FUTURE WORK.....	231
8 REFERENCES.....	233
POSTERS & PUBLICATIONS ORIGINATING FROM THIS STUDY	251

APPENDIX 1253
APPENDIX 2254
APPENDIX 3255
APPENDIX 4255
APPENDIX 5256
APPENDIX 6259
APPENDIX 7259
APPENDIX 8260
APPENDIX 9260

LIST OF FIGURES

Figure 1. Schematic representation of the human epidermis (adapted from Fuchs and Raghaven, 2002).	2
Figure 2. Representation of the structure of the stratum corneum (modified from Tobin, 2006).	6
Figure 3. The melanosomal organelles (Raposo and Marks, 2002).	15
Figure 4. A schematic diagram of a modified melanogenic pathway (adapted from Tobin, 2004).	17
Figure 5. Diagram depicting the post-translational processing of POMC (taken from Yeo et al, 2000).	19
Figure 6. A) Schematic representation of the polyunsaturated fatty acid, Arachidonic Acid, showing its 20 carbon backbone and 4 double bonds. B) Schematic representation of a phospholipid that incorporates arachidonic acid (adapted from Chakraborti, 2002).	25
Figure 7. Arachidonic acid mobilization (adapted from Fitzpatrick and Soberman, 2001).	26
Figure 8. Diagram depicting the formation of Prostaglandin G ₂ (PGG ₂) and prostaglandin H ₂ (PGH ₂) from arachidonic acid (adapted from Granstrom, 1984).	28
Figure 9. Biosynthetic pathways of prostanoids (adapted from Granstrom, 1984).	29
Figure 10. Diagram depicting the formation of 5-, 8-, 12- or 15-hydroxy-5, 8, 10, 14-icosatetraenoate (5-, 8-, 12- or 15-HETE) from arachidonic acid (adapted from Granstrom, 1984).	33
Figure 11. Diagram showing the entire electromagnetic spectrum (source NASA).	34
Figure 12. Schematic representation of the effect of Ultraviolet radiation (UVR) on deoxyribonucleic acid (DNA) (adapted from Pattison and Davies, 2006).	38
Figure 13. Schematic representation of the effect of Ultraviolet radiation (UVR) on proteins (adapted from Pattison and Davies, 2006).	40
Figure 14. Schematic diagram showing the consequences of the formation of lipid hydroperoxide from the peroxidation of lipids caused by the effects of Ultraviolet UVR (adapted from Pattison and Davies, 2006).	42
Figure 15. A) Schematic representation of mitochondria. B) Overview of mitochondrial ROS production (adapted from Murphy, 2009).	43
Figure 16. Schematic representation of immunohistochemical staining using a biotinylated secondary antibody and streptavidin-horseradish peroxidase system.	54
Figure 17. Schematic representation of single immunofluorescence using a fluorescein-conjugated secondary antibody.	57
Figure 18. Schematic representation of double immunofluorescence using a fluorescein-(FITC) and a tetramethyl rhodamine (TRITC) conjugated-secondary antibody.	58
Figure 19. Plate set-up for the Prostaglandin E ₂ and D ₂ MOX immunoassay.	68
Figure 20. Neutrophil infiltration into the skin (dermis and epidermis) before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	79
Figure 21. Neutrophil infiltration into the skin of individuals with SPT-1 (dermis and epidermis) before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	80
Figure 22. Photomicrographs showing neutrophil infiltration into the skin of an individual with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	81

Figure 23. Neutrophil infiltration into the skin of individuals with SPT-4 (dermis and epidermis) before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	82
Figure 24. Photomicrographs showing neutrophil infiltration into the skin of an individual with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	83
Figure 25. CD3 ⁺ lymphocyte infiltration into the skin (dermis and epidermis) before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	84
Figure 26. CD3 ⁺ lymphocyte infiltration into the skin of individuals with SPT-1 (dermis and epidermis) before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	85
Figure 27. Photomicrographs showing CD3 ⁺ lymphocyte infiltration into the skin of an individual with SPT-1 (dermis and epidermis) before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	86
Figure 28. CD3 ⁺ lymphocyte infiltration into the skin of individuals with SPT-4 (dermis and epidermis) before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	87
Figure 29. Photomicrographs showing CD3 ⁺ lymphocyte infiltration into the skin of an individual with SPT-4 (dermis and epidermis) before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	88
Figure 30. Photomicrographs showing CD4 ⁺ lymphocyte infiltration into the skin (dermis and epidermis) before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	90
Figure 31. Photomicrographs showing the absence CD8 ⁺ lymphocyte infiltration into the skin (dermis and epidermis) before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	91
Figure 32. NOS-1 expression in the dermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	92
Figure 33. NOS-1 expression in the suprabasal layer of the epidermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	93
Figure 34. NOS-1 expression in the basal layer of the epidermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	93
Figure 35. NOS-1 expression in the dermis in individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	94
Figure 36. NOS-1 expression in the suprabasal layer of the epidermis in individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	94
Figure 37. NOS-1 expression in the basal layer of the epidermis in individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	95
Figure 38. Photomicrographs showing NOS-1 expression in the skin of an individual with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	96
Figure 39. NOS-1 expression in the suprabasal layer of the epidermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	97
Figure 40. NOS-1 expression in the basal layer of the epidermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	97

