SERUM 25-HYDROXYVITAMIN D CONCENTRATION IN RELATION TO MELANOMA PROGNOSIS

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Abstract

Sun exposure, specifically solar ultraviolet radiation (UVR), is adversely associated with melanoma incidence, yet exposure to ultraviolet radiation is an important step in the pathway to vitamin D synthesis. *In vitro* studies suggest that cultured melanoma cells proliferate at a slower rate in response to the hormonal form of vitamin D, 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), and can synthesise $1,25(OH)_2D$ from the main circulating form of vitamin D, 25-hydroxyvitamin D (25(OH)D). Similarly, *in vivo* trials have found that calcitriol ($1,25(OH)_2D$) can suppress melanoma growth and inhibit metastasis in immunosuppressed rodents. Several epidemiological studies have suggested a link between higher sun exposure and improved melanoma prognosis, yet only a small body of research has been conducted to investigate if there is a relationship between vitamin D status and melanoma prognosis.

The QUT Melanoma Study examined the relationship between serum 25(OH)D concentration at time of diagnosis and the histopathological features of melanoma that are associated with prognosis. Prior to wider-excision surgery, 100 eligible melanoma patients were recruited to this cross-sectional study in Brisbane, Australia. Blood was obtained for serum 25(OH)D analysis at time of recruitment and each patient completed a self–report questionnaire and consented to a physical examination to collect data on known risk factors for melanoma. Logistic regression modelling was used to obtain odds ratios for associations between vitamin D (continuous, lowest quartile, 50 nmol/L, highest quartile) and the prognostic indicators for melanoma of Breslow thickness, Clark level and mitotic activity, while controlling for potential confounders.

In multivariable analysis adjusted for age, sex, ITA (Individual Topography Angle) value, BMI (Body Mass Index) and season at time of diagnosis, a statistically significant association was observed between vitamin D status at the time of melanoma diagnosis and Breslow thickness. Melanomas with Breslow thickness of 0.75mm or greater were considerably more common in patients with insufficient vitamin D status (< 50 nmol/L) compared to those with sufficient vitamin D status (≥ 50 nmol/l) (OR = 3.82, 95% *CI:* 1.03, 14.14, *P* = 0.04). This association strengthened when the lower quartile of serum 25(OH)D (< 45.25 nmol/L) was compared to all other quartiles (OR = 5.03, 95% CI: 1.32, 19.09, *P* = 0.02). No significant relationships were found between vitamin D status (insufficient versus sufficient) and either Clark level (Level 2–5 versus Level 1) or mitosis (present versus absent), and there was no evidence that higher levels of serum 25(OH)D (≥ 69.8 nmol/L) were protective for prognosis compared to lower levels (< 69.8 nmol/L).

While possible mechanisms responsible for this association are yet to be elucidated, the suggestion that sufficient vitamin D status may be associated with a better prognosis for melanoma could have wide ranging implications for public health messaging with respect to both melanoma and vitamin D. There will be inherent difficulties in devising an appropriate public health message that balances the need for sufficient vitamin D status while at the same time avoiding too much sun exposure – and the risk of developing melanoma. Until further research can define the optimal level of vitamin D necessary for a good melanoma prognosis, it would appear sensible for all to ensure that they maintain serum 25(OH)D levels greater than 50 nmol/L, particularly those at a greater risk of developing melanoma.

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List of Abbreviations

ASM	Age-standardised mortality rate
BMI	Body mass index
C	Celsius
CI	confidence interval
CT	Computerised tomography
DALY	Disability-adjusted life year
IARC	International Agency for Research on Cancer
in situ	only occupying the epidermis
IOM	Institute of Medicine
IU	International units
ITA	Individual topography angle
≤	equal to, or less than
≥	equal to, or greater than
LM	lentigo maligna melanoma
melanoma	cutaneous melanoma
mm	millimetre
MRI	Magnetic resonance imaging
nm	nanometre
nmol/L	nanomoles per litre
NM	nodular melanoma
OR	odds ratio
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
PET	Positive Emission Tomography

PSC	Purposeful Selection of Covariates
PTH	parathyroid hormone
QIMR	Queensland Institute of Medical Research (Brisbane, Australia)
QUT	Queensland University of Technology (Brisbane, Australia)
RR	Relative risk
SEER	Surveillance, Epidemiology and End Results
7-DHC	7-dehydrocholesterol
S-100β	S100 calcium binding protein β
SSM	superficial spreading melanoma
T1	Pre-operative visit
T2	Post-operative visit
25(OH)D	25-hydroxyvitamin D
μg/L	micrograms per litre
UVR	Ultraviolet radiation
vs.	versus

Glossary of Terms

Related to vitamin D:

7-DHC	7-dehydrocholesterol (provitamin D_3): a sterol in the cell membrane of keratinocytes
previtamin D_3	produced from 7-DHC after exposure to UVB
vitamin D_2	vitamin D (ergocalciferol): derived from plant sources
vitamin D_3	vitamin D (cholecalciferol): produced following a temperature dependent thermal isomerisation of previtamin D_3
25(OH)D	25-hydroxyvitamin D: produced after vitamin D_2 or D_3 is hydroxylated in the liver. It is also known as calcidiol.
$1,25(OH)_2D$	1,25-dihydroxyvitamin D: produced when 25(OH)D is hydroxylated in the kidney or other tissues. It is commonly referred to as calcitriol.

Related to melanoma:

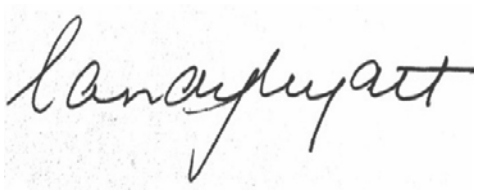
Breslow thickness	the vertical depth of the melanoma as measured from the top layer of the epidermis (or if ulcerated from the base of the ulcer) to the deepest invasive cell
Clark level	the level of anatomic invasion of the melanoma within the skin
Mitotic rate	the number of cells exhibiting mitosis per square millimetre (cell division)
Ulceration	the melanoma has breached the epidermis

Related to spectrophotometry:

L*	"lightness" of the skin measured on a scale from 0 (black) to 100 (perfect white)
a*	relates to colour ranging from red to green
b*	relates to colour from blue to yellow
Hue angle	basic tint of the colour: calculated from a* and b*
Chroma	saturation or intensity of colour tint: calculated from a* and b*
ITA value	a "value" for skin type: calculated from L* and b* to generate a value ranging from 90° (lightest or fairest) to -90° (darkest or black)
ITA group	skin type grouped according to ITA value

Statement of Original Authorship

The work contained in this thesis has not been previously submitted to meet requirements for an award at this or any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.

Signature: 

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Chapter 1: Introduction

This chapter outlines the problem statement (section 1.1) and the significance of the research (section 1.2), and identifies the aim (section 1.3). Section 1.4 defines the scope of the research and defines the terms that are used. Finally, section 1.5 provides an outline for the remaining chapters of the thesis.

1.1 PROBLEM STATEMENT

Queensland, the "Sunshine State" of Australia, has the highest incidence of cutaneous melanoma in the world.[1] Melanoma, a cancer originating from the melanocytes, or pigment forming cells found in the basal layer of the skin, presents a major global public health problem.[2] It is the fourth most common cancer diagnosed in Australia[3], the most common cause of cancer in Australians aged 15–24 years,[4] and in the United States of America it is the most common cancer in the 20–29 year old age group.[5] Surgery is the most effective form of treatment for melanoma; however prognosis is poor with no curative treatment currently available once the disease spreads to the regional lymph nodes and other organs.[6-8]

The exact cause of melanoma is unknown. Environmental factors, especially sun exposure, and genetic factors are strongly linked to the disease, while previous history of other skin cancers, increased number of naevi and fair skin phenotype are also known risk factors.[9,10] Exposure to solar ultraviolet radiation (UVR) is considered to be the major risk factor for melanoma in fair-skinned populations,[11-13] yet melanoma can form on parts of the body that are not exposed to the sun[14] and is diagnosed more often in indoor workers than outdoor workers.[15-17]

Some epidemiological research has suggested that UVR exposure, particularly recreational exposure, is associated with increased survival rates from melanoma.[18] Further, it has also been suggested that the possible protective effects of exposure to UVR against mortality from melanoma might be due to the positive effects of vitamin D on various aspects of cancer biology,[19] as approximately 90% of the human requirement of vitamin D is obtained through exposure to solar UVR.[20]

Cell biology research has shown that cultured melanoma cells proliferate more slowly in response to the active form of vitamin D (1,25-dihydroxyvitamin D)[21] and that 1,25-dihydroxyvitamin D induces apoptosis in human melanoma cell lines *in vitro.*[22] Recently, several epidemiological studies conducted in the Northern Hemisphere have reported that lower levels of serum 25-hydroxyvitamin D (the inactive biomarker of circulating vitamin D) were associated with a poorer outcome for patients with invasive melanoma.[23-25]

A large Australian study observed that 31% of participants were deficient (< 50 nmol/L) in vitamin D[26] and a small study conducted in Brisbane (the Queensland capital) suggested that almost one-third of that population – living in a high ambient UVR environment - had low vitamin D status (25 nmol/L–50 nmol/L).[27] If low vitamin D status at time of melanoma diagnosis does adversely affect prognosis, these findings from Queensland – highest melanoma incidence, high prevalence of vitamin D deficiency, link between vitamin D status and melanoma prognosis – taken together, would have considerable public health significance.

1.2 SIGNIFICANCE OF RESEARCH

No study has been conducted in Australia to investigate if vitamin D status at time of diagnosis is associated with melanoma prognosis. This represents a gap in the knowledge that may inform why some patients have a better prognostic outcome from melanoma than others.

1.3 AIM

The aim of this study was to identify, and explore, the nature of any relationship between serum 25-hydroxyvitamin D concentration (25(OH)D) of patients with newly diagnosed melanoma in Queensland, and the outcome for those patients as predicted by histopathological features of the melanoma that are known to be associated with prognosis.

1.4 SCOPE

Patients aged 18 years or older, who presented with a diagnosis of melanoma to either one of two plastic surgeons in Brisbane, Australia over a 12 month period, and who satisfied the inclusion and exclusion criteria, were invited to participate in the study. The prognostic indicators for melanoma of Breslow thickness, Clark level, mitotic rate and ulceration were obtained from the histopathology reports. To obtain serum 25(OH)D concentrations that were indicative of the participant's "usual" levels, blood samples were taken at the time of the first appointment with the surgeon (prior to the wider-excision surgery); that is before sun exposure behaviour may have changed in response to the diagnosis. As a matter of protocol, patients with a histologically proven provisional diagnosis of melanoma (following a biopsy usually performed by a general practitioner/primary care physician) are seen by either

surgeon within four days of their initial diagnosis, and wider-excision surgery routinely occurs within seven days of that appointment.

1.5 OVERVIEW OF THE THESIS

In Chapter 2, a literature review examines current knowledge on both melanoma and vitamin D, before research investigating relationships between melanoma prognosis and serum 25(OH)D concentration is found to be limited to three studies conducted in Germany and England. These studies are summarised and discussed before the need to address certain methodological issues such as timing of blood samples is argued and areas that await research are identified.

Chapter 3 draws on the conclusions of the previous chapter to establish the research question and then develop an appropriate method for conducting a study that will answer the question and fulfil the study aim. The research instruments and the method of their application are then described. Data analysis methods are discussed and a risk-factor model is developed and a step-by-step explanation of the model-building process outlined.

The study results are delivered in two chapters. Chapter 4 provides descriptive and summary statistics relating to the outcomes, main exposure and all covariates considered to be potential confounders of the relationship between serum 25(OH)D concentration and melanoma prognosis. This chapter sets the scene for the risk factor modelling to examine the association between serum 25(OH)D and the histological indicators of melanoma prognosis, that is presented in Chapter 5.

The results from both chapters 4 and 5 are then evaluated and discussed in Chapter 6 in terms of the contribution they make to existing knowledge on the relationship

between 25(OH)D concentration and melanoma prognosis. Recommendations for areas where this research could be extended in the future and possible improvements to the research design and limitations of the study are suggested, before final conclusions are drawn in Chapter 7.

Chapter 2: Literature Review

2.1 INTRODUCTION

To investigate if 25(OH)D concentration is associated with melanoma prognosis, electronic databases were searched up to January 2013 using the keywords vitamin D, 25(OH)D, 25-hydroxyvitamin D, VDR, vitamin D receptor, melanoma, cutaneous melanoma and CMM, in varying combinations and truncations. Only peer reviewed journal articles were retrieved and the reference lists from these articles were then searched for any hereto, unidentified studies. Following this initial retrieval, weekly alerts were set up on these databases: PubMed, Science Direct, HighWire Press (all journals) (aacrjnls-mailer@alerts.stanford.edu) and Web of Science and the same system of searching reference lists for further articles was used to locate new material. The Queensland University of Technology library retrieved and delivered documents that were too old to be available online. Articles were not constrained by language and two articles written in German required translation. Books relating to both vitamin D and melanoma were also examined.

This chapter will describe and discuss the findings of that search to identify what is currently understood about the relationship between vitamin D and melanoma prognosis. Initially skin biology, structure and function will be described, followed by a brief introduction to solar ultraviolet radiation and cancers of the skin. Epidemiology of cutaneous melanoma will then be explored and the burden of the disease in Queensland contextualised in relation to Australia, globally and to other cancers and over time. Melanoma aetiology will be discussed and the known risk factors identified. After examining clinical and histopathological diagnosis,

classification, treatment and management of the disease, the potential role of vitamin D will be introduced. Vitamin D nomenclature, production, availability and activation are then described in detail before research investigating links between vitamin D and melanoma is reviewed. Studies that have specifically examined the relationship between vitamin D and prognosis for melanoma are identified and discussed. Finally, a summary of findings is presented before the chapter concludes by identifying potential areas for further research.

2.2 SKIN BIOLOGY, STRUCTURE AND FUNCTION

2.2.1 Skin biology and function

The skin, the largest and most visible human organ, varies in thickness from less than 0.5 mm on the eyelids to 6 mm between the shoulder blades.[28] Covering the entire body, the skin has a range of functions that support survival by shielding the internal organs and tissues from physical, chemical and microbial attack.[29] Physical attack, such as punctures, abrasions and absorption, are deterred by the laminar structure of the skin, pathogens are repelled by the immune cells of the skin and maintenance of body homeostasis is ensured by the ability of the skin to sweat.[30] The skin also protects the body from the harmful effects of solar UVR and is the site for initialising the production of vitamin D.[28]

2.2.2 Skin structure

The skin has two main layers (see Figure 2.1); the epidermis and dermis. Directly underneath the dermis is a layer of connective tissue, the subcutaneous layer, which, although not technically part of the skin, is often referred to as such.[31]

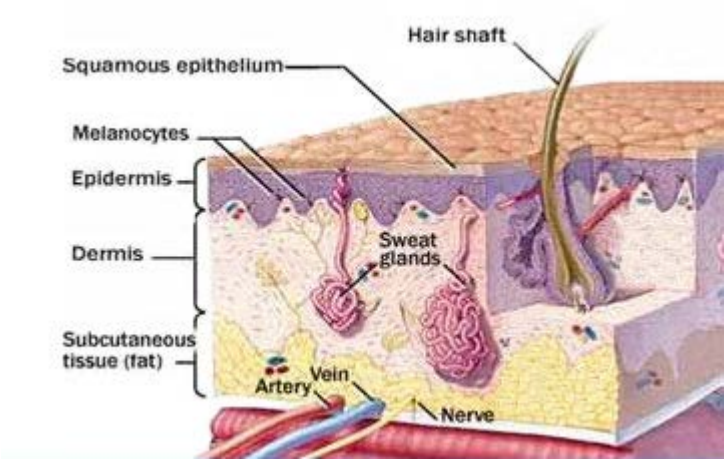

Figure 2.1. Cross-section of human skin consisting of the epidermis and the dermis, with the underlying subcutaneous layer.

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Epidermis

The epidermis (see Figure 2.2), on average only 0.1 mm thick, protects the deeper layers of the skin and is composed of several types of epithelial cells: keratinocytes, melanocytes, Langerhans cells and Merkel cells.[28] Keratinocytes make up approximately 90% of the epidermal cells, produce a tough protein (keratin) and are the main structural element of the outer skin.[30] Melanocytes produce the polymeric pigment, melanin, which contributes to skin colour and plays an important role in protecting the keratinocytes from exposure to UVR.[30] Langerhans cells play a role in immunity and the Merkel cells connect to nerve endings responsible for detection of light touch and pressure.[31,33,34]

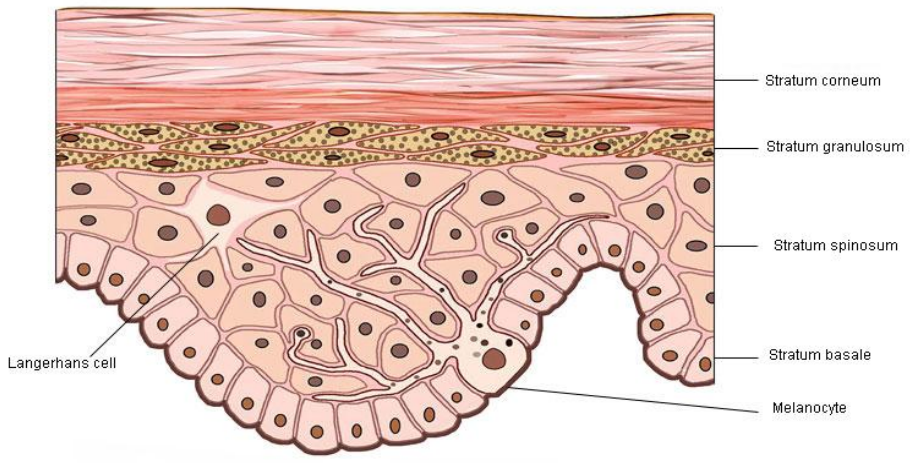

Figure 2.2. Cross-section of the epidermis showing the four main layers and identifying melanocytes and Langerhans cells.

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The epidermal cells are found in four distinct layers. From deepest to most superficial they are the stratum basale, stratum spinosum, stratum granulosum and the stratum corneum. Keratinocytes in the stratum basale undergo mitosis and migrate from this layer to be keratinised before being pushed to the surface.[30] The stratum corneum consists of up to 30 layers of dead keratinocytes arranged in overlapping layers to give the skin it's tough, waterproof characteristics.[28] The dead cells are continually shed to be replaced by new squamous cell keratinocytes pushing up from lower layers.[29]

Melanocytes, located in the stratum basale and matrix of the hair bulb, make dendritic connections with keratinocytes[34,36] and convert the amino acid tyrosine into melanin pigments which are released in melanosomes and transferred to the keratinocytes to make the brown pigment, melanin.[30,34] The amount of melanin produced is regulated by UVR exposure, genetic expression of enzymes such as

tyrosinase, and hormones (such as the α-melanocyte stimulating hormone)[37] and determines individual skin colour.[38]

Dermis

The dermis consists of fat, collagen, elastin fibres, salts, water, sebaceous glands, sweat glands, hair follicles, nerves and blood vessels.[30] This dense, strong skin layer is replenished by the rich blood supply that also supplies the multiplying cells in the basal layer of the epidermis.[28] Collagen comprises the majority of the dermis and gives the skin strength and the ability to scatter visible light and the sweat glands are responsible for temperature regulation, or homeostasis.[31]

Subcutaneous layer

The innermost layer of the skin connects the dermis above to the underlying organs. The subcutaneous layer consists predominately of loose fibrous connective tissue, fat and blood vessels.[30,31] Fat stored in this layer represents an energy source for the body, is an insulation layer against variability in outside temperatures, and also acts to cushion the body.[28]

2.3 SOLAR ULTRAVIOLET RADIATION (UVR)

Sun exposure from a young age is known to be a significant risk factor for melanoma, particularly in fair-skinned people,[11,39] yet sunlight is fundamental for life on Earth. UVR is the region of the solar radiation spectrum associated with skin cancer, in particular the long wavelength UVA (315–400 nm) and the shorter UVB (280–315 nm).[40] UVC (200–280 nm) does not reach Earth's surface as it is absorbed by oxygen and ozone in the upper atmosphere.[41] Approximately 90 to 95% of solar UVR energy reaching the Earth's surface is UVA.[42] With a longer wavelength than

UVB, UVA can penetrate deeper into skin and it is estimated that 19 to 50% of solar UVA reaches the depth of melanocytes**,** whereas only 9 to 14% of solar UVB reaches the melanocytes.[43] This long-wavelength property of UVA also allows it to pass through most automobile, office and household windows, whereas UVB is blocked by window glass.[44] Similarly, some research suggests that a substantially greater percent transmission of UVA, as compared with UVB, occurs through cotton fabrics.[45]

2.3.1 UVA versus UVB

The two UV spectra also have different biological effects. UVB is about 1,000 times more effective than UVA in inducing sunburn,[43] while UVA, on the other hand, is much more potent in inducing immediate pigment darkening (IPD) and persistent pigment darkening (PPD).[44] Both UVA and UVB cause biologic damage, with the formation of single oxygen radicals that subsequently lead to DNA-strand breakage, nucleotide damage and mutations.[44]

2.3.2 Skin response to UVR

Humans have adopted mechanisms to moderate the harmful effects of solar UVR, including the production of UV absorbing pigments such as melanin.[46] While the amount of melanin produced by the melanocytes is substantially increased after chronic UVB exposure,[34] the ratio of the two main types of melanoma pigment: eumelanin and pheomelanin, are critical in the determination of an individual's photosensitivity.[47-49] Eumelanin acts as a sunscreen to protect the skin from UVR induced damage, while in contrast pheomelanin is photosensitising and may promote carcinogenesis.[33,47] The ratio of epidermal eumelanin to pheomelanin therefore affects the susceptibility of the human skin to UVR damage.[47] Various studies have also documented that the darker the skin is, the lower the percentage of UVR

transmitted, thus explaining (albeit very simply), why dark skinned people can live free from skin cancer in the equatorial regions.[50,51]

2.4 CANCERS OF THE SKIN

The majority of skin cancers are caused by inappropriate exposure (according to skin type) to solar UVR.[17] The three main skin cancers: Basal cell carcinoma (BCC), Squamous cell carcinoma (SCC) and cutaneous melanoma, originate in the epidermis and take their names from corresponding cell types: basal cells, squamous cells and melanocytes.[52] BCCs and SCCs are combined as a group known as non-melanoma skin cancers (NMSC) and are the most commonly reported cancers in Australia.[53]

Melanoma accounts for less than five percent of all detected skin cancers.[54] Nevertheless, as they are rapidly growing, appear at any age, and on any part of the body, they are the most aggressive of all skin cancers due to their high likelihood of metastasis.[55] The melanoma usually presents as a freckle or mole that has changed size, shape, colour or texture, or a new spot that is very dark in colour.[56] Melanomas are often brown or black; yet this is not always the case as they can also be devoid of colour (amelanotic melanoma).[57]

2.4.1 Melanoma cell of origin theory

In response to solar UVR, epidermal keratinocytes regulate the survival, differentiation, motility and proliferation of melanocytes, stimulating them to produce melanin when the skin is exposed to the sun.[37] Melanin is responsible for making the skin tan (or brown) and for protecting the deeper layers of the skin from the harmful effects of the sun.[58] Melanocytes, derived from the neural crest during embryonic development,[59] are found in the epidermis, the eye, and in the epithelia of

the nose, oropharynx, urinary tract, anus and vagina.[60] The traditional model of melanoma development is that mature epidermal melanocytes are the cells of origin responsible for tumour development. This view is based on pathological features that suggest melanomas develop in the epidermis and then invade the dermis.[61]

This traditional view is now being challenged by research showing that subpopulations of tumour stem cells (TSCs) are present in solid cancers.[62,63] TSCs are capable of self-renewing, and although they comprise only a small part of a tumour, they have been shown to have the ability to generate new tumour nodules. Melanomas, like other solid tumours, have a subpopulation of TSCs, and with research identifying that tumour cells require an accumulation of genetic and phenotypic changes over extended periods of time, there is support for the theory that melanoma may develop from such a precursor cell that has acquired malignant mutations over time.[62]

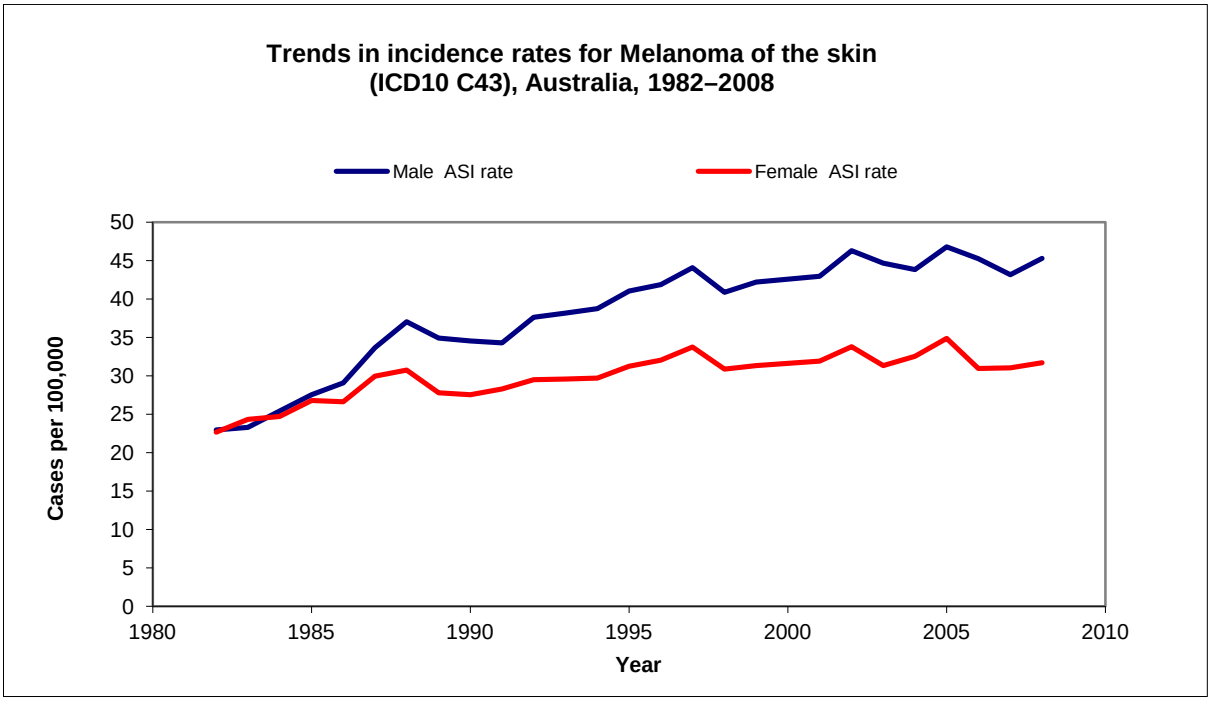
2.5 EPIDEMIOLOGY OF MELANOMA

2.5.1 Current incidence trends

The first reported mention of melanoma was by Hippocrates in the fifth century, B.C.[64] Melanoma now contributes significantly to the public health burden of many countries with the incidence rising globally since the 1950s.[65-67] The most recent global estimates are that there were approximately 200,000 new cases of invasive cutaneous melanoma diagnosed in 2008, with the disease reportedly the sixth most frequent cancer diagnosed in westernised countries of mainly Caucasian populations.[65]

Australia

In 2008, Australia had the highest age-standardised incidence rate for cutaneous melanoma (37 cases per 100,000 people) of any country in the world.[68] This rate was more than 12 times higher than the average world rate of three cases per 100,000 people.[68] With the exception of a levelling off period in the early to mid 1990s, Australian incidence rates, for both males and females (see Figure 2.3), have been steadily increasing over the past three decades[68] and show similar temporal patterns to data from countries in Europe and the United Kingdom.[6,69]



Note: Age-standardised to the Australian Population.
ASI: Age-standardised incidence rate

Figure 2.3. Incidence rate trends for melanoma in Australia for 1982–2008.

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The incidence rates for Australian males doubled from 27 cases per 100,000 males in 1982, to 57 cases per 100,000 males in 2007, while in the same time period the rate for females increased by 47% from 26 cases per 100,000 females to 38 cases per 100,000 females.[68]

Recent data indicate that Australian incidence rates may be stabilising due in part to a slight decline in incidence among young females (aged 25–44 years), and males in general.[65] In males, the risk of being diagnosed by age 75 is 1 in 24 or 1 in 15 by age 85, and in females the risks are 1 in 35 by age 75 and 1 in 24 by age 85.[68] In the ten-year period from 1998–2008, the annual average increase in incidence rates for melanoma was 1% per person, while in the period 2006–2008 the increase was 0.6%.[54]

United States of America

By comparison, in the United States in 2004, the equivalent lifetime risk was 1 in 55 for men and 1 in 82 for women.[70] The latest National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) data for 2005–2009 reports age-adjusted incidence rates of 27.2 per 100,000 males and 16.7 per 100,000 females.[5]

Queensland

Queensland, Australia's most north-eastern state, has the highest age-standardised incidence rates for melanoma in the world, with 83.2 cases per 100,000 for males and 53 cases per 100,000 for females reported in 2008.[1,3] For Queensland males,[3] the approximate risk of being diagnosed with melanoma by age 75 is 1 in 18 increasing to 1 in 11 by age 85. In females,[1] the risks of being diagnosed with melanoma are lower at 1 in 26 by the age of 75 years and 1 in 19 by the age of 85 years.

Queensland incidence in comparison to other states and countries

Data (see Table 2.1) from the most recent Cancer Incidence in Five Continents publication from the International Agency for Research on Cancer (IARC), shows that for the period 1998 to 2002, the reported melanoma incidence for Queensland was more than three times that of any country in Europe.[71]

Table 2.1

Selected IARC figures for comparative melanoma incidence 1998–2002

Country	**Age-standardised (world) incidence (per 100,000)**	
	Male	**Female**
Queensland, Australia	55.8	41.1
New South Wales, Australia	38.5	26.5
Victoria, Australia	27.3	23.4
New Zealand	34.8	31.4
US SEER 14 registries non-Hispanic whites	19.4	14.4
Vaud, Switzerland	16.6	19.6
Norway	14.2	14.6
Sweden	11.9	12.1
Denmark	11.9	14.1
Saarland, Germany	8.1	7.8
Yorkshire, England	6.9	8.9
Serbia	3.8	4.8
Latvia	3.2	4.2
Belarus	2.7	3.5

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2.5.2 Burden of disease

Melanoma is the fourth most common cancer diagnosed in Australia,[3] representing 9.6% of all cancers diagnosed (see Figure 2.4). It is also the most common cause of cancer in the 15-24 year age group.[4,54]

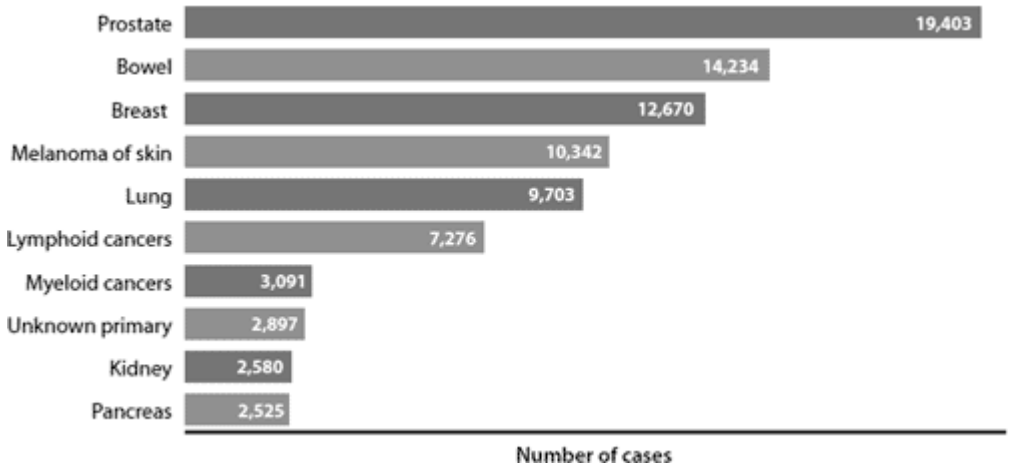


Figure 2.4. The 10 most commonly diagnosed cancers in Australia, 2007.
[68]

In 2007, melanoma was ranked eighth in Australia's causes of cancer burden of disease and was estimated by the Australian Institute of Health and Welfare (AIHW) to have accounted for 22,300 disability adjusted life years (DALYS) in 2010.[68] 16,800 of the DALYS were lost due to premature death ,while the remainder were healthy life years lost due to disability or disease.[68] Males reportedly suffered the greater burden of disease from melanoma, with more than twice the number of DALYs (15,300) of females (7,000).[68]

2.5.3 Mortality from melanoma

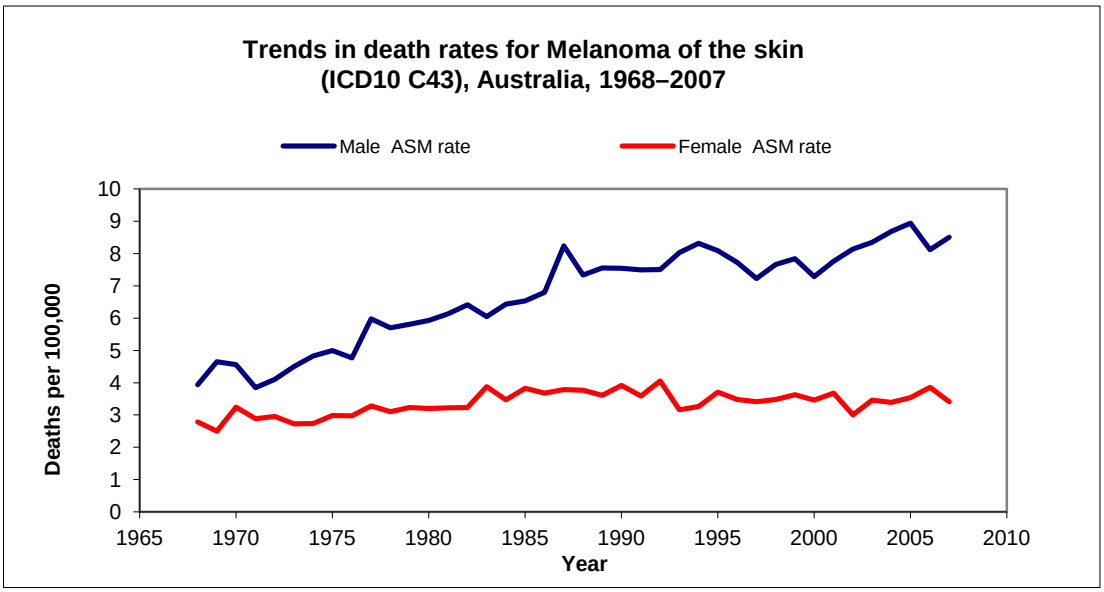
Absolute mortality rate

IARC[72] reported an estimated 46,000 deaths from melanoma globally in 2008. The Australian Cancer Incidence and Mortality books reveal that in 2007 there were 1,279 deaths from melanoma, which represented 3.2% of all cancer deaths in Australia.[54]

Temporal trends in mortality rate

While the incidence of melanoma worldwide is steadily increasing, mortality data from Australia, the United Kingdom, Scotland, France and Sweden, indicate that

death rates from melanoma are increasing more slowly.[54,69,73] In Australia,[74] the age-standardised mortality rate (ASM) for melanoma increased from 3.3 deaths per 100,000 in 1968 to 5.7 deaths per 100,000 in 2007. This increase was driven largely by the increasing mortality rates in males. Thus, while ASM rates for females have remained quite steady over the past four decades (from 2.8 deaths per 100,000 females in 1968), rates for males have slowly increased from 3.9 per 100,000 males (see Figure 2.5).[54] In 2007, the ASM rates for melanoma were higher for males (8.5 deaths per 100,000 males) than for females (3.4 deaths per 100,000 females).[54]



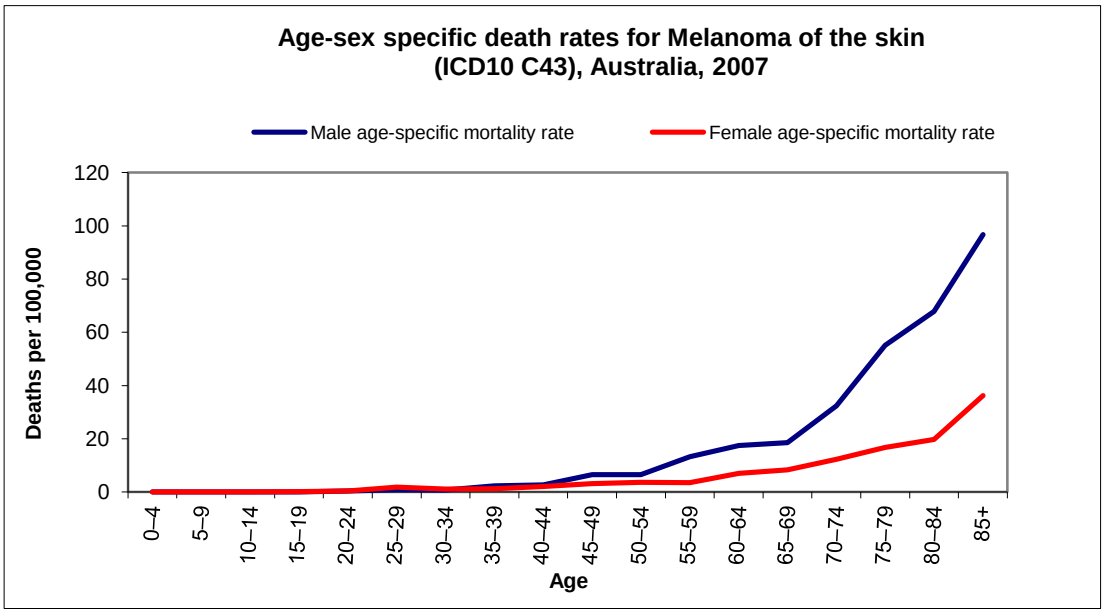
Note: Age-standardised to the Australian Standard Population 2001
ASM: Age-standardised mortality rate

Figure 2.5 Australian trends in mortality rates for cutaneous melanoma (1968-2007).[54]

The approximate doubling in the melanoma mortality rate in Australian males between 1968 and 2007 is similar to that seen in Scotland (albeit at much lower absolute levels): in the 25-year period from 1979-2003, melanoma mortality rates for Scottish males doubled from 1.1 to 2.4 deaths per 100,000 males, while the mortality

rates for Scottish females remained relatively unchanged at 1.5 deaths per 100,000 females.[69] This has led to the suggestion that a 1980s melanoma awareness campaign may be responsible for the relatively slow rise in Scotland's melanoma mortality rate compared to the incidence rate, due to earlier detection and excisions of thinner lesions.[69] However, this does not explain the lack of change in the female mortality rate.

Further, in Australia melanoma mortality rates increase with increasing age, more so for males than for females (see Figure 2.6).[54]



Note: Age-standardised to the Australian Standard Population 2001

Figure 2.6. Death rates for cutaneous melanoma by age and sex (2007).

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A more detailed examination of Australian temporal trends according to age group, shows that, in Australia from 1950–2002, mortality rates for males and females younger than 55 years decreased, there was a stabilisation of mortality rates for the

55–79 years age group and a three to four percent increase per annum in persons over the age of 80 years.[75]

2.5.4 Survival from melanoma

Approximately one quarter of all melanomas diagnosed in Australia in the period 1982–2002 were classified as Clark level 1 (*in situ*) tumours.[76] The detection of proportionally more *in situ* and thin melanomas (less than one millimetre) has positively impacted on survival from melanoma in Australia.[76] The 20 year survival rate[77] for thin melanomas is 96%. Localised (*in situ*) melanomas are reported to have a 99% survival rate five years after diagnosis,[74] while in comparison, thicker melanomas (greater than four millimetres) are reported to have a five-year survival rate of 55%.[74,78] This is similar to data from Scotland where five-year survival rates for thicker melanomas (greater than four millimetres) are reported to be 52.4% for males and 48.3% for females.[69]

In Australia in the five-year period from 2006–2010, females (94%) had a higher survival compared to males (89%) with no difference noted according to remoteness or socioeconomic status.[74] Nevertheless, overall five-year survival rates improved only marginally, from 88% in 1980 to 90% in 2004.[79] With incidence rates increasing in the same period, these trends are most likely related to earlier detection of proportionally more *in situ* and thinner lesions,[76] or possibly, some benefits of the primary prevention programs that have been in place nationally in Australia since the early 1980s.

2.5.5 Melanoma risk factors

In the 1950s sunlight was first suggested as the main environmental agent for melanoma due to the higher incidence of the disease in fair-skinned peoples.[12,13]

Considerable evidence has always supported the shorter wave UVB radiation (280–315 nm) as the responsible risk factor for melanoma, however more recently the longer wave, UVA radiation (315–400 nm) has also been implicated.[43,80] In 2006, a systematic review conducted by IARC determined that UVR emitted by tanning beds was associated with melanoma risk.[81] Subsequent to that report, in 2010 Lazovich et al[82] observed statistically significant increases in risk (OR = 4.44, 95% CI: 2.45, 8.02) in tanning beds emitting predominantly UVA radiation.

