

# bradscholars

## The role of eicosanoids in the human skin's response to ultraviolet radiation.

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## 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 E<sub>2</sub> (PGE<sub>2</sub>) 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 PGE<sub>2</sub>, 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 PGE<sub>2</sub> 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<sup>+</sup> 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<sup>+</sup> 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 (PLA<sub>2</sub>, COX-1, cPGES) to produce PGE<sub>2</sub>. 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 PGE<sub>2</sub> 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.

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## **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 (CO<sub>2</sub>)  
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 (FADH<sub>2</sub>)  
Fluorescein Isothiocyanate (FITC)  
Gas Chromatography (GC)  
Goat Serum (GS)  
G-Protein Coupled Receptor (GCPR)  
Guanine Diphosphate (GDP)  
Guanine Triphosphate (GTP)  
Hair Follicle Melanocytes (HFMs)  
Heneicosanoic Acid (C21:0)  
Hour (hr)  
Hydrochloric Acid (HCl)  
Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>)  
Hydroperoxyarachidonate (HPETE)  
Hydroxy-5, 8, 10, 14-eicosatetraenoate (HETE)  
Hydroxyl Radical ( $\cdot$ OH)  
Immunoglobulin E (IgE)  
Inducible Nitric Oxide Synthase (iNOS)  
Inositol Triphosphate (IP<sub>3</sub>)  
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 (B<sub>0</sub>)  
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 (O<sub>2</sub>)  
p38 Mitogen-Activated Protein Kinase (p38)  
Peroxisome Proliferator-Activated Receptor gamma (PPAR $\gamma$ )  
Peroxy Nitrite (ONOO-)  
Phosphate Buffered Saline (PBS)  
Phosphatidylinositol (PI)  
Phosphatidylinositol-3, 4, 5-Triphosphate (PIP<sub>3</sub>)  
Phospholipase-A<sub>2</sub> (PLA<sub>2</sub>)  
Polymerase Chain Reaction (PCR)  
Polyoma Enhancer Activator 3 (PEA3)  
Polyvinylidene difluoride (PVDF)  
Potassium Carbonate (K<sub>2</sub>CO<sub>3</sub>)  
Potassium Chloride (KCl)  
Proliferating Cell Nuclear Antigen (PCNA)  
Pro-opiomelanocortin (POMC)

Prostaglandin B<sub>2-d4</sub> (PGB<sub>2-d4</sub>)  
Prostaglandin E Synthase (PGES)  
Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>)  
Prostaglandin F<sub>2α</sub> (PGF<sub>2α</sub>)  
Prostaglandin G<sub>2</sub> (PGG<sub>2</sub>)  
Prostaglandin H<sub>2</sub> (PGH<sub>2</sub>)  
Prostaglandin I<sub>2</sub> (PGI<sub>2</sub>)  
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 (<sup>1</sup>O<sub>2</sub>)  
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 (O<sup>2-</sup>)  
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 (H<sub>2</sub>O)

$\alpha$ -Melanocyte Stimulating Hormone ( $\alpha$ -MSH)

$\alpha$ -Tocopherol (vitamin E)

$\beta$ -Endorphin ( $\beta$ -END)

$\beta$ -Melanocyte Stimulating Hormone ( $\beta$ -MSH)

$\mu$ -Opiate Receptor ( $\mu$ -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 <sub>2</sub> <i>d4</i> (PGB <sub>2</sub> <i>d4</i> )   | Cayman Chemical                    | Tyne and Wear | UK          |
| Prostaglandin D <sub>2</sub> (PGD <sub>2</sub> ) Methoxylamine (MOX) Enzyme Linked Immunosorbant Assay (ELISA) | Cayman Chemical                    | Tyne and Wear | UK          |
| Prostaglandin E <sub>2</sub> (PGE <sub>2</sub> ) 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<br>Synthesis Super Mix | Invitrogen                            | Paisley       | UK |
| Synthetic Melanin                                   | Sigma                                 | Poole         | UK |
| Tetramethylethylenediamine<br>(TEMED)               | Sigma                                 | Poole         | UK |
| Thymol  | Sigma                                 | Poole         | UK |
| Toluene   | Acros Organics<br>(Fisher Scientific) | Loughborough  | UK |
| Trimethylpentane                                    | Acros Organics<br>(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 |
| $\beta$ -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 <sup>2</sup>               | 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 <sup>2</sup> )                             | Corning Life Sciences    | Schiphol-Rijk   | The Netherlands |
| Culture Flasks (25 cm <sup>2</sup> )                              | Corning Life Sciences    | Schiphol-Rijk   | The Netherlands |
| Culture Flasks (75 cm <sup>2</sup> )                              | 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<br>Pagnell   | UK      |
| Needles (0.6 x 25 mm)                                 | NUNC (Fisher<br>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<br>Giles | UK      |
| Polymerase Chain Reaction<br>(PCR) Tubes              | Geneflow                    | Fradley              | UK      |
| Polyvinylidene difluoride (PVDF)<br>Transfer Membrane | GE Healthcare               | Chalfont St<br>Giles | UK      |
| Power Pack  | Bio-Rad<br>Laboratories     | Bath                 | UK      |
| Refrigerated Centrifuge                               | Dupont                      | Stevenage            | UK      |
| Solid Phase Extraction (SPE)<br>Cartridges            | Phenomenex                  | Macclesfield         | UK      |
| Spectrophotometer                                     | Agilent<br>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<br>Cytoplasmic Phospholipase<br>A <sub>2</sub> | 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<br>Forward | TGCCCAGCTCCTGGCCCGCC<br>GCTT     | Sigma   | Poole | UK      |
| Cyclooxygenase-1<br>Reverse | GTGCATCAACACAGGCGCCT<br>CTTC     | Sigma   | Poole | UK      |
| Cyclooxygenase-1<br>Forward | TGCCCAGCTCCTGGCCCGCC<br>GC       | Sigma   | Poole | UK      |
| Cyclooxygenase-1<br>Reverse | GTGCATCAACACAGGCGCCT<br>CTT      | Sigma   | Poole | UK      |
| Cyclooxygenase-2<br>Forward | TTCAAATGAGATTGTGGGAA<br>AATTGCT  | Sigma   | Poole | UK      |
| Cyclooxygenase-2<br>Reverse | AGATCATCTCTGCCTGAGTA<br>TCTTT    | Sigma   | Poole | UK      |
| Cyclooxygenase-2<br>Forward | TTCAAATGAGATTGTGGAAA<br>AAATTGCT | Sigma   | Poole | UK      |
| Cyclooxygenase-2<br>Reverse | AGATCATCTCTGCCTGAGTA<br>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).*