Figure 41. NOS-1 expression in the dermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	98
Figure 42. Photomicrographs showing NOS-1 expression in the skin of an individual with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	99
Figure 43. NOS-2 expression in the suprabasal layer of the epidermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	100
Figure 44. NOS-2 expression in the basal layer of the epidermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	100
Figure 45. NOS-2 expression in the dermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	101
Figure 46. NOS-2 expression in the suprabasal layer of the epidermis in individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	101
Figure 47. NOS-2 expression in the basal layer of the epidermis in individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	102
Figure 48. NOS-2 expression in the dermis in individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	102
Figure 49. Photomicrographs showing NOS-2 expression in the skin of an individual with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	103
Figure 50. NOS-2 expression in the suprabasal layer of the epidermis in individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	104
Figure 51. NOS-2 expression in the basal layer of the epidermis in individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	104
Figure 52. Photomicrographs showing NOS-2 expression in the skin of an individual with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	105
Figure 53. NOS-3 expression in the suprabasal layer of the epidermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	106
Figure 54. NOS-3 expression in the basal layer of the epidermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	106
Figure 55. NOS-3 expression in the dermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	107
Figure 56. NOS-3 expression in the basal layer of the epidermis in individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	107
Figure 57. NOS-3 expression in the dermis in individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	108
Figure 58. NOS-3 expression in the suprabasal layer of the epidermis in individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	108
Figure 59. Photomicrographs showing NOS-3 expression in the skin of an individual with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	109

Figure 60. NOS-3 expression in the suprabasal layer of the epidermis in individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	110
Figure 61. NOS-3 expression in the basal layer of the epidermis in individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	110
Figure 62. NOS-3 expression in the dermis in individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	111
Figure 63. Photomicrographs showing NOS-3 expression in the skin of an individual with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	112
Figure 64. NOS-1 expression in epidermal melanocytes.	114
Figure 65. NOS-2 expression in epidermal melanocytes.	115
Figure 66. NOS-3 expression in epidermal melanocytes.	116
Figure 67. 12-LOX expression in the epidermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	118
Figure 68. 12-LOX expression in the dermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	119
Figure 69. 12-LOX expression in the epidermis of individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	119
Figure 70. 12-LOX expression in the dermis of individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	120
Figure 71. Photomicrographs showing 12-LOX expression in the skin of an individual with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	121
Figure 72. 12-LOX expression in the epidermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	122
Figure 73. 12-LOX expression in the dermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	122
Figure 74. Photomicrographs showing 12-LOX expression in the skin of an individual with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	123
Figure 75. Expression of 12-LOX in epidermal melanocytes.	124
Figure 76. 15-LOX-2 expression in the epidermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	125
Figure 77. 15-LOX-2 expression in the dermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	125
Figure 78. 15-LOX-2 expression in the dermis of individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	126
Figure 79. 15-LOX-2 expression in the epidermis of individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	126
Figure 80. Photomicrographs showing 15-LOX-2 expression in the skin of an individual with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	127
Figure 81. 15-LOX-2 expression in the epidermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	128
Figure 82. 15-LOX-2 expression in the dermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	128

Figure 83. Photomicrographs showing 15-LOX-2 expression in the skin of an individual with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	129
Figure 84. Expression of 15-LOX-2 in human epidermal melanocytes (EM) and melanoma cells.	131
Figure 85. COX-1 expression in the epidermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	133
Figure 86. COX-1 expression in the dermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	133
Figure 87. COX-1 expression in the epidermis of individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	134
Figure 88. COX-1 expression in the dermis of individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	134
Figure 89. Photomicrographs showing COX-1 expression in the skin of an individual with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	135
Figure 90. COX-1 expression in the epidermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	136
Figure 91. COX-1 expression in the dermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	136
Figure 92. Photomicrographs showing COX-1 expression in the skin of an individual with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	137
Figure 93. Expression of COX-1 mRNA and protein in epidermal melanocytes (EM).	139
Figure 94. COX-2 expression in the suprabasal layer of the epidermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	140
Figure 95. COX-2 expression in the basal layer of the epidermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	140
Figure 96. COX-2 expression in the dermis before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	141
Figure 97. COX-2 expression in the basal layer of the epidermis of individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	142
Figure 98. COX-2 expression in the suprabasal layer of the epidermis of individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	142
Figure 99. COX-2 expression in the dermis of individuals (n=6) with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	142
Figure 100. Photomicrographs showing COX-2 expression in the skin of an individual with SPT-1 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	143
Figure 101. COX-2 expression in the suprabasal layer of the epidermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	144
Figure 102. COX-2 expression in the basal layer of the epidermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	145