The highest level of evidence describing major risk factors for melanoma is provided by three systematic meta-analyses reported by Gandini et al[9,10,83] in 2005. After conducting a systematic literature review of all observational studies on melanoma and risk factors published prior to September 2002, Gandini et al obtained studies that were independent, comparable, from populations that were homogenous for the risk factor under review, and which provided sufficient information to estimate relative risks.[9,10,83] Relative risks (RR) were extracted from 46 studies for common and atypical naevi, RR for sun exposure from 57 studies, and RR for family history, actinic damage and phenotypic factors from 60 studies.[9,10,83] Pooled estimates for the risk factors of interest were obtained using fixed and random effects models, between study variation and bias was explored by sub-group analysis and meta-regression, and publication bias and reliability of results was investigated by sensitivity analysis. Studies included in the meta-analyses were conducted in various regions of the world including North and South America, Europe, Israel and Australasia.

The meta-analyses[9,10,83] identified the following factors as the strongest predictors of cutaneous melanoma risk:

- skin phenotype (fair versus dark: RR = 2.06, 95% CI: 1.68, 2.52)
- skin type (I versus IV: RR = 2.09, 95% CI: 1.67, 2.58)
- number of common naevi: (greater than 100 naevi versus less than 15 naevi: RR = 6.89, 95% CI: 4.63, 10.25)
- number of atypical naevi (5 versus 0: RR = 6.36, 95% CI: 3.80, 10.33)
- presence of actinic damage, including solar lentigos and elastosis (RR = 2.02, 95% CI: 1.24, 3.29)
- presence of pre-malignant and skin cancer lesions (RR = 4.28, 95% CI: 2.80, 6.55)
- positive family history of melanoma (RR = 1.74, 95% CI: 1.41, 2.14).

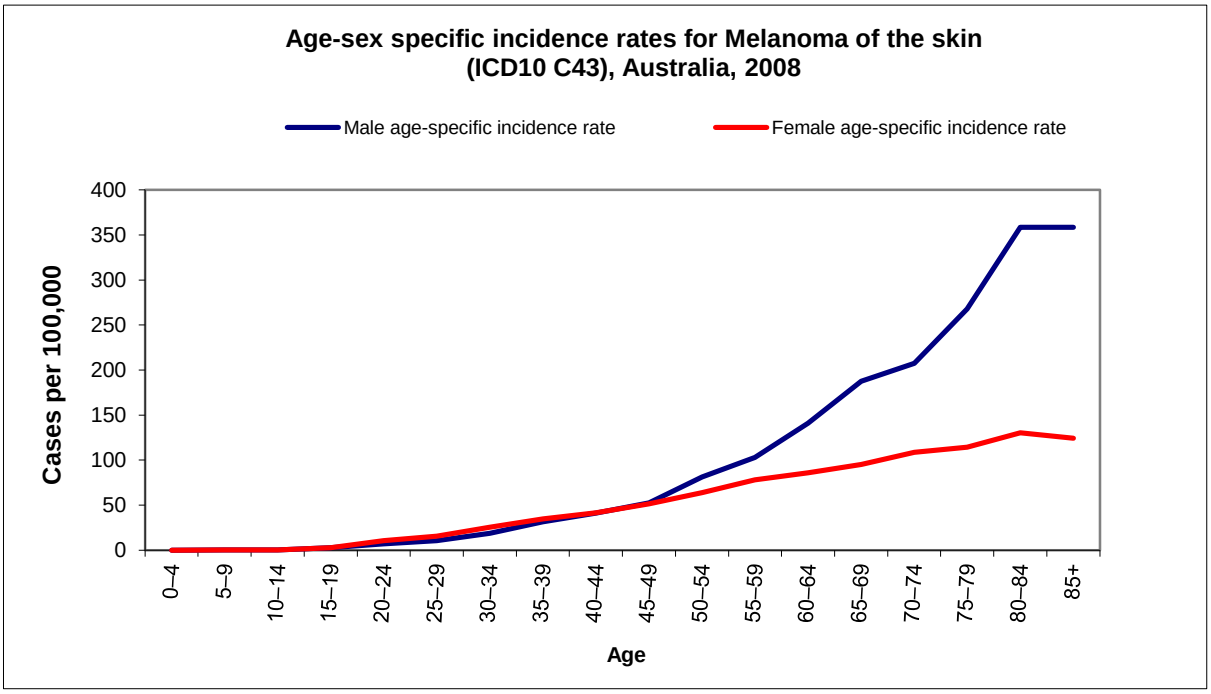
Higher levels of sun exposure were also identified by Gandini et al,[10] as a strong risk factor for melanoma, with varying relative risks dependent on latitude, skin phenotype and phototype, and sunburn history. Gandini et al[10] acknowledge that while the measurement of sun exposure is inconsistent across studies and may be subject to recall bias from both cases and controls, intermittent sun exposure is positively associated with melanoma risk whereas high continuous UVR exposure is inversely associated. The researcher's caveat for supporting intermittent sun exposure as a risk factor for melanoma, is that this was suggested by "well conducted" studies.[10] Sunburn history was also noted to be a risk factor, however Gandini et al report that histological type, particularly lentigo maligna melanoma (commonly associated with chronic UVR exposure), influenced the estimated RR.[10]

Although Gandini et al[83] investigated skin phototype as a possible risk factor for melanoma, none of the studies included in their meta-analysis reported results from studies that used objective measurement tools. Instead, studies reported skin type

using the Fitzpatrick classification, or definitions used for "skin type" were based on the skin's reaction to sunlight. An Italian study[84] examined objective measures of constitutive skin colour in relation to melanoma risk and found that skin brightness (L*) of an unexposed body site is associated with an increased melanoma risk (OR = 1.20, 95% CI: 1.12, 1.30). In 2002, a case-control study conducted by Brenner et al[84] used a reflectance spectrophotometer to assess constitutive skin colour of 183 diagnosed melanoma cases and 179 controls. Measurements were taken on the buttock (as an "unexposed" site), and although the study population included participants of Italian heritage, all recorded measurements met the criteria for low pigmentation (L* > 60).[84] The association between increased melanoma risk and L* persisted after the authors adjusted for other potential confounders. Others have also used reflectance spectrophotometry to show that cutaneous melanin density of another unexposed body site - the upper, inner arm, is a strong predictor of melanoma risk, particularly for males.[85]

Demographic risk factors

As with most cancers, age is one of the strongest risk factors for melanoma.[86] In Australia for example, the risk of developing melanoma in 2008 was 1 in 909 for 30–34 year olds, 1 in 485 for 40–44 year olds and 1 in 129 for 70–74 year olds.[54] While the incidence of melanoma increases dramatically with age (see Figure 2.7), this is particularly noticeable in males.[54,68]



Note: Age-standardised to the Australian Standard Population 2001

Figure 2.7. Australian age-sex specific incidence rates for melanoma (2008).

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It should be noted that while melanoma is predominantly found in white skinned populations, a recent large cohort study[87] identified that age, male sex and sunburn phenotypes may also be risk factors for melanoma in non-white populations (excluding African Americans).

Latitude

Whiteman and Green (2005) have suggested country of residence is another important determinant of the absolute risk of a melanoma occurring in a fair-skinned person.[86] The role of solar UVR in the development of melanoma is, in general, supported by examination of global melanoma incidence figures that show a clear latitude gradient of increasing incidence with decreasing latitude.[71,88-90] Within Australia (see Table 2.1, page 17), the potential influence of latitude is clearly demonstrated in the decreasing incidence rates from north to south.[3,71] This is not the case in Western Europe however, where the European national cancer registries

report that the highest incidence rates are found in the Scandinavian countries.[6,71] This is possibly related to the higher use of sun beds and sunny holidays in these populations living in low ambient UVR locations.

Patterns of sun exposure

Studies have reported that the incidence of melanoma is higher in those exposed to UVR intermittently, rather than those exposed to a smaller quantity of UVR every day.[52,91-93] As previously reported, a meta-analyses of 57 studies by Gandini et al[10] showed a positive association with intermittent sun exposure (RR = 1.61, 95% CI: 1.31, 1.99), and an inverse association for high continuous sun exposure patterns (RR = 0.95, 95% CI: 0.87, 1.04); although the latter was not statistically significant. This may explain the higher melanoma incidence in indoor workers[15,16,94] compared to outdoor workers (with high cumulative UVR exposure) and the small proportion of melanomas that arise in areas of the body rarely exposed to the sun, such as the buttocks.[95] Gandini et al[10] have suggested that the development of melanomas on intermittently exposed body sites (rather than continuously exposed), especially in younger people, may be due to the relatively unprotected skin allowing high transmission of UVR to the melanocytes.

Divergent pathways theory

The occurrence of melanomas on skin sites not commonly exposed to the sun, particularly in younger people and indoor workers, but with a predilection to sun exposed sites in older populations, has led to a "divergent pathways" theory to explain melanoma development on different body sites.[96-98] Queensland researchers have found that the prevalence of naevi and solar keratosis differs significantly between patients with truncal melanomas and those with melanomas of the head and neck.[98] Melanomas of the head and neck, rather than the trunk, were more likely to

be detected in people with fewer naevi (OR = 0.34, 95% CI: 0.15, 0.79), more than 20 solar keratoses (OR = 3.61, 95% CI: 1.42, 9.17), and with a tendency to have a prior history of surgical excisions to remove solar-induced skin lesions (OR = 1.87, 95% CI: 0.89, 3.92).[98] People with melanomas of the head or neck were also statistically significantly more likely to have medium (OR = 2.27, 95% CI: 1.04, 4.99) or higher (OR = 3.70, 95% CI: 1.52, 8.98) levels of occupational sun exposure than those of people with melanoma of the trunk.[98]

These findings suggest that there are two pathways to melanoma development. Melanomas of the head and neck are related to cumulative sun damage (consistent with their occurrence in older age groups), whereas melanomas of the trunk are associated with melanocyte proliferation due to skin phenotype, specific genetic mutations, and intermittent sun exposure.[98]

Genetic susceptibility

Recently, researchers investigating the role of genetics in melanoma development have identified certain gene mutations associated with primary melanomas particularly with respect to the melanocortin receptor 1 gene (*MC1R)*, proto-oncogene B-Raf (*BRAF)*, v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog *(KIT)* and *NRAS* genes.[99-102] Inherited mutations in genes encoded by the CDKN2A locus have also been associated with high melanoma risk (particularly in the context of familial melanoma), yet the reported prevalence is very low.[103,104]

2.6 CLINICAL AND HISTOPATHOLOGICAL DIAGNOSIS OF MELANOMA

2.6.1 Clinical diagnosis

Melanomas are more likely to be detected by the patient (44%), than by doctors (25.3%) or partners (18.6%).[105] Signs and symptoms of melanoma can include itching, changes in size, shape or colour of an existing skin lesion, or in more advanced cases, bleeding and crusting. Trained medical practitioners will apply the ABCD(E) rule (Asymmetry, Border irregularity, Colour variation, large Diameter [and Evolution]) to initially identify suspicious lesions.[106] Identification is aided by dermoscopy, a technique that allows clear and magnified vision of features of skin lesions unable to be seen with the naked eye.[107] Complete surgical excision of the suspected lesion, with a clear two millimetre margin confirmed by histological examination, is the recognised gold standard in the clinical diagnosis of a melanoma.[108,109]

2.6.2 Histopathological reporting of melanoma

The aim of the histopathology report is to provide the information needed for the clinician to optimally manage the patient. Macroscopic and microscopic examination of the excised specimen by a pathologist gives dimensions and visual descriptions of the specimen, identifies the correct diagnosis of primary melanoma, and verifies complete excision of the tumour.[8] In addition, histopathology describes the melanoma type, the tumour thickness (Breslow), the anatomical level (Clark level) of the tumour, and the presence of ulceration or mitosis. Other features often included in the histological report (cell type, solar elastosis, vascular invasion, regression) are relevant for studying the epidemiology and pathogenesis of the tumour.[8]

Immunohistochemistry is often performed during pathological examination of the specimen.[110] As S100 protein, a neuronal protein found in glial and Schwann cells and also in malignant melanocytes, is expressed by most melanoma cells, S100 staining of the entire excised specimen offers a more accurate means of assessing dermal and neural invasion, tumour thickness, and peripheral margins.[110,111]

2.6.3 Histopathological features of melanoma

In 1969, Clark et al[56] reported that most melanomas arose in epidermal melanocytes, as opposed to originating in existing naevi, and that the macroscopic appearance, defined microscopic characteristics, location and age, were determinants in identifying the various types of melanoma. The three main subtypes reported are superficial spreading melanoma (SSM), nodular melanoma (NM) and lentigo maligna melanoma (LM) or Hutchinson's melanotic freckle.[56] SSM, characterised by a relatively long initial flat phase, is the most common melanoma type and has been associated with intermittent small bouts of UVR exposure.[8,56] NM, on the other hand, presents as a fast growing nodular lesion that is more common in older people and is more likely to be found on the head and neck than on other areas of the body.[56,112] Like SSM, lentigo maligna melanomas also have a long initial flat phase, but these are more likely to present in outdoor workers, on chronically sun-damaged skin, and on the faces of older patients.[8,56,113]

Clark et al[56] also reported that based on the depth of invasion, each melanoma had a predicable biological behaviour pattern. They identified five anatomic levels of invasion that were considered to provide an accurate prognosis[56]:

- Clark level I: the melanoma occupies only the epidermis.
- Clark level II: the melanoma penetrates to the papillary dermis (the layer under the epidermis)
- Clark level III: the melanoma fills the papillary dermis and impinges on the reticular dermis, the next layer down.
- Clark level IV: the melanoma penetrates into the reticular or deep dermis
- Clark level V: the melanoma invades the subcutaneous fat.

Melanoma are still classified according to the Clark levels, however due in part to the fact that levels III and IV vary greatly in thickness[114] and that interpretation of levels is subjective and prone to inter-reporter variation,[115] Breslow thickness, the microscopic measurement of the distance in millimetres between the upper layer of the epidermis and the deepest point of tumour spread, is now regarded as the most important prognostic factor for melanoma, followed by identification of the tumour as a primary melanoma and confirmation that it is completely excised.[116]

2.6.4 Classification and staging

Melanomas are often classified into stages according to the thickness of the tumour, whether it has ulcerated, has lymph node involvement or whether there are metastases.[116] Lymph node involvement and metastases are confirmed by clinical staging techniques. The system of melanoma classification used today is based on the 2002 publication of the American Joint Committee on Cancer (AJCC) and the International Union against Cancer (UICC).[8] Early melanomas (Stages I and II) are localised, while more advanced melanomas (Stages III and IV) indicate metastatic spread to other parts of the body.[117]

2.6.5 Treatment and management of melanoma

Treatment for primary melanoma is a wide local excision of the melanoma including the surrounding skin and subcutaneous tissues.[8] The size of the wider excision is based on the size and depth of the visible lesion, with the aim to completely excise all *in situ* and invasive melanoma cells.[8] As previously mentioned, histopathology, the only clinically reliable tool for a definitive diagnosis of melanoma,[108] should confirm that the wider excision is complete. If lymph and systemic metastases are present on clinical staging, the life expectancy of the patient declines dramatically.[58,118] Any investigations performed after diagnosis are therefore aimed at the detection of regional or systemic disease.[8]

Nevertheless, a growing body of evidence now suggests that such investigations, which include chest x-rays, Computerised tomography (CT), Magnetic resonance imaging (MRI) and Positive Emission Tomography (PET) scans, cannot be recommended due to lack of any data indicating that they alter outcomes or are cost-effective. [119-122] Management of melanoma, and in particular the detection of lymph node metastases, is therefore limited to clinical examination of the lymph nodes at regular surveillance visits.

Tumour biomarkers

The use of tumour biomarkers in predicting metastatic spread of melanoma at an early stage of the disease has previously been considered questionable, yet they have recently been attracting attention as a possible adjunct to clinical examination in the follow up stage of melanoma surveillance.[123,124] S100 proteins, are found in the cytoplasm and nucleus of many cells, and are involved in the regulation of cellular processes such as cell cycle progression and differentiation.[125] Several studies have reported a correlation between serum levels of S100β [123,125,126] or S100ββ[124], and

relapse and survival from melanoma, with serum concentrations of S100β thought to reflect proliferation of melanoma cells and tumour mass.

2.6.6 Melanoma prognosis

Studies over the last 30 years have determined that the prognosis for patients with a primary melanoma (as opposed to a secondary metastasis) depends on a number of variables.[116,117,127-130] A study of 17,600 melanoma patients by Balch et al in 2001 demonstrated that survival was most powerfully predicted by Breslow thickness and ulceration.[116] Level of invasion (Clark level) was also found to be significant within the category of thin melanomas (≤ 1 mm).[116] Conversely, two later studies found that mitotic rate was second only to Breslow thickness as an important prognostic variable.[130,131] The 2003 study by Azzola et al[130] examined records of 3661 patients in the Sydney (Australia) Melanoma Unit database, while the study by Thompson et al[131] in 2011 analysed records of 13,296 patients in the AJCC melanoma staging database.

UVR exposure and melanoma prognosis

Few studies have explored the association between sun exposure and melanoma prognosis. While considering that UVR exposure is the main environmental risk factor for melanoma, it is rather paradoxical that researchers have suggested that melanoma may in fact be more benign (have a better prognosis) if associated with high ambient UVR exposure.[18] One of the first studies to suggest a link between higher sun exposure and improved melanoma prognosis found that patients with a history of high sun exposure, independent of the melanoma body site, mitotic rate, thickness and early detection, had a 40–60% lower mortality rate over 5 years than those reporting a history of low sun exposure.[18] Berwick et al[18] also reported that the presence of solar elastosis (a histological marker of chronic sun damage) was

statistically significantly inversely associated with fatality from melanoma (HR = 0.5; 95% CI: 0.3, 0.9). While this association was not found in three other studies,[132-134] earlier studies had found similar results.[129,135,136] One explanation for these findings could be that chronic sun exposure and sun damage are in fact in some way protective for aggressive melanoma.

Another study[137] found that a summer diagnosis was associated with a significant protective effect on survival from melanoma (Relative fatality = 0.72; 95% CI: 0.65, 0.81). This effect was independent of histologic indicators of prognosis, including Breslow thickness.[137] Boniol et al[137] reported that melanomas diagnosed in winter were thicker than those diagnosed in summer, and in an Italian study of 260 melanoma patients, Rosso et al[138] found that patients who had taken more sunny holidays prior to their diagnosis had a better survival. Another European study[139] estimated the average number of sunlight hours in 36 European cities, and found that lower melanoma mortality rates occurred in countries with the sunniest climates. It has been suggested that the mechanism responsible for these results could be that recent UVR exposure prior to melanoma diagnosis is indirectly protective for survival as a result of the anti-neoplastic effects of vitamin D on the tumour.[19,88,140]

2.7 VITAMIN D

Vitamin D is a fat-soluble steroid hormone precursor, named in the early 20th century after the discovery that cod-liver oil prevented the formation of rickets in children.[141-143] The term vitamin D refers to two molecules: vitamin D_2 (ergocalciferol) and vitamin D_3 (cholecalciferol).[144] The majority of vitamin D (90% or more) in many populations is obtained by exposure to sunlight (vitamin D_3),

leaving only a small amount of the vitamin to be obtained through diet or supplementation (vitamin D_3 or vitamin D_2).[145,146] Vitamin D is important in bone metabolism and is now thought to be important for a range of other physiological functions.

2.7.1 Cutaneous production of vitamin D

The stratum basale is the site for the UVR-mediated photosynthesis of vitamin D_3 when 7-dehydrocholesterol (7-DHC) in the cell membrane of keratinocytes is exposed to UVR in the UVB spectrum (280 – 315 nm).[31,147] 7-DHC, a sterol in the cholesterol biosynthetic pathway: lanosterol → lathosterol→ 7-dehydrocholesterol→ cholesterol,[148,149] differs from cholesterol by one additional double bond located in the steroid structure.[150] 7-DHC absorbs UVB, causing the B-ring to open with the formation of previtamin D_3.[151] After approximately 2 hours, previtamin D_3 undergoes a thermal isomerisation reaction at skin temperature to form vitamin D_3, which is stored in the fat cells in the dermis before entering the blood stream where it is bound to the vitamin D binding protein.[152]

7-DHC is found throughout the entire epidermis and dermis, however the highest concentrations of 7-DHC per milligram of lipid are located in the stratum basale.[148,151] Two studies[153,154] have reported vitamin D_3 production per unit area of epidermis irradiated. In 1958 Wheatley et al[153] found that 400 IU was produced from irradiation of three square inches of epidermis, while in the same year Bekemeier[154] reported that 4 to18 IU of vitamin D_3 were synthesised within three hours from the irradiation of one square centimetre of white human skin. Factors influencing the cutaneous production of vitamin D_3 from UVR exposure include factors that affect ambient UVB levels; time of day, season, cloud cover, latitude, altitude; and individual-level factors, such as orientation to the sun, body surface area exposed,

skin pigmentation (melanin density) and use of sunscreen (at recommended levels).[152] It has also been suggested that levels of 7-DHC decline with age,[155] thus decreasing the capacity of human skin to produce vitamin D_3.

2.7.2 Vitamin D in the diet

A small amount of vitamin D in the form of either vitamin D_3 (animal sources) or vitamin D_2 (plant sources) is obtained through the diet. Oily fish such as salmon, mackerel and tuna are good sources of vitamin D, as are cod liver oil and irradiated mushrooms.[147,152] Very small amounts of vitamin D can be obtained through eggs, meat and dairy products, and for some populations in the United States and Europe, supplemented foods are the major dietary source.[156] In 1997 an "adequate intake" (AI) was suggested for vitamin D[157], but there were no Recommended Daily Allowances (RDI) until these were set by the United States Food and Nutrition Board in 2010.[158] These recommendations are based on low exposure to sunlight and suggest an adequate intake of 400 IU for babies, 600 IU for ages 1 to 70 and 800 IU for those aged over 70.[158]

2.7.3 Vitamin D activation

25-hydroxyvitamin D (25(OH)D)

Both ergocalciferol and cholecalciferol are transported via the bloodstream to the liver, where they are hydroxylated to form 25-hydroxyvitamin D (25(OH)D), also commonly referred to as calcidiol.[159] 25(OH)D circulates in serum and is a good biomarker of the status of both skin synthesis and dietary intake of vitamin D due to its ease of measurement and its long half-life in circulation (estimated between three and five weeks).[147,160,161] For these reasons it is the universally accepted indicator of vitamin D status.[162,163] This biologically inactive form of vitamin D is subsequently

hydroxylated in the kidneys, with some additional production in other tissues, to form calcitriol, or 1,25-dihydroxyvitamin D ($1,25(OH)_2D$).[20]

1,25-dihydroxyvitamin D ($1,25(OH)_2D$)

$1,25(OH)_2D$ serum concentration is very tightly regulated and maintained at low circulating levels despite large changes in 25(OH)D concentration.[164] The main physiological function of $1,25(OH)_2D$, under the control of the parathyroid hormone (PTH), is to maintain adequate levels of calcium in the circulation.[165] $1,25(OH)_2D$ also regulates phosphorus absorption in the small intestine and is essential for bone development.[145] Vitamin D metabolic enzymes have now been found in a range of other cell types, so that active $1,25(OH)_2D$ can be synthesised and act at the local cell level to have local effects, for example, on immune function.[165]

2.8 THE VITAMIN D RECEPTOR (VDR)

Intracellularly, $1,25(OH)_2D$ binds to a nuclear vitamin D receptor (VDR).[161] Present on nearly every cell in the human body, the VDR is an intracellular hormone receptor specifically for vitamin D.[166] The VDR gene is located on chromosome 12q12-q14.[166] VDRs have been found in many cancer cells including those from tumours of the prostate, breast, colon, cervix, bladder, pancreas, pituitary and thyroid glands.[167,168] VDRs have also been found in normal melanocytes and in cultured melanoma cells.[169]

2.9 OPTIMAL VITAMIN D CONCENTRATION

Historical

Rickets is probably the most commonly known effect of vitamin D deficiency and was first described[141] by the Greek historian Herodotus (485–426 BC). In 1822, the Polish researcher, Sniadecki, observed that children living in the city of Warsaw had a much higher rate of rickets than children from the rural areas in Poland, and suggested that disease may be caused by lack of exposure to sunshine.[170] Later that century, Palm recommended that children should sunbathe to prevent rickets,[141] and then in the early 20th century it was shown that cod-liver oil also contained something that could cure rickets.[171]

Current recommendations

Vitamin D status is often classified as "deficient", "insufficient", "sufficient" or "optimal". There remains considerable controversy around the optimal level of 25(OH)D necessary for good health. While some suggest the optimal serum level of 25(OH)D should reflect the concentration necessary to suppress the parathyroid hormone, this level has been reported to vary widely from 20 nanomoles per litre (nmol/L) to 110 nmol/L.[172-174] Others have suggested optimal levels of 37.5 nmol/L[175], 75 nmol/L[176] and 80 nmol/L.[177] Based on a review of associations with a wide range of disease outcomes, and requirements for bone health, the United States Institute of Medicine (IOM) recommended in 2011 that a serum 25(OH)D level of 50 nmol/L should be considered sufficient.[178] Further, while there appeared to be a general consensus that a serum concentration of 25(OH)D of less than 25 nmol/L was classed as deficiency,[144,179] the IOM concluded that less than 30 nmol/L constituted risk of deficiency.[178] Blurring this disagreement is the wide variability in measurement results dependent on the assays and laboratory techniques used.[180-182]

Sub-optimal vitamin D and health

Vitamin D deficiency and insufficiency have been associated with a wide variety of chronic health problems. A review of ecological, case-control and cohort studies found evidence that higher concentrations were beneficial for autoimmune, cardiovascular and cerebrovascular diseases, bone health, muscle strength and type 2 diabetes.[183] Results from the Third National Health and Nutrition Examination Survey (NHANES) prospective cohort study suggest that both low and high levels of serum 25(OH)D may be associated with an increase in all-cause mortality.[184]

2.10 VITAMIN D AND CANCER

Vitamin D is considered to be a vital component of cellular networks that inhibit the proliferation of cells and encourage apoptosis.[140] Recent research in rodents suggests that optimal levels of 25(OH)D may actually decrease the risk of contracting some cancers, as well as limiting their invasive capacity and ability to metastasise.[167]

Many observational studies[185-187] have been published on the association between vitamin D status and risk for various cancers, but in particular breast, prostate and colorectal cancer. Meta-analyses of these studies have confirmed a link between low vitamin D status and increased risk of colorectal cancer, with the findings in relation to breast cancer equivocal.[188,189] Nevertheless, the World Health Organisation's (WHO) 2008 report on vitamin D and cancer[160] concluded that results at that time from observational studies were mixed for breast cancer, showed no link to prostate cancer risk, but did support a link to colorectal cancer. The WHO suggested that further cohort studies were warranted.

In relation to skin cancers, one case-control study in American males found an association between high levels of 25(OH)D and a reduced risk of NMSC.[190] Conversely, in a case-control study of American women, those in the highest quartile serum of 25(OH)D were more likely to have an increased risk of BCC (OR = 2.07, 95% CI = 1.52–2.80, *P* for trend < 0.0001) and SCC (OR = 3.77, 95% CI = 1.70–8.36, *P* for trend < 0.0002), than those in the lowest quartile.[186]

2.11 VITAMIN D AND MELANOMA

2.11.1 Cell biology research

In the early 1980s, intracellular receptors (VDR) for vitamin D ($1,25(OH)_2D$) were identified in both melanoma cell lines and in melanoma tissue in rodents.[21] Colston et al (1981) also demonstrated that $1,25(OH)_2D$ inhibited proliferation of melanoma cells both *in vitro* and *in vivo*.[21] Later in the 20th century, $1,25(OH)_2D$ was identified as inhibiting invasiveness[191] and inducing differentiation in melanoma cell lines.[192] In other laboratory-based trials, cultured melanoma cells synthesized $1,25(OH)_2D$ from 25(OH)D[169] and the $1,25(OH)_2D$ molecule was shown to induce apoptosis in human melanoma cell lines *in vitro*.[22] Similarly, *in vivo* trials have found that vitamin D ($1,25(OH)_2D$) can suppress melanoma growth and inhibit metastasis in immunosuppressed rodents.[191,193]

2.11.2 Genetic research

Several researchers have hypothesised that polymorphisms in the VDR gene may affect the risk of developing melanoma.[194,195] However a meta-analysis of six studies that investigated the association between VDR polymorphisms and melanoma risk found conflicting results and the authors suggested that further population based

studies were needed to fully address this area.[19] More recently (in 2009), Randerson-Moor et al[196] provided additional evidence to suggest that both the VDR and vitamin D may have a small role to play in melanoma risk and could possibly contribute to poorer outcomes after disease diagnosis. Research to analyse the expression pattern of the VDR in melanoma is limited to one retrospective study that also examined tissue from other skin lesions and normal skin.[197] All participants in the study were white, and although age and sex were reported, questionnaire data were not recorded. In addition to examining VDR expression, the study used histological staining to quantify solar elastosis. The VDR expression in melanoma cells was correlated with type, level, thickness and mitotic rate, with data in relation to ulceration not reported.[197] Findings from this study showed that VDR expression in melanoma tissue and the surrounding skin was significantly reduced when compared to normal skin. The main limitation of this study was that the technique used a brown/ black stain, Diaminobenzidine, rather than a red stain, which could have resulted in melanin being confused with the VDR.

2.11.3 Epidemiological research

Results from epidemiological studies investigating relationships between sun exposure and melanoma generally support a beneficial role for vitamin D in both melanoma incidence and melanoma prognosis.

Vitamin D and melanoma risk

Few studies have evaluated the association between vitamin D intake and melanoma risk and results have been conflicting. An early case-control study by Weinstock et al[198] found no association between vitamin D intake and melanoma risk. In 2009, a large cohort study (n > 65,000, including 455 participants who had developed melanoma) investigated the association between dietary and supplemental vitamin D

intake and melanoma risk.[199] This study found there was no evidence of reduced melanoma risk in association with either the highest quartile of dietary vitamin D intake (RR = 1.31; 95% CI: 0.94, 1.82), 10-year average supplemental vitamin D intake (RR = 1.13; 95% CI: 0.89, 1.43), or a combination of dietary and supplemental intake (RR = 1.05; 95% CI: 0.79,1.40).[199] Similarly, a study including 176 women with melanoma in the Women's Health Initiative cohort (n = 36,282) also found no association with vitamin D intake.[200] Conversely, in case-control studies using food frequency questionnaires, Millen et al and Vinceti et al both observed an inverse relationship between vitamin D intake from diet and melanoma risk.[201,202] Millen et al[201] recruited 497 patients newly diagnosed with melanoma and 561 controls and concluded that persons with higher intake of vitamin D had reduced risk of melanoma (OR = 0.66, 95% CI = 0.42, 1.02). Vinceti et al[202] studied 380 cases and 719 controls and found that an increase in vitamin D intake of 1ug (1 ug = 40 IU) per day was associated with an odds ratio for melanoma risk of 0.85 (95% CI 0.74, 0.97).

Studies examining the association between serum 25(OH)D levels and melanoma risk are scarce. In a small sample (n = 14) of melanoma patients, Reichrath and Querings[203] found that all patients had "sufficient' 25(OH)D levels (range: 50–125 nmol/L). A larger case-control study of Finnish male smokers[204] also found a lack of association between serum 25(OH)D levels and melanoma risk, as did a case-control study (cases: *n* = 92, controls: *n* = 276) in Northern England.[196]

Vitamin D and melanoma prognosis

In accordance with the work of Berwick et al,[18] where it was suggested that higher vitamin D status may be associated with decreased mortality after melanoma diagnosis, a number of both experimental and observational studies have now been

conducted to investigate this possible relationship. A retrospective cohort study (*n* = 217) conducted in the United Kingdom concluded that vitamin D supplements may be protective for melanoma prognosis (OR = 0.6; 95% CI: 0.4, 1.1).[24] While the results were only marginally non-significant, these results did not include dietary vitamin D intake. The findings of that study were tested in a survival analysis of 872 melanoma patients with lesions of Breslow thickness greater than 0.75 mm who were recruited in northern England over a six year period and followed-up for, on average, 4.7 years.[24] Clinical and histopathological data, a questionnaire and serum samples collected three to six months after surgery, enabled Newton-Bishop et al (2009) to also examine associations between 25(OH)D levels and Breslow thickness, relapse-free survival (RFS) and overall survival (OS).[24]

The authors concluded that higher serum 25(OH)D levels at sampling were associated with thinner tumours ($P = 0.002$) and were protective for relapse and death from melanoma[24]. A 20 nmol/L increase in serum 25(OH)D was associated with decreased risk of relapse (HR = 0.79; 95% CI: 0.64, 0.96; $P = 0.01$) or death (HR = 0.83; 95% CI: 0.68, 1.02), i.e. improved RFS and OS. The authors stated that although adjusted for age, sex, site, thickness and body mass index (BMI) this inverse relationship may have been due to unmeasured confounders.[24] As sun avoidance behaviour after melanoma diagnosis is relatively common[205,206] and the half-life of 25(OH)D is approximately three to five weeks,[147,160,161] it is possible (or even likely) that the 25(OH)D levels recorded in this study may be reflective of early changes in sun behaviour, and might not accurately represent the 25(OH)D levels at the time of diagnosis.

Another prospective study looked at the association between direct and indirect measures of vitamin D status, namely serum 25(OH)D levels and UVR exposure, on

the incidence and prognosis for melanoma.[25] Using similar methodology to Newton-Bishop et al, the German study[25] of 205 patients diagnosed with invasive melanoma collected clinical, histopathological and questionnaire data. Serum samples were obtained between October and April, only (despite recruitment at diagnosis throughout the year). The authors found that serum 25(OH)D levels were reduced in Stage IV melanoma patients as compared to those with Stage 1a or 1b disease (P = 0.006), but they were unable to comment on the role of 25(OH)D in melanoma pathogenesis and prognosis due to retrospectivity and sample size.[25]

A more recent study, also from Germany, recruited melanoma patients approximately six months after diagnosis and determined their serum 25(OH)D at that time, and after a further six months.[23] The investigators[23] concluded that lower 25(OH)D levels were associated with increased tumour thickness (< 1mm; ≥ 1–4 mm; > 4 mm: coefficient -1.45, P = 0.028) and higher AJCC stage (coefficient: -.79, P = 0.036).

2.12 SUMMARY

The highest global incidence rate for melanoma is found in Queensland, Australia.[54] Melanoma is the most aggressive skin cancer given its ability to grow rapidly in a vertical manner and then metastasise to other organs.[55] Exposure to solar UVR is considered one of the major risk factors for melanoma, along with fair skin phenotype, family history of melanoma, older age, increased numbers of common and atypical naevi, and country of residence.[9,10,83,98] Prognosis for melanoma is best predicted by Breslow thickness, mitotic rate, presence of ulceration, and Clark level.[116,131]

Studies looking at the relationship between sun exposure and melanoma have found that higher sun exposure and evidence of solar dermal damage were inversely associated with fatality from melanoma[18] and that melanomas diagnosed in summer had a better prognosis than those diagnosed in winter.[137] The results from these studies, and others, have prompted suggestions that sun exposure may have a beneficial effect on melanoma prognosis and has led to the hypothesis that the positive effect of sun exposure on melanoma prognosis could be mediated by improved vitamin D status.[19,140]

In vitro and *in vivo* studies have shown that vitamin D can suppress melanoma growth in melanoma cell lines[22] and in mice.[191,193] Vitamin D, a fat-soluble hormone derived predominantly from exposure of the skin to UVR in the UVB spectrum, is required to maintain calcium and phosphorous homeostasis. Vitamin D status is universally measured by serum 25-hydroxyvitamin D (25(OH)D) levels. Serum 25(OH)D has a long half-life in the circulation (estimated between three and five weeks)[147,160,161] and these levels are regarded as a biomarker of the status of both cutaneous synthesis and dietary intake of vitamin D. Although there is wide disagreement on optimal levels and wide variability in measurement techniques, low levels of serum 25(OH)D have previously been associated with the incidence of some cancers.[188,207,208]

Vitamin D intake was not found to have any association with melanoma risk in one study,[199] while another study[24] found that vitamin D supplementation had a beneficial effect on melanoma prognosis. While few epidemiological studies have investigated the potential link between vitamin D and melanoma prognosis, the authors of the latter study subsequently conducted a prospective cohort study and concluded that higher serum 25(OH)D levels at sampling were associated with lower

Breslow thickness and a lower rate of metastases.[24] This research, in conjunction with results from a study published in the same year[25] and a more recent study published in 2012,[23] both showing reduced levels of serum 25(OH)D in patients with advanced melanoma, adds some weight to the hypothesis mentioned above. That is: that vitamin D, or its proxy, recent sun exposure has a positive effect on melanoma prognosis.

The three studies that have investigated this possibility have paved the way for further research to build on their knowledge base. There are some missing links in the puzzle. First, thin melanomas (< 0.75 mm) and *in situ* melanomas have not been adequately studied to date. These form a large proportion of all melanomas diagnosed, and to accurately understand the real relationship between vitamin D and melanoma prognosis, serum 25(OH)D levels in patients with both thin and thicker tumours need to be investigated. Secondly, serum 25(OH)D levels at "diagnosis" with melanoma have not as yet been ascertained. To achieve that, and to minimise potential bias, serum would need to be obtained prior to wider excision surgery (before the patient's sun behaviour could potentially change in response to the melanoma diagnosis). To avoid the problems previously mentioned with respect to measurement variability, all serum samples should be analysed in one batch. Additionally, a number of factors that could potentially confound or mediate the association between serum 25(OH)D and melanoma prognosis, including skin pigmentation and phenotypic characteristics, and use of sunscreen do not appear to have not been measured in the studies conducted to date. Future studies could consider implementing methods to measure such factors.

Finally, all studies identified to date were conducted in the higher latitudes of the Northern Hemisphere. Latitude is both a potential risk factor for melanoma[71,88] and

also one of the factors that can influence cutaneous production of vitamin D_3 from UVR exposure.[152] To further enhance our understanding of the relationship between 25(OH)D levels and melanoma prognosis, it is appropriate to investigate whether these results:

1. from Northern Hemisphere research are comparable to those from research conducted in the lower latitudes of the Southern Hemisphere, in a region already identified to have high prevalence of vitamin D insufficiency
2. are similar when 25(OH)D levels at time of diagnosis are considered, and
3. are true for *in-situ* and thin (< 0.75 mm) melanomas, as well as for thicker melanomas.

Chapter 3: Research Design and Analytic Approach

3.1 INTRODUCTION

In Chapter 2, a review of the literature found that three studies had investigated the relationship between serum 25(OH)D level and melanoma prognosis. These studies provide evidence to suggest that a relationship does potentially exist and in doing so have built a solid foundation for further research in this area. An opportunity to increase the existing body of knowledge was identified. The review also found that both season at the time of diagnosis, and serum levels of S-100β, have been previously associated with melanoma prognosis.

This chapter commences by reviewing and considering the methods and research instruments used in previous studies into relationships between serum 25(OH)D level and melanoma prognosis, before outlining the criteria considered pivotal in conducting a study that would address the study aim, while overcoming some of the limitations of previous research. The criteria will also allow associations between melanoma prognosis and season at time of diagnosis, and serum levels of S-100β, to be investigated.

An observational, cross-sectional study is selected as the desired study design and the study sample and study procedure identified. The data collection instruments and the methods used for their implementation are then described in detail. It will be noted that in some areas of interest (such as life time sun exposure) more than one instrument is used with the aim of counteracting any potential bias that may occur with, for example, recall of past events. Management of data, including the use of

any coding practices is then described. Methods of data analysis are discussed and the model selection is explained. A step-by-step explanation of the model building process is subsequently provided.

3.2 REVIEW OF METHODS USED IN PREVIOUS STUDIES

The three studies previously described (pages 42-43) all used a prospective cohort study design and recruited patients with histologically proven cutaneous melanomas of various stage and type. One of the research groups[25] also incorporated a small case-control study into their study design. Measures of exposure varied between the studies. Newton-Bishop et al[24] administered a self-report questionnaire 3 to 6 months after diagnosis. The questionnaire included items on drug intake, dietary supplementation, age, height and weight, but it is unclear if information such as skin type, number of naevi, history of NMSC, sunburn history, family history of melanoma and lifetime sun exposure was collected.[24] Blood samples for serum 25(OH)D analysis were taken between 3 and 6 months after surgery, and histopathology reports and clinical notes were accessed to obtain details of the primary tumour and relapse data for 872 melanoma patients with melanomas of Breslow thickness greater than 0.75 mm.[24]

Nurnberg et al[25] recruited 205 melanoma patients and 141 healthy volunteer controls for their study. Data from the volunteer controls were included in some analyses to determine if results relating to serum 25(OH)D levels and questionnaire items differed between the two groups. The self-administered questionnaire, completed by approximately 25% of both groups (melanoma group: $n = 58$ and volunteer group: $n = 42$), provided information on skin type, height, weight, history of sun exposure,

sunscreen use and painful sunburns. An objective measure of lifetime sun exposure or skin type does not appear to have been used. Nurnberg et al[25] report that all participant's blood samples were taken between October and April to minimise seasonal variation in 25(OH)D levels (although participants were recruited following melanoma diagnosis throughout the year). The clinical and histopathological data obtained for this study were used to allocate patients to a melanoma "stage". Melanomas classified as Clark Level 1 (*in situ*) were not represented in this study.