Figure 103. COX-2 expression in the dermis of individuals (n=6) with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	145
Figure 104. Photomicrographs showing COX-2 expression in the skin of an individual with SPT-4 before (0 hrs) and after (4, 24 and 72 hrs) a single dose of UVR (120 mJ/cm ²).	146
Figure 105. Presence of COX-2 mRNA but absence of COX-2 protein in epidermal melanocytes (EM).	148
Figure 106. Morphology of epidermal melanocytes derived from individuals with SPT-1 under baseline conditions.	152
Figure 107. Morphology of epidermal melanocytes derived from individuals with SPT-4 under baseline conditions.	153
Figure 108. Melanin content in epidermal melanocytes derived from individuals with SPT-1 under baseline conditions.	154
Figure 109. Melanin content of epidermal melanocytes derived from individuals with SPT-4 under baseline conditions.	155
Figure 110. Melanin content of epidermal melanocytes derived from individuals with either SPT-1 or SPT-4 shown A) individually and B) grouped into SPT-1 or SPT-4.	155
Figure 111. Dopa-oxidase activity of tyrosinase and expression of tyrosinase in epidermal melanocytes (EM) from 8 different individuals under baseline conditions.	157
Figure 112. Expression of tyrosinase related protein-1 (TRP-1) and dopachrome tautomerase (DCT) in epidermal melanocytes (EM) from 8 different individuals under baseline conditions.	158
Figure 113. Production of prostanoids by epidermal melanocytes (EM) under baseline (B) conditions and 24 hrs post 10 μ M arachidonic acid stimulation (AA).	161
Figure 114. Production of PGE ₂ by epidermal melanocytes (EM).	162
Figure 115. Production of PGD ₂ by epidermal melanocytes (EM).	163
Figure 116. Production of PGE ₂ by epidermal melanocytes (EM).	165
Figure 117. Expression of phosphorylated cPLA ₂ (PcPLA ₂) in epidermal melanocytes (EM) under baseline conditions.	167
Figure 118. Expression of cyclooxygenase-1 (COX-1) in epidermal melanocytes (EM) under baseline conditions.	169
Figure 119. Expression of COX-1 by epidermal melanocytes (EM) derived from 3 different individuals under baseline (B) conditions and in response to 24 hrs incubation with 10 μ M arachidonic acid (AA).	171
Figure 120. Expression of Cytoplasmic Prostaglandin E Synthase (cPGES) by epidermal melanocytes (EM) from 8 different individuals under baseline conditions.	173
Figure 121. Effect of UVR on epidermal melanocyte (EM) viability.	176
Figure 122. Effect of 55 mJ/cm ² UVR on PGE ₂ production by epidermal melanocytes.	177
Figure 123. Effect of 73 mJ/cm ² UVR on epidermal melanocyte (EM) morphology.	178
Figure 124. Effect of 73 mJ/cm ² UVR on epidermal melanocyte (EM) morphology.	179
Figure 125. Effect of 73 mJ/cm ² UVR on PGE ₂ production by epidermal melanocytes.	180
Figure 126. PGE ₂ production by epidermal melanocytes in response to arachidonic acid and UVR with arachidonic acid.	182
Figure 127. Malondialdehyde formation on protein from epidermal melanocytes 24 hrs post 73 mJ/cm ² UVR.	183
Figure 128. Effect of 48 hrs incubation with 50 μ M H ₂ O ₂ on PGE ₂ production by epidermal melanocytes.	184

Figure 129. Effect of 48 hrs incubation with 50 μM H_2O_2 on COX-1 expression in epidermal melanocytes (EM).	185
Figure 130. Effect of 24 hrs pre-incubation with 10 μM melatonin on PGE_2 production by epidermal melanocytes in response to a) 55 mJ/cm^2 UVR and b) 48 hrs incubation with 50 μM H_2O_2	188
Figure 131. Effect of arachidonic acid (AA) and luzindole on a) melanin content and b) PGE_2 production by epidermal melanocytes.	190
Figure 132. Expression of Mel-1A receptors in epidermal melanocytes (EM).	192
Figure 133. Effect of UVR, melatonin and luzindole on arachidonic acid (AA) levels in epidermal melanocytes.....	195
Figure 134. Effect of UVR and melatonin on linoleic and eicosatrienoic acid levels in epidermal melanocytes.....	197
Figure 135. Effect of UVR and melatonin on docosatetraenoic acid levels in epidermal melanocytes.....	199
Figure 136. Proposed effect of UVR on NF- κB activity, 12-lipoxygenase (12-LOX) expression, 15-lipoxygenase (15-LOX) expression, neutrophil infiltration and elastin fragment formation in the skin of individuals with either SPT-1 or with SPT-4.....	208
Figure 137. Proposed effect of UVR on $\text{CD}3/4^+$ T-cell infiltration into the skin of individuals with either SPT-1 or SPT-4, and the involvement of Langerhans cells and regulatory T-cells.	211