It is not clear how Gambichler et al[23] obtained data for their study, however age, sex, body mass index (BMI), season and skin type were ascertained and serum was collected for 25(OH)D analysis up to six months after diagnosis.

3.3 AIM, OBJECTIVES AND RESEARCH QUESTION

Based on the review of the literature, the aim, objectives and the research question for the QUT Melanoma Study were:

3.3.1 Aim

To identify, and explore the nature of any relationship between the serum 25(OH)D level at diagnosis of patients newly diagnosed with melanoma in Queensland, and the outcome for those patients as predicted by histopathological features of the melanoma that are known to be associated with prognosis.

3.3.2 Objectives

The primary objectives of this research were to:

1. Recruit a cohort of patients newly diagnosed with melanoma from an area with high melanoma incidence, including those with *in situ* (Clark Level 1) tumours

2. Examine vitamin D status (serum 25(OH)D concentration) at time of melanoma diagnosis in relation to histopathological features (Breslow thickness, Clark level, presence of ulceration and mitotic rate) previously shown to be predictive of prognosis, after adjustment for possible confounding factors.

Secondary objectives were to:

3. Describe seasonal variation in vitamin D status within the sample
4. Identify any relationship between S-100β level and the histopathological features of the tumours.

3.3.3 Research question

The research question to answer was:

What is the relationship between serum 25(OH)D concentration at time of diagnosis with melanoma and histopathological features of melanoma that are associated with prognosis in an "at risk" population.

3.3.4 Conceptual design

To add to the existing knowledge in the area and to answer the research question, the following criteria were considered pivotal in the study's conceptual design:

1. Accessibility to melanoma patients that was feasible and reliable
2. Patients with both non-invasive and invasive melanoma to be recruited
3. Recruitment to take place over a 12 month period to allow the effect of season on the relationship between serum 25(OH)D and melanoma prognosis to be investigated
4. Primary outcome data (histopathological features of the melanoma related to prognosis) to be obtained from histopathology reports of both biopsy and wider excision specimens

5. To minimise disease-related bias:
    a. Patients with melanoma to be recruited prior to the wider-excision of their melanoma, before sun exposure behaviour potentially changes in response to melanoma diagnosis
    b. Blood samples for serum 25(OH)D analysis to be obtained from melanoma patients as soon as possible after diagnosis, and prior to surgery for the wider-excision of the melanoma
    c. Data on demographics and risk factors for melanoma such as sun exposure history, latitudes of residence during life, family history of melanoma, history of NMSC, history of sunburns in childhood and sun protection habits to be obtained via a questionnaire administered prior to wider excision surgery
6. To minimise bias due to assay variability, serum samples to be analysed in a single batch for 25(OH)D and S-100β
7. To minimise recall bias, other known risk factors for melanoma to be measured objectively where feasible (spectrophotometric skin type, researcher-assessed eye and hair colour and naevi count, histological solar elastosis for cumulative sun damage), and by validated questionnaire where this is more appropriate (e.g. hair colour as a teenager, tanning and burning tendencies, lifetime sun exposure).

3.4 STUDY DESIGN

An observational, cross-sectional study design was chosen to ensure that all criteria for fulfilling the objectives of the research were satisfied.

3.4.1 Study sample (Objective 1)

All melanoma patients presenting to two Brisbane medical clinics over a twelve month period and who met the inclusion and exclusion criteria were invited to participate in the study. The small number of patients diagnosed with melanoma precluded random sampling, so convenience (purposeful) sampling was used. An estimated sample size of 100 participants was determined to be feasible based on:

- historical data from the two clinics regarding the number of patients presenting annually with melanoma
- the time frame available for the study
- financial limitations
- resource limitations (manpower).

Plastic surgeons

Although cutaneous melanoma is commonly diagnosed from excision or shave biopsies performed by primary care physicians or dermatologists, good clinical practice recommends that further wider excision of the lesion is then indicated down to, but not including the deep fascia (unless it is involved).[8] For patient comfort and to contain bleeding, this surgery is normally performed as day surgery and often by plastic surgeons. Therefore, two plastic surgeons in Brisbane, Australia were approached, agreed to assist with the research and consented for their patients to be recruited to the study (the researcher is a registered nurse and has worked for one of the surgeons for 14 years). Patients with a positive biopsy report, and who met the exclusion and inclusion criteria (below), were invited to participate in this study.

Inclusion criteria:

- patients with a histologically proven cutaneous melanoma (non-invasive and invasive)

- aged 18 years or older
- able to read and understand English.

Exclusion criteria:

- diagnosis made from metastatic melanoma or lymph node tissue
- reporting of pre-existing conditions that could alter the vitamin D pathway such as: history of cirrhosis of the liver, chronic liver disease or history of kidney disease
- transplant recipients
- patients on high dose calcium therapy (which could impact the metabolism of vitamin D).[209]

3.4.2 Study procedure

The study protocol (see Appendix A) stated that the surgeon, having determined that the patient satisfied the criteria above, would advise the patient of their eligibility to take part in the QUT Melanoma Study. I did not speak to patients in advance of their appointment with the surgeon and the patient was treated and regarded first and foremost as a patient of the surgeon, and secondly as a study participant. Similarly, it was a privilege to have access to the intimate workings of such busy surgeries and I ensured that I was as unobtrusive as possible at all times. 102 melanoma patients presented to the surgeons during the 12-month period from July 2010 to July 2011. All were informed of the study and 100 consented to participate.

3.4.3 Recruitment

Initially, each patient was given a letter to read by the surgeon (see Appendix B), but time constraints in these busy practices meant that this was not feasible and both surgeons independently asked if this protocol could be deleted, preferring to verbally

introduce the study. To ensure that I was on hand to speak to any prospective participants, the practice managers of each practice contacted me in advance of all appointments made for melanoma patients. Both surgeries have a policy of seeing probable melanoma patients as a matter of urgency (within 1-3 days) following the initial phone call to arrange an appointment; therefore most appointments are made before-hours, after-hours or squeezed in whenever possible around previously scheduled patients. At the initial appointment, the patient is often confused, upset and shocked by their diagnosis and by their rapid escalation through the medical system. The methods used in this study, and their application, were therefore designed with the patient foremost in mind to minimise any further time or emotional burden on the patient. An overview of how this was implemented is illustrated in Figure 3.1.

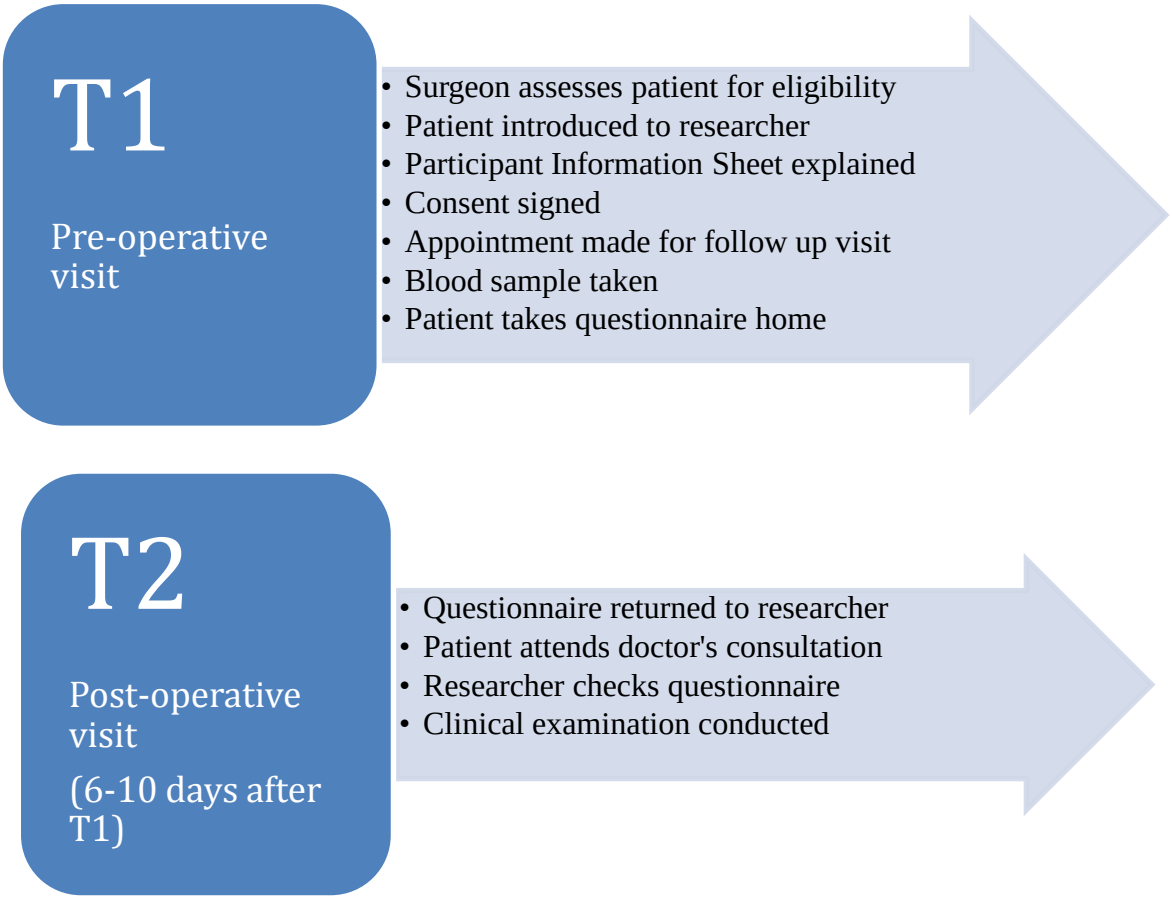

Figure 3.1. Overview of study procedure.

T1: Pre-operative visit

After consenting verbally to the surgeon to release a copy of their pathology report, patients were introduced to me at the end of their initial consultation with the surgeon. Prior to signing a *consent form* (see Appendix C), the patients and any family or friends present, were taken to a quiet area in the surgery and I re-introduced myself before describing the study, the patient's potential involvement and the options for consent to all or various parts of the study. This time was useful for the patient to ask questions, not only about the study but also about the forthcoming chain of events: surgery, post-operative care, return visits etc.

The patient was then given a detailed *participant information sheet* (see Appendix D) that reiterated the contents of the previous discussion and included background information on the study, on vitamin D, and on melanoma. The *participant information sheet* also clearly set out the protocol and timeframe for the collection of data to ensure that prior to consenting to take part in the study patients were well informed of any extra burden this may impose on them. Patients were reassured about confidentiality, assigned an identifying (ID) number from M001 to M100 and all documentation was then coded with this number.

The patient signed the consent form and was then given a copy of that, the participant information sheet and a coded *QUT Melanoma Study Questionnaire* (see Appendix E) in a clear plastic folder clearly labelled with their ID number and with my business card attached. It is normal practice for patients to return to the surgeon's rooms six to ten days post-operatively for removal of sutures (it should be noted that both plastic surgeons who recruited participants to the study adhere to these same time protocols). Participants were asked if this post-operative appointment would be a suitable time for them to return their questionnaire and to undergo the *QUT*

Melanoma Study Clinical Examination (see Appendix F), thus ensuring that all study protocols could be completed during routine appointment times. Participants were then accompanied to a commercial pathology company's collection room (located in the same building) for the study's pre-operative blood test. In many cases (71%), pre-operative blood tests had already been ordered by the surgeon and the *QUT Melanoma Study* sample was drawn without requiring any further venepuncture.

T2: Post-operative visit

At the first post-operative visit the *QUT Melanoma Study Questionnaire* was checked for any missing or unclear data and the patient had any queries clarified before the *QUT Melanoma Study Clinical Examination* was conducted. In the event that participants had forgotten to bring the questionnaire to the appointment (6%), a stamped self-addressed envelope was provided to allow quick and easy return of the form.

3.4.4 Recruitment risk management strategies

Recruitment commenced in July 2010 with one surgeon, however in November 2010 with the regime established and streamlined, recruitment began from both surgeons simultaneously. During the recruitment period, constant communication within the study team, including reviews every two weeks, ensured that cohort recruitment remained on track. In the event that the established regime was not achieving adequate study numbers, two further surgeons had also been approached prior to the commencement of the study with a request to be on "stand by". Although recruitment was on target, consideration was given to increasing the estimated sample size by also recruiting patients from those surgeons. We (the study team) decided this would not be feasible given that the time already involved in one person recruiting patients and collecting data was substantial.

There was no loss to follow up between T1 and T2 as these visits were undertaken as part of usual care. Melanoma patients are very aware of the need to adhere to the strict melanoma surveillance procedures that are in place and rarely miss a scheduled appointment. The same risk management strategies that work so efficiently in the surgeon's clinics to avoid failure to return for a post-operative appointment were incorporated in the QUT Melanoma Study. These included:

- making the participant aware that the researcher was available at any time to discuss the study
- ensuring that at T1 they received a business card with all contact details attached to the front of a personalised study pack
- ringing them on the day if they missed the appointment
- reducing participant burden by scheduling T2 to coincide with the normal scheduled melanoma post-operative visit
- accompanying all participants to any additional tests (e.g. scans) that had been ordered at T1.

3.5 DATA COLLECTION INSTRUMENTS

A copy of all instruments is provided in the Appendices to this document.

3.5.1 Questionnaire

The QUT Melanoma Study Questionnaire, comprising 58 questions, was developed and used by the Queensland Institute of Medical Research (QIMR) in over 700 participants in their 'Causes of Melanoma Study'. Professor David Whiteman very kindly consented for the questionnaire to be used, thus ensuring that by maintaining the consistency of study questions, valid comparisons between the two studies could be made if desired.[210]

The self-report questionnaire included questions on:

- demographics – date and place of birth, sex, education, marital status
- city of birth and all cities and towns lived in, from primary school to present day
- the anatomic location of melanoma
- history of the study melanoma
- frequency of skin examinations
- skin phenotype – hair and eye colour as a teenager, tanning and burning propensity
- time spent in the sun as a school child
- hair style (covering ears) during school years
- naevus and freckle burden as a teenager
- sunburns during primary and secondary school years, and since leaving school
- occupational history
- adult occupational and recreational sun exposure behaviour
- sun protection as an adult
- skin cancer history and skin cancer screening
- smoking history
- family history of melanoma.

Melanomas were reported by the participant in the questionnaire as occurring in seven general sites (including back, chest, head) and also in fourteen more specific sites (for example: “side of neck” and “back of neck” were more specific to the neck region). Propensity to sunburn was determined by the response of the skin to being in the strong sun for 30 minutes in the middle of the day for the first time in summer according to a five point scale ranging from never burning to always burning. To elicit tanning propensity, the participant was asked to indicate their level of tan after

spending several weeks at the beach in the strong sun: no tan, light tan, moderate tan or deeply tanned. Naevus burden as a teenager was recorded by ticking one of four pictures depicting a person with varying numbers of naevi: none, few, some, or many. Four faces depicting no freckles to many freckles were used as a visual guide to answer the question "When you were a teenager, how many freckles (for face and arms) did you have at the end of summer?"

The questionnaire measured the time spent in the sun as a school child by asking identical questions for primary school and secondary school. Using a five point scale of never, rarely, sometimes, usually and always or almost always, the questions asked how often they were outdoors in the sun in summer after school, and on weekends and holidays. Adult sun behaviour data were obtained by participants reporting the following information for each year of life from the time they left high school to diagnosis (questionnaire page 12): type of job, place of work (city/town), age of starting and stopping work in that place, number of days worked in the job and amount of time spent in the sun in summer on work days and non-work days at each job (mostly indoors, outdoors for 1–4 hours, mostly outdoors).

Adult sunscreen use at two time points, five years ago and ten years ago, was assessed for three body areas: 1) face, head and neck, 2) hands or forearms, and 3) other parts of the body; and according to the number of days per week sunscreen was applied. Six response options of none, occasional, 1–2 days, 3–4 days, 5–6 days, and every day, were provided. Sunburn (during primary school, high school and since leaving school), described as blistering, soreness for two days or more, or peeling, had six levels of response from never, 1 to 5, 6 to 10, 11 to 20, 21 to 50, and more than 50 sunburns.

Previous history of NMSC was ascertained by asking how many skin cancers had been either cut out, frozen or burnt off, treated with radium or in other ways. History of sunspots was determined in the same manner.

Any areas of uncertainty that may have arisen in relation to the questionnaire were resolved at the clinical examination visit. The clinical examination also allowed an opportunity to revisit some items included in the questionnaire to check any items that may have caused recall problems: in particular family history of melanoma, numbers of lesions previously removed and outdoor activities since completing high school. Visual aids (see Appendices G and H) were used to confirm teenage hair and eye colour.

3.5.2 Clinical examination

The QUT Melanoma Study Clinical Examination followed a protocol developed and tested by Professor David Whiteman and others at QIMR. I conducted all examinations at T2, after first undertaking training to update skills and gain confidence in conducting accurate and effective skin examination (with thanks to Dr Marcia Davis, a dermatologist who has conducted in excess of 600 clinical examinations at QIMR).

Clinical examination data were collected on:

- the anatomic location of melanoma
- number of current naevi
- naevi previously removed
- current freckle, solar lentigo and solar keratosis burden
- determination of skin type by spectrophotometry
- height and weight

- current eye colour.

Further data were collected at the clinical examination on:

- family history of melanoma
- supplement use.

The location of the study melanoma was identified to confirm that this was in agreement with the histopathology report. The number of naevi was quantified by examination of the head, neck, trunk and upper limbs (from the hipline up) to identify any naevi 2 mm or greater in diameter. Freckle and solar lentigo density was identified for three regions: the forearms, the face and the shoulders, according to a four-point scale of none, minimal, moderate and heavy. Solar keratoses were quantified for the dorsum of hand, dorsum of forearm, forehead, cheek, nose and chin for both sides of the body. If more than 50 keratoses were present at any one sub-site, this was recorded as 50+. Weight was measured using electronic scales and height was obtained from patient's driver's licences. Eye colour was compared to a standard eye colour chart (see Appendix H), while answers to questions regarding family history of melanoma and use of vitamin D or calcium supplements were recorded as yes or no. If vitamin D supplementation was obtained from a multivitamin formula, this was only recorded as "yes" if the formula contained greater than 200 IU of vitamin D. Spectrophotometry was used to gain an objective measure of skin type and is described below.

Spectrophotometry

Reflectance spectrophotometry measures reflected light in the range of 360–740nm (visual spectrum) and uses colorimetry to assess skin colour. Measurements of skin reflectance, obtained from parts of the body that are both exposed (dorsal forearm and forehead) and not usually exposed to the sun (upper inner arm), were recorded

using a portable hand-held spectrophotometer: Konica Minolta CM-2500d (Minolta Camera Company, Ltd., Osaka, Japan). Measurements were recorded in the three dimensional colour space known as the CIELAB (Commission Internationale d'Eclairge 1976) L*a*b* colour space.[211] The value of L ("lightness") is measured using a scale from 0 (black) to 100 (perfect white), a* relates to colour ranging from red to green and b* relates to colour from blue to yellow.[211] The protocol for the spectrophotometer use is included in Appendix I.

3.5.3 Serum for 25(OH)D and S-100β (Objectives 2, 3 and 4)

To fulfil the second, third and fourth objectives of the study, prior to wider excision surgery a blood sample for measurement of serum concentrations of 25(OH)D and S-100β was obtained from all participants by a trained phlebotomist in the rooms of a commercial pathology company. The blood was drawn, centrifuged, the serum aliquoted and identified by ID number only, and then transported to QUT's Kelvin Grove Campus in a cold box and stored at -80°C in the AusSun laboratory (Room E210, Kelvin Grove Campus).

All serum analyses were performed at the AusSun laboratory at QUT in Brisbane, Australia. Concentration of 25(OH)D was measured as a single batch using a commercial chemiluminescent immunoassay (LIAISON® 25_OH Vitamin D TOTAL assay: DiaSorin, Inc., Stillwater, MN). This assay measures total 25(OH)D including 25(OH)D_2 (ergocalciferol) and 25(OH)D_3 (cholecalciferol). Intra-assay variability was 3–6%. Corresponding values for inter-assay variability were 6–9%. The laboratory that performed the testing is a participant in the Vitamin D External Quality Assessment Scheme (DEQAS). Serum S-100β levels were analysed using LIAISON® Sangtec®100 (DiaSorin, Saluggia, Italy), a frequently used assay that

has been shown to have good reproducibility and is reported to have the highest sensitivity and accuracy when compared to other assays.[125,212,213]

3.5.4 Histopathology (Objective 2)

Data regarding the histopathological features of the cutaneous melanoma were obtained from the participant's biopsy and wider excision histopathology reports. All definitive (wider) excisions were reported by dermatopathologists from Sullivan Nicolaides Pathology, one of Australia's largest pathology practices. All dermatopathologists, led by Dr David Weedon, are Fellows of the Royal College of Pathologists Australia (FRCPA). Sullivan Nicolaides Pathology is quality certified to ISO standards.

Outcome data obtained were:

- Breslow thickness (millimetres)
- Clark level (levels 1–5)
- mitotic rate (per square millimetre)
- ulceration (presence or absence).

Additional data also obtained from the histopathology reports included:

- melanoma type
- melanoma site
- extent, or presence of, solar elastosis.

Solar elastosis is not always routinely included in melanoma histopathology reports; therefore a comment was added to all wider-excision pathology request forms requesting that the pathologist describe the extent of solar elastosis observed in the specimen.

3.6 DATA MANAGEMENT

Data from the questionnaire and clinical examination, blood samples and histopathology reports, were initially recorded in Excel (Microsoft Office 2007) in pre-coded numeric format with some exceptions:

- place names, which were subsequently allocated a latitude using World Atlas[214]
- certain data from the histopathology reports, including melanoma type, anatomic site of melanoma and degree of solar elastosis were entered verbatim as string variables, and then subsequently recoded into numeric variables.

Routinely, all data collected during one week was recorded in Excel that week to minimise data entry error caused by repetitious entry of large quantities of data. The data entry process was audited by re-entering data from all IDs ending in 6 (i.e. M006, M016, M026 etc.) after 10 questionnaire or clinical examination data had been entered.

At the completion of the twelve month recruitment period, the Excel data files were copied to the statistical analysis program, Stata IC Version 11.2.[215] Data cleaning and consistency checking was then undertaken before analysis commenced. Frequency distributions of all variables were run to check for missing, invalid and incongruous values. Continuous variable values (age, serum 25(OH)D, S-100β, Breslow, spectrophotometry readings, height, weight, BMI, number of naevi) were checked to ensure they were within a plausible range. I personally undertook all data collection, management, entry and cleaning.

3.7 DATA ANALYSIS PREPARATION

Before commencing analysis many variables required recoding and, or, regrouping.

3.7.1 Questionnaire data

Age was used as a continuous variable and also regrouped as a two-level categorical variable with a cut-point of 60 years to best represent the sample distribution (median age = 62.5 years, mean age = 61.4 years). Information generated from the questions relating to sun exposure after school and on the weekends during primary and high school was aggregated to form two groups of "low" and "high" exposure. Sunscreen use was regrouped from six to three categories by combining: a) never and occasional, b) 1–2 and 3–4 days per week, and c) 5–6 and 7 days per week. Combined with scores from the three anatomic areas for sunscreen use:

- face or head or neck
- hands or forearms, and
- other parts of the body,

two variables were created for sunscreen use.

1. sunscreen use five years ago, and

2. sunscreen use ten years ago.

Sunburn has been previously considered as both a dichotomous and an ordinal categorical variable.[216,217] Whiteman and Green[216] also report that wide variation exists in the reporting of sunburns particularly with respect to life-periods and quantification of exposure. Sunburns were reported as a categorical variable in the three life-periods explored in the questionnaire: primary school, secondary school and after school (adult), quantified according to the range in number of burns reported (see Table 3.1).

While decisions regarding most recoding were based on the logical grouping of the different levels of response, other responses, such as eye colour (personal communication with Dr Guy D'Mellow, FRACS, Ophthalmologist), were regrouped based on professional advice (see Table 3.1). Some categories were also collapsed due to small numbers in specific levels.

Table 3.1
Regrouping of categorical covariates

Covariate	**Likert scale used in questionnaire**	**Recoding**
"How long ago did someone notice a problem with the spot?"	< 3months	< 3 months
	3–6 months	3–12 months
	6–12 months	1–5 years
	1–5 years	
	> 5years	
	Don't remember	Don't remember = missing
Freckle burden as teenager, and	none	none
Naevi burden as teenager	few	few/some
	some	many
	many	
Burning tendency	never	never/rarely burn
	rarely	sometimes burn
	sometimes	always burn
	mostly	
	always	
Tanning tendency	tans deeply	tans deeply/moderately
	tans moderately	tans lightly/ does not tan
	tans slightly	
	does not tan	
"How often were you outdoors in the sun after school / on weekends and holidays in summer?" and "How often did you wear a hat at those times"	never	never, rarely or sometimes
	rarely	usually & always
	sometimes	
	usually	
	always	
	never	
Primary school sunburns,	0–5	0–5 burns
Secondary school sunburns, and	6–10	6–20 burns
Adult sunburns	11–20	21–50+
	21–50	
	> 50	
Sunscreen use as adult	never	never/occasional
1) 5 years ago	occasional	1–4 days a week
2) 10 years ago	1–2 days a week	5–7 days a week
	3–4 days a week	
	5–6 days a week	
	every day	
Hair colour as young teenager	black	black/dark brown
	dark brown	light brown
	auburn/strawberry blonde	auburn/strawberry blonde/blond/red
	blond	
	red	
	light brown	
	other	
Eye colour	blue	blue/grey
	grey	hazel/green
	hazel	brown
	green	
	brown	
Education	primary school	primary/secondary school
	secondary school	trade/technical/college diploma
	trade certificate	university degree
	technical/college diploma	
	university degree	
	other	

Sun exposure variable

A new variable for Reported Adult Sun Exposure was generated from the questionnaire. Participants reported the following information for each year of life from the time they left high school (see page 12 of Appendix E):

- place of work (city/town)
- age of starting and stopping work in that place
- number of days worked in the job
- amount of time spent in the sun in summer on work days and non-work days at each job (1 = mostly indoors, 2 = outdoors for 1–4 hours, 3 = mostly outdoors).

Eighteen years of age was classed as "adult" and any work reported prior to that age was omitted from any calculation. Locations or cities were recoded with their relevant absolute latitude.

Sun exposure work was calculated for workdays at each job as follows:

Sun exposure time in years [Age finished job minus age commenced job]
X
Latitude effect (90 – latitude)/90)
X
Workdays sun exposure time (number of days worked x amount of time outdoors in sun)

Sun exposure non-work was calculated for non-work days at each job as follows:

Sun exposure time in years (Age finished job – age commenced job)
X
Latitude effect (90 – latitude)/90
X
Non-work days sun exposure time (number of days worked x amount of time outdoors in sun)

The resulting *sun exposure work* and *sun exposure non-work* values for each job were then combined to give a *Cumulative adult workday sun exposure* total and a *Cumulative adult non-work day sun exposure* total. *Reported Adult Sun Exposure*

was determined as the total of *Cumulative adult workday sun exposure* and *Cumulative adult non-workday sun exposure* and was analysed as a continuous and categorical variable. As the values of the *Reported Adult Sun Exposure* variable were not normally distributed, the median (308.1) was used as the cut-off for "low" and "high" adult sun exposure.

3.7.2 Clinical examination data

Body Mass Index (BMI) was calculated from weight and height and was grouped for analysis to those < 25 kg/m^2, 25–29.99 kg/m^2, and those ≥ 30 kg/m^2; i.e. normal, overweight or obese. *Naevus burden* was grouped into 0–9 naevi, 10–39 naevi, and ≥ 40 naevi to reflect low, medium and high burden. Scores for *freckling* and *solar lentigo density* of the three sites were regrouped from four groups to three: "nil", "few or some", and "many". Number of *solar keratoses* was treated as a continuous variable.

Spectrophotometry

Spectrophotometry measurements of L* a* and b* were reported from each of 3 sites: the left upper inner arm (an unexposed body site), the left dorsal forearm and the central forehead, as individual continuous measurements. While lightness of skin is reported by L* and measured from 0 (black) to 100 (perfect white), the three measurements are more commonly used in skin pigmentation research according to previously defined combinations of these values to represent hue, chroma and ITA value.[211] The hue angle[211] represents the basic tint of the skin colour ranging from red (0°) to yellow (90°) and was calculated in Stata as h^o = arctangent(b*/a*)*57.3. Chroma[84] represents the saturation, or intensity of that colour tint and was calculated as $C^* = \sqrt{(a^{*2} + b^{*2})}$. Chroma scores are seen to increase as intensity of colour increases.[84] The L* and b* values from the upper inner arm were used to calculate a

variable for "skin-type", the individual topography angle (ITA) value.[211] Using the formula:

ITA = [arctangent((L*-50)/ b*)]*180/3.14159

values ranging from 90° to -90° were generated and used to form six "skin type" groups (see Table 3.2).

Table 3.2 *ITA skin types*

ITA value	Skin type
$> 55^o$ to $\leq 90^o$	Very light/very fair
$> 41^o$ to $\leq 55^o$	Light/fair
$> 28^o$ to $\leq 41^o$	Intermediate/medium
$> 10^o$ to $\leq 28^o$	Tanned/olive
$> -30^o$ to $\leq 10^o$	Brown/dark
$> -90^o$ to $\leq -30^o$	Dark/black

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Only the three lightest ITA skin types were represented by the participants in this study. ITA skin type was included in the analyses as an ordinal categorical variable and hue, chroma and ITA value were included as continuous variables. ITA value was chosen as the representative variable for "skin type" in all modelling (reported in Chapter 5).

3.7.3 Serum 25(OH)D and serum S-100β

Serum 25(OH)D concentration (nmol/L) was reported as a continuous measurement in the raw data file. *A priori* I aimed to examine the markers of melanoma prognosis in relation to 25(OH)D as a continuous measure, to allow examination of the linear association between adverse outcomes and increasing levels of 25(OH)D. I also examined the effect of low vitamin D status: using both the 50 nmol/L cut-point endorsed by the IOM[178] and the lowest quartile of 25(OH)D (less than 45.25 nmol/L)

in this sample. Furthermore, I investigated whether high vitamin D status (here the highest quartile (equal to or greater than 69.8 nmol/L), since only a few participants had 25(OH)D levels of 75 nmol/L or higher) was associated with melanoma prognostic indicators. Both low and high vitamin D status were investigated to identify if there was, in effect a dose-response i.e. an adverse effect of low 25(OH)D concentration or a beneficial effect of high 25(OH)D concentration.

Serum S-100β level was reported and analysed as a continuous measurement. I had also intended to analyse S-100β as a binary variable of less than, or greater than or equal to 0.15 µg/L (range for healthy men and women), however as only one sample (0.24 µg/L) was greater than the cut-off point this was not possible.

3.7.4 Histopathology

Data for the outcome variables were recorded in the raw data files as follows:

- Breslow thickness: continuous (millimetres)
- Clark level: ordinal categorical (Levels 1–5)
- mitotic rate: continuous (number of mitosis per mm^2)
- ulceration: binary (yes/no).

Breslow thickness

Initially it was anticipated that Breslow thickness should be analysed as a continuous variable, but due to the large number of *in situ* tumours (43%), Breslow thickness was recoded as a binary outcome (< 0.75 mm and ≥ 0.75 mm). This reflects the common cut-off depths for prognosis as reported in the literature.[219]

Clark level

A small number (n = 2) of Clark level 5 melanomas were reported. To allow comparison to be made between *in situ* and invasive tumours, Clark level was recoded as a dichotomous categorical variable of Level 1 and Levels 2–5.

Mitosis and ulceration

Mitotic activity was reported in 23% of the melanomas. Mitotic rate, recorded in the raw data files as a continuous variable with zero representing "no mitotic activity", was subsequently recoded as a binary outcome (absent or present). Ulceration was reported in only 5% of the tumours; therefore due to insufficient cell size it was not included as an outcome variable.

Melanoma type

Eleven different melanoma types and subtypes were described in the histopathology reports and recorded in the raw data Excel spreadsheet. Initially grouping was based on the method proposed by Clark et al,[56] of three types ranging from least to most malignant, i.e. lentigo maligna melanoma, superficial spreading melanoma and nodular melanoma. Some lesions did not fit into that grouping, necessitating the formation of a fourth group: "other melanoma". This group included situations where two types of melanoma were reported within a single lesion (e.g. "malignant melanoma of superficial spreading and lentigo maligna types") and others that were simply reported as "malignant melanoma" and other sub types.

Thus the four groups were:

- Lentigo maligna melanoma (LM)
- Superficial spreading melanoma (SSM)
- Nodular melanoma (NM)
- Other melanoma.

If participants had been diagnosed with more than one melanoma simultaneously, only the first melanoma reported in the histopathology for the biopsy specimen was included in the analysis.

Melanoma site

To simplify the raw data relating to the anatomic site of the melanoma, this was aggregated to form four anatomic regions commonly used to describe melanoma locales: head and neck, trunk, upper and lower extremities.[219] Any shoulder lesions were classified as "upper extremity".

Solar elastosis

Although all histopathological analyses were performed in one commercial pathology laboratory, the terminology used in some histopathology reports to comment on the degree of solar elastosis varied slightly from that requested, thus requiring some regrouping. For example; "nil", "no evidence of", and "negligible", were regrouped into "absent". Solar elastosis was subsequently recoded as a dichotomous variable of absent and present.

3.8 DATA ANALYSES

Summary descriptive statistics of frequency, measures of central tendency (mean and median), and dispersion (standard deviation and range) were reported for continuous variables, and number (*n*) and percentages (%) were reported for categorical variables. Frequency histograms were examined in Stata for all continuous variables to check for normality of distribution. Relationships between variables were tested using Chi-squared tests, Fisher's exact test (if there was less than n = 5 in any cells), Kruskal-Wallis and independent t-tests. Multicollinearity checks were undertaken by examining simple pair-wise Pearson (r) correlations between the variables. Cut-off

values for "r" that confirm multicollinearity range from 0.7 [220] to 0.9.[221] "r" $\geq$ 0.9 was used as the cut-off in this study and then further checks were undertaken for collinearity using the Variance Inflation Factor (Stata command "vif") to check that all values were less than 10.[222]

As the sample size was n = 100, percentages were reported to one decimal place according to guidelines adopted from Lang and Secic.[223] All summary statistics (mean, standard deviation, median, range) were reported to one decimal point, with the exception of S-100β (values here were all less than one) that was reported to two decimal points. 95% Confidence Intervals (95% CI), coefficients and *P*-values were reported to two decimal places. The only exception to this rule was when the *P*-value was less than 0.001, in which case it was reported as $P < .001$.

3.9 MODEL AND VARIABLE SELECTION

Focussing on the aim of the study, an epidemiological approach to the analysis was used to examine the independent association between surrogate measures of melanoma prognosis (the outcomes) and serum 25(OH)D concentration at diagnosis, while adjusting for potential confounding factors. A subsidiary analysis explored the predictors of melanoma prognosis (here Breslow thickness, Clark level and presence of mitosis) within these data. Missing values were managed by excluding all the data on those people for the specific analysis using that variable. However, there were very few missing values.

Association between 25(OH)D and melanoma prognosis

I first examined the bivariate association between 25(OH)D concentration and each covariate under consideration using linear regression. Next, using logistic regression

I undertook a bivariate analysis of the association between each outcome variable and all covariates under consideration. In order to control for any potential confounding effects, potential confounders were identified if they were significant at $\alpha < 0.2$ in the bivariate analyses for both the outcome under investigation and 25(OH)D. A confounder is an independent risk factor for the outcome; it has a statistical association with the exposure of interest and it is not on the causal pathway between the exposure of interest and the outcome.[224] The measure of association between an exposure and an outcome can become distorted by the presence of another variable–the confounder.

To adjust for age (in years) and sex, both variables were also identified for inclusion in the multivariable models regardless of their level of significance in the bivariate analyses. Significant covariates were identified in the preliminary multivariable models (of the outcome and 25(OH)D concentration) if they were either a logical confounder or if their inclusion in the model changed the coefficient of the main exposure of interest (vitamin D) by 5% or greater (indicating a confounding effect). These significant covariates were then adjusted for in the final models.

To ensure that the non-linear scale ratio on which odds ratios are measured did not impact on the 5% change rule, the raw coefficient, rather than the odds ratio for vitamin D, was used to gauge if there was a 5% or greater change. However, final results were reported as Adjusted Odds Ratios (AOR).

Best predictors of melanoma prognosis

The aim of this process was to build a predictive model of melanoma prognosis. As in many model-building situations in epidemiological studies the most significant problem faced in analysing these study data was to select, from a very large number

of covariates, those that should be in the model. Covariates were purposefully selected, initially by their "significance" in a bivariate analysis at $\alpha < 0.2$ level. Then using a purposeful selection of covariates (PSC) approach outlined by Hosmer and Lemeshow,[225] a preliminary multivariable analysis was undertaken as each potentially important covariate was introduced one at a time into the model. A synopsis follows.

Step 1: Bivariate analysis

Step 1 began with a bivariate analysis of the association between the outcome and each covariate under consideration using binary logistic regression.

Step 2: Identify covariates for preliminary multivariable model

Covariates with *P*-values of less than 0.2 in the bivariate analyses were identified, and other variables considered likely to be "important" were flagged.

Step 3: Fit preliminary multivariable model

Covariates identified in Step 2 were fitted into a preliminary multivariable model. Variables with *P*-values of 0.2 or greater were then removed from the model.

Step 4: Selection of variables for best fitting preliminary model

Individual covariates were reinserted into the preliminary multivariable model and likelihood ratio tests were used to examine whether included covariates improved the model fit. Covariates were retained if the *P*-value for the likelihood ratio test was less than 0.05.

Step 5: Reintroduction of previously excluded variables

Covariates that were not considered for inclusion in the preliminary multivariable model, but considered "important" were added back into this model (one at a time). Improvement of the model was again tested using likelihood ratio tests.

Step 6: Examine linearity of the continuous covariates included in the preliminary model

The *fracpoly, compare* command in Stata was used to identify the best parametric form.

Step 7: Effect modification

Using a multiplicative term in the model, the preliminary multivariable model was then examined to identify if the magnitude of the effect was different for different groups (for example, males and females).

Step 8: Fit main effects model

To determine the best fit for the main effects model, the likelihood ratio test was used to examine whether the inclusion of identified covariates improved the model.

3.10 ETHICS

Prior to commencement of the research, all of the documentation for the QUT Melanoma Study was submitted, via a National Ethics Application Form (NEAF), to the Human Research Ethics Committee (HREC) of the Queensland University of Technology (EC00171). The study received approval from the HREC, confirming that it met the requirements of the NHMRC National Statement on Ethical Conduct in Human Research (Approval number: 0900000681) (see Appendix J). All participants signed written informed consent prior to participation in the QUT Melanoma Study.

3.11 CONFIDENTIALITY

The de-identified hardcopy data for each participant, i.e. completed questionnaire and clinical examination results, were stored by I.D. number in a sequential filing system that was easily accessible to the researcher. To maintain total confidentiality, the hard copies of the histopathology reports and the originals of the consent forms were stored in a locked filing cabinet in a separate office accessible only by swipe card. Electronic data files were all de-identified.

3.12 SUMMARY

Based on the conclusions of the literature review, a cross sectional exploratory study, designed to identify the relationship between the serum 25(OH)D concentration of patients with newly diagnosed melanoma, and their outcome as predicted by histopathological features of the melanoma that are associated with prognosis, was implemented. The study design overcame the perceived limitations of previous studies and allowed the study objectives to be fulfilled by:

- recruiting patients with both non-invasive and invasive melanomas (Objective 1)
- obtaining blood samples for vitamin D and S-100β analysis prior to wider-excision surgery (Objectives 2 and 4)
- recruiting the sample over a twelve month period (Objective 3)
- obtaining copies of the histopathology reports for both the biopsy and wider excision surgeries (Objective 2)
- administering a comprehensive questionnaire (demographics, sun exposure history, sun protection habits, history of skin cancer) (Objective 2), and
- performing a clinical examination of each participant, including a naevi count and spectrophotometry (Objective 2).

To answer the research question and address the study objectives, data were subsequently analysed in Stata IC Version 11.2 [215] to obtain descriptive statistics, and using the outcome measures of Breslow thickness (≥ 0.75 mm vs. < 0.75 mm), Clark level (Levels 2–5 vs. Level 1) and mitosis (present vs. absent), I examined vitamin D (continuous, lowest quartile, 50 nmol/L, highest quartile) as a risk factor for these outcomes. I further used a purposeful selection of covariate model building process to develop a predictive model for Breslow thickness. The results of the data analyses will be discussed in the following chapters.

Chapter 4: Preliminary results

4.1 INTRODUCTION

Results will be presented in two chapters. As discussed in Chapter 3, risk factor modelling was used to answer the research question: "What is the relationship between serum 25(OH)D concentration at time of diagnosis with melanoma, and histopathological features of melanoma that are associated with prognosis?" A purposeful selection of covariates model building process was used to develop a predictive model for Breslow thickness to elucidate the key predictors.

In this chapter all relevant statistics considered necessary to "set the scene" for the modelling processes are presented. The data presented are not simply descriptive. To gain a more comprehensive understanding of the sample, relationships between variables of interest are also explored, allowing the secondary study objectives of:

- describing seasonal variation in vitamin D status within the sample, and
- identifying the relationship between S-100β level and the histopathological features of the tumours,

to be achieved.