LIST OF TABLES

Table 1. Table of reagents used in this project.....	xxii
Table 2. Table of equipment and consumables used in this project.....	xxv
Table 3. Details of primary antibodies used in this project.....	xxvii
Table 4. Details of secondary antibodies used in this project.	xxviii
Table 5. Details of blocking peptides used in this project.....	xxviii
Table 6. Table of primers used in the Polymerase Chain Reaction in this project.....	xxix
Table 7. Table of biological material used in this project.	xxx

ABBREVIATIONS

12-lipoxygenase (12-LOX)
15-lipoxygenase (15-LOX)
3, 4-dihydroxy-L-phenylalanine (L-DOPA)
4, 6-diaminidino-2-phenylindole (DAPI)
5, 6-dihydroxyindole (DHI)
5, 6-dihydroxyindole-2-carboxylic acid (DHICA)
5-lipoxygenase (5-LOX)
8-lipoxygenase (8-LOX)
Acetylcholinesterase (AChE)
Adenosine Triphosphate (ATP)
Adrenocorticotrophic Hormone (ACTH)
Amino Ethyl-Carbazole (AEC)
Arachidonic Acid (AA)
Basement Membrane Zone (BMZ)
Basic Fibroblast Growth Factor (bFGF)
Blank (Blk)
Bovine Serum Albumin (BSA)
Butylated Hydroxytoluene (BHT)
Calcitonin Gene Related Peptide (CGRP)
Carbon Dioxide (CO₂)
Cell Adhesion Molecules (CAMs)
Complementary Deoxyribonucleic Acid (cDNA)
Cyclic-Adenosine Monophosphate (cAMP)
Cyclooxygenase-1 (COX-1)
Cyclooxygenase-2 (COX-2)
Deoxyribonucleic Acid (DNA)
Diacylglycerol (DAG)
Diethyl Pyrocarbonate (DEPC)
Dimethylsulphoxide (DMSO)
Donkey Serum (DS)
Dopachrome Tautomerase (DCT)

Endothelin-1 (ET-1)
Enhanced Chemi-Luminescence (ECL)
Enzyme Linked Immunosorbant Assay (ELISA)
Ethylenediaminetetraacetic acid (EDTA)
Extracellular Matrix (ECM)
Fatty Acid Methyl Ester (FAME)
Fetal Calf Serum (FCS)
Flavin Adenine Dinucleotide (FADH₂)
Fluorescein Isothiocyanate (FITC)
Gas Chromatography (GC)
Goat Serum (GS)
G-Protein Coupled Receptor (GPCR)
Guanine Diphosphate (GDP)
Guanine Triphosphate (GTP)
Hair Follicle Melanocytes (HFMs)
Heneicosanoic Acid (C21:0)
Hour (hr)
Hydrochloric Acid (HCl)
Hydrogen Peroxide (H₂O₂)
Hydroperoxyarachidonate (HPETE)
Hydroxy-5, 8, 10, 14-eicosatetraenoate (HETE)
Hydroxyl Radical (\cdot OH)
Immunoglobulin E (IgE)
Inducible Nitric Oxide Synthase (iNOS)
Inositol Triphosphate (IP₃)
Interleukin (IL)
Langerhans Cells (LC)
Leukemia Inhibitory Factor (LIF)
Liquid Chromatography/Electrospray Ionization-Mass Spectrometry/Mass Spectrometry (LC/ESI-MS/MS)
Lysophosphatidylcholine (LPC)
Maximum Binding (B₀)
Melanocortin-1 Receptor (MC-1R)

Melatonin 1A Receptor (MEL-1A)
Melatonin 1B Receptor (MEL-1B)
Messenger Ribonucleic Acid (mRNA)
Methoxylamine (MOX)
Methoxylamine Hydrochloride (MOX HC)
Microphthalmia Induced Transcription Factor (MITF)
Minute (min)
Multiple Reaction Monitoring (MRM)
Neutrophil Elastase (NE)
Nicotinamide Adenine Dinucleotide (NADH)
Nitric Oxide (NO)
Nitric Oxide Synthase-1 (NOS-1)
Nitric Oxide Synthase-2 (NOS-2)
Nitric Oxide Synthase-3 (NOS-3)
Non-Specific Binding (NSB)
Nuclear Factor- $\kappa\beta$ (NF- $\kappa\beta$)
Oculocutaneous Albinism Type 1 (OCA1)
Optimal Cutting Temperature (OCT)
Oxygen (O₂)
p38 Mitogen-Activated Protein Kinase (p38)
Peroxisome Proliferator-Activated Receptor gamma (PPAR γ)
Peroxy Nitrite (ONOO-)
Phosphate Buffered Saline (PBS)
Phosphatidylinositol (PI)
Phosphatidylinositol-3, 4, 5-Triphosphate (PIP₃)
Phospholipase-A₂ (PLA₂)
Polymerase Chain Reaction (PCR)
Polyoma Enhancer Activator 3 (PEA3)
Polyvinylidene difluoride (PVDF)
Potassium Carbonate (K₂CO₃)
Potassium Chloride (KCl)
Proliferating Cell Nuclear Antigen (PCNA)
Pro-opiomelanocortin (POMC)

Prostaglandin B_{2-d4} (PGB_{2-d4})
Prostaglandin E Synthase (PGES)
Prostaglandin E₂ (PGE₂)
Prostaglandin F_{2α} (PGF_{2α})
Prostaglandin G₂ (PGG₂)
Prostaglandin H₂ (PGH₂)
Prostaglandin I₂ (PGI₂)
Protein Kinase A (PKA)
Protein Kinase C-β (PKCβ)
Protein Kinase-C (PKC)
Proteinase-Activated Receptor 2 (PAR2)
Reactive Oxygen Species (ROS)
Receptor for Activated C-Kinase-1 (RACK-1)
Ribonucleic Acid (RNA)
Second (sec)
Singlet Oxygen (¹O₂)
Skin Phototype (SPT)
Skin Phototype-1 (SPT-1)
Skin Phototype-4 (SPT-4)
Sodium Dodecyl Sulphate (SDS)
Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)
Sodium Hydroxide (NaOH)
Solid Phase Extraction (SPE)
Stem Cell Factor (SCF)
Streptavidin Horseradish-Peroxidase (SHRP)
Superoxide Anion (O²⁻)
Tetramethyl Isothiocyanate (TRITC)
Tetramethylethylenediamine (TEMED)
Total Activity (TA)
Tris-acetate-EDTA (TAE)
Tris-EDTA (TE)
Tumor Necrosis Factor-alpha (TNFα)
Tyrosinase Related Protein-1 (TRP-1)

Ultrafast Internal Conversion (UIC)

Ultraviolet Radiation (UVR)

Upstream Transcription Factor-1 (USF-1)

Urocanic Acid (UCA)

UVA (Ultraviolet Radiation with wavelength 320-400 nm)

UVB (Ultraviolet Radiation with wavelength 280-320 nm)

UVC (Ultraviolet Radiation with wavelength 200-280 nm)

Water (H₂O)

α -Melanocyte Stimulating Hormone (α -MSH)

α -Tocopherol (vitamin E)

β -Endorphin (β -END)

β -Melanocyte Stimulating Hormone (β -MSH)

μ -Opiate Receptor (μ -OR)

REAGENTS

Table 1. Table of reagents used in this project.

Reagent	Company	City	Country
Acetic Acid	Sigma	Poole	UK
Acetone	Fisher Scientific	Loughborough	UK
Acetonitrile	Fisher Scientific	Loughborough	UK
Acrylagel	Flowgen Bioscience	Nottingham	UK
Amino Ethyl-Carbazole (AEC) Chromagen	Vector	Peterborough	UK
Agarose	Sigma	Poole	UK
Ammonium Persulphate	Sigma	Poole	UK
Arachidonic Acid	Cayman Chemical	Tyne and Wear	UK
Basic Fibroblast Growth Factor	TEBU-bio	Peterborough	UK
Boron Trifluoride	Sigma	Poole	UK
Butylated Hydroxytoluene	Sigma	Poole	UK
Bis-acrylagel	Flowgen Bioscience	Nottingham	UK
Bovine Serum Albumin	Sigma	Poole	UK
C21 Internal Standard (Heneicanoic Acid)	Sigma	Poole	UK
Chloroform	Fisher Scientific	Loughborough	UK
Ciprofloxacin	Bayer	Berkshire	UK
4', 6-diaminidino-2-phenylindole (DAPI)	Vector	Peterborough	UK
Developer	Sigma	Poole	UK
Di Sodium Hydrogen Orthophosphate	Sigma	Poole	UK
Dichloromethane	Fisher Scientific	Loughborough	UK
Dimethylsulphoxide	Sigma	Poole	UK
Donkey Serum	Invitrogen	Paisley	UK
Ethylenedaiminetetraacetic acid (EDTA)	Sigma	Poole	UK
Endothelin-1	Sigma	Poole	UK
Ethanol	Fisher Scientific	Loughborough	UK
Ethidium Bromide	Sigma	Poole	UK
Fatty Acid Methyl Ester (FAME) Cocktail	Supelco	Belleforte, PA	USA
Fast Cycling Polymerase Chain Reaction (PCR) Kit	Qiagen	Sussex	UK
Fast Green	Sigma	Poole	UK
Fetal Calf Serum	Invitrogen	Paisley	UK
Fixer	Sigma	Poole	UK
Fungizone	Gibco (Invitrogen)	Paisley	UK
Geneticin	Gibco (Invitrogen)	Paisley	UK
Glacial Acetic Acid	BDH (VWR)	Lutterworth	UK
Glycergel	DAKO	Cambridgeshire	UK