The results of the modelling processes will then be reported in Chapter 5.

Chapter 4 commences by describing, in Section 4.2 the study setting, response rate and general descriptive participant characteristics. Section 4.3 examines data related to the exposures of interest, before melanoma-related data are examined in Section 4.4. Both sections present relevant descriptive data before interrelationships between variables within each section, including age and sex, are explored. Section 4.5

reports on relevant relationships between sun exposure-related variables and melanoma-related variables.

4.2 DESCRIPTIVE STATISTICS – GENERAL

4.2.1 Setting

This study was conducted in Brisbane, Queensland, Australia. Brisbane is geographically situated at Latitude 27.5° South, Longitude 153° East and experiences a mean maximum temperature of 26.4°C (80°F) and mean minimum of 16.2°C (61°F) (Winter: 11°C to 21°C, Summer: 20°C to 28°C, Spring and Autumn: 15°C to 25°C).

4.2.2 Response rate

106 patients with cutaneous melanoma presented to the two surgeons during the study period. Of these, 102 fulfilled the inclusion and exclusion criteria and were approached to take part in the study; 100 patients consented to participate. Due to patient privacy regulations the only data available for the two patients who declined to participate was that they were female. Histopathology from both the biopsy and wider excision surgeries was obtained for all participants, blood samples for serum 25(OH)D and S-100β analyses were collected prior to wider excision for the entire sample, 99% of participants completed the questionnaire and 98% took part in the clinical examination.

4.2.3 Participant characteristics

Study participants had a median age of 62.5 and a mean age of 61.4 years. The youngest patient was 26 years old and the oldest 89 years. Males were marginally over-represented (56%) compared to females (44%). 89% of the sample was born in

Australia and 61% in Queensland. Three quarters (76%) were married or in a de-facto relationship and 67% had completed technical or tertiary education. Four patients (4%) had not attended high school and had moved into the work force at age 14. Ever smoking was reported by 40%, while 6% were current smokers. Average BMI was 27.28 kg/m^2 (SD = 4.68). 65% of participants were either overweight or obese.

4.3 EXPOSURE-RELATED DATA

4.3.1 Descriptive data

Overall, the QUT Melanoma Study sample had "sufficient" serum 25(OH)D levels, reporting a mean level of 58.2 nmol/L (see Table 4.1). Naevi burden and Reported Adult Sun Exposure were not normally distributed (see Table 4.1), and as described in Section 3.7.2 (page 69) naevi burden was subsequently analysed as an ordinal categorical variable and the median, rather than the mean was used as the threshold to categorise Reported Adult Sun Exposure in later analyses.

Table 4.1
Summary descriptive statistics (frequency, mean, standard deviation, median and range) for continuous exposure variables

Exposure (n)	**Mean**	**Std. Dev.**	**Median**	**Min.**	**Max.**
Serum 25(OH)D [nmol/L] (100)	58.2	19.2	58.6	15.8	114.0
Height [centimetres] (97)	171.2	9.2	171.5	150.0	189.0
Weight [kilograms] (97)	80.0	15.8	80.0	53.0	117.0
BMI (97)	27.3	4.7	26.8	19.6	42.5
Naevi burden at diagnosis (98)	34.0	41.0	19.5	0.0	210.0
Inner arm L* (98)	69.2	2.7	69.6	61.2	76.3
Inner arm a* (98)	5.7	1.5	5.7	1.6	10.5
Inner arm b* (98)	15.9	2.4	15.8	10.4	22.1
Forearm L* (98)	56.9	5.6	56.7	43.6	69.8
Forearm a* (98)	9.8	1.9	9.6	4.4	14.5
Forearm b* (98)	18.0	2.5	17.9	7.3	24.0
Forehead L* (98)	61.1	3.4	61.3	52.9	69.1
Forehead a* (98)	14.2	2.9	13.9	6.4	22.4
Forehead b* (98)	16.3	2.0	16.5	11.2	21.2
Inner arm chroma (98)	16.9	2.5	16.8	10.6	24.4
Inner arm hue (98)	70.1	4.4	70.7	58.5	82.6
Forearm chroma (98)	20.6	2.4	20.7	14.4	76.6
Forearm hue (98)	61.4	6.1	61.7	29.7	72.0
Forehead chroma (98)	21.8	2.4	22.0	14.6	70.7
Forehead hue (98)	49.3	7.0	50.0	35.4	35.4
ITA value (98)	50.3	6.8	50.2	28.1	67.5
Reported Adult Sun Exposure (96)#	342.7	186.3	308.1	56.7	1206.0

Refer page 68

Summary descriptive statistics (number and percentage) are provided in Table 4.2 for skin phenotype variables and other categorical variables that have previously been identified as potential risk factors for melanoma. Few participants reported having many freckles (4%) or moles (5.1%) as a teenager and less than one quarter (22.2%) reported teenage hair colour that was blonde, red, auburn or strawberry blonde. The percentage of participants in each sunburn category remained similar across the three time periods (primary school, secondary school and as an adult).

Table 4.2
Summary descriptive statistics for categorical exposure variables

Characteristics (*n*)	**Categories**	***n* (%)**
Freckle density as teenager (99)	Nil	37 (37.4)
	Few/some	58 (58.6)
	Many	4 (4.0)
Naevus density as teenager (98)	Nil	27 (27.5)
	Few/some	66 (67.4)
	Many	5 (5.1)
Hair colour as teenager (99)	Black/ dark brown	48 (48.5)
	Light brown	29 (29.3)
	Blonde, red, auburn/sb*	22 (22.2)
Eye colour (98)	Blue/grey	36 (36.7)
	Green/hazel	48 (49.0)
	Brown	14 (14.3)
Freckle density at diagnosis (98)	Nil	63 (64.3)
	Few/some	21 (21.4)
	Many	14 (14.3)
Primary school sunburns (99)	0–5 burns	40 (40.4)
	6–20 burns	44 (44.4)
	20+ burns	15 (15.2)
Secondary school sunburns (95)	0–5 burns	39 (41.1)
	6–20 burns	44 (46.3)
	20+ burns	12 (12.6)
Adult sunburns (99)	0–5 burns	45 (45.5)
	6–20 burns	39 (39.4)
	20+ burns	15 (15.1)

*strawberry blonde

Summary statistics provided in Table 4.3 show that 70% of the sample had ITA Type 2 skin colour (light/fair skin), yet 73.7% reported that they tan deeply or moderately after spending several weeks at the beach in the sun without any sun protection. Over half (53%) of the participants reported that they mostly or always burn after unprotected exposure to the midday sun for 30 minutes for the first time in summer and almost three-quarters (72.6%) reported high levels of sun exposure during their school years.

Examination of serum 25(OH)D levels in relation to categories of the other variables (see Table 4.3) showed that there was very little difference between males and

females for 25(OH)D level (P = 0.91), older adults had higher 25(OH)D levels (P = 0.37) than the younger adults, while those with the lightest skin type had lower 25(OH)D levels (P = 0.08) than the other skin types.

Table 4.3
Summary descriptive statistics (frequency, mean, median, standard deviation and range) of serum 25(OH) concentration (nmol/L) for categorical variables of interest

			Serum 25-hydroxyvitamin D concentration (nmol/L)			
Characteristics (*n*)	**Categories**	***n* (%)**	**Mean**	***SD***	**Median**	**Range**
Sex (100)	Male	56 (56.0)	58.4	17.6	59.3	21.1–114.0
	Female	44 (44.0)	57.9	21.3	57.5	15.8–104.0
Age group (100)	< 60 years	40 (40.0)	56.1	17.8	55.2	20.9–104.0
	≥ 60 years	60 (60.0)	59.6	20.1	59.7	15.8–114.0
BMI (97)	< 25.0	34 (35.0)	60.0	18.4	61.3	20.9–104.0
	25–29.9	36 (37.0)	59.3	22.1	58.4	15.8 –114.0
	≥ 30	27 (28.0)	53.4	16.3	51.3	24.5–89.6
Burning tendency (99)	Rarely/never	16 (16.2)	55.9	20.8	49.9	15.8–101.0
	Sometimes	30 (30.3)	60.3	21.5	59.9	20.9–114.0
	Mostly/always	53 (53.5)	57.5	17.7	58.6	21.1–104.0
Tanning tendency (99)	Tan deeply/moderately	73 (73.7)	58.8	18.2	58.6	15.8–114.0
	Tan slightly/ does not tan	26 (26.3)	56.2	22.4	58.4	20.9–104.0
ITA skin type (98)	Very light/very fair	20 (21.0)	53.4	18.1	58.4	21.1–85.7
	Light/fair	70 (71.0)	58.0	17.6	57.5	20.9–104.0
	Intermediate/medium	8 (8.0)	67.5	31.8	60.8	15.8–114.0
School-years sun	Low	26 (27.4)	57.4	22.7	57.5	15.8–104.0
exposure (95)	High	69 (72.6)	58.0	18.1	57.8	20.9–114.0
Reported Adult Sun	Low	48(50.0)	57.6	20.0	53.7	15.8–114.0
Exposure (96)	High	48 (50.0)	59.9	19.3	59.2	20.9–104.0

Summary descriptive statistics for serum 25(OH)D concentration at season of diagnosis were also examined and are presented in Figure 4.1. The lowest average seasonal levels of 25(OH)D were reported during winter (52 .0 nmol/L) and the highest in summer (62.3 nmol/L).

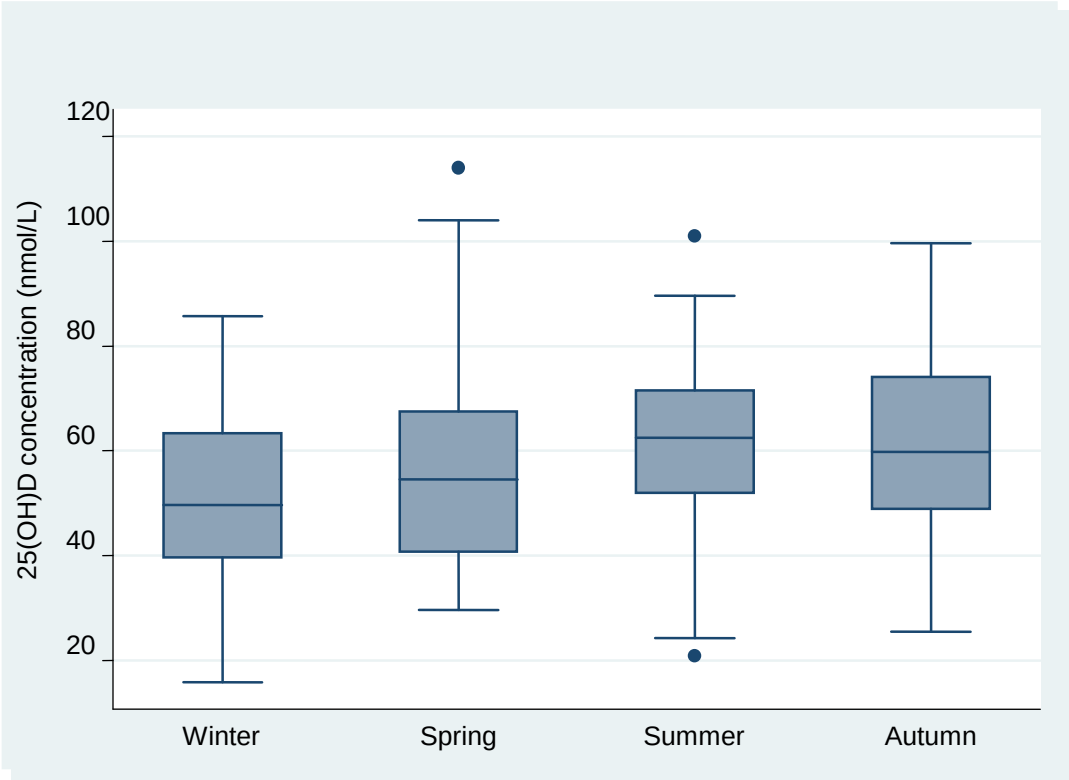


Figure 4.1. Serum 25(OH)D concentration (nmol/L) by season of diagnosis with melanoma

Examination of the descriptive statistics for the exposure variables has provided some indications of the characteristics of the study sample. I now present the results of analyses of relevant inter-relationships between the various exposure variables.

4.3.2 Interrelationships between exposure variables and age and sex

As discussed in Chapter 2 (section 2.5), male sex and increasing age are both risk factors for being diagnosed with melanoma. Males are also reported to have higher mortality rates for melanoma when compared to females. Bivariate relationships between continuous exposure variables and sex and age (≥ 60 years vs. < 60 years) are presented in Table 4.4 (significant associations have been highlighted). Significantly higher naevi burden at diagnosis was seen in males compared to females (P = 0.03). The older age group (60 years or older) were significantly more likely to have fewer naevi (P = 0.03), higher mean levels of Reported Adult Sun

Exposure ($P < 0.001$), lower mean ITA value ($P = 0.03$) and higher chroma (greater intensity of colour) of an unexposed body site ($P < 0.001$), than those aged less than 60 years.

Table 4.4
Relationships (logistic regression) between continuous exposure variables and sex and age (≥ 60 years vs. < 60 years)

	Males	**Females**		**Age (< 60 years)**	**Age (≥ 60 years)**	
Exposure (n)	**Mean (SD)**	**Mean (*SD*)**	***P***	**Mean (*SD*)**	**Mean (*SD*)**	***P***
Naevi burden at diagnosis (98)	42.0 (49.9)	24.2 (22.0)	**0.03**	48.7 (47.4)	24.2 (32.5)	**0.03**
BMI (97)	27.7 (3.5)	26.8 (5.9)	0.37	27.9 (5.9)	26.9 (3.6)	0.28
Inner arm L* (98)	68.9 (2.9)	69.5 (2.3)	0.29	69.2 (2.9)	69.2 (2.5)	0.91
Inner arm a* (98)	5.9 (1.5)	5.5 (1.4)	0.16	5.6 (1.6)	5.8 (1.4)	0.44
Inner arm b* (98)	15.7 (2.5)	16.0 (2.3)	0.53	14.9 (2.6)	16.5 (1.9)	< **0.001**
Forearm L* (98)	53.7 (4.6)	61.0 (3.7)	< **0.001**	58.2 (5.7)	56.0 (5.4)	0.06
Forearm a* (98)	10.2 (2.0)	9.3 (1.7)	0.24	9.6 (2.2)	9.9 (1.7)	0.59
Forearm b* (98)	17.2 (2.4)	19.1 (2.2)	< **0.001**	18.2 (2.3)	17.9 (2.6)	0.49
Forehead L* (98)	60.2 (3.3)	62.1 (3.2)	**0.01**	61.4 (3.0)	60.8 (3.6)	0.43
Forehead a* (98)	14.7 (2.8)	13.5 (2.9)	**0.04**	13.8 (3.0)	14.5 (2.8)	0.27
Forehead b* (98)	16.1 (2.0)	16.6 (2.0)	0.20	16.3 (2.2)	16.4 (1.9)	0.86
Inner arm chroma (98)	16.9 (2.6)	17.0 (2.4)	0.80	16.0 (2.8)	17.6 (2.0)	< **0.001**
Inner arm hue (98)	69.4 (4.6)	71.1 (3.9)	0.06	69.4 (4.5)	70.6 (4.2)	0.20
Forearm chroma (98)	20.1 (2.2)	21.3 (2.4)	**0.01**	20.7 (2.6)	20.5 (2.2)	0.71
Forearm hue (98)	59.3 (6.6)	64.1 (3.9)	< **0.001**	62.3 (5.3)	60.8 (6.5)	0.25
Forehead chroma (98)	22.0 (2.5)	21.6 (2.2)	0.49	21.5 (2.5)	22.0 (2.3)	0.36
Forehead hue (98)	47.8 (6.3)	51.2 (7.3)	**0.02**	50.0 (7.6)	48.8 (6.5)	0.42
ITA value (98)	50.1 (7.5)	50.6 (6.1)	0.74	52.1 (7.8)	49.1 (5.9)	**0.03**
Reported Adult Sun Exposure (96)	356.9 (221.1)	323.7 (221.1)	0.39	243.4 (127.8)	407.8 (190.7)	< **0.001**

Sex was significantly associated with tanning tendency ($\chi^2 = 6.26$, $P = 0.02$), with a higher percentage of males compared to females reporting tanning deeply or moderately after spending several weeks at the beach in the strong sun (see Table

4.5). Sex was also significantly associated with sun exposure during school years (χ^2 = 6.51; *P* = 0.01) and with applying sunscreen five years ago (Fisher's exact = 0.03). Males reported higher sun exposure during their school years and to using sunscreen more infrequently five years ago, than females. Age group (≥ 60 years vs. < 60 years) was significantly associated with solar lentigo burden at time of diagnosis (Fisher's exact = 0.02) and Reported Adult Sun Exposure (χ^2 = 11.0; *P* < 0.001).

Table 4.5
Relationships (χ^2, or Fisher's exact) between categorical exposure variables and sex and age (≥ 60 years vs. <60 years)

	Males	**Females**		**Age (< 60 years)**	**Age (≥ 60 years)**	
Exposure (*n*)	***n* (%)**	***n* (%)**	***P***	***n* (%)**	***n* (%)**	***P***
BMI (97)			0.11			0.66
< 25.0	14 (35.9)	20 (34.5)		14 (35.9)	20 (34.5)	
25–29.9	12 (30.8)	24 (41.4)		12 (30.8)	24 (41.4)	
≥ 30	13 (33.3)	14 (24.1)		13 (33.3)	14 (24.1)	
Season at diagnosis (100)			0.23			0.75
Winter	12 (21.4)	17 (38.6)		14 (35.0)	15 (25.0)	
Spring	17 (30.4)	8 (18.2)		9 (22.5)	16 (26.7)	
Summer	11 (19.6)	9 (20.5)		7 (17.5)	13 (21.6)	
Autumn	16 (28.6)	10 (22.7)		10 (25.0)	16 (26.7)	
Naevi burden at diagnosis (98)			0.52			**< 0.001**
0–9	13 (23.6)	12 (27.9)		6 (15.0)	19 (32.8)	
10–39	25 (45.5)	20 (46.5)		16 (40.0)	29 (50.0)	
40 +	17 (30.9)	11 (25.6)		18 (45.0)	10 (17.2)	
Solar lentigo density at diagnosis (98)			0.42			**0.02**
Nil	6 (10.9)	6 (14.0)		8 (20.0)	4 (6.9)	
Few/some	19 (34.6)	17 (39.5)		18 (45.0)	18 (31.0)	
Many	30 (54.5)	20 (46.5)		14 (35.0)	36 (62.1)	
Burning tendency (99)			0.07			0.27
Rarely/never	12 (21.8)	4 (9.1)		4 (10.0)	12 (20.3)	
Sometimes	19 (34.6)	11 (25.0)		11 (27.5)	19 (32.2)	
Mostly/always	24 (43.6)	29 (65.9)		25 (62.5)	28 (47.5)	
Tanning tendency (99)			**0.01**			0.49
Tan deeply/moderately	46 (83.6)	27 (61.4)		28 (70.0)	45 (76.3)	
Tan slightly/does not tan	9 (16.4)	17 (38.6)		12 (30.0)	14 (23.7)	
ITA skin type (98)			0.47			0.05
Very light/very fair	12 (21.8)	8 (18.6)		13 (32.5)	7 (12.1)	
Light/fair	37 (67.3)	33 (76.7)		24 (60)	46 (79.3)	
Intermediate/medium	6 (10.9)	2 (4.7)		3 (7.5)	5 (8.6)	
School-years sun exposure (95)			**0.01**			0.98
Low	9 (17.0)	17 (40.5)		11 (27.5)	15 (27.3)	
High	44 (83.0)	25 (59.5)		29 (72.5)	40 (72.7)	
Reported Adult Sun Exposure (96)			0.65			**< 0.001**
Low	31 (56.4)	25 (61.0)		30 (79.0)	26 (44.8)	
High	24 (43.6)	16 (39.0)		8 (21.0)	32 (55.2)	
Sunscreen use 5 years ago (99)			**0.03**			0.33
Most of time	1 (1.8)	6 (13.6)		5 (12.5)	2 (3.4)	
Sometimes	15 (27.3)	16 (36.4)		12 (30.0)	19 (32.2)	
Rarely/never	39 (70.9)	22 (50.0)		23 (57.5)	38 (64.4)	
Sunscreen use 10 years ago (99)			0.09			0.67
Most of time	0 (0.0)	3 (6.8)		2 (5.0)	1 (1.7)	
Sometimes	14 (25.5)	14 (31.8)		12 (30.0)	16 (27.1)	
Rarely/never	41 (74.5)	27 (61.4)		26 (65.0)	42 (71.2)	
Solar elastosis (48)			0.58			0.45
Absent	7 (25.9)	7 (33.3)		7 (35.0)	7 (25.0)	
Present	20 (74.1)	14 (66.7)		13 (65.0)	21 (75.0)	

4.3.3 Interrelationships between sun-related variables

Further analyses were conducted to examine whether there was any relationship between levels of self-reported sun exposure at either school age, or as an adult, and the presence of solar elastosis (see Table 4.6). Due to the small sample size of melanomas reported by histology for solar elastosis, 95% Confidence Intervals are also included in this table. No significant associations were identified.

Table 4.6
Associations (Fisher's exact) between reported levels of sun exposure and presence of solar elastosis

	Solar elastosis		
Characteristic (*n*)	**Present *n* (%; 95% CI)**	**Absent *n* (%; 95% CI)**	***P***
School-years sun exposure (46)			0.68
Low	5 (62.5; 48.5, 76.5)	3 (37.5; 23.5, 51.5)	
High	27 (71.1; 58.0, 84.2)	11 (28.9; 15.8, 42.0)	
Reported Adult Sun Exposure (47)			0.26
Low	13 (61.9; 48.0, 75.8)	8 (38.1; 24.2, 52.0)	
High	20 (76.9; 64.9, 88.9)	6 (23.1; 11.1, 35.1)	

Of the self-reported skin phenotype characteristics, only burning tendency (P = 0.03) and tanning tendency (P = 0.04) when unprotected in the summer sun were significantly associated with ITA skin type (see Table 4.7).

Table 4.7
Associations (Fisher's exact) between ITA skin type and skin phenotype characteristics

	ITA skin type			
Characteristic (*n*)	**Very light/very fair** ***n* (%)**	**Light/fair** ***n* (%)**	**Intermediate/ medium** ***n* (%)**	***P***
Burning tendency (98)				**0.03**
Rarely/never	1 (5.0)	10 (14.0)	4 (50.0)	
Sometimes	5 (25.0)	22 (32.0)	3 (38.0)	
Mostly/always	14 (70.0)	38 (54.0)	1 (12.0)	
Tanning tendency (98)				**0.04**
Tans deeply/moderately	11 (55.0)	53 (76.0)	8 (100.0)	
Tans slightly/does not tan	9 (45.0)	17 (24.0)	0 (0.0)	
Freckle density as teenager (98)				0.71
Nil	5 (25.0)	27 (39.0)	4 (50.0)	
Few/some	14 (70)	40 (57.0)	4 (50.0)	
Many	1 (5.0)	3 (4.0)	0 (0.0)	
Hair colour as teenager (98)				0.47
Black/dark brown	8 (40.0)	34 (49.0)	6 (75.0)	
Light brown	6 (30.0)	20 (28.0)	2 (25.0)	
Blond, red, s/b*, auburn	6 (30.0)	16 (23.0)	0 (0.0)	
Eye colour (98)				0.65
Blue/grey	8 (40.0)	24 (34.0)	4 (50.0)	
Green/hazel	10 (50.0)	36 (51.0)	2 (25.0)	
Brown	2 (10.0)	10 (15.0)	2 (25.0)	
Naevi burden at diagnosis (98)				0.92
0–9	6 (30.0)	17 (24.3)	2 (25.0)	
10–39	8 (40.0)	34 (48.6)	3 (37.5)	
40 +	6 (30.0)	19 (27.1)	3 (37.5)	

* strawberry blonde

4.3.4 Interrelationships between exposure variables and serum 25(OH)D

Serum 25(OH)D concentration was initially analysed in bivariate modelling as a continuous variable and is reported as such in the bivariate modelling in Chapter 5. Table 4.8 is provided to examine any relationships between vitamin D status categorised as "sufficient" (according to the threshold of 50 nmol/L) and sun exposure related factors. Season at diagnosis was significantly associated with vitamin D sufficiency ($\chi^2 = 8.56$, $df = 3$, $P = 0.04$), and proportionally more participants diagnosed with melanoma during summer and autumn had sufficient vitamin D status (≥ 50nmol/L) than those diagnosed in the other seasons of the year. No significant associations with other variables emerged.

Table 4.8
Associations (χ^2, or Fisher's exact) between vitamin D status (< 50 nmol/L vs. ≥ 50 nmol/L) and variables of interest

	Vitamin D status		
	< 50 nmol/L	**≥ 50 nmol/L**	
Characteristic (*n*)	***n* (%)**	***n* (%)**	***P***
Sex			0.95
Male (56)	20 (35.7)	36 (64.3)	
Female (44)	16 (36.4)	28 (63.6)	
Age group			0.87
< 60 years (40)	14 (35.0)	26 (65.0)	
≥ 60 years (60)	22 (36.7)	38 (63.3)	
Education			0.48
University degree (44)	18 (40.9)	26 (59.1)	
Diploma/trade cert. (23)	6 (26.1)	17 (73.9)	
Primary/secondary school (32)	12 (37.5)	20 (62.5)	
Season at diagnosis			**0.04**
Winter (29)	15 (51.7)	14 (48.3)	
Spring (25)	11 (44.0)	14 (56.0)	
Summer (20)	3 (15.0)	17 (85.0)	
Autumn (26)	7 (26.9)	19 (73.1)	
Body Mass Index			0.32
< 25.0 (34)	10 (29.4)	24 (70.6)	
25–29.9 (36)	13 (36.1)	23 (63.9)	
≥ 30 (27)	13 (48.2)	14 (51.8)	
ITA skin type			0.58
Intermediate/medium (8)	2 (25.0)	6 (75.0)	
Light/fair (70)	25 (35.7)	45 (64.3)	
Very light/very fair (20)	9 (45.0)	11 (55.0)	
Reported Adult Sun Exposure			0.83
Low (48)	18 (37.5)	30 (62.5)	
High (48)	17 (35.4)	31 (64.6)	
Solar elastosis			0.16
Absent (14)	8 (57.1)	6 (42.9)	
Present (34)	12 (35.3)	22 (64.7)	
Tanning tendency			0.80
Tan deeply/moderately (73)	26 (35.6)	47 (64.4)	
Tan slightly/does not tan (26)	10 (38.5)	16 (61.5)	
Burning tendency			0.40
Rarely/never (16)	8 (50.0)	8 (50.0)	
Sometimes (30)	9(30.0)	21 (70.0)	
Mostly/always (43)	19 (35.9)	34 (64.1)	
Sunscreen use 5 years ago			0.93
Most of time (7)	3 (42.9)	4 (57.1)	
Sometimes (31)	11 (35.5)	20 (64.5)	
Rarely/never (61)	22 (36.1)	39 (63.9)	
Sunscreen use 10 years ago			0.48
Most of time (3)	2 (66.7)	1 (33.3)	
Sometimes (28)	11 (39.3)	17 (60.7)	
Rarely/never (68)	23 (33.8)	45 (66.2)	

4.4 MELANOMA-RELATED DATA

4.4.1 Descriptive data

In this section descriptive data are presented for melanoma and related factors, including anatomic site, histological features, season at diagnosis, history of NMSC, family history of melanoma and serum S-100β.

Superficial spreading melanoma (SSM) was the most common form of melanoma in this study (see Figure 4.2), comprising 54% (*n* = 54) of all cases.

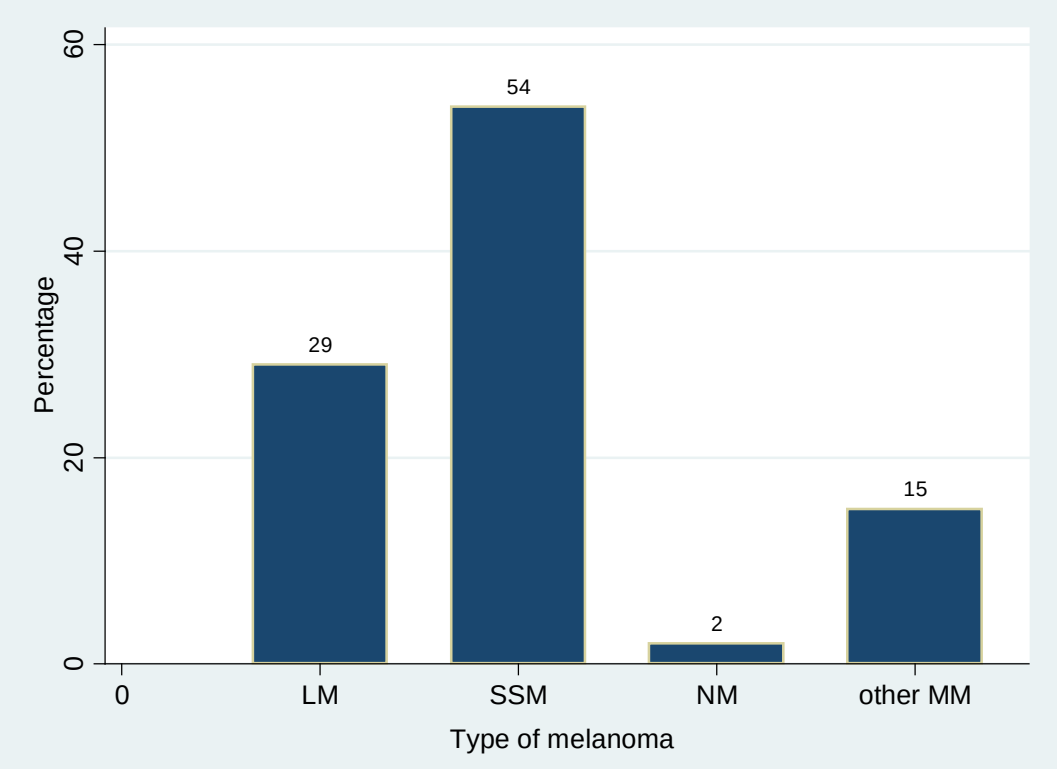


Figure 4.2. Percentage distribution of the different types of melanoma in the QUT Melanoma Study

LM: lentigo maligna melanoma; SSM: superficial spreading melanoma; NM: nodular melanoma; other MM: all other melanomas.

Approximately one third (37%) of all study melanomas were reported on the trunk, while the upper extremities were the least common site (12%) for melanoma (see Figure 4.3).

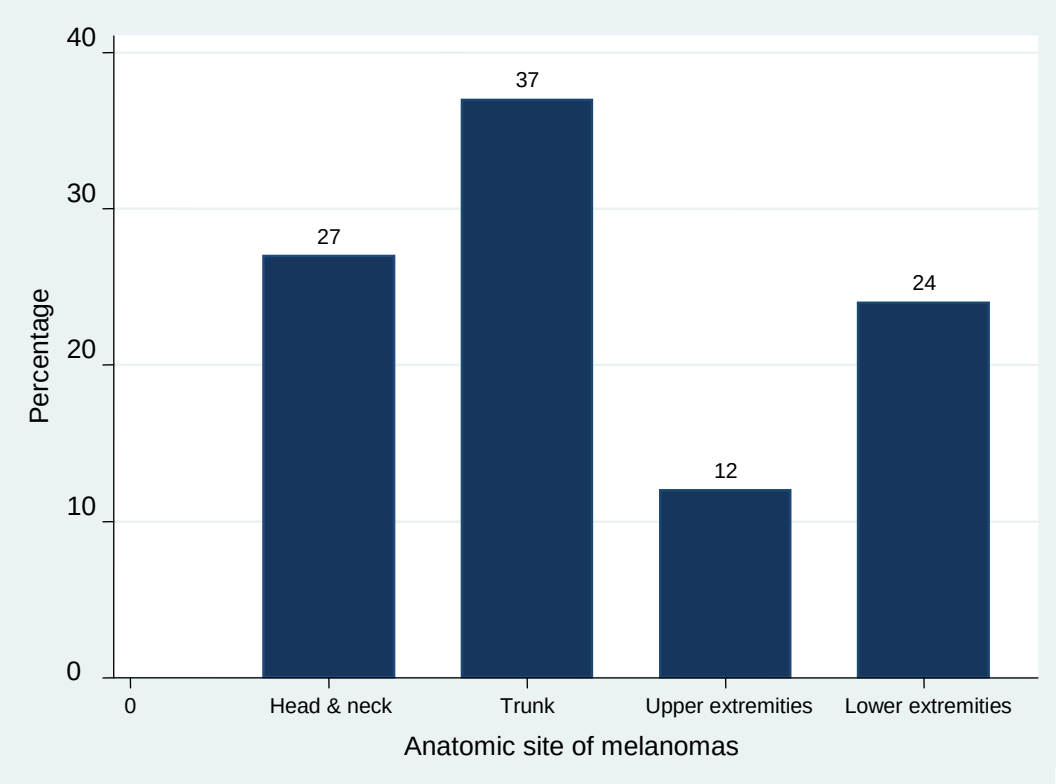


Figure 4.3. Percentage distribution of melanomas by anatomic site

Breslow thickness of melanomas ranged from 0 mm (43%) to 7.8 mm; ulceration was reported in 5% of cases and 4% of patients were diagnosed simultaneously with two melanomas.

On examination at diagnosis, 51.1% of participants had a high (many) solar lentigo density. More than half (56%) of the sample had a previous history of NMSC, one-third (33%) had a family history of melanoma, 57% of melanomas had been observed in the three months prior to excision and almost half (46.4%) of

participants had a doctor check their skin three or more times in the three years before diagnosis (see Table 4.9).

Table 4.9
Summary descriptive statistics for categorical variables related to melanoma

Characteristics (*n*)	**Categories**	***n* (%)**
Breslow thickness (100)	< 0 75 mm	83 (83.0)
	≥ 0.75 mm	17 (17.0)
Clark level (100)	Level 1	45 (45.0)
	Level 2	36 (36.0)
	Level 3	8 (8.0)
	Level 4	9 (9.0)
	Level 5	2 (2.0)
Mitosis	Absent	77 (77.0)
	Present	23 (23.0)
Naevi burden at diagnosis (98)	0–9	25 (25.5)
	10–39	45 (45.9)
	40 +	28 (28.6)
Solar lentigo density at diagnosis (98)	Nil	12 (12.2)
	Few/some	36 (36.7)
	Many	50 (51.1)
Solar elastosis (48)	Absent	14 (29.2)
	Present	34 (70.8)
Sunscreen use 5 years ago (99)	Most of time	7 (7.1)
	Sometimes	31 (31.3)
	Rarely/never	61 (61.6)
Sunscreen use 10 years ago (99)	Most of time	3 (3.0)
	Sometimes	28 (28.3)
	Rarely/never	68 (68.7)
History of NMSC (99)	No	43 (43.4)
	Yes	56 (56.6)
Previous melanoma (98)	No	85 (86.7)
	Yes	13 (13.3)
Family history of melanoma (99)	No	66 (66.7)
	Yes	33 (33.3)
History of any internal cancer (97)	No	83 (85.6)
	Yes	14 (14.4)
Time since lesion was noticed (99)	< 3 months	57 (57.6)
	3–12 months	27 (27.3)
	> 1 year	15 (15.1)
Skin checks by doctor in last 3 years (99)	Never	25 (25.3)
	1 or 2 times	28 (28.3))
	3 or more times	46 (46.4)

4.4.2 Melanoma type in relation to sex, age, site and prognostic indicators

Superficial spreading melanoma (SSM) was the most common type of melanoma diagnosed in both males (44.6%) and females (65.9%). Lentigo maligna melanomas (LM) were more common in males than females (see Figure 4.4), but sex was not significantly associated with melanoma type ($P = 0.15$).

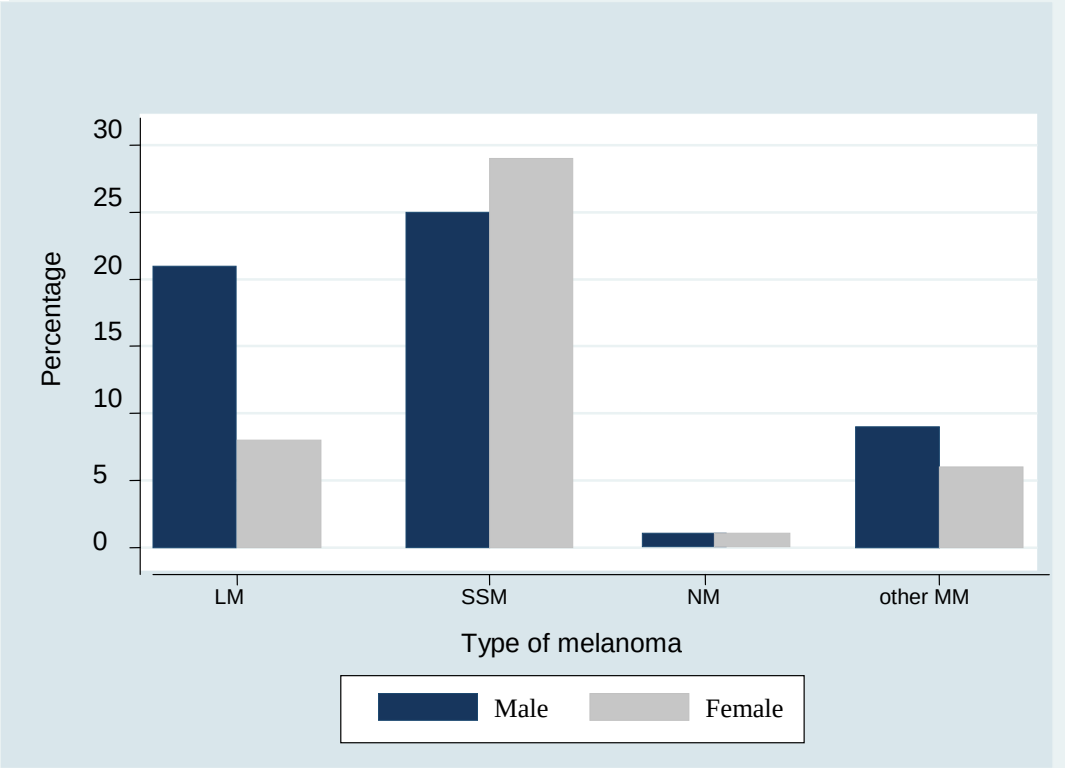


Figure 4.4. Percentage distribution (frequency distribution) of melanoma types according to sex.

Participants with SSM ranged in age from 31 years to 88 years. The lowest mean age for any melanoma type was 54 years in patients with nodular melanoma, although here the sample size ($n = 2$) was very small (see Table 4.10). There was little variation in the mean age of males and females according to melanoma type. Melanoma type was not statistically associated with age ($F(3) = 0.38$, $P = 0.76$).

Table 4.10
Mean age (SD) and sex of patients according to melanoma type

Melanoma type (*n* = 100)	**Frequency (%)**	**Mean age (*SD*)**	**Mean age (males) (*SD*)**	**Mean age (females) (*SD*)**
Lentigo maligna	29 (29.0)	62.9 (10.0)	64.1 (9.4)	59.5 (11.2)
Superficial spreading	54 (54.0)	60.9 (13.4)	61.8 (13.8)	60.1 (13.2)
Nodular	2 (2.0)	54.0 (1.4)	55.0 (-)	53.0 (-)
Others	15 (15.0)	61.5 (15.1)	60.2 (16.2)	63.5 (14.4)

Statistically significant associations were noted between melanoma type and the outcome measures of Breslow thickness (χ^2 =18.63, *df* = 3, *P* < 0.001), Clark level (χ^2 = 16.93, *df* = 3, *P* < 0.001) and mitosis (χ^2 = 10.74, *df* = 3, *P* = 0.01) (see Table 4.11). All lentigo maligna melanomas had a Breslow thickness less than 0.75 mm and both nodular melanomas had a Breslow thickness equal to or greater than 0.75 mm (see Table 4.11).

Table 4.11
Associations (χ^2 or Fisher's exact) between melanoma type and histological characteristics of the melanomas

	Melanoma types				
	LMM	**SSM**	**NM**	**Other**	
Characteristics	***n* (%)**	***n* (%)**	***n*(%)**	***n* (%)**	***P***
Breslow thickness					< 0.001
< 0.75 mm	29 (34.9)	44 (53.1)	0 (0.0)	10 (12.1)	
≥ 0.75 mm	0 (0.0)	10 (58.8)	2 (11.8)	5 (29.4)	
Clark level					< 0.001
Level 1	22 (48.9)	19 (42.2)	0 (0.0)	4 (8.9)	
Level 2–5	7 (12.7)	35 (63.7)	2 (3.6)	11(20.0)	
Mitosis					0.01
Absent	25 (32.5)	43 (55.8)	0 (0.0)	9 (11.7)	
Present	4 (17.4)	11 (47.8)	2 (8.7)	6 (26.1)	

4.4.3 Anatomic site of melanomas in relation to sex, age, melanoma type and prognostic indicators

In males, melanomas were reported more often on the head and neck (34%) and trunk (41%); in females the trunk (32%) and legs (32%) were the most common sites. Figure 4.5 displays the percentage distribution of the anatomic sites of the melanomas according to sex and shows that a higher percentage of the melanomas of the head and neck, and trunk, were found in males, while the females reported a higher percentage of melanomas on the arms and legs. In the QUT Melanoma Study, the anatomic site of the melanomas was significantly associated with sex ($\chi^2 = 6.18$, $df = 1$, $P = 0.01$).