Glycerol	Fisher Scientific	Loughborough	UK
Glycine	Sigma	Poole	UK
Goat Serum	Invitrogen	Paisley	UK
Hexane	Fisher Scientific	Loughborough	UK
Hydrochloric Acid	Sigma	Poole	UK
Hydrogen Peroxide	Sigma	Poole	UK
Isopropanol	Fisher Scientific	Loughborough	UK
3, 4-dihydroxy-L-phenylalanine (L-DOPA)	Sigma	Poole	UK
L-Glutamine	Gibco (Invitrogen)	Paisley	UK
Luminol	Biochemika (FLUKA)	Buchs	Switzerland
Luzindole	Sigma	Poole	UK
Magnesium Sulphate	Sigma	Poole	UK
Methanol	Fisher Scientific	Loughborough	UK
Methyl Formate	Acros Organics (Fisher Scientific)	Loughborough	UK
Meyers Haematoxylin	Sigma	Poole	UK
Minimum Essential Amino Acids	Gibco (Invitrogen)	Paisley	UK
Minimum Essential Medium	Gibco (Invitrogen)	Paisley	UK
Nitrogen	BOC Gases	Guildford	UK
Optimal Cutting Temperature Compound	Tissue-Tek (Sakura Finetek)	Illinois	USA
P-coumaric	Sigma	Poole	UK
Penicillin/Streptomycin	Gibco (Invitrogen)	Paisley	UK
Prostaglandin B ₂ <i>d4</i> (PGB ₂ <i>d4</i>)	Cayman Chemical	Tyne and Wear	UK
Prostaglandin D ₂ (PGD ₂) Methoxylamine (MOX) Enzyme Linked Immunosorbant Assay (ELISA)	Cayman Chemical	Tyne and Wear	UK
Prostaglandin E ₂ (PGE ₂) ELISA	Cayman Chemical	Tyne and Wear	UK
Poly-L-lysine	Sigma	Poole	UK
Potassium Carbonate	Sigma	Poole	UK
Potassium Chloride	Sigma	Poole	UK
Potassium Dihydrogen Orthophosphate	Sigma	Poole	UK
Protease Inhibitor Cocktail	Sigma	Poole	UK
Reagent A	Bio-Rad Laboratories	Bath	UK
Reagent B	Bio-Rad Laboratories	Bath	UK
Reagent S	Bio-Rad Laboratories	Bath	UK
RNeasy Isolation Kit	Qiagen	Sussex	UK
RPMI 1640	Gibco (Invitrogen)	Paisley	UK
Serum Free Keratinocyte	PromoCell	Heidelberg	Germany

Medium			
Sodium Bicarbonate	Sigma	Poole	UK
Sodium Chloride	Sigma	Poole	UK
Sodium Dodecyl Sulphate	Sigma	Poole	UK
Sodium Hydroxide	Sigma	Poole	UK
Sodium Sulphate	Sigma	Poole	UK
Superscript III First Strand Synthesis Super Mix	Invitrogen	Paisley	UK
Synthetic Melanin	Sigma	Poole	UK
Tetramethylethylenediamine (TEMED)	Sigma	Poole	UK
Thymol	Sigma	Poole	UK
Toluene	Acros Organics (Fisher Scientific)	Loughborough	UK
Trimethylpentane	Acros Organics (Fisher Scientific)	Loughborough	UK
Trizma Base	Sigma	Poole	UK
Tris-Acetate	Sigma	Poole	UK
Tris-Base	Sigma	Poole	UK
Trypsin/EDTA	Gibco (Invitrogen)	Paisley	UK
Tween-20	Sigma	Poole	UK
Ultrapure Water	Cayman Chemical	Tyne and Wear	UK
β -Mercaptoethanol	Sigma	Poole	UK

EQUIPMENT AND CONSUMABLES

Table 2. Table of equipment and consumables used in this project.