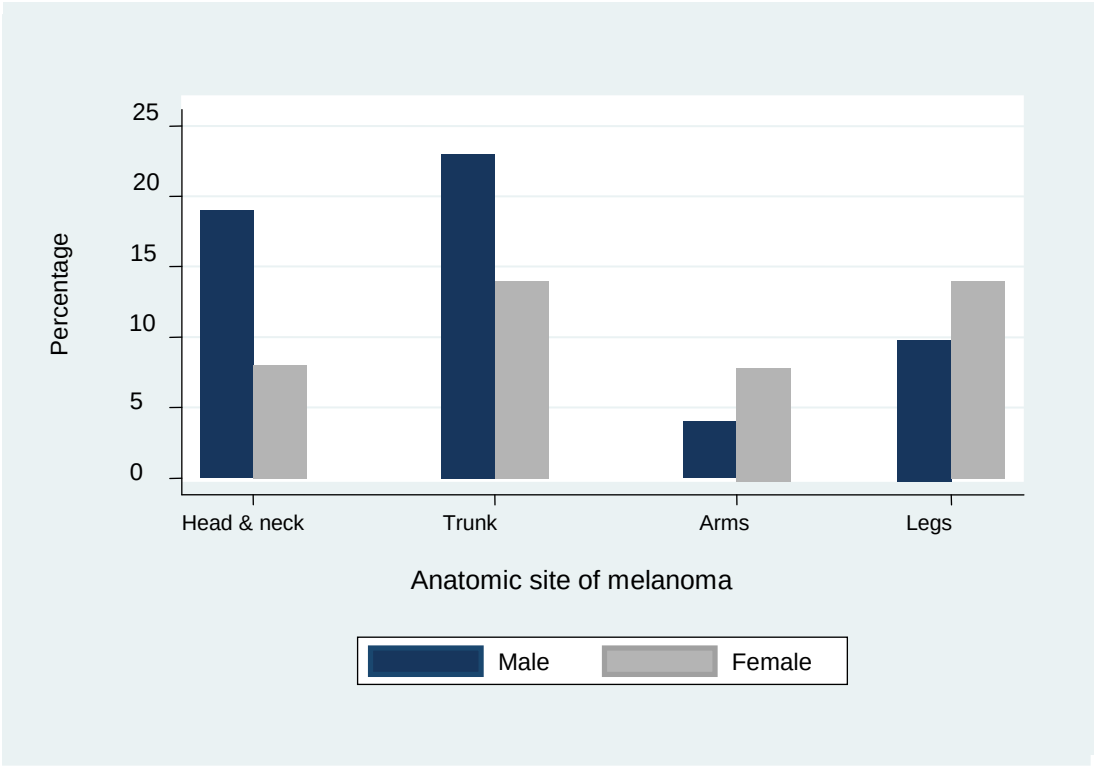


Figure 4.5. Percentage distribution (frequency distribution) of the anatomic sites of melanomas according to sex.

Melanomas on the upper extremities were reported at the lowest mean age (55.8 years) and melanomas on the trunk at the highest mean age (65.9 years) (see Table 4.12).

Table 4.12
Mean age (SD) and sex of participants according to anatomic site of melanoma

		Mean age (*SD*)		
Anatomic site	**Frequency (%)**	**Sample**	**Males**	**Females**
Head and neck	27 (27.0)	58.7 (14.3)	57.0 (15.6)	62.6 (10.4)
Trunk	37 (37.0)	65.9 (10.1)	67.5 (9.5)	63.1 (10.9)
Upper extremities	12 (12.0)	55.8 (8.4)	57.5 (5.2)	55.0 (9.8)
Lower extremities	24 (24.0)	60.5 (14.1)	62.2 (10.1)	59.2 (16.6)

Melanoma anatomic site was also significantly associated with age group (≥ 60 years vs. < 60 years) ($\chi^2 = 8.07$, $df = 3$, $P = 0.04$) and 75.7% of melanomas located on the trunk were reported in the older age group (≥ 60 years).

Both nodular melanomas were found on the upper extremities; 62% of all head and neck melanomas were lentigo maligna melanomas, and 88% of melanomas on the lower extremities were of the superficial spreading type (see Figure 4.6). There was a significant association between anatomic site and type of melanoma ($\chi^2 = 44.05$, df = 9, $P < 0.001$).

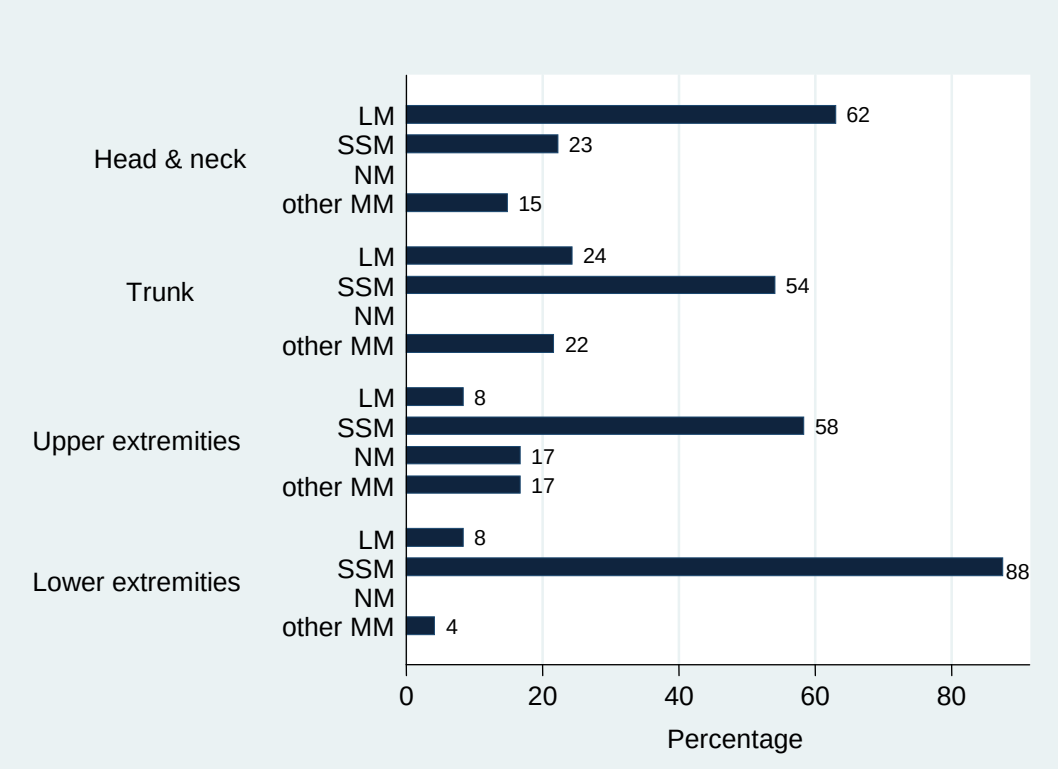


Figure 4.6. Percentage distribution of each melanoma type according to anatomic site.

The anatomic site of the melanomas was not significantly associated with the outcome measures of Breslow thickness, Clark level or mitosis (see Table 4.13).

Table 4.13
Associations (χ^2, or Fisher's exact) between anatomic site and histological characteristics of the melanomas

	Anatomic site				
	Head & neck	**Trunk**	**Upper extremities**	**Lower extremities**	
Characteristics	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***P***
Breslow					0.69
< 0.75 mm	24 (28.9)	31 (37.4)	9 (10.8)	19 (22.9)	
≥ 0.75 mm	3 (17.6)	6 (35.3)	3 (17.7)	5 (29.4)	
Clark level					0.39
Level 1	14 (31.1)	19 (42.2)	4 (8.9)	8 (17.8)	
Level 2–5	13 (23.6)	18 (32.7)	8 (14.6)	16 (29.1)	
Mitosis					0.38
Absent	19 (24.7)	32 (41.5)	9 (11.7)	17 (22.1)	
Present	8 (34.8)	5 (21.8)	3 (13.0)	7 (30.4)	

4.4.4 Interrelationships and associations between S-100β and sex, age and histological characteristics of the study melanomas

The fourth objective of the QUT Melanoma study was to identify any relationship between S-100β level and histological features of the melanomas. Mean and median serum S-100β levels were 0.05 µg/L (*SD* = 0.04; range < 0.02 to 0.24). Summary descriptive statistics for serum S-100β levels and variables of interest are displayed in Table 4.14. Crude associations analysed using the General Linear Model, are also displayed. Melanoma type, mitosis (present vs. absent) and age group (≥ 60 years vs. < 60 years) were noted to be significantly associated with S-100β level.

Table 4.14
Summary descriptive statistics (frequency, mean, median, standard deviation and range) of serum S-100β (µg/L) and associations (General Linear Model) for variables of interest

		Serum S-100β concentration (µg/L)				
Characteristic	***n* (%)**	**Mean**	***SD*.**	**Median**	**Range**	***P***
Sex						0.09
Male	56 (56.0)	0.05	0.02	0.05	< 0.02 – 0.10	
Female	44 (44.0)	0.06	0.05	0.05	< 0.02 – 0.24	
Age group						0.05
< 60 years	40 (40.0)	0.04	0.03	0.04	< 0.02 – 0.14	
≥ 60 years	60 (60.0)	0.06	0.04	0.05	< 0.02 – 0.24	
Anatomic site						0.22
Head & neck	27 (27.0)	0.05	0.03	0.05	< 0.02 – 0.10	
Trunk	37 (37.0)	0.05	0.02	0.05	< 0.02 – 0.09	
Upper extremities	12 (12.0)	0.06	0.04	0.06	< 0.02 – 0.14	
Lower extremities	24 (24.0)	0.06	0.05	0.05	< 0.02 – 0.24	
Melanoma type						0.05
LM	29 (29.0)	0.05	0.03	0.05	< 0.02 – 0.10	
SSM	54 (54.0)	0.05	0.05	0.05	< 0.02 – 0.14	
NM	2 (2.0)	0.06	0.01	0.06	0.05 – 0.06	
Other melanomas	15 (15.0)	0.07	0.05	0.06	< 0.02 – 0.24	
Breslow thickness						0.17
< 0.75 mm	83 (83.0)	0.05	0.03	0.05	< 0.02 – 0.14	
≥ 0.75 mm	17 (17.0)	0.06	0.05	0.05	< 0.02 – 0.24	
Clark level						0.06
Level 1	45 (45.0)	0.04	0.02	0.04	< 0.02 – 0.10	
Level 2–5	55 (55.0)	0.06	0.04	0.06	< 0.02 – 0.24	
Mitosis						0.05
Absent	77 (77.0)	0.03	0.03	0.05	< 0.02 – 0.14	
Present	23 (23.0)	0.06	0.04	0.06	< 0.02 – 0.24	

A higher than normal level of S-100β (≥ 15 µg/L) was found in one patient (1%), a female aged 89 years who presented with a Level 4, 3.98 mm thick, ulcerated lesion (described as a "malignant melanoma") on the lower leg. The histopathologist reported the presence of seven mitotic figures per square millimetre. Her serum 25(OH)D level was 85.7 nmol/l.

4.5 RELATIONSHIPS BETWEEN VARIABLES FROM SECTIONS 4.3 AND 4.4

In this section, I present initial data for relationships between variables from the sun exposure related data and the melanoma related data. Table 4.15 presents descriptive statistics and crude associations for serum 25(OH)D concentration, melanoma site and histological characteristics. A non-significant association between higher mean serum 25(OH)D concentration and Breslow thickness less than 0.75 mm is noted.

Table 4.15
Summary descriptive statistics (frequency, mean, median, standard deviation and range) of serum 25(OH)D (nmol/L) and associations (General Linear Model) with histological characteristics of the melanomas

		25-Hydroxyvitamin D concentration (nmol/L)				
Characteristic (*n*)	***n* (%)**	**Mean**	***SD*.**	**Median**	**Range**	***P***
Anatomic site (100)						0.87
Head & neck	27 (27.0)	56.8	16.6	58.6	21.1–99.6	
Trunk	37 (37.0)	59.1	17.1	58.9	37.9–114.0	
Upper limbs	12 (12.0)	64.3	23.5	61.6	24.3–104.0	
Lower limbs	24 (24.0)	55.1	22.9	54.9	15.8–101.0	
Melanoma type (100)						0.55
LM	29 (29.0)	59.9	17.7	59.8	21.1–99.6	
SSM	54 (54.0)	57.5	20.6	58.6	15.8–114.0	
Nodular	2 (2.0)	73.3	43.5	73.3	42.5–104.0	
Other melanomas	15 (15.0)	55.2	14.3	52.7	32.8–85.7	
Breslow thickness (100)						0.08
< 0.75 mm	83 (83.0)	59.7	18.0	59.0	15.8–114.0	
≥ 0.75 mm	17 (17.0)	50.7	23.4	42.5	24.3–104.0	
Clark level (100)						0.22
Level 1	45 (45.0)	60.8	20.7	59.6	15.8–114.0	
Level 2–5	55 (55.0)	56.0	17.9	53.3	24.3–104.0	
Mitosis (100)						0.47
Absent	77 (77.0)	58.9	18.8	58.6	15.8–114.0	
Present	23 (23.0)	55.6	20.9	57.8	24.5–104.0	

Interrelationships between solar elastosis and anatomic site

After noting that there was no association between the absence or presence of solar elastosis and levels of previous sun exposure (Table 4.6, page 91), I investigated whether there was any evidence of a relationship between solar elastosis and the anatomic site of the melanoma. While no significant associations were noted, all melanomas (n = 7) situated on the upper extremities (and histologically examined for solar elastosis) showed evidence of solar elastosis (see Table 4.16).

Table 4.16
Presence of solar elastosis and association (Fisher's exact) with the anatomic site of melanoma

	Solar elastosis		
Anatomic site (n =48)	**Absent** ***n* (%; 95% CI)**	**Present** ***n* (%; 95% CI)**	***P***
Head & neck	2 (25.0; 12.8, 37.3)	6 (75.0; 62.8, 87.3)	0.11
Trunk	9 (47.4; 33.3, 61.5)	10 (52.6; 38.5, 66.7)	
Upper extremities	0 (0.0; 0.0, 0.0)	7 (100.0; 1.0, 1.0)	
Lower extremities	3 (21.4; 9.0, 33.0)	11 (78.6; 67.0, 90.2)	

Interrelationships between ITA skin type and prognostic outcome measures for melanoma

Although ITA value will be examined at all bivariate stages of the analysis in Chapter 5, the details of the relationship between ITA skin type and the three outcome measures are presented in Table 4.17. Of particular interest is that no melanomas with Breslow thickness greater than or equal to 0.75 mm were reported in participants with an intermediate skin type. Breslow thickness (P = 0.03) and mitosis (P = 0.02) were both significantly associated with ITA skin type.

Table 4.17
ITA skin type and associations (Fisher's exact) with the three outcome measures for melanoma prognosis

Outcome (n = 100)	ITA skin type: Intermediate n (%)	Light/fair n (%)	Very light/very fair n (%)	P
Breslow thickness				0.03
< 0.75 mm	8 (100.0)	61 (87.1)	13 (65.0)	
≥ 0.75 mm	0 (0.0)	9 (12.9)	7 (35.0)	
Clark level				0.24
Level 1	5 (62.5)	33 (47.1)	6 (30.0)	
Level 2–5	3 (37.5)	37 (52.9)	14 (70.0)	
Mitosis				0.02
Absent	8 (100.0	57 (81.4)	11 (55.0)	
Present	0 (0.0)	13 (18.6)	9 (45.0)	

4.6 SUMMARY

Chapter 4 has presented descriptive statistics for the study population, main exposures of interest, the melanomas and has also explored relationships between variables of interest. The following points provide a brief summation of the findings:

- mean age of participants: 61.4 years
- mean 25(OH)D concentration: 58.2 nmol/L
- mean BMI: 27.34 kg/m^2
- most common type of melanoma: superficial spreading melanoma (SSM)

In addition, the third and fourth study objectives have been achieved:

- Season at diagnosis was found to be significantly associated (P = 0.04) with serum 25(OH)D concentration
- S-100β level was significantly associated with melanoma type and mitosis.

Chapter 5 will now address the research question and examine the predictors of Breslow thickness.

Chapter 5: Results of Model Building

5.1 INTRODUCTION

Chapter 5 presents the risk factor model building process designed to answer the research question: “What is the relationship between serum 25(OH)D level at time of diagnosis with melanoma, and histopathological features of melanoma that are associated with prognosis?” An analysis of the key predictors of Breslow thickness is also provided in this chapter.

Relationship between serum 25(OH)D level and melanoma prognosis

The process, as outlined in Chapter 3, involved building models for each of the outcome variables of Breslow thickness, Clark level and mitosis in relation to measures of vitamin D. As discussed in section 3.9, covariates satisfying the conditions of a confounder were identified for inclusion in the preliminary multivariable models. Age (in years) and sex were retained in all models. 25(OH)D was first modelled as a continuous variable, and then according to cut-points for low vitamin D status and high vitamin D status (as described in section 3.7.3). If the addition of any covariate to the preliminary multivariable models (outcome and vitamin D) resulted in a change to the vitamin D coefficient of 5% or more, this was regarded as evidence of confounding, and that covariate was then retained in the multivariable model with age (in years) and sex. In order to provide results that were more clinically relevant, all subsequent analyses report serum 25(OH)D per 10 nmol/L, rather than by one nmol/L. Preliminary analyses are presented in terms of beta coefficients; final results of the modelling are presented as adjusted odds ratios (AOR).

The chapter begins by examining bivariate associations between 25(OH)D concentration and each covariate. The results for the risk factor modelling to examine the association between serum 25(OH)D and each prognostic outcome commences with the Breslow thickness model, before presenting the results for Clark level and mitosis. Results from the risk factor modelling for categories of vitamin D status are also presented (at the conclusion of each 25(OH)D concentration examination). The chapter concludes by presenting a predictive model for Breslow thickness.

5.2 BIVARIATE ANALYSIS OF SERUM 25(OH)D CONCENTRATION AND COVARIATES

The bivariate associations between the main outcome of interest, serum 25 (OH)D concentration (continuous variable) and each covariate were examined using the General Linear Model (see Table 5.1). Coefficients represent the mean difference in 25(OH)D concentration per unit increase in the covariate from the referent. Nine spectrophotometric skin pigmentation variables (including ITA value), BMI, adult sunburns, solar elastosis, when the spot was noticed and taking supplements were all associated with serum 25(OH)D concentration at $\alpha < 0.2$ level. As discussed in Section 3.7.2 (page 70), ITA value was chosen as the representative covariate for "skin type" in all modelling.

Table 5.1
Bivariate linear regression for serum 25(OH)D concentration and all covariates

Variable	Coefficient	95% *CI*	*P*
Age	0.17	-.14, 0.47	0.28
Sex			
Male		referent	
Female	-.43	-8.16, 7.29	0.91
Melanoma type			
LMM		referent	
SSM	-2.39	-11.22, 6.44	0.59
NM	13.34	-14.72, 41.40	0.35
Other	-4.76	-16.96, 7.45	0.44
Anatomic site			
Head & neck		referent	
Trunk	2.29	-7.42, 12.0	0.64
Upper extremities	7.43	-5.88, 20.74	0.27
Lower extremities	-1.68	-12.44, 9.08	0.76
Season at diagnosis			
Winter		referent	
Spring	6.40	-3.93, 16.69	0.22
Summer	10.28	-.70, 21.27	0.07
Autumn	9.82	-.39, 20.02	0.06
S-100β	65.97	-42.25, 174.20	0.23
ITA value	-.66	-1.22, -.11	0.02
Inner arm L*	-1.33	-2.77, 0.10	0.07
Inner arm a*	2.83	0.22, 5.44	0.03
Inner arm b*	1.72	0.11, 3.32	0.04
Forearm L*	-.94	-1.61, -.27	<0.001
Forearm a*	3.25	1.33, 5.16	<0.001
Forearm b*	0.49	-1.08, 2.05	0.54
Forehead L*	-.10	-1.25, 1.06	0.87
Forehead a*	0.52	-.82, 1.86	0.44
Forehead b*	-.06	-2.01, 1.89	0.95
Inner arm chroma	1.80	0.28, 3.32	0.02
Inner arm hue	-.42	-1.31, 0.47	0.35
Forearm chroma	1.47	-.15, 3.09	0.07
Forearm hue	-.69	-1.33, -.07	0.03
Forehead chroma	0.54	-1.11, 2.20	0.52
Forehead hue	-.18	-.74, 0.38	0.54
BMI	-.72	-1.55, 0.11	0.09
Naevi burden at diagnosis	-.04	-.13, 0.06	0.45
Freckle density at diagnosis			
Low		referent	
Medium	6.67	-2.97, 16.30	0.17
High	1.95	-9.34, 13.24	0.73
Solar lentigo density at diagnosis			
Low		referent	
Medium	4.69	-8.13, 17.51	0.47
High	1.94	-10.42, 14.31	0.76
Eye colour			
Brown		referent	
Green/hazel	2.98	-8.67, 14.62	0.61
Blue/grey	6.32	-5.76, 18.40	0.30
Burning tendency			
Rarely/never burns		referent	
Sometimes burns	4.39	-7.56, 16.33	0.47
Mostly/always burns	1.56	-9.44, 12.57	0.78
Tanning tendency			
Tans deeply/moderately		referent	

Variable	Coefficient	95% *CI*	*P*
Tans slightly/does not tan	-2.55	-11.33, 6.23	0.57
Hair colour as teenager			
Black/dark brown		referent	
Light brown	-3.73	-12.79, 5.32	0.42
Blonde, red, auburn, s/b*, blonde	1.77	-8.14, 11.68	0.72
Naevus density as teenager			
Nil		referent	
Few/some	1.13	-7.70, 9.97	0.80
Many	-7.91	-26.75, 10.92	0.41
Freckle density as teenager			
Nil		referent	
Few/some	0.54	-7.60, 8.67	0.90
Many	-4.46	-24.81, 15.90	0.67
School-years sun exposure			
Low		referent	
High	0.57	-8.32, 9.46	0.90
Reported Adult Sun Exposure (continuous)	53.16	44.88, 61.45	0.19
Primary school sunburns			
0–5 burns		referent	
6–20 burns	0.66	-7.72, 9.04	0.88
> 20 burns	-6.88	-18.49, 4.74	0.24
Secondary school sunburns			
0–5 burns		referent	
6–20 burns	-1.21	-9.69, 7.27	0.78
> 20 burns	-7.72	-20.45, 5.00	0.23
Adult sunburns			
0–5 burns		referent	
6–20 burns	-5.54	-13.88, 2.81	0.19
> 20 burns	-8.42	-19.79, 2.95	0.15
Solar elastosis			
Absent		referent	
Present	9.63	-3.69, 22.94	0.15
Sunscreen use 10 years ago			
Wore sunscreen most of time		referent	
Wore sunscreen sometimes	12.96	-10.35, 36.27	0.27
Rarely/never wore sunscreen	8.63	-14.00, 31.26	0.45
Sunscreen use 5 years			
Wore sunscreen most of time		referent	
Wore sunscreen sometimes	0.97	-15.13, 17.06	0.91
Rarely/never wore sunscreen	-3.66	-19.00, 11.69	0.64
When was spot noticed			
Less than 3 months		referent	
3–12 months	1.68	-7.17, 10.53	0.70
> 12months	-10.25	-21.25, 0.74	0.07
Family history of melanoma			
No		referent	
Yes	3.43	-4.75, 11.62	0.41
History of previous melanoma			
No		referent	
Yes	-1.75	-13.18, 9.68	0.76
History of other internal cancer			
No		referent	
Yes	-3.24	-13.86, 7.39	0.55
Take supplements			
No		referent	
Yes	-8.92	-18.38, 0.53	0.06
Ever smoked			
No		referent	

Variable	Coefficient	95% *CI*	*P*
Yes	1.95	-5.93, 9.83	0.63
Education			
Primary/Secondary school		referent	
Trade/ Technical certificate	6.46	-3.41, 16.32	0.20
Tertiary education	4.01	-4.90, 12.92	0.37

*strawberry blond

5.3 BRESLOW THICKNESS MODEL

5.3.1 Step 1: Bivariate association between Breslow thickness and vitamin D

Breslow thickness was not normally distributed due to the large number (43%) of *in situ* melanomas and was modelled as a categorical variable reflecting "thin" tumours (< 0.75 mm) and "thick" tumours (≥ 0.75 mm). A Breslow thickness of less than 0.75 mm is the referent category in all analyses. Results are reported as beta-coefficients, 95% confidence intervals and *P*-values based on a logit model. Higher 25(OH)D concentration was inversely associated with Breslow thickness (beta = -.03, 95% CI = -.06, 0.00, $P = 0.08$) .

5.3.2 Step 2: Examination of each covariate in relation to the outcome, Breslow thickness

Bivariate associations between Breslow thickness (≥ 0.75 mm vs. < 0.75 mm) and all covariates are presented in Table 5.2. Covariates identified as significantly associated with Breslow thickness at $\alpha < 0.2$ level were S-100β, ITA value, inner arm chroma and BMI.

Table 5.2
Bivariate logistic regression analysis for Breslow thickness (≥ 0.75 mm vs. < 0.75 mm) in relation to all covariates

Variable	Coefficient	95% *CI*	*P*
Age	-.01	-.05, 0.03	0.62
Sex			
Male		referent	
Female	0.43	-.61, 1.48	0.41
Melanoma type			0.24 (Fisher's)
Anatomic site			
Head & neck		referent	
Trunk	0.48	-1.04, 1.92	0.56
Upper extremities	0.98	-.79, 2.75	0.28
Lower extremities	0.74	-.81, 2.30	0.35
Season of diagnosis			
Winter		referent	
Spring	0.68	-.72, 2.08	0.34
Summer	0.10	-1.60, 1.66	0.91
Autumn	0.13	-1.71, 1.52	0.87
S-100β	9.39	-4.00, 22.78	0.17
ITA value	0.11	0.02, 0.21	0.02
Inner arm L*	0.14	-.08, 0.36	0.23
Inner arm chroma	-.35	-.60, -.10	< 0.001
BMI	0.12	0.00, 0.23	0.05
Naevi burden at diagnosis	-.00	-.02, 0.01	0.72
Freckle density at diagnosis			
Low		referent	
Medium	-.12	-1.52, 1.27	0.86
High	0.37	-1.08, 1.81	0.62
Solar lentigo density at diagnosis			
Low		referent	
Medium	-1.13	-2.66, 0.40	0.15
High	-1.12	-2.56, 0.32	0.13
Eye colour			
Brown		referent	
Green/hazel	-.17	-1.64, 1.30	0.82
Blue/grey	-.78	-2.43, 0.87	0.35
Burning tendency			
Rarely/never burns		referent	
Sometimes burns	-.41	-2.04, 1.23	0.63
Mostly/always burns	-.12	-1.57, 1.33	0.87
Tanning tendency			
Tans deeply/moderately		referent	
Tans slightly/does not tan	0.64	-.49, 1.77	0.27
Hair colour as teenager			
Black/dark brown		referent	
Light brown	0.98	-.20, 2.16	0.10
Blonde, red, auburn, s/b*, blonde	-.36	-2.04, 1.33	0.68
Naevus density as teenager			
Nil		referent	
Few/some	0.14	-1.10, 1.38	0.83
Many	0.36	-2.07, 2.80	0.77
Freckle density as teenager			
Nil		referent	
Few/some	-.70	-1.81, 0.41	0.22
Many	0.19	-2.20, 2.58	0.88

Variable	Coefficient	95% *CI*	*P*
School-years sun exposure			
Low		referent	
High	-.04	-1.20, 1.29	0.95
Reported Adult Sun Exposure (continuous)	0.00	-.00, .00	0.51
Primary school sunburns			
0–5 burns		referent	
6–20 burns	0.07	-1.11, 1.26	0.91
> 20 burns	0.35	-1.19, 1.88	0.66
High school sunburns			
0–5 burns		referent	
6 – 20 burns	0.20	-.96, 1.36	0.73
> 20 burns	-.69	-2.92, 1.53	0.54
Adult sunburns			
0–5 burns		referent	
6–20 burns	-.64	-1.92, 0.65	0.33
> 20 burns	0.52	-.86, 1.90	0.46
Solar elastosis			
Absent		referent	
Present	0.25	-1.49, 1.99	0.77
Sunscreen use 10 years ago			0.64 (Fisher's)
Sunscreen use 5 years			
Wore sunscreen most of time		referent	
Wore sunscreen sometimes	-.44	-2.87, 1.99	0.72
Rarely/never wore sunscreen	0.38	-1.82, 2.60	0.73
When was spot noticed			
Less than 3 months		referent	
3–12 months	0.07	-1.12, 1.25	0.91
> 12months	-1.09	-3.23, 1.05	0.32
Family history of melanoma			
No		referent	
Yes	-.47	-1.70, 0.74	0.44
History of previous melanoma			0.12(Fisher's)
History of other internal cancer			
No		referent	
Yes	0.38	-1.02, 1.79	0.59
Take supplements			
No		referent	
Yes	-.71	-1.90, 0.49	0.25
Ever smoked			
No		referent	
Yes	-.15	-1.25, 0.96	0.80
Education			
Primary/Secondary school		referent	
Trade/ Technical certificate	0.50	-.93, 1.92	0.50
Tertiary education	0.78	-.47, 2.03	0.22

*strawberry blond

5.3.3 Step 3: Selection of potential confounders

Potential confounders were first identified according to whether they were associated with the exposure AND the outcome ($P < 0.2$) in the bivariate analyses (see Table

5.3). Apart from age and sex (retained for reasons noted previously), ITA value and BMI were the only covariates identified.

Table 5.3
Age, sex and covariates flagged at $\alpha < 0.2$ level in serum 25(OH)D concentration AND Breslow thickness (≥ 0.75 mm vs. < 0.75 mm) bivariate analyses

Covariates	**25(OH)D** ***P***	**Breslow** ***P***
Age	0.28	0.62
Sex	0.91	0.41
ITA value	0.02	0.02
BMI	0.09	0.05

5.3.4 Step 4: Examine effect of potential confounding variables on association between 25(OH)D concentration and Breslow thickness

Table 5.4 shows the effect on the 25(OH)D coefficient of introducing each of the potential confounders (identified in Table 5.3) into the model containing only Breslow thickness (outcome) and 25(OH)D concentration (exposure). As can be seen in Table 5.4, inclusion of either ITA value or BMI caused significant changes in the 25(OH)D coefficient, confirming these covariates as confounders of the association between Breslow thickness and 25(OH)D concentration. Although the inclusion of age and sex into the basic model did not change the 25(OH)D coefficient, they were retained in the model as previously noted. Thus, in relation to the outcome of Breslow thickness the preliminary model included 25(OH)D, age, sex, ITA value and BMI.

Table 5.4
Effect of potential confounders on the association between Breslow thickness (≥ 0.75 mm vs. < 0.75 mm) and 25(OH)D concentration (per 10 nmol/L)

Model (*n*=98)	**Coefficient 25(OH)D**	**% change in 25(OH)D coefficient**
Breslow, 25(OH)D	-.293	
Breslow, 25(OH)D, age	-.286	-2.39
Breslow, 25(OH)D, sex	-.282	-3.75
Breslow, 25(OH)D, ITA value	-.255	-12.97
Breslow, 25(OH)D, BMI	-.238	-18.43

5.3.5 Step 5: Reassessing variables initially excluded

The confounding effect of other key factors previously identified in other studies as being important predictors of either vitamin D status or melanoma risk, was tested and is summarised in Table 5.5. Only season at diagnosis was noted to have a greater than 5% effect on the 25(OH)D coefficient.

Table 5.5
Examination of additional potential confounders on the association between Breslow thickness (≥ 0.75 mm vs. < 0.75 mm) and 25(OH)D concentration (per 10 nmol/L)

Model (*n*=98)	**Coefficient 25(OH)D**	**% change in 25(OH)D coefficient**
* Preliminary model	-.212	
* Preliminary model, season at diagnosis	-.245	15.57
* Preliminary model, naevi burden at diagnosis	-.212	0.00
* Preliminary model, primary school sunburns	-.211	1.42

* Preliminary model = Breslow thickness, 25(OH)D, age, sex, ITA value, BMI

5.3.6 Step 6: Final model for the association between 25(OH)D concentration and Breslow thickness

On the basis of these analyses, the final model contained age, sex, ITA value, BMI and season at diagnosis. The results of the final model, for the association between 25(OH)D concentration (per 10 nmol/L) and Breslow thickness (≥ 0.75 mm vs. < 0.75 mm) are shown in Table 5.6.

Table 5.6
Relationship of 25(OH)D concentration (per 10 nmol/L) to Breslow thickness (≥ 0.75 mm vs.< 0.75 mm), with adjustment for confounding factors

Model (*n* = 98)	**Crude OR**	**95% CI**	***P***	**Adjusted OR[a]**	**95% CI**	***P***
25(OH)D	0.75	0.54, 1.02	0.07	0.78	0.55, 1.11	0.17

[a] adjusted for age, sex, ITA value, BMI, season at diagnosis

5.3.7 Step 7: Effect modification

I assessed whether the association (above) between 25(OH)D concentration and Breslow thickness (≥ 0.75 mm vs. < 0.75 mm) varied according to age or sex. No significant interaction was noted (age: z = -.99, P = 0.32; sex: z = 0.82, P = 0.41).

5.3.8 Final Breslow thickness and serum 25(OH)D models

At the completion of the risk factor modelling process and after carefully examining and accounting for potential confounding factors (including age and sex), I determined that ITA value, BMI, and season at diagnosis (and therefore blood draw) were the only confounding factors in the association between 25(OH)D concentration at time of diagnosis and Breslow thickness (≥ 0.75 mm vs. < 0.75 mm), an indicator of melanoma prognosis.

I found that the risk of greater Breslow thickness decreased by 22% per 10 nmol/L increase in serum 25(OH)D concentration. While this was not statistically significant (P = 0.17), the results were suggestive of an adverse effect of lower 25(OH)D concentration, i.e. an association with higher Breslow thickness. As noted in the Methods, I had decided a priori to also investigate the effect of low vitamin D status and high vitamin D status. To explore any possible adverse effect of low vitamin D status, I examined both the lower quartile compared to all other quartiles, and vitamin D insufficiency (compared to vitamin D sufficiency), as defined by the IOM[178]. To explore a possible beneficial effect of higher vitamin D status, the highest quartile of 25(OH)D concentration was examined in comparison to all other quartiles. The crude and adjusted results for these analyses are presented in Table 5.7 (n = 98).

After adjusting for age, sex, ITA value, BMI and season at diagnosis (the factors previously identified as confounders in the 25(OH)D concentration analysis), vitamin D insufficiency was significantly associated with a higher risk of having a Breslow thickness greater than 0.75 mm.

Table 5.7
Final models (crude and adjusted) of vitamin D status at three different thresholds for "sufficiency" and Breslow thickness (≥ 0.75 mm vs. < 0.75 mm)

Vitamin D status	**Crude OR**	**95% CI**	***P***	**Adjusted OR[a]**	**95% CI**	***P***
≥ 45.25 nmol/L	Reference			Reference		
< 45.25 nmol/L	5.30	1.72, 16.40	0.01	5.03	1.32, 19.09	0.02
≥ 50 nmol/L	Reference			Reference		
< 50 nmol/L	3.59	1.18, 10.93	0.03	3.82	1.03, 14.14	0.04
≥ 69.8nmol/L	Reference			Reference		
< 69.8 nmol/L	0.97	0.28, 3.34	0.96	0.83	0.19, 3.58	0.80

[a] adjusted for age, sex, ITA value, BMI, season at diagnosis

The results show that at time of diagnosis with melanoma, low vitamin D status defined as either the lowest quartile, or vitamin D insufficiency as defined by the IOM, was associated with increased odds of having a higher Breslow thickness. Although the adjusted ORs were in a protective direction, i.e. less than 1.00, for higher vitamin D status, there was no evidence of a significant protective effect.

5.4 CLARK LEVEL MODEL

5.4.1 Step 1: Bivariate association between Clark level and vitamin D

Clark level was analysed as a dichotomous categorical variable of Level 1 and Levels 2–5. Clark Level 1 is the reference category in all analyses. Results, reported with beta coefficients, 95% confidence intervals and *P*-values, are based on a logit model and shown in Table 5.8. Higher 25(OH)D concentration was not associated with Clark level (beta = -.01, 95% CI = -.03, 0.01, $P = 0.22$).

5.4.2 Step 2: Examination of each covariate in relation to the outcome, Clark level

Table 5.8 shows the results of the bivariate logistic regression for Clark level and all covariates. Anatomic site, season at diagnosis, S-100β, ITA value, inner arm chroma, solar lentigo density at diagnosis, eye colour, hair colour as a teenager, secondary school sunburns, when the spot was noticed, histories of previous melanoma and other internal cancers, taking supplements and education level were all associated with Clark level at $\alpha < 0.2$.

Table 5.8
Bivariate logistic regression of Clark level (Levels 2–5 vs. Level 1) and all covariates

Variable	Coefficient.	95% *CI*	*P*
Age	-.00	-.03,0.03	0.89
Sex			
Male		referent	
Female	0.30	-.50, 1.09	0.47
Melanoma type			0.01
Anatomic site			
Head & neck		referent	
Trunk	0.02	-.97, 1.01	0.97
Upper extremities	0.77	-.65, 2.19	0.29
Lower extremities	0.77	-.37, 1.90	0.19
Season of diagnosis			
Winter		referent	
Spring	1.59	0.37, 2.82	0.01
Summer	-.20	-1.35, 1.08	0.74
Autumn	0.36	-.70, 1.42	0.51
S-100β	12.96	-.54, 26.49	0.06
ITA value	0.05	0.01, 0.11	0.12
Inner arm L*	0.05	-.11, 0.20	0.56
Inner arm chroma	-.17	-.34, 0.01	0.06
BMI	0.01	-.81, 0.96	0.81
Naevi burden at diagnosis	-.00	-.01, 0.01	0.95
Freckle density at diagnosis			
Low		referent	
Medium	-.13	-1.12, 0.86	0.80
High	0.65	-1.10, 1.23	0.91
Solar lentigo density at diagnosis			
Low		referent	
Medium	-1.21	-2.67, 0.25	0.11
High	-.86	-2.32, 0.11	0.24
Eye colour			
Brown		referent	
Green/hazel	0.73	-.15, 1.61	0.10
Blue/grey	0.51	-.74, 1.76	0.42
Burning tendency			
Rarely/never burns		referent	
Sometimes burns	0.02	-1.20, 1.24	0.98
Mostly/always burns	-.14	-1.26, 0.99	0.81
Tanning tendency			
Tans deeply/moderately		referent	
Tans slightly/does not tan	0.61	-.32, 1.54	0.20
Hair colour as teenager			
Black/dark brown		referent	
Light brown	0.97	-.03, 1.96	0.06
Blonde, red, auburn, s/b*, blonde	-.37	-1.39, 0.65	0.48
Naevus density as teenager			
Nil		referent	
Few/some	-.04	-.94, 0.86	0.93
Many	-.63	-2.57, 1.31	0.53
Freckle density as teenager			
Nil		referent	
Few/some	-.38	-1.22, 0.45	0.37
Many	0.72	-.1.64, 3.07	0.55
School-years sun exposure			
Low		referent	
High	0.20	-.70, 1.11	0.66
Adult Reported Sun Exposure			

Variable	Coefficient.	95% *CI*	*P*
(continuous)	8.41	-.80, 0.80	1.00
Primary school sunburns			
0–5 burns		referent	
6–20 burns	0.36	-.51, 1.23	0.41
> 20 burns	-.50	-1.71, 0.70	0.41
Secondary school sunburns			
0–5 burns		referent	
6–20 burns	0.92	0.04, 1.81	0.04
> 20 burns	0.70	-.61, 2.01	0.30
Adult sunburns			
0–5 burns		referent	
6–20 burns	0.23	-.64, 1.10	0.60
> 20 burns	-.27	-1.43, 0.90	0.65
Solar elastosis			
Absent		referent	
Present	0.07	-1.19, 1.33	0.92
Sunscreen use 10 years ago			
Wore sunscreen most of time		referent	
Wore sunscreen sometimes	0.69	-1.82, 3.21	0.59
Rarely/never wore sunscreen	0.99	-1.46, 3.44	0.43
Sunscreen use 5 years			
Wore sunscreen most of time		referent	
Wore sunscreen sometimes	0.35	-1.30, 2.00	0.68
Rarely/never wore sunscreen	0.58	-.99, 2.17	0.47
When was spot noticed?			
Less than 3 months		referent	
3–12 months	0.69	-.29, 1.67	0.17
> 12 months	-1.19	-2.44, 0.07	0.06
Family history of melanoma			
No		referent	
Yes	-.55	-1.39, 0.29	0.20
History of previous melanoma			
No		referent	
Yes	-1.16	-2.42, 0.09	0.07
History of other internal cancer			
No		referent	
Yes	1.22	-.12, 2.57	0.07
Take supplements			
No		referent	
Yes	1.03	0.01, 2.06	0.05
Ever smoked			
No		referent	
Yes	0.03	-.76, 0.84	0.94
Education			
Primary/Secondary school		referent	
Trade/ Technical certificate	0.54	-.48, 1.55	0.30
Tertiary education	1.06	0.11, 2.02	0.03

*strawberry blond

5.4.3 Step 3: Selection of potential confounders

Variables associated with both 25(OH)D concentration AND Clark level at $P < 0.2$ in the bivariate analyses, were identified as potential confounders and are presented in Table 5.9.

Table 5.9
*Age, sex and covariates flagged at $\alpha < 0.2$ level in serum 25(OH)D concentration AND Clark level (Levels 2 –*5 *vs. Level 1*) *bivariate analyses*

Covariates	**25(OH)D** ***P***	**Clark level** ***P***
Age	0.28	0.89
Sex	0.91	0.47
Season at diagnosis	0.06	0.01
ITA value	0.02	0.12
Supplements	0.06	0.05

5.4.4 Step 4: Examine effect of potential confounding variables on association between 25(OH)D concentration and Clark level

Table 5.10 shows that the inclusion of either season at diagnosis, ITA value or supplements into the 25(OH)D concentration (exposure) and Clark level (outcome) model significantly changed the vitamin D coefficient at $\alpha \geq 0.05$. These covariates were therefore confirmed as confounders of the association between 25(OH)D and Clark level. Neither age nor sex changed the 25(OH)D coefficient of the basic model, but they were retained as noted previously. Thus the preliminary model for the outcome of Clark level, included 25(OH)D concentration, age, sex, season at diagnosis, ITA value and supplements.

Table 5.10
*Effect of potential confounders on the association between Clark level (Levels 2 –*5 *vs. Level 1*) *and 25(OH)D concentration (per 10 nmol/L)*

Model (*n*=98)	**Coefficient 25(OH)D**	**% change in 25(OH)D coefficient**
Clark, 25(OH)D	-.123	
Clark, 25(OH)D, age	-.125	0.81
Clark, 25(OH)D, sex	-.122	0.81
Clark, 25(OH)D, season at diagnosis	-.150	21.95
Clark, 25(OH)D, ITA value	-.088	-28.46
Clark, 25(OH)D, supplements	-.088	-28.46

5.4.5 Step 5: Reassessing variables initially excluded

The confounding effect of other key factors previously associated with either vitamin D status or melanoma risk was then tested. The effect of adding each of these variables to the preliminary model is shown in Table 5.11.

Table 5.11
*Examination of additional potential confounders on the association between Clark level (Levels 2 –*5 *vs. Level 1*) *and 25(OH)D concentration (per 10 nmol/L)*

Model (*n*=98)	**Coefficient 25(OH)D**	**% change in 25(OH)D coefficient**
* Preliminary model	-.038	
* Preliminary model, BMI	-.042	0.53
* Preliminary model, naevi burden at diagnosis	-.036	-7.89
* Preliminary model, primary school sunburns	-.072	92.11

* Preliminary model = Clark level, 25(OH)D, age, sex, season at diagnosis, ITA value, supplements

5.4.6 Step 6: Final model for the association between 25(OH)D concentration and Clark level

On the basis of these analyses, the final model contained age, sex, season at diagnosis, ITA value, supplements, naevi burden at diagnosis and primary school sunburns. The result of the final model for the association between 25(OH)D

concentration (per 10 nmol/L) and Clark level (Levels 2–5 vs. Level 1) is presented in Table 5.12.

Table 5.12
*Relationship of 25(OH)D concentration (per 10 nmol/L) to Clark level (Levels 2 –*5 *vs. Level 1*), *with adjustment for confounding factors*

Model (*n* = 98)	**Crude OR**	**95% CI**	***P***	**Adjusted OR[a]**	**95% CI**	***P***
25(OH)D	0.88	0.72, 1.09	0.26	0.93	0.71, 1.22	0.60

[a] adjusted for age, sex, season at diagnosis, ITA value, supplements, BMI, naevi burden at diagnosis, primary school sunburns

5.4.7 Effect modification

The association between Clark level and vitamin D concentration was examined to identify if it differed according to age or sex. No significant interaction was noted (age: z = -.93, P = 0.35; sex: z = 1.16, P = 0.25).

5.4.8 Final Clark level and serum 25(OH)D models

At the conclusion of the modelling process, season at diagnosis, ITA value, supplements, BMI, naevi burden at diagnosis and number of sunburns during primary school were confounders in the association between 25(OH)D concentration at time of diagnosis and Clark level (Level 2–5 vs. Level 1). Although I found that the risk of deeper Clark levels decreased by 7% per 10 nmol/L increase in 25(OH)D concentration, the association was not significantly significant (P = 0.60). Based on these results it would appear that in this study there was no suggestion that deeper Clark levels were associated with lower serum 25(OH)D concentration at time of diagnosis.

As described previously in the Breslow thickness model, I further investigated if there was any effect of low and high vitamin D status on Clark level, adjusting for the factors identified as confounders in the association between 25(OH)D

concentration and Clark level (season at diagnosis, ITA value, supplements, BMI, naevi burden at diagnosis, primary school sunburns), and age and sex. The results are presented in Table 5.13 (n = 98).

Table 5.13
Final models (crude and adjusted) of vitamin D status at three different thresholds for "sufficiency" at time of diagnosis and Clark level (Levels 2–5 vs. Level 1)

Vitamin D status	**Crude OR**	**95% CI**	***P***	**Adjusted OR[a]**	**95% CI**	***P***
≥ 45.25 nmol/L	Reference			Reference		
< 45.25 nmol/L	1.64	0.64, 4.18	0.30	1.28	0.38, 4.33	0.69
≥ 50 nmol/L	Reference			Reference		
< 50 nmol/L	1.77	0.76, 4.11	0.19	1.83	0.62, 5.44	0.27
≥ 69.8nmol/L	Reference			Reference		
< 69.8 nmol/L	2.05	0.81, 5.23	0.13	1.56	0.48, 5.12	0.46

[a] adjusted for age, sex, season at diagnosis, ITA value, supplements, BMI, naevi burden at diagnosis, primary school sunburns

The results show that there was no significant association between low, insufficient or higher vitamin D status, and Clark level in the QUT Melanoma Study.

5.5 MITOSIS MODEL

5.5.1 Step 1: Bivariate association between presence of mitosis and vitamin D

The presence of mitosis was reported in 23% of the melanomas. Mitosis was modelled as a binary variable with two categories: present or absent. Results (beta coefficients, 95% confidence intervals and *P*-values) for the bivariate analysis between mitosis and each covariate are reported in Table 5.14.

Higher 25(OH)D concentration was not associated with presence of mitosis (beta = -.01, 95% CI = -.03, 0.02, $P = 0.46$).

5.5.2 Step 2: Examination of each covariate in relation to the outcome, presence of mitosis

Results for this step are presented in Table 5.14. They show that anatomic site, season at diagnosis, S-100β, ITA value, BMI, naevi burden at diagnosis and burning tendency were associated with mitosis at $\alpha < 0.2$.

Table 5.14
Bivariate logistic regression for mitosis (absent vs. present) and all covariates

Variable	Coefficient	95% *CI*	*P*
Age	0.01	-.03, 0.05	0.62
Sex			
Male		referent	
Female	-.50	-1.47, 0.47	0.31
Melanoma type			0.02 (Fisher's)
Anatomic site			
Head & neck		referent	
Trunk	-.99	-2.24, 0.26	0.12
Upper extremities	-.23	-1.78, 1.31	0.77
Lower extremities	-.02	-1.23, 1.18	0.97
Season at diagnosis			
Winter		referent	
Spring	0.94	-.26, 2.14	0.13
Summer	-.85	-2.57, 0.86	0.33
Autumn	-.09	-1.42, 1.23	0.89
S-100β	12.60	-.64, 25.83	0.06
ITA value	0.12	0.03, 0.20	0.01
Inner arm L*	0.11	-.08, 0.30	0.25
Inner arm chroma	-.34	-.57, -.12	< 0.001
BMI	0.09	0.01, 0.20	0.07
Weight	0.03	-.00, 0.06	0.09
Height	0.02	-.04, -.07	0.54
Naevi burden at diagnosis	-.01	-.03, 0.01	0.19
Freckle density at diagnosis			
Low		referent	
Medium	0.18	-.99, 1.36	0.76
High	0.43	-.88, 1.74	0.52
Solar lentigo density at diagnosis			
Low			
Medium		referent	
High	-.51	-2.08, 1.06	0.53
	0.05	-1.40, 1.50	0.94
Eye colour			
Brown		referent	
Green/hazel	0.15	-.87, 1.18	0.77
Blue/grey	-.54	-2.23, 1.15	0.53
Burning tendency			
Rarely/never burns		referent	
Sometimes burns	1.70	-.48, 3.88	0.13
Mostly/always burns	1.58	-.53, 3.70	0.14
Tanning tendency			0.00
Hair colour as teenager			
Black/dark brown		referent	
Light brown	-.26	-1.31, 0.78	0.62
Blonde, red, auburn, s/b*, blonde	-.34	-2.69, 2.02	0.78
Naevus density as teenager			
Nil		referent	
Few/some	-.26	-1.31, 0.78	0.62
Many	-.34	-2.69, 2.02	0.78
Freckle density as teenager			
Nil		referent	
Few/some	-.57	-1.57, 0.42	0.26
Many	0.99	-1.10, 3.08	0.35
School years sun exposure			
Low		referent	
High	0.15	-.98, 1.28	0.79

Variable	Coefficient	95% *CI*	*P*
Reported Adult Sun Exposure (continuous)	0.00	-.00, 0.00	0.12
Primary school sunburns			
0–5 burns		referent	
6–20 burns	0.57	-.48, 1.62	0.29
> 20 burns	0.16	-1.34, 1.67	0.83
Secondary school sunburns			
0–5 burns		referent	
6–20 burns	-.44	-1.49, 0.61	0.41
> 20 burns	-.54	-2.22, 1.14	0.53
Adult sunburns			
0–5 burns		referent	
6–20 burns	-.80	-1.89, 0.28	0.15
> 20 burns	-.49	-1.91, 0.93	0.50
Solar elastosis			
Absent		referent	
Present	0.42	-1.05, 1.90	0.57
Sunscreen use 10 years ago			0.50
Sunscreen use 5 years			
Wore sunscreen most of time		referent	
Wore sunscreen sometimes	0.74	-1.53, 3.00	0.52
Rarely/never wore sunscreen	0.49	-1.72, 2.69	0.67
When was spot noticed			0.33(Fisher's)
Less than 3 months		referent	
3–12 months	0.07	-.98, 1.12	0.89
> 12months	-1.52	-3.63, 0.60	0.16
Family history of melanoma			
No		referent	
Yes	-.09	-1.10, 0.93	0.86
History of previous melanoma			
History of other internal cancer			
No			
Yes		referent	
	1.14	-.05, 2.33	0.06
Take supplements			
No		referent	
Yes	-.18	-1.33, 0.97	0.76
Ever smoked			
No		referent	
Yes	0.03	-.94, 0.99	0.96
Education			
Primary/Secondary school		referent	
Trade/ Technical certificate	0.84	-.36, 2.04	0.17
Tertiary education	0.57	-.57, 1.70	0.33

*strawberry blonde

5.5.3 Step 3: Selection of potential confounders

Potential confounders that were associated with both 25(OH)D concentration AND mitosis at $P < 0.2$ in the bivariate analyses, are presented in Table 5.15.

Table 5.15
Age, sex and covariates flagged at $\alpha < 0.2$ level in serum 25(OH)D concentration AND mitosis bivariate analyses

Covariates	25(OH)D *P*	Mitosis *P*
Age	0.28	0.62
Sex	0.91	0.31
Season at diagnosis	0.06	0.13
ITA value	0.02	0.01
BMI	0.09	0.07
Reported adult sun exposure	0.19	0.12
Adult sunburns	0.15	0.15

5.5.4 Step 4: Examine effect of potential confounders on association between 25(OH)D concentration and mitosis

Table 5.16 shows the effect on the 25(OH)D coefficient when each covariate identified in Table 5.15 is introduced into the model of the outcome, mitosis (present vs. absent) and 25(OH)D concentration (exposure). All covariates (with the exception of age and sex) caused the 25(OH)D coefficient to change significantly, confirming that they were confounders of the association between 25(OH)D concentration and mitosis. Therefore, the preliminary model included 25(OH)D, age, sex, season at diagnosis, ITA value, BMI, reported adult sun exposure and adult sunburns.

Table 5.16
Effect of potential confounders on the association between mitosis (present vs. absent) and 25(OH)D concentration (per 10 nmol/L)

Model (*n*=95)	Coefficient 25(OH)D	% change in 25(OH)D coefficient
Mitosis, 25(OH)D	-.094	
Mitosis, 25(OH)D, age	-.097	3.19
Mitosis, 25(OH)D, sex	-.098	4.26
Mitosis, 25(OH)D, season at diagnosis	-.080	-14.89
Mitosis, 25(OH)D, ITA value	-.023	-75.53
Mitosis, 25(OH)D, BMI	-.052	-44.68
Mitosis, 25(OH)D, reported adult sun exposure	-.128	36.17
Mitosis, 25(OH)D, adult sunburns	-.120	27.66

5.5.5 Step 5: Reassessing variables initially excluded

The confounding effect of other key factors previously associated with either vitamin D status or melanoma risk, was then tested by adding each of these variables to the preliminary model (shown in Table 5.17).

Table 5.17
Examination of additional potential confounders on the association between mitosis (present vs. absent) and 25(OH)D concentration (per 10 nmol/L)

Model (*n*=95)	**Coefficient 25(OH)D**	**% change in 25(OH)D coefficient**
* Preliminary model	-.030	
* Preliminary model, naevi burden at diagnosis	-.041	36.67
* Preliminary model, primary school sunburns	-.033	10.00

* Preliminary model = mitosis (present vs. absent), 25(OH)D, age, sex, season at diagnosis, ITA value, BMI, reported adult sun exposure, adult sunburns

5.5.6 Step 6: Final model for the association between 25(OH)D concentration and mitosis

On the basis of these analyses, the final model contained age, sex, season at diagnosis, ITA value, BMI, reported adult sun exposure, adult sunburns, naevi burden at diagnosis, and primary school sunburns. The results of the final model, for the association between 25(OH)D concentration (per 10 nmol/L) and mitosis (present vs. absent) are shown in Table 5.18.

Table 5.18
Relationship of 25(OH)D concentration (per 10 nmol/L) to Mitosis (present vs. absent), with adjustment for confounding factors

Model (*n* = 95)	**Crude OR**	**95% CI**	***P***	**Adjusted OR[a]**	**95% CI**	***P***
25(OH)D	0.91	0.71, 1.17	0.47	0.96	0.70, 1.33	0.82

[a] adjusted for age, sex, season at diagnosis, ITA value, BMI, reported adult sun exposure, adult sunburns, naevi burden at diagnosis, primary school sunburns

Possible effect modification due to age or sex was examined, but no significant interaction was noted (age: z = -1.72, P = 0.09; sex: z = 0.44, P = 0.66).

5.5.7 Final mitosis and serum 25(OH)D models

After examining and accounting for potential confounding factors (including age and sex), season at diagnosis, ITA value, BMI, reported adult sun exposure, adult sunburns, naevi burden at diagnosis, and primary school sunburns were found to be the only confounding factors in the association between 25(OH)D concentration at time of diagnosis with melanoma and mitosis (present vs. absent).

The results show that there was a 4% reduction in risk for the presence of mitosis per 10 nmol/L increase in serum 25(OH)D concentration, but this was not statistically significant ($P = 0.82$). I therefore concluded that in the QUT Melanoma Study there was no association between 25(OH)D concentration at time of diagnosis and mitosis. The effect of low vitamin D status and high vitamin D status (using the lower quartile, IOM "insufficiency" and the higher quartile) was then examined as described in section 5.3.8. The crude and adjusted results for these analyses are presented in Table 5.19 ($n = 95$). The adjusted model included any factors previously identified as confounders (in the mitosis and 25(OH)D concentration analysis).

After adjusting for age, sex, season at diagnosis, ITA value, BMI, reported adult sun exposure, adult sunburns, naevi burden at diagnosis, and primary school sunburns, vitamin D insufficiency was not associated with a higher risk of having presence of mitosis and there was no evidence of a significant protective effect of higher vitamin D status.

Table 5.19
Final models (crude and adjusted) of vitamin D status at three different thresholds for "sufficiency" at time of diagnosis and mitosis (present vs. absent)

Vitamin D status	Crude OR	95% CI	*P*	Adjusted OR[a]	95% CI	*P*
≥ 45.25 nmol/L	Reference			Reference		
< 45.25 nmol/L	2.47	0.89, 6.80	0.08	2.21	0.56, 8.68	0.26
≥ 50 nmol/L	Reference			Reference		
< 50 nmol/L	1.60	0.61, 4.21	0.34	2.12	0.72, 6.19	0.17
≥ 69.8nmol/L	Reference			Reference		
< 69.8 nmol/L	0.40	0.27, 2.39	0.70	0.40	0.10, 1.69	0.21

[a] adjusted for age, sex, season at diagnosis, ITA value, BMI, reported adult sun exposure, adult sunburns, naevi burden at diagnosis, primary school sunburns

5.6 PREDICTIVE MODELLING

At the conclusion of the risk factor modelling, Breslow thickness (≥ 0.75 mm vs. < 0.75 mm) was the only histological outcome variable for melanoma prognosis significantly associated with vitamin D insufficiency. I now provide an analysis of the key predictors of Breslow thickness in this dataset, using the PSC approach outlined in the Methods.

Step 1: Bivariate analysis

A bivariate analysis of the association between Breslow thickness (≥ 0.75 mm vs. < 0.75 mm) and each covariate under consideration was undertaken using binary logistic regression and is provided previously in Section 5.3 (pages 112–113).

Step 2: Identification of covariates for preliminary multivariable model

Covariates significant at $P < 0.2$ in the bivariate analysis and other covariates considered likely to be "important" were identified (see Table 5.20).

Table 5.20
Covariates identified for inclusion in preliminary multivariable models

Covariates	**Breslow *P***
Significant at $P < 0.2$	
25(OH)D concentration	0.08
S-100β	0.17
ITA value	0.02
BMI	0.05
Solar lentigo density at diagnosis	0.13
Hair colour as teenager	0.10
Considered important	
Age	0.62
Sex	0.41
Naevi burden at diagnosis	0.72

Step 3: Fit preliminary multivariable model

All covariates identified in Table 5.20 as significant at P < 0.2 were then fitted into the preliminary multivariable model for Breslow thickness. Table 5.21 shows the *P*-value of the covariates in that model.

Table 5.21
Effect of covariates in the multivariable model

Model (*n*=98)	***P*-value of covariate**	***P*-value of model**
		0.03
25(OH)D	0.32	
S-100β	0.11	
ITA value	0.10	
BMI	0.34	
Solar lentigo density at diagnosis	0.75	
Hair colour as teenager	0.38	

As described in the Methods, a *P* value greater than 0.2 was used as the criteria for removing covariates from the model. Based on this criterion, S-100β and ITA value were the only covariates retained in the model.

Step 4: Selection of variables for best fitting preliminary model

Individual covariates excluded in the previous step were then reinserted (one covariate at a time) into the preliminary multivariable model of S-100β and ITA value, and likelihood ratio tests performed to examine if their inclusion improved the model fit (see Table 5.22). Covariates were considered to improve the model fit if the *P*-value for the likelihood ratio test was < 0.05.

Table 5.22
Effect of covariates on the preliminary multivariable model of S-100β *and ITA value*

Model (*n*=98)	***P*-value of likelihood ratio test**
25 (OH)D	0.09
BMI	0.11
Solar lentigo density at diagnosis	0.40
Hair colour as teenager	0.07
Age	0.85
Sex	0.25
Naevi burden at diagnosis	0.81

The results presented in Table 5.22 show that none of the covariates improved the model fit, and the preliminary multivariable model was therefore S-100β and ITA value.

Step 5: Reintroduce covariates previously excluded

Age, sex and naevi burden at diagnosis, although excluded after the bivariate analysis, were identified as being "important", and were then added back, one at a time, into the preliminary multivariable model. Improvement of the model was again tested using likelihood ratio test (see Table 5.23).

Table 5.23
Effect of excluded covariates on the preliminary multivariable model of S-100β *and ITA value*

Model (*n*=98)	***P*-value of likelihood ratio test**
Age	0.60
Sex	0.32
Naevi burden at diagnosis	0.95

Examination of Table 5.23 shows that none of the covariates significantly improved the model (at $P < 0.05$).

Step 6: Examine linearity of continuous covariates included in the preliminary model

To ascertain whether S-100β and ITA were linear in the logit, the *fracpoly,* compare command in Stata was used. Fractional polynomial modelling confirmed that there was no evidence of significant deviation from a linear fit.

Step 7: Effect modification

Using a multiplicative term in the model, the preliminary effects model of S-100β and ITA value was then examined to identify if the magnitude of the effect was different for males and females. The *P*-value for the interaction term was 0.13, indicating that there was no effect modification due to sex.

Step 8: Main effects model

To determine the best fit for the main effects model, the likelihood ratio test was used to examine whether the inclusion of identified covariates improved the model. At the conclusion of the predictive modelling process, the best predictors for Breslow thickness in this study (*P*-value for model = 0.01) were ITA value (*P*= 0.01) and low vitamin D status (*P* = 0.01).

Chapter 6: Discussion

6.1 INTRODUCTION

The aim of this research project was to identify and explore the nature of any relationship between the serum 25(OH)D level at diagnosis of patients at the time of diagnosis with melanoma, and the outcome for those patients as predicted by the histopathological indicators of melanoma prognosis. To my knowledge this is the first Australian study to do so. In this chapter the study findings will be discussed in the context of current theory, current practice and the conduct of the QUT Melanoma study. Strengths and limitations will be acknowledged and any potential biases identified. Formal conclusions will not be drawn to this chapter, as these will be reserved for the final chapter.

6.2 OVERALL STUDY FINDINGS

The QUT Melanoma study found that although there was no significant association between 25(OH)D concentration at time of diagnosis and any of the prognostic indicators for melanoma, the risk of greater (≥ 0.75 mm) Breslow thickness was observed to decrease by 22% per 10 nmol/L increase in serum 25(OH)D concentration (P = 0.17). Further investigations found that lower vitamin D status, either in absolute terms or relative to others in the study cohort, was adversely associated with Breslow thickness. After adjustment for age, sex, ITA value, BMI and season at diagnosis, vitamin D insufficiency, whether this was as less than an absolute cut-off of 50 nmol/L (OR = 3.82, 95% *CI*: 1.03, 14.14, P =0.04) or the lowest quartile of 45.25 nmol/L (OR = 5.03, 95% *CI:* 1.32, 19.09, P = 0.02), was significantly associated with an increased odds of having a Breslow thickness of 0.75

mm or greater. The importance of low vitamin D status to Breslow thickness was confirmed in a predictive model.

I had anticipated that the results for vitamin D status and Clark level (Levels 2–5 vs. Level 1) might be quite similar to that seen for Breslow thickness, simply due to the fact that the basement membrane was breached in both comparative groups i.e. those with Breslow thickness of greater than 0.75 mm and those with Clark levels of 2–5. The lack of association between vitamin D insufficiency and higher Clark level, but positive association between vitamin D insufficiency and greater Breslow thickness may reflect the assignment to different categories of a number of Clark level 2 tumours that were less than 0.75 mm in depth - the inclusion of these is the only difference between the Clark model and the Breslow thickness model. This suggestion is supported by results from additional analysis of Clark Level as an ordinal categorical variable with three categories of Level 1, Level 2 and Levels 3–5 (see Appendix K). Here there was an adverse association with vitamin D insufficiency that approached statistical significance: OR = 2.17, 95% CI: 0.96, 4.94, $P = 0.06$. Further larger studies would be needed to explore these associations with adequate statistical power.

Higher levels of serum 25(OH)D were also examined for association with melanoma prognosis, using the highest quartile of 69.8 nmol/L as the threshold for “higher levels”. Modelling with Breslow thickness, Clark level and mitosis suggested that there was no effect on prognosis of higher levels of serum 25(OH)D. These results are consistent with an hypotheses that it is insufficient vitamin D status at time of diagnosis with melanoma that is associated with a poorer prognosis, rather than high vitamin D status being associated with better prognosis.

Interpretation of overall study findings

The results from the QUT Melanoma study add to the mounting body of evidence that supports sufficient vitamin D status as beneficial for good health, wellbeing, cancer incidence and prognosis. The overall findings are in agreement with those of three previously identified Northern Hemisphere studies that have investigated the association between serum 25(OH)D levels and melanoma prognosis. The prospective cohort study by Newton-Bishop et al,[24] reported that higher Breslow thickness was associated with lower serum 25(OH)D levels in patients with invasive melanoma, while a more recent study by Gambichler et al[23] found that decreased serum 25(OH)D levels were associated with increased tumour thickness and stage. Similarly, in their 2009 case-control study, Nurnberg et al[25] identified that patients with advanced melanoma were at a higher risk of having serum 25(OH)D levels lower than 50 nmol/L.

When comparing my results to these studies, the three key differences in study design should be revisited. First, in this cross-sectional study, melanoma stage (see Chapter 2, page 30) was not identified as a determinant of prognosis; instead the known histological prognostic features of melanoma - Breslow thickness, Clark level and mitosis - were used as proxies for prognosis. Secondly, in contrast to other studies, The QUT Melanoma study included melanoma *in situ* as well as invasive melanomas, and thirdly, serum samples for 25(OH)D were obtained at diagnosis; i.e. within approximately four days of the provisional diagnosis of melanoma by histopathological report and prior to wider-excision surgery, rather than at various times after diagnosis. The last point is considered to be the most notable difference, as patients diagnosed with melanoma have been shown to have both increased sun

awareness and sun protection behaviour[205,206] and any ascertainment of vitamin D status after diagnosis may reflect this behaviour change.

While studies investigating associations between vitamin D and melanoma prognosis are limited, the QUT Melanoma Study findings also agree with recent epidemiological studies of associations between serum 25(OH)D levels and prognosis for other cancers.[226-229] In a retrospective study of 197 patients diagnosed with gastric cancer, Ren et al[228] found that post-diagnosis vitamin D insufficiency (< 50 nmol/L) was associated with poorer prognosis for gastric cancer (P = 0.02), while Peppone et al[229] examined blood taken from 194 women 30.1 days (mean) prior to breast cancer surgery and reported that patients with worse prognostic indicators (OR = 3.15, 95% CI = 1.05, 9.49) and more aggressive cancers (P = 0.04) had lower mean serum 25(OH)D levels compared to women with less aggressive disease. Similar findings have been reported in prostate cancer.

Gilbert et al[226] investigated the association between 25(OH)D concentration and advanced versus localised prostate cancer (n = 1,447) and found that higher levels of serum 25(OH)D were protective for advanced prostate cancer (OR = 2.33, 95% CI = 1.26, 4.28), while in 2011, Fang et al[227] had provided evidence that higher pre-diagnostic 25(OH)D levels may be associated with localised prostate cancer stage.

Although the results of this cross-sectional study are suggestive of insufficient vitamin D status as a risk factor for thicker melanomas, causality cannot be inferred. I considered if the association could be due to reverse causality, with deeper tumours causing low vitamin D status (as an indicator of the health status of the individual). In the design of the QUT Melanoma Study, I aimed to minimize the risk of reverse causality by taking serum for 25(OH)D measurement at the time of diagnosis, before

disease-related changes in sun exposure behaviour – and therefore 25(OH)D concentration – had occurred.

The strongest association between vitamin D status and melanoma prognosis in this study, was in relation to Breslow thickness. There are a number of possible pathways through which vitamin D could affect melanoma thickness. For example, cell biology research has shown that vitamin D ($1,25(OH)_2D$) can inhibit tumour invasiveness in melanoma cell lines *in vitro*[191] and suppress melanoma growth in rodents.[191,193] Thus, having sufficient serum 25(OH)D levels could inhibit the vertical progression of the melanoma by adjusting the 1,25(OH)D concentration in the surrounding microenvironment. Notably however, in this study, high vitamin D status was not associated with thinner tumours.

Here I measured serum 25(OH)D as a marker of vitamin D status. Yet there is considerable evidence that local production of the active hormone occurs and may be of primary importance to suppression (or not) or tumour growth.[230] As such, serum 25(OH)D is only a crude proxy for levels of the active hormone both systemically and locally, and as a marker for activity of the vitamin D pathway.

The vitamin D receptor (VDR) is a vital link in that pathway,[165,231] as the mediator for the action of 1,25(OH)D. As mentioned in Chapter 2, others[197] have shown that vitamin D receptor (VDR) expression is significantly decreased in melanoma metastases and melanoma when compared to normal skin, and that normal skin surrounding melanomas has also been observed to have significantly decreased VDR expression. This may signify that the reduction or absence of VDRs is implicated in the progression of melanoma, and it would be therefore intriguing to identify any relationships between VDR expression patterns of both melanoma tissue and surrounding healthy skin (from the wider-excision) from the QUT Melanoma Study

samples, and the corresponding serum 25(OH)D concentrations obtained at time of diagnosis, in relation to markers of melanoma prognosis.

Future implications of main finding

A case-control study recruiting melanoma patients as both the cases (Breslow thickness greater than 0.75 mm) and controls (melanoma *in situ* or melanomas with Breslow thickness less than 0.75 mm) from the approximately 1500 melanoma patients presenting to all plastic surgeons in Brisbane over a 12 month period, would have the potential to provide more definitive evidence to either support or refute the findings generated by this study. Yet the larger sample size, while providing more statistical power, would still not confirm if insufficient vitamin D status is actually a risk factor for, or a potential outcome of poor melanoma prognosis. The exact processes responsible will only be elucidated once the biological interactions between vitamin D, melanoma and other factors are further understood.

Based on the single serum sample obtained at diagnosis I cannot extrapolate from my study data that vitamin D supplementation after diagnosis would be beneficial for melanoma patients in terms of disease progression. Indeed, there is no evidence in this study that high 25(OH)D levels were associated with benefit, although shifting those with vitamin D insufficiency to a sufficient status may be worthwhile. That would require a randomized trial of vitamin D supplementation in patients with melanoma. While this would not address the question of why low levels of serum 25(OH)D prior to, or at diagnosis with melanoma might be related to melanoma prognosis, it would, if successful prove to be invaluable as a treatment for all patients diagnosed with melanoma. In the meantime, if we were to assume that the odds ratio reported in the QUT Melanoma Study approximates the relative risk (melanoma is a rare disease) and that the prevalence of the risk exposure in the population (low

vitamin D status) is 30%, approximately 18% of the incidence of thick melanomas in people with melanoma can be attributable to insufficient vitamin D status (less than 50 nmol/L). This suggests that rather than wait for further studies to be undertaken, it may be prudent to simply encourage the population to maintain an adequate vitamin D status while practicing sensible sun safety. This would be particularly applicable to those at a higher risk of developing melanoma.

6.3 SERUM 25(OH)D CONCENTRATION IN CONTEXT WITH OTHER STUDIES

Approximately one-third (36%), of the QUT Melanoma study population was observed to be vitamin D deficient (25(OH)D concentration less than 50 nmol/L). Nurnberg et al[25] found 78.1% of patients had a serum 25(OH)D concentration less than 50 nmol/L and Gambichler et al[23] reported 73.8%. Newton-Bishop et al[24] found that 64% of their study participants had levels less than 60 nmol/L. The three studies all obtained serum for 25(OH)D at various times after diagnosis.[23-25] The timing of blood sampling; i.e. at diagnosis, to ensure that sun exposure patterns did not change in response to the diagnosis of melanoma, may partly explain the difference between the reported percentage of patients with less than optimal (50 nmol/L) serum 25(OH)D levels in the QUT Melanoma study, compared to other studies.[23-25]

The sub-tropical study setting (27° South) of the QUT Melanoma study is a further possible explanation for the lower prevalence of vitamin D deficiency, as the other studies had been conducted at latitudes higher than 49° North. According to data from the United States and Canada,[232] cutaneous production of vitamin D is minimal at and above latitude 42° North from November to February, or from mid-October until mid-April from latitude 52° North and higher. One of the studies that examined

serum 25(OH)D status in relation to melanoma prognosis[25] collected serum samples during this time frame, so that the high levels of vitamin D insufficiency reported are perhaps unsurprising. Median 25(OH)D levels were reported by Nurnberg et al[25] and Gambichler et al[23] of 35.75 nmol/L and 30.75 nmol/L, respectively, while a study of older Finnish men also obtained blood samples from previously diagnosed melanoma patients at a similar time of the year, and reported median serum 25(OH)D levels of 33.1 nmol/L.[204] In comparison, the median level serum 25(OH)D concentration in the QUT Melanoma Study was 58.6 nmol/L.

Seasonal variation

Another possible explanation for the difference between vitamin D status and median 25(OH)D levels reported in the QUT Melanoma study compared to other studies, is that serum was obtained over a 12 month period. This enabled the study's third objective of describing seasonal variation in vitamin D status in the sample to be investigated. Modest seasonal variation was seen in serum 25(OH)D levels; mean serum 25(OH)D levels were, as to be expected, highest in summer (62.3 nmol/L) and lowest in Winter (52 nmol/L), although this difference was not statistically significant ($P = 0.09$). The lack of significance may be a reflection of the sample size, as vitamin D status ($\geq$ 50 nmol/L vs. < 50 nmol/L) was significantly associated ($\chi^2 = 8.56$, $df = 3$, $P = 0.04$) with season.

In situ melanomas

The difference in the proportion of the sample with vitamin D insufficiency in the QUT Melanoma Study compared to other studies was not due to the inclusion in the former of *in situ* melanomas, as there was no statistically significant difference in vitamin D status according to whether the melanoma had breached the basement membrane or not ($\chi^2 = 1.81$, $df = 1$, $P = 0.18$).

Vitamin D assays

It should not be overlooked, and is important to note, that the differences in serum 25(OH)D levels between the studies may also be due at least in part, to poor agreement between the various assays used for vitamin D analysis. This has been widely reported[182,233] and until standardised measures are agreed, inter-assay variability will remain an issue when comparing studies.

6.4 SERUM 25(OH)D LEVELS IN COMPARISON TO AUSTRALIAN DATA

The distribution of vitamin D status in the QUT Melanoma Study was comparable to that reported in a large Australian study (Australian Diabetes, Obesity and Lifestyle study (AusDiab)) of 11,247 adults.[26] In that Australia-wide study, vitamin D deficiency (< 50 nmol/L) was observed in 31% of participants compared to 36% of the QUT Melanoma Study participants.[26] Mean (63 nmol/L) and median (60 nmol/L) serum 25(OH)D concentrations were higher in the AusDiab study[26] as compared to the QUT Melanoma study (58.2 nmol/L and 58.6 nmol/L respectively). Serum 25(OH)D levels are reported to decrease with increasing age, so at least part of the difference in 25(OH)D levels between the two studies may be explained by the difference in age distribution of participants in the two studies, as participants in the QUT Melanoma Study (mean age = 61.4 years) were, on average, 13 years older than those in the AusDiab study (mean = 47.6 years in males, 48.9 years in females).[26] In addition, participants in the QUT Melanoma Study were recruited from the Brisbane area, unlike the AusDiab participants who were recruited Australia-wide. Variation in 25(OH)D assays across Australia is widely reported[234,235] and may also have accounted for some of the differences seen between the studies.

6.5 SERUM 25(OH)D LEVELS AND COVARIATES

In bivariate analyses, the only covariates significantly associated ($P = 0.05$) with serum 25(OH)D concentration were seven of the "skin type" variables generated from the spectrophotometric output (see Table 5.1, page 109). While these crude data must be interpreted with caution, some variables negatively associated with serum 25(OH)D concentration are suggestive of increased serum 25(OH)D levels in participants with greater sun exposure to an exposed body site and also in those participants with an intermediate skin type. While an intermediate skin type is still in the "light" skin range, this result probably reflects that these skin types may spend more time outdoors due to their better sun tolerance.

Others have looked at the association between 25(OH)D levels and ITA value in multiethnic samples of New Zealanders, with contrasting results. Nessvi et al[236] reported an increase of 2–3 nmol/L per 10% increase in ITA value (this association almost disappeared after adjusting for ethnicity), while Rockell et al[237] found a 5% decrease in 25(OH)D for every 10° increase in ITA value. Yet to the best of my knowledge there has not been any prior research conducted to identify associations between the specific spectrophotometric skin pigmentation characteristics (L*, a*, b*, chroma and hue) and serum 25(OH)D levels. While not an original objective of the QUT Melanoma study, these data provide some hereto unknown insight (albeit at the crude level) into the relationship between skin phototype and vitamin D status, but more research needs to be undertaken before any association is clearly understood. Interestingly, none of the self-report measures of skin phenotype were associated with serum 25(OH)D concentration, even at the $\alpha < 0.2$ level.

6.6 SKIN TYPE CHARACTERISTICS

6.6.1 Spectrophotometry and melanoma prognosis: bivariate associations

One particularly interesting outcome of the QUT Melanoma Study was the apparent association between two of the prognostic indicators and various measures of skin phototype. Detailed analysis of skin type in relation to melanoma prognosis is outside the scope of this current thesis, so the bivariate associations that I consider here require confirmation in more detailed analyses. Significant positive bivariate associations were observed with respect to the ITA value (skin colour value) of the unexposed upper arm and both Breslow thickness (OR = 1.12, $P = 0.02$) and mitosis (OR = 1.12, $P = 0.01$), suggesting that as ITA values increased (and the skin became lighter) prognosis was poorer. While it is not possible to comment on the relationship between the ITA groups and either Breslow thickness, or mitotic activity due to empty cells, no participant with an intermediate skin type presented with a melanoma with either a Breslow thickness equal to or greater than 0.75 mm, or with mitotic activity present.

Although there is an abundance of studies reporting fair skin type as a risk factor for melanoma, the observations seen in this study, albeit in a modest sample size, do suggest a possible trend to a protective effect of a darker skin type for melanoma *prognosis* and may warrant further investigation in a larger sample, or a more detailed analysis in this sample to adjust for confounding. In the predictive modelling undertaken in this study, the importance of ITA value to Breslow thickness was confirmed.

While ITA value was chosen as the representative variable of objective skin type, other variables derived from the spectrophotometer readings were associated, at the

bivariate level, with melanoma prognosis. Chroma (the saturation or intensity of colour) of the inner arm was negatively associated with Breslow thickness (beta = -0.35, 95% CI: -0.60, -0.10, $P < 0.001$) and mitosis (beta = -0.34, 95% CI: -0.57, -0.12, $P < 0.001$). These results indicate that the odds of a poorer prognosis increased as the skin colour intensity decreased, or became fairer. Others[84] have found that chroma of an unexposed site is associated with increased risk for melanoma at a bivariate level, and I suggest that, based on results from the QUT Melanoma study, chroma could also be a risk factor for prognosis and does warrant further investigation in larger studies or further analysis in this study.

In other studies, L* (lightness coefficient of the skin) has been validated as being highly associated with the melanin content of the skin.[238] While it was not possible to determine the melanin content of the skin in the QUT Melanoma study, L* at the inner arm was not associated at the bivariate level with any of the outcome measures of prognosis: Breslow thickness, Clark level or mitosis. This may be explained in part by the fairly homogenous study sample in terms of depth of skin colour, as all study participants met the criteria for "low pigmentation" (L* > 60).

6.6.2 Self-report measures of skin phenotype and melanoma prognosis

Certain skin phenotype characteristics are considered to be risk factors for melanoma incidence and also for cutaneous production of serum 25(OH)D, yet only one self-report measure of skin phenotype ("hair colour as a teenager" in the Clark level model) was significantly associated (beta = 0.88, $P = 0.05$) with an outcome measure of melanoma prognosis. In retrospect, rather than examine the relationship between individual self-report measures of skin phenotype in relation to melanoma prognosis, there may have been more benefit in amalgamating the self-report measures and allocating each participant to one of the Fitzpatrick skin types, as the sample size

may not have been sufficiently large to support analyses of the individual characteristics.

6.6.3 Spectrophotometry versus self-report

Bivariate relationships between ITA skin types and self-report measures of skin phenotype were examined (see Table 4.7, page 92) to identify if there was agreement between the two measures. The self-report measures of propensity to burn ($P = 0.03$) and propensity to tan ($P = 0.04$) were the only reported phenotypic characteristics significantly associated with ITA skin type groups. Even though 71% of the sample was classified as having a light/fair skin type, 73.7% reported that they would tan deeply or moderately after spending several weeks at the beach in the sun without any sun protection. These two statistics appear contradictory, possibly highlighting unreliability of self-report measures with respect to skin phenotype characteristics.[239] Spectrophotometry clearly has value as an objective measure of skin pigmentation, although self-report remains important as a measure of perceived skin type – with importance for perceived need for use of sun protection – and for sun sensitivity, which is only loosely related to sun colour.[240]

6.7 SERUM S-100β LEVELS

The fourth study objective was to identify any relationships between S-100β levels and the histopathological features of the tumours. The median serum S-100β levels of 0.05 µg/L were similar (0.06 µg/L) to those obtained by others.[213] High levels ($\geq$ 0.15 µg/L) of S-100β were found in one patient (1%). Elevated serum S-100β levels are known to correlate with melanoma progression and poor prognosis,[124,125,241] therefore as per the assay manufacturer's instructions, the

suggested threshold of 0.15 µg/L was used to reflect non-pathological levels of S-100β. If 0.20 µg/L had been used as the threshold in this study, as others have previously done,[241] the results would not have changed, as 99% of participants had levels below both thresholds.