Item	Company	City	Country
8-Well Chamber Slides	NUNC (Fisher Scientific)	Loughborough	UK
96-Well Culture Plates	Corning Life Sciences	Schiphol-Rijk	The Netherlands
Autosampler	Agilent Technologies	California	USA
Blot Module-XCell II	Invitrogen	Paisley	UK
Counting Chamber-Depth 0.1 mm 1/400 mm ²	Hawksley	Lancing	UK
Coverslips	VWR	Lutterworth	UK
Cryostat	Leica	Wetzlar	Germany
Cryovials (1 ml)	NUNC (Fisher Scientific)	Loughborough	UK
Culture Dish	Invitrogen	Paisley	UK
Culture Flasks (225 cm ²)	Corning Life Sciences	Schiphol-Rijk	The Netherlands
Culture Flasks (25 cm ²)	Corning Life Sciences	Schiphol-Rijk	The Netherlands
Culture Flasks (75 cm ²)	Corning Life Sciences	Schiphol-Rijk	The Netherlands
Dewar (Arpege 70)	Air Liquide	Maine Laville	France
Electrophoresis Chamber-NOVEX Mini-Cell	Invitrogen	Paisley	UK
Electrospray Ionization Triple Quadruple Ultima Mass Spectrometer	Micromass	Manchester	UK
Eppendorf Tubes (1.5 ml)	Invitrogen	Paisley	UK
Gas Chromatography Column	Phenomenex	Macclesfield	UK
Gel Cassettes (1.0 mm)	Invitrogen	Paisley	UK
Gel Comb (15 well)	Invitrogen	Paisley	UK
Glass Pipettes	Fisher	Loughborough	UK
Heat Block	Thermoscientific	Cranlington	UK
Heraeus Incubator (Hera Cell)	DJB Labcare	Newport Pagnell	UK
High Performance Liquid Chromatography (HPLC) Pump (Alliance)	Waters	Elstree	UK
Hydrogen Generator (HG200)	CLAIND	Lenno	Italy
Inverted Microscope (CKX41)	Olympus	Watford	UK
Isopropanol Bath	Nalgene (Fisher)	Loughborough	UK

	Scientific)		
Light Meter	Waldmann		UK
Mass Spec Column	Phenomenex	Macclesfield	UK
Microscope	Nikon		UK
Microscope slides	BDH (VWR)	Lutterworth	UK
MSE Centrifuge (Harrier 15/80)	DJB Labcare	Newport Pagnell	UK
Needles (0.6 x 25 mm)	NUNC (Fisher Scientific)	Loughborough	UK
Orbital Shaker	Grant Instruments	Cambridge	UK
PAP Pen	Invitrogen	Paisley	UK
Parafilm	Pechiney	Illinois	USA
PCR Electrophoresis Tank	Invitrogen	Paisley	UK
Photographic Film	Sigma	Poole	UK
Photographic Film Wallet	Sigma	Poole	UK
Plate Reader	GE Healthcare	Chalfont St Giles	UK
Polymerase Chain Reaction (PCR) Tubes	Geneflow	Fradley	UK
Polyvinylidene difluoride (PVDF) Transfer Membrane	GE Healthcare	Chalfont St Giles	UK
Power Pack	Bio-Rad Laboratories	Bath	UK
Refrigerated Centrifuge	Dupont	Stevenage	UK
Solid Phase Extraction (SPE) Cartridges	Phenomenex	Macclesfield	UK
Spectrophotometer	Agilent Technologies	California	USA
Square Plastic Dishes	Invitrogen	Paisley	UK
Syringes (1 ml)	Invitrogen	Paisley	UK
Techne TC-512 Thermocycler	Witec		Germany
Ultraviolet (UV) Lamp	Philips		UK
Universal Tubes (25 ml)	Invitrogen	Paisley	UK
Vacuum Manifold	Phenomenex	Macclesfield	UK
Vortexer	Grant Instruments	Cambridge	UK
Water Bath	Grant Instruments	Cambridge	UK

PRIMARY ANTIBODIES

Table 3. Details of primary antibodies used in this project.

Antibody Specificity	Type	Company	Dilution			
			ICC	IHC	WB	IF
Neutrophil Elastase	Monoclonal	NovoCastra		1:70		
CD3	Monoclonal	NovoCastra		1:40		
CD4	Monoclonal	NovoCastra		1:40		
CD8	Monoclonal	NovoCastra		1:20		
Nitric Oxide Synthase-1	Polyclonal	Santa Cruz	1:100	1:100		1:50
Nitric Oxide Synthase-2	Polyclonal	Santa Cruz	1:200	1:100		1:50
Nitric Oxide Synthase-3	Polyclonal	Santa Cruz	1:100	1:100		1:50
12-Lipoxygenase	Polyclonal	Abcam	1:200		1:2000	
15-Lipoxygenase-2	Polyclonal	Abcam	1:100			
Cyclooxygenase-1	Polyclonal	Santa Cruz			1:100	
Cyclooxygenase-1	Polyclonal	Alexis	1:100			
Cyclooxygenase-2	Polyclonal	Alexis	1:200			1:50
Cyclooxygenase-2	Polyclonal	Cayman			1:50	
Malondialdehyde	Polyclonal	Santa Cruz			1:300	
Tyrosinase	Polyclonal	Santa Cruz			1:75	
Dopachrome Tautomerase	Polyclonal	Santa Cruz			1:100	
Tyrosinase Related Protein-1	Polyclonal	Santa Cruz			1:1000	
Actin	Polyclonal	Santa Cruz			1:500	
Phosphorylated Cytoplasmic Phospholipase A ₂	Polyclonal	Santa Cruz	1:100		1:300	1:50
Prostaglandin E Synthase	Polyclonal	Cayman	1:100		1:100	1:50
Mel-1A	Polyclonal	Santa Cruz	1:100		1:100	
Mel-1B	Polyclonal	Santa Cruz	1:100		1:100	
NKI/beteb (gp-100)	Polyclonal	Monosan	1:50			1:50

ICC (immunocytochemistry), *IHC* (immunohistochemistry), *WB* (Western Blotting), *IF* (immunofluorescence).