As all serum samples were obtained prior to wider excision of the tumour, it was anticipated that the S-100β levels reported in the QUT Melanoma study could reflect, in part, the inflammatory response to the diagnostic biopsy or alternatively the presence of the primary tumour. A previous study had reported that serum collected with the primary tumour still *in situ* correlated with the Breslow thickness of the tumour.[242] This may be the explanation for the lack of a significant association between Breslow thickness and S-100β levels ($P = 0.17$) seen here.

On the basis of the single serum sample obtained at diagnosis, it was not possible to report on S-100β in relation to disease progression; but only on prognosis by using the proxies of Breslow thickness, Clark level and mitotic activity. While S-100β levels were found to be significantly associated with mitotic activity ($P = 0.05$), the results were not clinically meaningful as only one percent of the samples reached a pathological level. This is an indication that more power was needed to reach any conclusion about the utility of S-100β as a marker of melanoma prognosis when using the histopathological proxies for prognosis. Regardless of whether the histopathological indicators for diagnosis are to be used as proxies for prognosis in future studies, or melanoma patients are observed for disease progression over time, examination of S-100β serum levels at regular intervals after diagnosis is suggested.

6.8 PUBLIC HEALTH

General implications

From a public health perspective it was pleasing to note that almost half (46.5%) of the study sample had three or more full body skin checks by a doctor in the past 3 years, with 74% reporting they had had at least one full body skin check by a doctor in that time. This compares favourably to the 35.3% of Queensland melanoma patients who reported having a whole-body skin examination in the 3 years before diagnosis in the large survey by McPherson et al,[105] and could be interpreted as an indication that public health messages with respect to skin examinations are being heeded. The regularity with which participants in the QUT Melanoma Study undertook full body skin checks, in addition to 57% reporting that their "spot" was noticed less than 3 months prior to excision, may explain the large number of *in situ* lesions (43%) observed in the study. This proportion is slightly higher than the Queensland figures for 2002 which show that 36% all melanomas diagnosed in that year were melanoma *in situ.*[76]

6.9 LIMITATIONS

There are several limitations to this study. Much of the self-report information relating to adult and school years sun exposure required significant long-term recall of places, dates, sunburns and sun behaviour patterns. Others[160] have previously commented on the potential for bias in this area. The clinical examination at T2 provided an opportunity to minimise potential bias in some of these "problem" areas. For example, self-reported recall of eye and hair colour in teenage years was assisted by use of visual aids (see Appendices G and H). The use of spectrophotometry to

gain an objective measure of skin phenotype characteristics was also a valuable tool to minimise potential self-report bias.

The method of melanoma classification was a limiting factor with respect to the analysis of melanoma according to type, with 15% of all melanomas being necessarily included in a non-homogenous category of “other melanomas”. It is possible that this proportion may have been lower if the histopathologist had been a member of the study team and additional reporting could have been requested. Consideration was given to allocating melanomas with two reported types to the first type mentioned in the histopathology report, but in the interest of accuracy this was not followed through.

The recoding of variables with Likert scales greater in number than four meant that information with respect to extreme values or responses was lost, or assimilated into other groups. The collapsing of categories, while a standard part of analysis, was more a reflection of the small sample size. To limit any misclassification bias, wherever possible the methods used to recode these variables were based on methods used by others, or according to clinical advice. I acknowledge that it is possible that participants may have responded differently if given fewer choices in the first instance.

There is always the possibility that the observed association between serum 25(OH)D and Breslow thickness could be explained by the presence of confounding or bias. While there may be confounders for melanoma prognosis that are as yet unknown, the study controlled for factors that are known to influence vitamin D levels or that may affect melanoma prognosis, in a rigorous analytical process.

One area of potential measurement bias was identified and relates to serum 25(OH)D concentration. As discussed in the literature review there is a lack of agreement with respect to the most appropriate and reliable method to use for measurement of serum 25(OH)D concentration and until a "gold standard" method, and assay, is identified for this process, the potential will exist for conjecture with respect to correct measurements. This is more important in comparing absolute values across studies than in comparing results within a study, where the samples were assayed in batched analysis at study completion using a single assay within a single laboratory with excellent quality assurance performance.

There were several recorded measures that could not be used in analyses due to uncertainty concerning their reliability. Questionnaire self-report of previous solar keratoses and skin cancers removed or treated by a doctor, were subject to conjecture as many patients were unclear as to the actual nature of lesions that had been treated. For the same reason, age at time of diagnosis with the first skin cancer could not be included in analyses. In addition, during the clinical examination accurate quantification of solar keratoses was compromised as patients often presented with obvious signs of recent cryotherapy, or they reported having recently been to the dermatologist or GP and had "many" burnt off. For this reason, number of solar keratosis was also not included in any analyses.

The sample size of 100 participants limited the comparisons between various sub-groups and may be a possible explanation for the predominately null findings reported in this study. In addition, the study had low power to detect any effect modification, as in general the sample size required to detect this should be at least four times that required for the main effect.[243]

6.10 STRENGTHS

Initial concerns that as this would already be a stressful time for the patient, and any further intrusions could be seen as unwelcome, proved to be unfounded as the study had a very high participation rate with 98% of patients approached agreeing to participate in the QUT Melanoma study. The sample size (n = 100) was therefore representative of the population approached to participate in the study (n = 102). Based on recent data from the Queensland Government,[244] the study sample size (n = 100) represents approximately 9% of the 1172 melanomas diagnosed annually in the Brisbane area in 2007–2009, and thus can also be considered representative of melanoma patients in the Brisbane population.

Various attributes of the study melanomas confirm that the sample was representative with respect to the subtypes and anatomic sites of melanoma diagnosed in Queensland. The most common melanoma subtype seen in this study, superficial spreading melanoma (54%), was also the most commonly reported invasive melanoma subtype to the Queensland Cancer Registry in the period 1982–2008 (54.9%).[245] In the same period, the Queensland Cancer Registry reported that the trunk (33.5%) was the most common site for melanomas.[245] Likewise, in the QUT Melanoma study melanomas were most commonly found on the trunk (37%).

All serum samples were obtained at diagnosis and prior to wider-excision surgery over a 12 month recruitment period, which allowed for investigation of any effect of seasonal differences in serum 25(OH)D concentration on the relationships under investigation.

Epidemiological studies often rely on self -assessment of skin colour and skin phenotype characteristics as a subjective guide to skin type. A positive finding from

this study was the value of reflectance spectrophotometry to objectively assess skin colour type and its features of brightness, chroma and hue. All skin type covariates satisfying the criteria for inclusion in the preliminary multivariable models were obtained from the spectrophotometric readings (although only ITA value was used in multivariable models). It is unlikely that these readings could have generated any measurement bias given that one person using one spectrophotometer according to the study protocol performed all examinations.

Ultimately skin phototype (ITA value) was a confounder in the relationship between all the prognostic outcomes and vitamin D status, yet I was also able to demonstrate that there were significant associations between other skin colour variables and prognostic outcome variables and serum 25(OH)D concentration in bivariate analyses. Further investigations that modelled these variables, in place of ITA value, could provide further interesting insight into the role of skin phototype characteristics in the association between vitamin D and melanoma prognosis.

I was also able to measure the effect of a large number of possible confounders of the relationship between vitamin D status and melanoma prognosis. The failure to identify any significant associations in the Clark level and mitosis models, despite a number of risk factors common to both of these prognostic indicators and also to Breslow thickness, could have been due to low study power, or the true absence of association between the risk factors and Clark level and mitosis. This study was unable to distinguish between these explanations.

Chapter 7: Conclusion

In conclusion, the aim of this study has been to identify and explore the nature of the association between serum 25-hydroxyvitamin D level at time of diagnosis and the outcome for those patients as predicted by the histological indicators of melanoma prognosis. The QUT Melanoma Study, a cross-sectional, observational study of 100 melanoma patients in Brisbane, Queensland, provides new evidence to suggest there is a significant association between vitamin D insufficiency (< 50 nmol/L) at time of diagnosis with melanoma and thicker tumours (Breslow thickness ≥ 0.75 mm). The strength of this association increased when a lower threshold for vitamin D status (lower quartile of < 45.25 nmol/L) was examined. No evidence of any association between insufficient vitamin D status and the other prognostic markers of Clark level (Level 2-5 vs. Level 1) or mitosis (present vs. absent), or of any protective effect on melanoma prognosis of higher levels of serum 25(OH)D was observed. Given that thicker tumours are associated with a poorer prognosis; this finding suggests that insufficient vitamin D status at time of diagnosis with melanoma may be associated with a poorer prognosis.

Blood sampling within days of the provisional diagnosis and prior to the wider-excision of the tumour ensured that sun avoidance behavior did not impact on serum 25(OH)D levels and allowed for accurate reporting of the participant's vitamin D status prior to definitive wider-excision surgery.

From a public health perspective, the reduction of mortality from melanoma is paramount and can only be achieved if melanoma is prevented or is diagnosed when very thin and preferentially when *in situ*. The QUT Melanoma Study was *not*

designed to examine vitamin D status and the “risks” for developing melanoma, so comment cannot be made with respect of the potential role of vitamin D in the prevention of melanoma. The study was instead concerned with 25(OH)D concentration and the outcome for melanoma – survival. While the QUT Melanoma Study adds to the knowledge of how vitamin D is associated with melanoma prognosis, the mechanism behind this association is yet to be elucidated. I suggest that other unmeasured factors in the vitamin D pathway, such as the VDR, could play a role as a result of their absence or reduced numbers within the melanoma and surrounding skin. Further, sufficient serum 25(OH)D levels may inhibit vertical growth of melanomas beyond a depth of 0.75 mm, as has been shown in animal models. The results presented here suggest that, if the findings are causal, ensuring that all members of the population were vitamin D sufficient (greater than or equal to 50 nmol/L), would result in approximately 18% fewer thick melanomas – with their attendant poorer prognosis - being diagnosed.

Queensland has the highest incidence of melanoma in the world; therefore the suggestion that sufficient levels of vitamin D might be protective for poorer melanoma prognosis could have wide ranging implications for the public health message with respect to both melanoma and vitamin D – regardless of the mechanism responsible. There may be inherent difficulties in devising an appropriate public health message that balances the need for optimal vitamin D status while at the same time avoiding too much sun exposure – and the risk of developing melanoma. Messages would require tailoring and this would be particularly important for indoor workers, fairer skin types, those with a family history of melanoma and the elderly. For example, rather than relying on sun exposure for

maintenance of adequate 25(OH)D levels, those with fairer skin types may be best to use vitamin D supplements.

The mechanism of the potential benefit of sufficient levels of serum 25(OH)D for melanoma prognosis requires further study. Until then it would appear sensible for all Queenslanders to ensure that they maintain serum 25(OH)D levels greater than 50 nmol/L. This would be particularly pertinent for those at a higher risk of developing melanoma – not to avoid developing a melanoma but to possibly protect against having a thicker tumour and thus a poorer prognosis.

Reference list

1. Cancer Council Queensland. Queensland Cancer Registry. Cancer in Queensland Incidence and Mortality 1982-2006. 2009; http://www.cancerqld.org.au/page/research_statistics/queensland_cancer_registry/ Accessed 29/04/2012.

2. Garbe C, Leiter U. Melanoma epidemiology and trends. *Clinical Dermatology.* 2009;27:3-9.

3. Australian Institute of Health and Welfare. Cancer - Australian cancer incidence statistics update, Dec 2008. http://www.aihw.gov.au/cancer/index.cfm. Accessed 29/04/2012.

4. Australian Institute of Health and Welfare. AIHW Interactive Cancer Incidence Data Cubes. 2005; http://www.aihw.gov.au/cancer/data/datacubes/index.cfm. Accessed 14/09/2012.

5. Howlader N, Noone A, M. K, et al. Stat Bite U.S. Melanoma Incidence - SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations). *J. Natl. Cancer Inst.* 2012;16(104).

6. de Vries E, Coebergh JW. Cutaneous malignant melanoma in Europe. *Eur. J. Cancer.* 2004;40(16):2355-2366.

7. Thompson JA. Ten Years of Progress in Melanoma. *J. Natl. Compr. Canc. Netw.* 2012;10(8):932-935.

8. Australian Cancer Network Melanoma Guidelines Revision Working Party. *Clinical Practice Guidelines for the Management of Melanoma in Australia and New Zealand.* 2008.

9. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. *Eur. J. Cancer.* Jan 2005a;41(1):28-44.

10. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. *Eur. J. Cancer.* 2005b;41(1):45-60.

11. Armstrong B, Kricker A. How much melanoma is caused by sun exposure? *Melanoma Res.* 1993(3):395-401.

12. McGovern VJ. Melanoblastoma. *Med. J. Aust.* 1952;1(5):139-142.

13. Lancaster H. Some geograhical aspects of the mortality from melanoma in Europeans. *Med. J. Aust.* 1956(1):1082-1087.

14. Elwood JM, Gallagher RP. Body site distribution of cutaneous malignant melanoma in relationship to patterns of sun exposure. *Int. J. Cancer. Suppl.* 1998;78(3):276-280.

15. Beral V, Robinson N. The relationship of malignant melanoma, basal and squamous skin cancers to indoor and outdoor work. *Br. J. Cancer.* 1981(44):886-891.

16. Vagero D, Ringback G, Kiviranta HBJ. Melanoma and other tumors of the skin among office, other indoor and outdoor workers in Sweden 1961-1979. *Br. J. Cancer.* 1986;4(53):507–512.

17. Lee JAH. Melanoma. In: Schottenfeld D, ed. *Cancer epidemiology and prevention*. Philadelphia: W. B. Saunders; 1982:984-995.

18. Berwick M, Armstrong BK, Ben-Porat L, et al. Sun exposure and mortality from melanoma. *J. Natl. Cancer Inst.* 2005;97(3):195-199.

19. Mocellin S, Nitti D. Vitamin D receptor polymorphisms and the risk of cutaneous melanoma: a systematic review and meta-analysis. *Cancer.* 2008;113(9):2398-2407.

20. Holick MF. Vitamin D: A millenium perspective. *J. Cell. Biochem.* 2003;88(2):296-307.

21. Colston K, Colston M, Feldman D. 1,25-dihydroxyvitamin D3 and malignant melanoma: the presence of receptors and inhibition of cell growth in culture. *Endocrinology.* 1981;108:1083-1086.

22. Seifert M, Rech M, Meineke V, Tilgen W, Reichrath J. Differential biological effects of 1,25-dihydroxyvitamin D_3 on melanoma cell lines in vitro. *The Journal of steroid biochemistry and molecular biology.* 2004;89-90(1-5):375-379.

23. Gambichler T, Bindsteiner M, Höxtermann S, Kreuter A. Serum 25-hydroxyvitamin D serum levels in a large German cohort of melanoma patients. *Br. J. Dermatol.* 2012;168(3):625–628.

24. Newton-Bishop JA, Beswick S, Randerson-Moor J, et al. Serum 25-hydroxyvitamin D3 levels are associated with breslow thickness at presentation and survival from melanoma. *J. Clin. Oncol.* 2009;27:5439-5444.

25. Nurnberg B, Graber S, Gartner B, et al. Reduced serum 25-hydroxyvitamin D levels in stage IV melanoma patients. *Anticancer Res.* 2009;29(9):3669-3674.

26. Daly R, Gagnon C, Lu ZX, et al. Prevalence of vitamin D deficiency and its determinants in Australian adults aged 25 years and older: a national, population-based study. *Clin. Endocrinol. (Oxf.).* 2012;77(1):26-35.

27. Kimlin MG, Harrison S, Nowak M, Moore M, Brodie A, Lang C. Does a high UV environment ensure adequate vitamin D status? *Journal of Photochemistry and Photobiology B: Biology.* 2007;89(2-3):139-147.

28. Saladin KS. *Anatomy and Physiology.* 5th ed. New York: McGraw-Hill; 2010.

29. Jablonski NG. The evolution of human skin and skin colour. *Annual Review of Anthropology.* 2004;33(1):585-623.

30. Patton K, Thibodeau G. *Anatomy & Physiology.* 7 ed. St. Louis: Mosby Elsevier; 2010.

31. Seeley R, Stephens T, Tate P. *Anatomy & Physiology.* 8 ed. New York: McGraw-Hill; 2008.

32. Mayo Clinic. Mayo Foundation for Medical Education and Research. 2008; http://www.mayoclinic.com/health/skin.

33. Stepien K, Dzierzega-Lecznar A, Kurkiewicz S, Tam I. Melanin from epidermal human melanocytes: Study by Pyrolytic GC/MS. *J. Am. Soc. Mass Spectrom.* 2009;20(3):464-468.

34. Hearing VJ. Biochemical control of melanogenesis and melanosomal organization. *Journal of Investigative Dermatology Symposium Proceedings.* 1999;4(1):24-28.

35. New Zealand Dermatological Society Incorporated. 2012; http://www.dermnetnz.org/doctors/principles/images/epidermis.pdf. Accessed 12-02-2011.

36. Zaidi MR, Day C-P, Merlino G. From UVs to Metastases: Modeling Melanoma Initiation and Progression in the Mouse. *J. Invest. Dermatol.* 2008;128(10):2381-2391.

37. Walker G, Hacker E. Ultraviolet light as a modulator of melanoma development, . In: Murph M, ed. *Research on Melanoma - A Glimpse into Current Directions and Future Trends,*: InTech; 2011.

38. Abdel-Malek ZA, Scott M, Suzuki I, et al. The Melanocortin-1 Receptor is a key regulator of human cutaneous pigmentation. *Pigment Cell Res.* 2000;13(Supplement 8):156-162.

39. International Agency for Research on Cancer Expert Group (IARC). IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. *Solar and Ultraviolet Radiation.* 1992;55 227.

40. International Commission on Non-Ionizing Radiation Protection (ICNIRP). Guidelines on limits of exposure to ultraviolet radiation of wavelenghts between 180nm and 400nm (incoherent optical radiation). *Health Phys.* 2004;87(2):171-176.

41. English DR, Armstrong BK, Kricker A, Fleming C. Sunlight and cancer. *Cancer Causes & Control.* 1997;8(3):271-283.

42. Parisi AV, Wong JCF. An estimation of biological hazards due to solar radiation. *Journal of Photochemistry and Photobiology B: Biology.* 2000;54(2-3):126-130.

43. Wang SQ, Setlow R, Berwick M, et al. Ultraviolet A and melanoma: A review. *J. Am. Acad. Dermatol.* 2001;44(5):837-846.

44. Jhappan C, Noonan FP, Merlino G. Ultraviolet radiation and cutaneous malignant melanoma. *Oncogene.* 2003;22(20):3099-3112.

45. Wang SQ, Kopf AW, Marx J, Bogdan A, Polsky D, Bart RS. Reduction of ultraviolet transmission through cotton T-shirt fabrics with low ultraviolet

protection by various laundering methods and dyeing: clinical implications. *J. Am. Acad. Dermatol.* May 2001;44(5):767-774.

46. Loomis WF. Skin-pigment regulation of vitamin-D biosynthesis in man. *Science.* 1967;157(3788):501-506.

47. Zanetti R, Prota G, Napolitano A, et al. Development of an intergrated method of skin phenotype measurement using the melanins. *Melanoma Res.* 2001(6):551-557.

48. Prota G. Melanins, melanogenesis and melanocytes: looking a their functional significance from the chemist's viewpoint. *Pigment Cell & Melanoma Research.* 2000;13:283-293.

49. Ito S. A chemist's view of melanogenesis. *Pigment Cell Res.* 2003;16:230-236.

50. Jablonski NG, Chaplin G. Skin deep. *Sci. Am.* 2003;13(2):72-79.

51. Parra EJ. Human pigmentation variation: Evolution, genetic basis, and implications for public health. *Am. J. Phys. Anthropol.* 2007;134(S45):85-105.

52. Armstrong BK, Kricker A. The epidemiology of UV induced skin cancer. *J. Photochem. Photobiol. B.* 2001;63(1-3):8-18.

53. Australian Institute of Health and Welfare and Australasian Association of Cancer Registries. Cancer in Australia: an overview, 2006. 2007; http://www.aihw.gov.au/publications/index.cfm/title/10476. Accessed 14/03/2012.

54. Australian Institute of Health and Welfare. ACIM (Australian Cancer Incidence and Mortality) Books. 2012; http://www.aihw.gov.au/cancer/data/acim_books/index.cfm. Accessed 21/11/2012.

55. Maddodi N, Setaluri V. Role of UV in cutaneous melanoma. *Photochem. Photobiol.* 2008;84(2):528-536.

56. Clark WH, Jr., From L, Bernardino EA, Mihm MC. The histogenesis and biologic behavior of primary human malignant melanomas of the skin. *Cancer Res.* 1969;29(3):705-727.

57. Adler M, White CJ. Amelanotic malignant melanoma. *Seminars in Cutaneous Medicine & Surgery.* 1997(16):122-130.

58. Gray-Schopfer V, Wellbrock C, Marais R. Melanoma biology and new targeted therapy. *Nature.* 2007;445(7130):851-857.

59. Rawles ME. Origin of melanophores and their role in development of colour patterns in vertebrates. *Physiol. Res.* 1948(28):383-408.

60. Chang AE, Karnell LH, Menck HR. The National Cancer database report on cutaneous and noncutaneous melanoma. *Cancer.* 1998;83(8):1664-1678.

61. Cramer SF. The origin of epidermal melanocytes. Implications for the histogenesis of nevi and melanomas. *Arch. Pathol. Lab. Med.* 1991;115(2):115-119.

62. Grichnik JM. Melanoma, Nevogenesis, and Stem Cell Biology. *J. Invest. Dermatol.* 2008;128(10):2365-2380.

63. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. *Nature Reviews.Cancer.* 2003;3(121):895-902.

64. Urteaga OB, Pack GT. On the antiquity of melanoma. *Cancer.* 1966;19(5):607-610.

65. Erdmann F, Lortet-Tieulent J, Schüz J, et al. International trends in the incidence of malignant melanoma 1953–2008—are recent generations at higher or lower risk? *Int. J. Cancer.* 2012;132(2):385-400.

66. Linos E, Swetter SM, Cockburn M, Colditz G, Clarke C. Increasing burden of melanoma in the United States. *J. Invest. Dermatol.* 2009;129(7):1666-1674.

67. Lens M, Dawes M. Global perspectives of contemporary epidemiological trends of cutaneous malignant melanoma. *Br. J. Dermatol.* 2004(150):179-185.

68. Australian Institute of Health and Welfare (AIHW) and Australasian Association of Cancer Registries (AACR). Cancer in Australia 2010: an overview. 2010; http://www.aihw.gov.au/WorkArea/DownloadAsset.aspx?id=6442472684. Accessed 14/06/2012.

69. MacKie RM, Bray C, Vestey J, et al. Melanoma incidence and mortality in Scotland 1979-2003. *Br. J. Cancer.* 2007;96(11):1772-1777.

70. Jemal A, Tiwari RC, Murray T, et al. Cancer Statistics, 2004. *CA. Cancer J. Clin.* 2004;54(1):8-29.

71. Curado M, Edwards B, Shin H, et al. Cancer Incidence in Five Continents, Vol IX. IARC Scientific Publications No. 160. ; 2007; Lyon.

72. Ferlay J, Bray F, Forman D, Mathers C, Parkin D. *Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10.* 2008.

73. MacKie RM, Hauschild A, Eggermont AM. Epidemiology of invasive cutaneous melanoma. *Ann. Oncol.* Aug 2009;20 Suppl 6:vi1-7.

74. Australian Institute of Health and Welfare (AIHW). Cancer survival and prevalence in Australia: period estimates from 1982 to 2010. Canberra: AIHW.; 2012.

75. Baade P, Coory M. Trends in melanoma mortality in Australia: 1950-2002 and their implications for melanoma control. *Aust. N. Z. J. Public Health.* 2005;29(4):383-386.

76. Coory M, Baade P, Aitken JF, Smithers M, McLeod R, Ring I. Trends for in situ and invasive melanoma in Queensland, Australia, 1982-2002. *Cancer Causes & Control.* 2006;17(1):21-27.

77. Green AC, Baade P, Coory M, Aitken JF, Smithers M. Population-based 20-Year survival among people diagnosed with thin melanomas in Queensland, Australia. *J. Clin. Oncol.* 2012;30(13):1462-1467.

78. Tracey E, Baker D, Chen W, Stavrou E, Bishop J. *Cancer in New South Wales: Incidence, Mortality and Prevalence 2005.* Sydney: Cancer Institute NSW;2007.

79. Tracey E, Barraclough H, Chen W, et al. *Survival from cancer in New South Wales: 1980 - 2003.* Sydney: Cancer Institute NSW Monograph;2007.

80. Autier P, J-F. D, Eggermont AM, Coebergh JW. Epidemiological evidence that UVA radiation is involved in the genesis of cutaneous melanoma. *Curr. Opin. Oncol.* 2011(23):189-196.

81. International Agency for Research on Cancer Working Group on artificial ultraviolet (UV) light and skin cancer (IARC). The association of use of sunbeds and cutaneous malignant melanoma and other skin cancers: A systemic review. *Int. J. Cancer.* 2006;120:1116-1122.

82. Lazovich D, Vogel R, Berwick M, Weinstock MA, Anderson K, Warshaw EM. Indoor tanning and risk of melanoma: A case-control study in a highly exposed population *Cancer Epidemiology Biomarkers and Prevention.* 2010;19(6):1557-1568.

83. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. *Eur. J. Cancer.* 2005c;41(14):2040-2059.

84. Brenner A, Lubin J, Calista D, Landi M. Instrumental measurements of skin color and skin ultraviolet light sensitivity and risk of Cutaneous Malignant Melanoma:

A case-control study in an Italian population. *Am. J. Epidemiol.* 2002;156(4):353-362.

85. Dwyer T, Blizzard L, Ashbolt R, Plumb J, Berwick M, Stankovich JM. Cutaneous melanin density of Caucasians measured by spectrophotometry and risk of malignant melanoma, basal cell carcinoma, and squamous cell carcinoma of the skin. *Am. J. Epidemiol.* 2002;155(7):614-621.

86. Whiteman DC, Green AC. A risk prediction tool for melanoma? *Cancer Epidemiology, Biomarkers & Prevention.* 2005;14(4):761-763.

87. Park SL, Le Marchand L, Wilkens LR, et al. Risk factors for malignant melanoma in white and non-white/non-African American populations: the multiethnic cohort. *Cancer Prev Res (Phila).* 2012;5(3):423-434.

88. Boniol M, De Vries E, Coebergh JW, Dore JF. Seasonal variation in the occurrence of cutaneous melanoma in Europe: influence of latitude. An analysis using the EUROCARE group of registries. *Eur. J. Cancer.* 2005;41(1):126-132.

89. Armstrong BK, Kricker A, English DR. Sun exposure and skin cancer. *Australian Journal of Dermatology.* 1997;38(Suppl. 1):S1-S6.

90. Whiteman DC, Green A. Melanoma and sun exposure: where are we now? *Int. J. Dermatol.* 1999(38):481-489.

91. Elwood JM. Melanoma and sun exposure: contrasts between intermittent and chronic exposure. *World J. Surg.* 1992;16(2):157-165.

92. Gandini S, Raimondi S, Gnagnarella P, Doré J-F, Maisonneuve P, Testori A. Vitamin D and skin cancer: A meta-analysis. *Eur. J. Cancer.* 2009;45(4):634-641.

93. Chang YM, Barrett JH, Bishop DT, et al. Sun exposure and melanoma risk at different latitudes: a pooled analysis of 5700 cases and 7216 controls. *Int. J. Epidemiol.* 2009;3(38):814-830.

94. Lee JAH, Strickland D. Malignant melanoma: social status and outdoor work. *Br. J. Cancer.* 1980(41):757-763.

95. Siskind V, Whiteman DC, Aitken JF, Martin NG, Green AC. An analysis of risk factors for cutaneous melanoma by anatomical site (Australia). *Cancer Causes & Control.* 2005(16):193-199.

96. Whiteman DC, Stickley M, Watt P, Hughes MC, Davis MB, Green AC. Anatomic site, sun exposure, and risk of cutaneous melanoma. *J. Clin. Oncol.* 2006;24(19):3172-3177.

97. Rivers JK. Is there more than one road to melanoma? *Lancet.* 2004;363(9410):728-730.

98. Whiteman DC, Watt P, Purdie DM, Hughes MC, Hayward NK, Green AC. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. *J. Natl. Cancer Inst.* 2003;95(11):806-812.

99. Liu W, Kelly JW, Trivett M, et al. Distinct clinical and pathological features are associated with the BRAFT1799A(V600E) mutation in primary melanoma. *J. Invest. Dermatol.* 2006;127(4):900-905.

100. Hacker E, Nagore E, Cerroni L, et al. NRAS and BRAF mutations in cutaneous melanoma and the association with MC1R genotype: findings from Spanish and Austrian populations. *J. Invest. Dermatol.* 2012. http://dx.doi.org/10.1038/jid.2012.385.

101. Rouzaud F, Kadekaro AL, Abdel-Malek ZA, Hearing VJ. MC1R and the response of melanocytes to ultraviolet radiation. *Mutat. Res.* 2005;571(1-2):133-152.

102. Flaherty K, Puzanov I, Sosman J, et al. Phase 1 study of PLX4032: Prrof of concept for V600E BRAF mutation as as therapeutic target in human cancer. 2009 ASCO Annual Meeting; 2009.

103. Goldstein AM, Chan M, Harland M, et al. Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. *J. Med. Genet.* 2007;2(44):99-106.

104. Berwick M, Orlow I, Hummer AJ, et al. The prevalence of CDKN2A germ-line mutations and relative risk for cutaneous malignant melanoma: an international population-based study. *Cancer Epidemiology Biomarkers & Prevention.* 2006;8(15):1520-1525.

105. McPherson M, Elwood M, English DR, Baade PD, Youl PH, Aitken JF. Presentation and detection of invasive melanoma in a high-risk population. *J. Am. Acad. Dermatol.* 2006;54(5):783-792.

106. Rigel DS, Friedman RJ, Kopf AW, Polsky D. ABCDE--an evolving concept in the early detection of melanoma. *Arch. Dermatol.* 2005;141(8):1032-1034.

107. Menzies SW, Zalaudek I. Why perform dermoscopy?: The evidence for its role in the routine management of pigmented skin lesions. *Arch. Dermatol.* 2006;142(9):1211-1212.

108. Greco M, Mitri MD, Chiriacò F, Leo G, Brienza E, Maffia M. Serum proteomic profile of cutaneous malignant melanoma and relation to cancer progression: Association to tumor derived alpha-N-acetylgalactosaminidase activity. *Cancer Lett.* 2009;283(2):222-229.

109. Scottish Intercollegiate Guidelines Network (SIGN). Guideline No 72. Cutaneous Melanoma: A National Clinical Guideline. Updated February 2004. 2003. http://www.sign.ac.uk/guidelines/fulltext/72/index.html. Accessed 20-05-2010.

110. Eng W, Tschen JA. Comparison of S-100 Versus Hematoxylin and Eosin Staining for Evaluating Dermal Invasion and Peripheral Margins by Desmoplastic Malignant Melanoma. *The American Journal of Dermatopathology.* 2000;22(1):26-29.

111. Gaynor R, Irie R, Morton DL, Herscman H. S100 protein is present in cultured human malignant melanomas. *Nature.* 1980(286):400-401.

112. Chamberlain AJ, Fritschi L, Giles G, Dowling JP, Kelly JW. Nodular type and older age as the most significant associations of thick melanoma in Victoria, Australia. *Arch. Dermatol. Res.* 2002;138(5):609-614.

113. Holman CD, Armstrong B, Heenan P. Relationship of cutaneous malignant melanoma to individual sunlight-exposure habits. *J. Natl. Cancer Inst.* 1986;76(3):403-414.

114. Hansen MG, McCarten AB. Tumor thickness and lymphocytic infiltration in malignant melanoma of the head and neck. *Am. J. Surg.* 1974(128):557.

115. Massi D, LeBoit PE. *Patterns of melanoma.* Darnstadt 2004.

116. Balch CM, Soong S-J, Gershenwald JE, et al. Prognostic factors analysis of 17,600 melanoma patients: Validation of the American Joint Committee on Cancer Melanoma Staging System. *J. Clin. Oncol.* 2001;19(16):3622-3634.

117. Balch CM, Buzaid AC, Soong SJ, et al. Final version of the American Joint Committee on Cancer staging system for cutaneous melanoma. *J. Clin. Oncol.* 2001;19(16):3635-3648.

118. Cummins DL, Cummins JM, Pantle H, Silverman MA, Leonard AL, Chanmugam A. Cutaneous malignant melanoma. *Mayo Clin. Proc.* 2006;81(4):500-507.

119. Bafounta M-L, Beauchet A, Chagnon S, Saiag P. Ultrasonography or palpation for detection of melanoma nodal invasion: a meta-analysis. *The Lancet Oncology.* 2004;5(11):673-680.

120. Fink AM, Holle-Robatsch S, Herzog N, et al. Positron emission tomography is not useful in detecting metastasis in the sentinel lymph node in patients with primary malignant melanoma stage I and II. *Melanoma Res.* 2004;14(2):141-145.

121. Terhune MH, Swanson N, Johnson TM. Use of Chest Radiography in the Initial Evaluation of Patients With Localized Melanoma. *Arch. Dermatol.* 1998;134(5):569-572.

122. Buzaid AC, Sandler AB, Mani S, et al. Role of computed tomography in the staging of primary melanoma. *J. Clin. Oncol.* 1993;11(4):638-643.

123. Tas F, Yasasever V, Duranyildiz D, et al. Clinical value of protein S100 and melanoma-inhibitory activity (MIA) in malignant melanoma. *Am. J. Clin. Oncol.* 2004;27(3):225-228.

124. Bolander A, Agnarsdottir M, Wagenius G, et al. Serological and immunohistochemical analysis of S100 and new derivatives as markers for prognosis in patients with malignant melanoma. *Melanoma Res.* 2008;18(6):412-419.

125. Harpio R, Einarsson R. S100 proteins as cancer biomarkers with focus on S100B in malignant melanoma. *Clin. Biochem.* 2004;37(7):512-518.

126. Smit LHM, Korse CM, Hart AAM, et al. Normal values of serum S-100B predict prolonged survival for stage IV melanoma patients. *Eur. J. Cancer.* 2005;41(3):386-392.

127. Vollmer RT. Malignant melanoma: A multivariate analysis of prognostic factors. *Pathol. Annu.* 1989(24):383-407.

128. MacKie RM, Aitchison T, Sirel JM, McLaren K, Watt DC. Prognostic models for subgroups of melanoma patients from the Scottish Melanoma Group database 1979-86, and their subsequent validation. *Br. J. Cancer.* 1995;71(1):173-176.

129. Barnhill R, Fine J, Rousch G, Berwick M. Predicting five-year outcome for patients with cutaneous melanoma in a population-based study. *Cancer.* 1996(78):427-432.

130. Azzola MF, Shaw HM, Thompson JF, et al. Tumor mitotic rate is a more powerful prognostic indicator than ulceration in patients with primary cutaneous melanoma. *Cancer.* 2003;97(6):1488-1498.

131. Thompson JF, Soong S-J, Balch CM, et al. Prognostic significance of mitotic rate in localized primary cutaneous melanoma: An analysis of patients in the multi-institutional American Joint Committee on Cancer Melanoma Staging Database. *J. Clin. Oncol.* 2011;29(21):2199-2205.

132. Larsen T, Grude T. A retrospective histological study of 669 cases of primary cutaneous malignant melanoma in clinical stage 1.6. The relation of dermal solar elastosis to sex, age and survival of the patient and to localization, histological type and level of invasion of the tumour. *Acta Pathol. Microbiol. Scand.* 1979;87A(5):361-366.

133. Sondergaard K, Schou G. Prognostic factors in primary cutaneous melanoma. *Am. J. Dermatopathol.* 1985;7 Suppl: p. 1-4.

134. Vollmer RT. Solar elastosis in cutaneous melanoma. *Am. J. Clin. Pathol.* 2007;128(2):260-264.

135. Knutson C, Hori J, Spratt JJ. *Melanoma.* Chicago: Year Book Med. Publishers Inc; 1971.

136. Heenan P, English DR, Holman CDJ, Armstrong B. Survival among patients with clinical stage 1 cutaneous malignant melanoma diagnosed in Western Australia in 1975/1976 and 1980.1981. *Cancer.* 1991;68(9):2079-2087.

137. Boniol M, Armstrong BK, Dore JF. Variation in incidence and fatality of melanoma by season of diagnosis in New South Wales, Australia. *Cancer Epidemiology, Biomarkers & Prevention.* 2006;15(3):524-526.

138. Rosso S, Sera F, Segnan N, Zanetti R. Sun exposure prior to diagnosis is associated with improved survival in melanoma patients: Results from a long-term follow-up study of Italian patients. *Eur. J. Cancer.* 2008;44(9):1275-1281.

139. Shipman A, Clark A, Levell N. Sunnier European countries have lower melanoma mortality. *Clinical & Experimental Dermatology.* 2011(36):544-547.

140. Osborne JE, Hutchinson PE. Vitamin D and systemic cancer: is this relevant to malignant melanoma? *Br. J. Dermatol.* 2002;147(2):197-213.

141. Hochberg Z, ed *Vitamin D and Rickets.* Basel: Karger; 2003. Savage MO, ed. Endocrine Development.

142. Stolzt VD, ed *New topics in vitamin D research.* 1 ed. New York: Nova Science Publishers; 2006. Stolzt VD, ed.

143. Park EA. The use if vitamin D preparations in the prevention and treatment of disease *Journal of The American Medical Association.* 1938;111(13):1179-1187.

144. Johnson MA, Kimlin MG. Vitamin D, aging, and the 2005 Dietary Guidelines for Americans. *Nutr. Rev.* 2006;64(9):410-421.

145. Holick MF. Vitamin D: The underappreciated D-lightful hormone that is important for skeletal and cellular health. *Current Opinion in Endocrinology and Diabetes.* 2002(9):87-98.

146. Glerup H, Mikkelsen K, Poulsen L, et al. Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. *J. Intern. Med.* 2000;247(2):260-268.

147. Wolpowitz D, Gilchrest BA. The vitamin D questions: How much do you need and how should you get it? *J. Am. Acad. Dermatol.* 2006;54(2):301-317.

148. Reinertson RP, Wheatley VR. Studies on the Chemical Composition of Human Epidermal Lipids. *The Journal Of Investigative Dermatology.* 1959;32(1):49-59.

149. Kelley R. Diagnosis of Smith-Lemli-Opitz Syndrome by gas chromatography or mass-spectrometry of 7-dehydrocholesterol in plasma, amniotic fluid and cultured skin fibroblasts. *Clin. Chim. Acta.* 1995(236):45-58.

150. Rog T, Vattulainen I, Jansen M, Ikonen E, Karttunen M. Comparison of cholesterol and its direct precursors along the biosynthetic pathway: Effects of cholesterol, desmosterol and 7-dehydrocholesterol on saturated and unsaturated lipid bilayers. *J. Chem. Phys.* 2008;129(15):154508.

151. Holick MF, MacLaughlin JA, Clark MB, et al. Photosynthesis of previtamin D_3 in human skin and the physiologic consequences. *Science.* 1980;210(4466):203-205.

152. Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. *Am. J. Clin. Nutr.* Dec 2004;80(6):1678S-1688S.

153. Wheatley VR, Reinertson RP, Sher D. The presence of vitamin D precursors in human epidermis. *The Journal Of Investigative Dermatology.* 1958;31(1):51-54.

154. Bekemeier H. Versuche zur maximalen antirachitischen UV -Aktivierung isolierter menschlicher Haut. *Acta biol. med. germ.* 1958;1:765-767.

155. MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D_3. *The Journal Of Clinical Investigation.* 1985;76(4):1536-1538.

156. Calvo MS, Whiting SJ, Barton CN. Vitamin D intake: A global perspective of current status. *The Journal of Nutrition.* 2005;135(2):310.

157. Vieth R, Fraser D. Vitamin D insufficiency: no recommended dietary allowance exists for this nutrient. *CMAJ: Canadian Medical Association Journal.* 2002;166(12):1541.

158. National Institutes of Health. Dietary supplement fact sheet: Vitamin D. 2010; http://ods.od.nih.gov/factsheets/VitaminD_HealthProfessional/. Accessed 12/08/2013, 2013.

159. Holick MF. Vitamin D status: measurement, interpretation and clinical application. *Ann. Epidemiol.* 2009;19(2):73-78.

160. International Agency for Research on Cancer (IARC). *Vitamin D and Cancer/ a report of the IARC Working Group on Vitamin D (IARC Working Group Reports; 5).* Lyon2008.

161. Lee JH, O'Keefe JH, Bell D, Hensrud DD, Holick MF. Vitamin D deficiency: An important, common, and easily treatable cardiovascular risk factor? *J. Am. Coll. Cardiol.* 2008;52(24):1949-1956.