SECONDARY ANTIBODIES

Table 4. Details of secondary antibodies used in this project.

Antibody Specificity	Conjugation	Company	Dilution			
			ICC	IHC	WB	IF
Anti-Mouse	Horseradish-peroxidase	Sigma			1:1000	
Anti-Rabbit	Horseradish-peroxidase	GE Healthcare			1:1000	
Anti-Goat	Horseradish-peroxidase	Serotec			1:600	
LSAB Kit (Anti-Mouse/Rabbit)	Biotin	Dako	X	X		
Anti-Rabbit	Fluorescein	Invitrogen				1:50
Anti-Goat	Fluorescein	Invitrogen				1:50
Anti-Mouse	Tetramethylrhodamine	Invitrogen				1:50

ICC (immunocytochemistry), IHC (immunohistochemistry), WB (Western Blotting), IF (immunofluorescence).

BLOCKING PEPTIDES

Table 5. Details of blocking peptides used in this project.

Partial Peptide Sequence From	Company	Dilution			
		ICC	IHC	WB	IF
Cyclooxygenase-1	Santa Cruz			1:100	
15-Lipoxygenase-2	Abcam	1:100			
Mel-1A	Santa Cruz	1:100			
Mel-1B	Santa Cruz	1:100			

ICC (immunocytochemistry), IHC (immunohistochemistry), WB (Western Blotting), IF (immunofluorescence).

PRIMERS

Table 6. Table of primers used in the Polymerase Chain Reaction in this project.

Target	Primer Sequence 5'-3'	Company	City	Country
Cyclooxygenase-1 Forward	TGCCCAGCTCCTGGCCCGCC GCTT	Sigma	Poole	UK
Cyclooxygenase-1 Reverse	GTGCATCAACACAGGCGCCT CTTC	Sigma	Poole	UK
Cyclooxygenase-1 Forward	TGCCCAGCTCCTGGCCCGCC GC	Sigma	Poole	UK
Cyclooxygenase-1 Reverse	GTGCATCAACACAGGCGCCT CTT	Sigma	Poole	UK
Cyclooxygenase-2 Forward	TTCAAATGAGATTGTGGGAA AATTGCT	Sigma	Poole	UK
Cyclooxygenase-2 Reverse	AGATCATCTCTGCCTGAGTA TCTTT	Sigma	Poole	UK
Cyclooxygenase-2 Forward	TTCAAATGAGATTGTGGAAA AAATTGCT	Sigma	Poole	UK
Cyclooxygenase-2 Reverse	AGATCATCTCTGCCTGAGTA TCTT	Sigma	Poole	UK
Actin Forward	CGCTGCGCTGGTCGTCGACA	Sigma	Poole	UK
Actin Reverse	GTCACGCACGATTTCCCGCT	Sigma	Poole	UK

BIOLOGICAL MATERIAL

Table 7. Table of biological material used in this project.

Subject Code	Skin Phototype	Age (yrs)	Gender	Sample Origin
23	1	32	F	Blister Roof
24	1	39	M	Blister Roof
25	1	36	F	Punch Biopsy
26	4	37	F	Punch Biopsy
27	1	44	F	Punch Biopsy
28	4	35	F	Punch Biopsy
29	1	29	F	Punch Biopsy
30	1	33	M	Blister Roof
31	4	22	M	Punch Biopsy
32	4	58	M	Punch Biopsy
33	4	52	F	Punch Biopsy
34	1	53	F	Blister Roof
35	1	36	F	Blister Roof
36	4	60	F	Blister Roof
37	1	27	F	Blister Roof
38	4	49	F	Punch Biopsy
39	4	42	M	Punch Biopsy
40	4	49	F	Blister Roof
41	1	31	M	Blister Roof
42	4	26	F	Blister Roof
43	4	51	F	Blister Roof
44	1	60	M	Punch Biopsy
45	4	26	M	Blister Roof
46	1	19	F	Punch Biopsy
47	1	31	F	Blister Roof
48	4	24	F	Blister Roof
49	4	39	M	Blister Roof
50	4	33	F	Blister Roof
51	1	32	M	Punch Biopsy
52	4	36	M	Blister Roof
53	1	50	F	Punch Biopsy
54	1	44	M	Punch Biopsy
55	1	34	M	Punch Biopsy
56	4	19	F	Punch Biopsy
F39	2/3	39	F	Breast Reduction
FM55	N/A	N/A	N/A	N/A
FM94	N/A	N/A	N/A	N/A
FM3	N/A	N/A	N/A	N/A

M (male), F (female), FM55, FM94 (Human melanoma cells), FM3 (Hamster melanoma cells), N/A (not applicable).