162. Adams JS, Clemens TL, Parrish JA, Holick MF. Vitamin-D synthesis and metabolism after ultraviolet irradiation of normal and vitamin-D-deficient subjects. *N. Engl. J. Med.* 1982;306:722-725.

163. Reichel H, Koeffler HP, Norman AW. The role of the vitamin D endocrine system in health and disease. *N. Engl. J. Med.* 1989(320):980-991.

164. Bouillon R. Vitamin D status in man.

165. Bouillon R, Carmeliet G, Verlinden L, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr. Rev.* 2008;29(6):726-776.

166. Raimondi S, Johansson H, Maisonneuve P, Gandini S. Review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. *Carcinogenesis.* 2009;30(7):1170-1180.

167. Bouillon R, Eelen G, Verlinden L, Mathieu C, Carmeliet G, Verstuyf A. Vitamin D and cancer. *J. Steroid Biochem. Mol. Biol.* 2006;102:156-162.

168. Haussler M, Whitfield G, Haussler C, et al. The nuclear vitamin D receptor: biological and molecular regulatory properties revealed. *J. Bone Miner. Res.* 1998;13(3):325-349.

169. Reichrath J, Rech M, Moeini M, Meineke V, Tilgen W, Seifert M. Modulation of Vitamin D-induced growth inhibition in melanoma cell lines: implications for an important function of vitamin D receptor (VDR) and 1,25-dihydroxyvitamin D_3-24-hydroxylase (24-OHase) expression, histonedeacetylation, and calpain activity. 3rd Satellite Symposium of the Working Group Dermato-Endocrinology of the German Society for Skin Research (ADF); 2005; Dresden.

170. Mozolowski W. Jedrzej Snaidecki (1768-1838). *Nature.* 1939(3612):121.

171. Mellanby T. The part played by an "accessory factor" in the production of experimental rickets. *Journal of Physiology (Proceedings of the Physiological Society).* 1918;52:X1-X2.

172. Dawson-Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth R. Estimates of optimal vitamin D status. *Osteoporos. Int.* 2005;16(7):713-716.

173. Lips P. Which circulating level of 25-hydroxyvitamin D is appropriate. *J. Steroid Biochem. Mol. Biol.* 2004;89-90(1-5):611-614.

174. Vieth R. What is the optimal vitamin D status. *Prog. Biophys. Mol. Biol.* 2006;92(1):26-32.

175. Nesby-O'Dell S, Scanlon K, Cogswell M. Hypovitaminosis D prevalence and determinants among African American and white women of reproductive age: Third National Health and Nutrition Examination Survey, 1988-1994. *Am. J. Clin. Nutr.* 2002(76):187-192.

176. Holick MF. High prevalence of vitamin D inadequacy and implications for heath. *Mayo Clin. Proc.* 2006(81):355-373.

177. Heaney RP. The Vitamin D requirement in health and disease. *The Journal of Steroid Biochemistry and Molecular Biology.* 2005;97(1–2):13-19.

178. Institute of Medicine (IOM). *Dietary reference intakes for calcium and vitamin D.* Washington DC: The National Academies Press;2011.

179. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am. J. Clin. Nutr.* 2006;84(1):18-28.

180. Holick MF. Vitamin D Status: Measurement, interpretation, and clinical application. *Ann. Epidemiol.* 2009;19(2):73-78.

181. Binkley N, Krueger D, Lensmeyer G. 25-hydroxyvitamin D measurement, 2009: A review for clinicians. *J. Clin. Densitom.* 2009;12(4):417-427.

182. Nowak M, Harrison S, Buettner PG, et al. Vitamin D status of adults from tropical Australia determined using two different laboratory assays: implications for public health messages. *Photochemical & Photobiological Sciences.* 2011(87):935-943.

183. Wang SQ. Epidemiology of vitamin D in health and disease. *Nutrition Research Reviews.* 2009(22):188-203.

184. Melamed ML, Michos ED, Post W, Astor B. 25-Hydroxyvitamin D Levels and the Risk of Mortality in the General Population. *Arch. Intern. Med.* 2008;168(15):1629-1637.

185. Mondul AM, Weinstein SJ, Männistö S, et al. Serum vitamin D and risk of bladder cancer. *Cancer Res.* 2010(70):9218-9223.

186. Liang G, Nan H, Qureshi AA, Han J. Pre-diagnostic plasma 25-hydroxyvitamin d levels and risk of non-melanoma skin cancer in women. *PLoS ONE.* 2012;7(4):e35211.

187. Giovannucci E. Vitamin D and cancer incidence in the Harvard cohorts. *Ann. Epidemiol.* 2009;19(2):84-88.

188. Garland CF, Gorham ED, Mohr SB, et al. Vitamin D and prevention of breast cancer: pooled analysis. *The Journal of steroid biochemistry and molecular biology.* 2007;103(3-5):708-711.

189. Gorham ED, Garland CF, Garland FC, et al. Optimal vitamin D status for colorectal cancer prevention: a quantitative meta analysis. *Am. J. Prev. Med.* 2007;32(3):210-216.

190. Tang JY, Parimi N, Wu A, et al. Inverse association between serum 25(OH) vitamin D levels and non-melanoma skin cancer in elderly men. *Cancer Causes Control.* 2009;3(21):387-391.

191. Yudoh K, Matsuno H, Kimura T. 1alpha, 25-Dihydroxyvitamin D_3 inhibits *in vitro* invasiveness through the extracellular matrix and *in vivo* pulmonary metastasis of B16 mouse melanoma. *J. Lab. Clin. Med.* 1999;133:120-128.

192. Danielsson C, Fehsel K, Polly P, Carlberg C. Differential apoptotic response of human melanoma cells to 1 alpha,25-dihydroxyvitamin D3 and its analogues. *Cell Death Differ.* 1998;5(11):946-952.

193. Eisman J, Barkla D, Tutton P. Suppression of *in vivo* growth of human cancer solid tumour xenographs by 1,25-dihydroxyvitamin D_3. *Cancer Res.* 1987;47:21-25.

194. Halsall JA, Osborne JE, Potter L, Pringle JH, Hutchinson PE. A novel polymorphism in the 1A promoter region of the vitamin D receptor is associated with altered susceptibilty and prognosis in malignant melanoma. *Br. J. Cancer.* 2004;91(4):765-770.

195. Santonocito C, Capizzi R, Concolino P, et al. Association between cutaneous melanoma, Breslow thickness and vitamin D receptor Bs*m*I polymorphism. *Br. J. Dermatol.* 2007;156(2):277-282.

196. Randerson-Moor JA, Taylor JC, Elliott F, et al. Vitamin D receptor gene polymorphisms, serum 25-hydroxyvitamin D levels, and melanoma: UK case-control comparisons and a meta-analysis of published VDR data. *Eur. J. Cancer.* 2009;45(18):3271-3281.

197. Brozyna AA, Jozwicki W, Janjetovic Z, Slominski AT. Expression of vitamin D receptor decreases during progression of pigmented skin lesions. *Hum. Pathol.* 2011;42(5):618-631.

198. Weinstock MA, Stampfer MJ, Lew RA, Willett WC, Sober AJ. Case-control study of melanoma and dietary vitamin D: Implications for advocacy of sun protection and sunscreen se. *J. Invest. Dermatol.* 1992;98(5):809-811.

199. Asgari MM, Maruti SS, Kushi LH, White E. A cohort study of Vitamin D intake and melanoma risk. *J. Invest. Dermatol.* 2009;129(7):1675-1680.

200. Tang JY, Fu T, LeBlanc E, et al. Calcium Plus Vitamin D Supplementation and the Risk of Nonmelanoma and Melanoma Skin Cancer: Post Hoc Analyses of the Women's Health Initiative Randomized Controlled Trial. *J. Clin. Oncol.* 2011;29(22):3078-3084.

201. Millen AE, Tucker MA, Hartge P, et al. Diet and melanoma in a case-control study. *Cancer Epidemiology, Biomarkers & Prevention.* 2004;13(6):1042-1051.

202. Vinceti M, Malagoli C, Fiorentini C, et al. Inverse association between dietary vitmain D and risk of cutaneous melanoma in a northern Italy population. *Nutr. Cancer.* 2011;63(4):506-513.

203. Reichrath J, Querings K. No evidence for reduced 25-hydroxyvitamin D serum levels in melanoma patients. *Cancer Causes Control.* 2004;15(1):97-98.

204. Major JM, Kiruthu C, Weinstein SJ, et al. Pre-diagnostic circulating vitamin d and risk of melanoma in men. *PLoS ONE.* 2012;7(4):e35112.

205. Freiman A, Yu J, Loutfi A, Wang B. Impact of melanoma diagnosis on sun-awareness and protection: efficacy of education campaigns in a high-risk population. *J. Cutan. Med. Surg.* 2004;8(5):303-309.

206. Idorn LW, Philipsen PA, Wulf HC. Sun exposure before and after a diagnosis of cutaneous malignant melanoma: estimated by developments in serum vitamin D, skin pigmentation and interviews. *Br. J. Dermatol.* 2011;165(1):164-170.

207. Garland C, Gorham E, Mohr S, Garland F. Vitamin D for cancer prevention - global perspective
Ann. Epidemiol. 2009;19(7):468-483.

208. Garland C. Symposium in print on the epidemiology of Vitamin D and cancer. *Ann. Epidemiol.* 2009;19:439.

209. Heaney RP. Vitamin D and calcium interactions: functional outcomes. *The American Journal of Clinical Nutrition.* 2008;88(2):541S-544S.

210. Mealing N, Banks E, Jorm L, Steel D, Clements M, Rogers K. Investigation of relative risk estimates from studies of the same population with contrasting response rates and designs. *BMC Med. Res. Methodol.* 2010;10(1):26.

211. Weatherall IL, Coombs BD. Skin color measurements in terms of CIELAB color space values. *J. Invest. Dermatol.* 1992;99(4):468-473.

212. Smit LHM, Korse CM, Bonfrer JMG. Comparison of four different assays for determination of S-100B. *Int. J. Biol. Markers.* 2005;20(1):34-42.

213. Domingo-Domenech J, Castel T, Auge J, et al. Prognostic implications of protein S-100B serum levels in the clinical outcome of high-risk melanoma patients. *Tumour Biol.* 2007(28):264-272.

214. Worldatlas.com. 2011; http://www.worldatlas.com/aatlas/latitude_and_longitude_finder. Accessed 29-10-2011.

215. *Stata Statistical Software: Release 11.* [computer program]. College Station, TX: StataCorp LP.; 2009.

216. Whiteman D, Green A. Melanoma and sunburn. *Cancer Causes Control.* 1994;5:564-572.

217. Dennis LK, Vanbeek MJ, Beane Freeman LE, Smith BJ, Dawson DV, Coughlin JA. Sunburns and risk of cutaneous melanoma: does age matter? A comprehensive meta-analysis. *Ann. Epidemiol.* 2008;18(8):614-627.

218. Del Bino S, Sok J, Bessac E, Bernerd F. Relationship between skin response to ultraviolet exposure and skin color type. *Pigment Cell Res.* 2006;19(6):606-614.

219. Breslow A. Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. *Ann. Surg.* 1970;172(5):902-908.

220. Belsley DA, Kuh E, Welsch RE. *Regression Diagnostics: Identifying Influential Data and Sources of Collinearity.* New York: Wiley Interscience; 1980.

221. Griffith DA, Amerhein. CG. *Multivariate statistical analysis for geographers.* Englewood Cliffs: Prentice Hall; 1997.

222. Neter J, Kutner MH, Nachtsheim CJ, Wasserman W, Chicago, Illinois, USA. *Applied linear regression models.* 3rd ed. Chicago: Times Mirror Higher Education Group; 1996.

223. Lang TA, Secic M. *How to Report Statistics in Medicine.* Philadelphia: American College of Physicans; 2003.

224. Webb P, Bain C, Pirozzo S. *Essential epidemiology: An introduction for students and health professionals.* New York: Cambridge University Press; 2005.

225. Hosmer D, Lemeshow S. Applied logistic regression. 2 ed: John Wiley & Sons, Inc.; 2004. Accessed 26-06-2012.

226. Gilbert R, Metcalfe C, Fraser WD, et al. Associations of circulating 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, and vitamin D pathway genes with

prostate-specific antigen progression in men with localized prostate cancer undergoing active monitoring. *Eur. J. Cancer Prev.* 2013;22(2):121-125.

227. Fang F, Kasperzyk JL, Shui I, et al. Prediagnostic plasma vitamin D metabolites and mortality among patients with prostate cancer. *PLoS ONE.* 2011;6(4):1-6.

228. Ren C, Qiu M-z, Wang D-s, et al. Prognostic effects of 25-hydroxyvitamin D levels in gastric cancer. *Journal of Translational Medicine.* 2012;10(16):1-7.

229. Peppone L, Rickles A, Janelsins M, Insalaco M, Skinner K. The association between breast cancer prognostic indicators and serum 25-OH vitmain D levels. *Ann. Surg. Oncol.* 2012(19):2590-2599.

230. Morris HA, Anderson PH. Autocrine and paracrine actions of vitamin d. *The Clinical biochemist. Reviews / Australian Association of Clinical Biochemists.* 2010;31(4):129-138.

231. DeLuca H. Overview of general physiologic features and functions of vitamin D. *Am. J. Clin. Nutr.* 2004(80):1689S–1696S.

232. Holick MF. McCollum Award Lecture, 1994: Vitamin D - New horizons for the 21st century. *Am. J. Clin. Nutr.* 1994;60:619-630.

233. Lips P, Chapuy M, Dawson-Hughes B, Pols H, Holick MF. An international comparison of serum 25-hydroxyvitamin D measurements. *Osteoporos. Int.* 1999(9):394-397.

234. Harrison S, Nowak M, Buettner PG, et al. Public health and clinical dilemmas resulting from imprecise vitamin D tests. *Journal of Rural and Tropical Public Health.* 2009;8:52-58.

235. Lai J, Lucas RM, Banks E, Ponsonby A-L. Variability in vitamin D assays impairs clinical assessment of vitamin D status. *J. Intern. Med.* 2012;42(1):43-50.

236. Nessvi S, Johansson L, Jopson J, et al. Association of 25-hydroxyvitamin D3 levels in adult New Zealanders with ethnicity, skin color and self-reported skin sensitivity to sun exposure. *Photochemical & Photobiological Sciences.* 2011;87(5):1173-1178.

237. Rockell JE, Skeaff C, Williams S, Green T. Association between quantitative measures of skin color and plasma 25-hydroxyvitamin D. *Osteoporos. Int.* 2008;19(11):1639-1642.

238. Shriver MD, Parra EJ. Comparison of narrow-band reflectance spectroscopy and tristimulus colorimetry for measurements of skin and hair color in persons of different biological ancestry. *Am. J. Phys. Anthropol.* 2000;112(1):17-27.

239. Rampen FH, Fleuren BA, de Boo TM, Lemmens WA. Unreliability of self-reported burning tendency and tanning ability. *Arch. Dermatol.* 1988;124(6):885-888.

240. Thieden E, Philipsen PA, Sandby-Moller J, Wulf HC. Sunscreen use related to UV exposure, age, sex, and occupation based on personal dosimeter readings and sun-exposure behavior diaries. *Arch. Dermatol. Res.* 2005;141(8):967-973.

241. Bouwhuis MG, Suciu S, Kruit W, et al. Prognostic value of serial blood S100B determinations in stage IIB-III melanoma patients: A corollary study to EORTC trial 18952. *Eur. J. Cancer.* 2010;47(3):361-368.

242. Abraha H, Fuller L, Du Vivier A, Higgins E, Sherwood R. Serum S-100 protein: a potentially useful prognostic marker in cutaneous melanoma. *Br. J. Dermatol.* 1997(137):381-385.

243. Smith PG, Day NE. The design of case-control studies: influence of confounding and interaction effects. *Int. J. Epidemiol.* 1984;13(3):356–365.

244. Queensland Government. *Melanoma in Queensland: An Overview 2012.* Queensland Health, Brisbane 2012.

245. Youl PH, Youlden DR, Baade PD. Changes in the site distribution of common melanoma subtypes in Queensland, Australia over time: implications for public health campaigns. *Br. J. Dermatol.* 2013;168:136-144.

Appendices

Appendix A

QUT Melanoma Study protocol

Appointment/Interview Guidelines

Appointment 1 (T1)

- Dr Cockburn/Cheng to give patient introductory letter
- If patient wishes to be involved in the study, CW to meet and greet patient
- Spend approximately 30 minutes with patient and give them a personal folder (identified with their study number) containing the PIS to read, the consent form to sign and the questionnaire to take home)
- Give patient a copy of the consent form to place in their study folder
- Make post-operative appointment and ask patient to bring the completed questionnaire with them to the post-operative appointment
- Take patient down to Sullivan & Nicolaides collection room for first blood collection and introduce them to phlebotomist
- If patients are ordered any other tests (e.g. scans), take them to the appropriate departments and organise tests for them

Appointment 2 (T2)

Prior to appointment

- Organise patient folder (USE **PENCIL** TO RECORD DETAILS)
- Ensure visual aids are present
- Check alcohol wipes and skin wipes are available

During appointment

- Clarify any questions the participant may have regarding the study
- Check completed survey and mark any queries with sticky tags
- Ensure spectrometer is turned on and is blank against the paper

Skin Reflectance Test

- Prior to conducting test, ask participant about use of tanning products over the last month. Explain why the use of these products will negate need for testing at this interview
- Wipe the skin sites with alcohol wipes and leave to dry for several minutes
- While skin is drying ask hair, eye and skin colour questions
- Take the readings at the left inner upper arm, left dorsum of forearm and the central forehead **(as per collection instruction guidelines)**
- Write these readings down by hand on the clinical examination form
- Explain, if asked, the meanings of these readings

Naevi Count

- Looking at freckle and lentigo counts
- Solar keratoses – red, scaly rough texture; need to touch to identify
- Naevi count
- Check eyes for naevi
- Check sun-exposed sites first always checking left side then right
- Ask participant to stand under good light and check hand, forearm, upper arm, and then check neck front then back
- Ask participant to remove shirt
- Examine shoulders and upper back (leave bra on and count by lifting up straps)
- Lower back – down to belt line/2.5 cms below umbilicus
- Progress around to stomach, making sure not to count lesions twice
- Count chest down to bra line, participant can put shirt back

Questions to ask:

- Melanoma family history
- solarium use
- anatomic site of melanoma
- hair as teenager (visual aids)
- solar keratoses and skin cancers (clarify if patient is aware of differences)

Appendix B

Introduction to study letter

The QUT Melanoma Study

Have you heard about vitamin D? Did you know that vitamin D levels in your blood may be affected by how much sun exposure you are getting? A new research study, The QUT Melanoma Study, is conducting a study to explore the relationship between sun exposure, vitamin D and melanoma in Australian adults.

Vitamin D has many roles in the human body and having adequate amounts of vitamin D is very important for building and maintaining a healthy skeleton throughout life. However, we also know that most of the vitamin D we need comes from the sun and the sun is considered to be a risk factor for the development of melanoma. In Queensland we have one of the highest rates of melanoma in the world, yet recent studies conducted in Brisbane have shown that almost one-third of the population may have low levels of vitamin D. This is one reason why we need to explore and understand more about the relationship between sun exposure, vitamin D and melanoma. The knowledge we gain may help in the fight against melanoma.

We would like to invite you to participate in The QUT Melanoma Study.

The QUT Melanoma Study has received full ethical approval (QUT ethics certificate number 0900000681).

We are inviting adults aged over 18 years, who live in Queensland and who have been newly diagnosed with melanoma, to join the study.
Could you please let us know whether or not you would like to learn more about the study by signing on the reverse of this page? If you indicate that you are interested in reading more, we will give you an information sheet that explains in more detail why we are conducting The QUT Melanoma Study and what is involved in participating.

QUT research student, Candy Wyatt, will then contact you shortly after this to arrange a meeting at your convenience.

If you do not want to take part in this study we will not contact you again.

Thank you for your consideration to support this important research.

Yours sincerely,

Professor Michael Kimlin and Candy Wyatt (PhD Student)
AusSun Research Laboratory
Institute of Health and Biomedical Innovation
Queensland University of Technology
60 Musk Avenue
Kelvin Grove QLD 4059

CONFIDENTIAL

Date: _____

Name (please print): _____

Address (please print): _____

_____

Phone numbers: _____

Email: _____

☐ **YES,** I would like to know more about this study

PLEASE NOTE*: Ticking this box does <u>not</u> commit you to anything at this stage.*

OR

☐ **NO,** I do not wish to be involved in this study.

THANK YOU

Appendix C

QUT Melanoma Study consent form

CONSENT FORM for QUT RESEARCH PROJECT

"The QUT Melanoma Study"

Research Team Contacts	
Professor Michael Kimlin (Principal Researcher): **Phone: 3138 5802** **Mobile: 0401 313 534** **Email**: m.kimlin@qut.edu.au	**Candy Wyatt** (Associate researcher/ PhD student): **Phone: 3138 0275** **Mobile: 0418 748 172** **Email**: candy.wyatt@qut.edu.au

Statement of consent

By signing below, you are indicating that you:

- have read and understood the information document regarding this project
- have had any questions answered to your satisfaction
- understand that if you have any additional questions you can contact the research team
- understand that you are free to withdraw at any time, without comment or penalty
- understand that you can contact the Research Ethics Coordinator on +61 7 3138 2091 or ethicscontact@qut.edu.au if you have concerns about the ethical conduct of the project
- agree to participate in the project
- agree / disagree for testing, storage and re-testing of serum samples (please indicate by placing a cross in the appropriate box)

☐ I agree to the use of my blood and tissue samples for this project only (specific consent).

☐ I agree to the use of my samples for this project and for closely related projects in the future (extended consent). In this event I understand that my samples will be de-identified.

☐ I wish to be contacted if you intend to use my stored samples for future research.

Name .....

Signature .....

Date ..... / ..... / .....

Appendix D

QUT Melanoma Study Participant Information Sheet

PARTICIPANT INFORMATION for QUT RESEARCH PROJECT

"The QUT Melanoma Study"

Research Team Contacts	
Professor Michael Kimlin (Principal Researcher): **Phone: 3138 5802** **Mobile: 0401 313 534** **Email:** m.kimlin@qut.edu.au	**Candy Wyatt** (Associate researcher/ PhD student): **Phone**: **3138 0275** **Mobile**: **0418 748 172** **Email:** candy.wyatt@qut.edu.au

Description

This study is being undertaken as part of a PhD project to look at the inter-relationships between sun exposure, vitamin D and melanoma. It is being conducted by a PhD research student based at the **Queensland University of Technology's** Institute of Health and Biomedical Innovation (IHBI) AusSun Research Laboratory – Candy Wyatt.

There is very little information about how sun exposure and vitamin D levels might affect the development of melanoma in humans. This is an important gap in our understanding, given the high sun exposure of Australians in general and our growing interest in the role that vitamin D plays in many health outcomes. In this study we aim to examine how factors such as individual sun exposure and sun protection habits, personal characteristics (like skin, eye and hair colour, and number of naevi/moles) and vitamin D levels affect melanoma development and characteristics.

The research team requests your assistance because we require the participants in this study to be adults aged 18 years and over, with a diagnosed melanoma.

Participation

Your participation in this study is voluntary. If you do agree to participate, you can withdraw from participation at any time during the study without comment or penalty. Your decision to participate will in no way impact upon your current or future relationship with QUT (for example, your grades if you are a student there), or with your treating medical practitioner.

If I decide to take part, what do I have to do?

Your involvement would include:

- the collection of a venous blood sample prior to your surgery,

- completing a survey to provide basic data about you – your age, gender, education etc – and also about your skin type, past sun-exposure history, sun protection habits and any history of skin cancer,
- an examination of your skin to count moles, freckles and sunspots (by a registered nurse)and
- an assessment of your skin colour at three body sites (your inner-upper arm, hand and face), using a hand-held spectrophotometer (a painless test that shines light on the skin and measures reflected light),
- having 2 more blood samples taken – one at 6 months and one at 12 months after your first sample was taken,
- giving consent for our research team to examine some tissue samples from your melanoma. This does not involve you directly. The pathologist would send us the samples after they have completed their examination of the excised specimen.

Who will take my blood and where will this be done?

You will be able to have your blood samples taken by trained personnel at any Sullivan & Nicolaides Pathology collection room. All other parts of the study will be conducted in your doctor's rooms at your convenience.

What happens to my blood and tissue samples?

Blood samples: All of the blood samples will be stored in a freezer at QUT until the end of the data collection phase of the study. At that time, one portion of the initial blood sample will be tested for the level of vitamin D and a tumour biomarker called S100BB. Another portion of that blood sample will remain stored for possible future research looking at genetic risk factors for melanoma. The samples collected at 6 and 12 months will also remain stored for possible future research looking at levels of vitamin D and S100BB in relation to your melanoma.

Tissue Samples will be examined under the microscope to look for a number of different cells and proteins that may be present in melanoma tissue.

All blood and tissue samples are stored securely, are only identified by a code and will be accessed only by qualified medical researchers.

Expected benefits

It is expected that this study will not benefit you. However, it may benefit the general population in the future.

Will I find out the results of research using my samples?

Due to the length of this study, the **overall results** will not be available for up to 18 months after you participate. Once these are available however, a summary will be posted on the AusSun website at http://www.uv.hlth.qut.edu.au/.

Individual vitamin D results will also be posted on the website as numbers (e.g. M 007). Use the number that is on the front of your "study folder" to identify your results, or simply contact **Candy Wyatt**. You are most welcome to share those results with your surgeon or General Practitioner.

Future research using your samples

After we have finished this particular study, **if you have agreed**, we will keep the information and remaining samples that you give us indefinitely. In the future we may match your information against other health registers and we may use your samples

for future biochemical or genetic studies. We may also contact you again to ask you to take part in a follow-up study but you will be under **no obligation** to do so. Any extra studies that use your samples will have to be approved by the Scientific and Ethics Committees at QUT and the institution carrying out the study, before your information and material can be used. Any information or material given to researchers will be identified by a code only, so it will not be possible for them to identify you in any way. You will not receive any notice of future uses of your information or samples, unless you have requested this on the consent form.

There is a chance that information derived from the samples that you are donating under this study may, in the future, have some commercial value, for example if they lead to the development of a commercial product. You will not be compensated for your participation in the study or for any future value that the sample you have given may be found to have. However, it is our intention that if money is generated as a result of research using your samples then some will be put into a special fund to be used for future research into skin cancer.

Risks of this study

There are no risks beyond normal day-to-day living associated with your participation in this project.

The project does require you to have venous blood samples taken by a person qualified to do so, and in some instances some minor adverse side effects and discomfort may occur. Minor risks are common to any venous blood sampling and include bleeding and/or minor bruising at the blood collection site, occasionally multiple punctures to locate veins, and temporary dizziness or fainting. In the very unlikely event that you experience one of the above side effects, prompt medical assistance will be provided.

Possible adverse effects of taking blood include bleeding and redness or swelling at the site, and in extreme cases fainting may occur. These risks will be minimised and managed by applying pressure to the puncture site and in the unlikely event of fainting, with prompt attention from clinical staff.

QUT provides for limited free counselling for research participants of QUT projects, who may experience discomfort or distress as a result of their participation in the research. Should you wish to access this service please contact the Clinic Receptionist of the QUT Psychology Clinic on +61 7 3138 0999. Please indicate to the receptionist that you are a research participant.

Confidentiality

All comments and responses are anonymous and will be treated confidentially. The names of individual persons are not required in any of the responses.

The privacy of all volunteers in this research program is taken very seriously. When you fill out a survey or donate a sample of blood to this study we will make every effort to protect your privacy.

- All your surveys and samples will be stored securely in such a way that they cannot immediately be identified as having come from you. They will be labeled with a unique barcode number so that they do not get confused with surveys or samples from someone else.

- Any identifying information (your full name, address etc.) will be stored separately from the samples and information you provide. Access to this identifying information is restricted to a small number of senior members of the study team.

- No information that could be used to identify you or your family will be included in any report on the results of the study.

Consent to Participate

We would like to ask you to sign a written consent form (enclosed) to confirm that you understand what participation in this study entails and your agreement to participate.

Questions / further information about the study

Melanoma, sometimes referred to as malignant melanoma, is a cancer of pigmented cells in the skin. The number of people in Australia with melanoma has been increasing every year and Queensland has the highest rate of melanoma in the world. Men and women are equally at risk from developing melanoma.

Ultraviolet radiation (UVR) reaches the earth from the sun. Excessive UV radiation from sun exposure is a major cause of various skin cancers.

Vitamin D is important for building and maintaining a healthy skeleton throughout life. Some vitamin D is obtained through diet, but most vitamin D is obtained from exposure to the sun. Not getting enough sun exposure can cause low vitamin D levels. Recent studies conducted in Brisbane have shown that almost a third of the population may have low levels of vitamin D.

How are vitamin D and UV from the sun linked? Vitamin D is produced when a substance in the skin cells is exposed to UVR from the sun. However, the amount of vitamin D produced is influenced by other factors such as skin colour, age, sunscreen use, season, time of day and latitude.

Please contact the researcher team members named above to have any questions answered or if you require further information about the study.

Concerns / complaints regarding the conduct of the study

QUT is committed to researcher integrity and the ethical conduct of research projects. However, if you do have any concerns or complaints about the ethical conduct of the project you may contact the QUT Research Ethics Coordinator on +61 7 3138 2091 or ethicscontact@qut.edu.au. The Research Ethics Coordinator is not connected with the research project and can facilitate a resolution to your concern in an impartial manner.
This study has received full ethical approval from the University Human Research Ethics Committee (Approval Certificate number: 0900000681)

Appendix E

QUT Melanoma Study questionnaire

The QUT Melanoma Study

Survey Booklet

Instructions

1. The survey takes about 30 minutes to complete, but the time does vary from person to person.
2. We suggest using a **pencil** so that you can make changes.
3. Take time with your answers. If you need a rest, please stop and then come back to the survey later.
4. Most of the questions ask you to tick a box for the answer that is best for you. Some ask you for a short written answer.
5. If at any time you are uncertain about what to do, or cannot answer a question, just leave it until you meet with our researcher and we will help you at that time. Otherwise you can ring Candy Wyatt (on 0418 748172) for help.
6. All information provided will be treated in **STRICT CONFIDENCE**.

Your Research Team:

Professor Michael Kimlin (Principal Researcher):
Phone: 3138 5802
Mobile: 0401 313 534
Email: m.kimlin@qut.edu.au

Ms Candy Wyatt (Associate Researcher/PhD student):
Phone: 3138 0275
Mobile: 0418 748 172
Email: candy.wyatt@qut.edu.au

THANK YOU FOR PARTICIPATING IN OUR STUDY.
We look forward to seeing you soon.

CONFIDENTIAL

AusSun RESEARCH LAB
Health • Education • Community

QUT ihbi Institute of Health and Biomedical Innovation

Section 1. A few personal details

1. **Your date of birth:** ☐☐/☐☐/☐☐
   (dd / mm / yy)

2. **Your current age:** ☐☐ years

3. **Sex:** ☐1 male ☐2 female

4. **Where were you born?**

Town/City	State/Province	Country

IF NOT AUSTRALIAN born, at what age did you come to live permanently in Australia? ☐☐ years

Section 2. Some questions about your melanoma

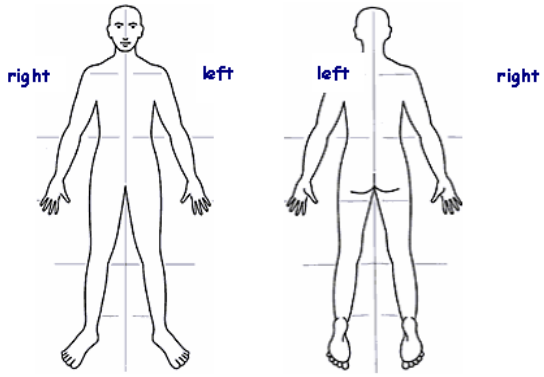
You recently had a melanoma treated. This study aims to find out more about what causes melanomas. First, we need to be sure that we know *exactly* where on your body that your melanoma was located.

5. **On what SIDE of your body was this melanoma found?** ☐1 Left ☐2 Right

6. A. **On which GENERAL body site was this melanoma found?** *Please tick ONE box under the "general site" column below.*

General site	*Can you please provide more detail?*	Specific site
☐1 Head	→	☐1 ears ☐2 scalp ☐3 cheek ☐4 nose ☐5 other
☐2 Neck	→	☐6 throat ☐7 side of neck ☐8 back of neck
☐3 Shoulders		
☐4 Back	→	☐9 upper back ☐10 middle back ☐11 lower back
☐5 Chest		
☐6 Abdomen *("tummy")*		
☐7 Other body site	→	☐12 Arms ☐13 Legs ☐14 Other

6. B. Draw on the figure below the position where the melanoma occurred:



The next questions are about how your melanoma was first discovered.

7. Did you already have a spot at the place where the melanoma started?

☐1 No, I did not have a spot at the place until the melanoma started.

☐2 Yes, I did have a spot there before it turned into a melanoma.

The spot was there for ☐☐ months or ☐☐ years before it changed

☐3 I don't remember

8. When did somebody first notice that something might be wrong with the spot. (That is, there was some concern that the spot might be a skin cancer). Was it

☐1 more than 5 yrs before it was cut out

☐2 between 1 to 5 years before it was cut out

☐3 between 6 months to 1 yr before it was cut out

☐4 between 3 to 6 months before it was cut out

☐5 less than 3 months before it was cut out

☐6 I don't remember

9. So, about what date was it that somebody noticed that something might be wrong with that spot? *(Please give your best estimate – even just the reporting year is very helpful)*

Day ☐☐ **Month** ☐☐ **Year** ☐☐☐☐

How sure are you of this date?

☐1 very sure ☐2 fairly sure (within a month) ☐3 not sure at all

10. What was it about the spot that made you (or someone else) think that something might be wrong? *(please tick all that apply)*

- ☐ A size
- ☐ B colour
- ☐ C irregular shape
- ☐ D lumpy / raised up
- ☐ E bleeding or crusting
- ☐ F pain
- ☐ G irritation / itch
- ☐ H just looked different to other spots
- ☐ I something else *(please specify)* _____
- ☐ J don't remember

11. What date did a doctor first examine this spot FOR THE VERY FIRST TIME?
(Please give your best estimate)

Day ☐☐ **Month** ☐☐ **Year** ☐☐☐☐

How sure are you of this date?

- ☐ 1 very sure
- ☐ 2 fairly sure (within a month)
- ☐ 3 not sure at all

12. What doctor was that?

- ☐ 1 General practitioner (GP)
- ☐ 2 doctor in a skin cancer clinic
- ☐ 3 public hospital doctor
- ☐ 4 dermatologist
- ☐ 5 surgeon / plastic surgeon
- ☐ 6 other doctor
- ☐ 7 don't remember

13. What did the doctor do about the spot? Did they:

- ☐ 1 give you the 'all clear'
- ☐ 2 decide to watch it for a while
- ☐ 3 treat it immediately
- ☐ 4 refer you to another doctor
- ☐ 5 something else
- ☐ 6 don't remember

Section 3. Some questions about your hair and skin

14. What was your <u>natural</u> hair colour when you were a young teenager? *Please tick one box*

☐ 1 Black ☐ 2 Dark brown ☐ 3 Auburn / strawberry blonde
☐ 4 Blond ☐ 5 Red ☐ 6 Light brown ☐ 7 Other (please specify)……

15. Have you ever had a beard?

☐ 1 No ☐ 2 Yes → **how many years have you had a beard (in total)** ☐☐ years

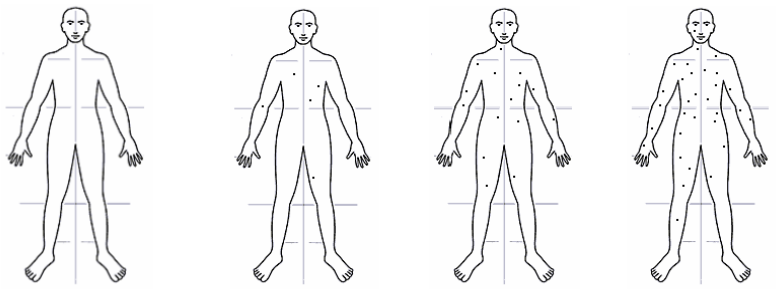
16. Has a doctor ever told you you have a skin condition called VITILIGO *("areas of whitening or loss of pigment on the surface of the skin")*

☐ 1 No ☐ 2 Yes → **how old were you when you were diagnosed** ☐☐ years old

17. Do you have grey hair?

☐ 1 No ☐ 2 Yes → **how old were you when you first went grey** ☐☐ years old

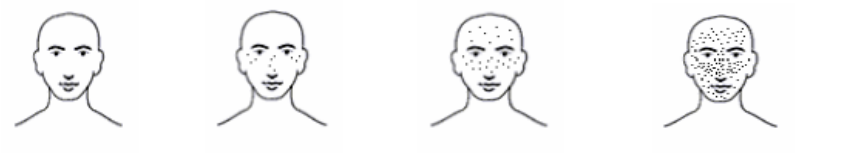
18. When you were a teenager, how many MOLES did you have on your skin? Match your answers with the pictures below. (*Moles are small brown, black or pink, either raised or flat skin markings that do not change after sun exposure*) *Please tick one box*



☐ 1 I had no moles ☐ 2 I had a few moles ☐ 3 I had some moles ☐ 4 I had many moles

19. When you were a teenager, how many FRECKLES did you have at the end of summer?
Match your answers with the pictures below for face and arms. *Please tick one box*

FACE (TICK ONE)



☐1 I had no freckles ☐2 I had a few freckles ☐3 I had some freckles ☐4 I had many freckles

Now, some questions about how your skin reacts when you are out in the sun. We are interested separately in burning and tanning.

Sunburn

Imagine you were in the strong sun for 30 minutes in the middle of the day for the first time in summer, without any protection such as sunscreen or clothing. We are interested in how much your skin would BURN.

20. Which of the following best describes what would happen to your skin?
Please tick one box

☐1 My skin would never burn
☐2 My skin would rarely burn
☐3 My skin would sometimes burn
☐4 My skin would mostly burn
☐5 My skin would always burn

Tanning

Now, *imagine* you spend several weeks at the beach in the strong sun, without any protection such as sunscreen or clothing. We are interested in how much your skin would TAN.

21. Which of the following best describes what would happen to your skin?
Please tick one box

☐1 My skin would not tan
☐2 My skin would tan lightly
☐3 My skin would tan moderately
☐4 My skin would tan deeply

Section 4. Primary School (age 5 years until high school age, about 13 years)

The next set of questions asks about the time when you were a child at primary or state school. We want to know about your sun exposure on school days and non-school days (holidays and weekends) during your primary school years.

*First, please tell us the name of the all the **primary schools** you attended, the place where this occurred and the age you started and stopped each primary school. (Secondary or high schools are detailed later.)*

Name of primary school	Town, state or country	Age start	Age stopped
1			
2			
3			
4			

Time spent outdoors

22. During all the years when you were at primary school, how often were you outdoors in the sun after school in summer? *Please tick one box*

☐1 Never ☐2 Rarely ☐3 Sometimes ☐4 Usually ☐5 Always or almost always

23. During all the years when you were at primary school, how often were you outdoors in the sun on weekends and holidays in summer? *Please tick one box*

☐1 Never ☐2 Rarely ☐3 Sometimes ☐4 Usually ☐5 Always or almost always

Hats

During all the years when you were at PRIMARY school, about how often did you wear a hat when you were outdoors and exposed to the sun…..

24. … on schooldays in summer? *Please tick one box*

☐1 Never ☐2 Rarely ☐3 Sometimes ☐4 Usually ☐5 Always or almost always

25. … on weekends and holidays in summer? *Please tick one box*

☐1 Never ☐2 Rarely ☐3 Sometimes ☐4 Usually ☐5 Always or almost always

Hair style

26. During the years when you were at primary school, when outdoors were your EARS usually

☐ 1 completely covered by hair ☐ 2 partly covered by hair ☐ 3 uncovered: no hair worn over ears at all

Sunburn

27. During all the years when you were at primary school (including all weekends and all holidays), how many times were you sunburned so badly that you developed either:

- blistering of the skin,
- soreness for 2 days or more,
- peeling of the skin? *Please tick one box*

☐ 1 More than 50 times

☐ 2 21 to 50 times

☐ 3 11 to 20 times

☐ 4 6 to 10 times

☐ 5 1 to 5 times

☐ 6 Never

Section 5. Secondary or High School

The next set of questions asks about when you were at secondary school. We want to know about your sun exposure when at school and when on holidays and weekends as a teenager during your secondary school years.

28. First, did you attend secondary school?

☐ 1 Yes ☐ 2 No, please go to **Section 6, *Time since leaving school***

*If **YES**, then please complete the following table. Please tell us the name of the all the **secondary schools** you attended, the place where this occurred and the age you started and stopped each secondary school.*

Name of secondary school	Town, state or country	Age start	Age stopped
1			
2			
3			
4			

Time spent outdoors

29. During all the years when you were at secondary school, how often were you outdoors in the sun after school in summer? *Please tick one box*

☐ 1 Never ☐ 2 Rarely ☐ 3 Sometimes ☐ 4 Usually ☐ 5 Always or almost always

30. During all the years when you were at secondary school, how often were you outdoors in the sun on weekends and holidays in summer? *Please tick one box*

☐ 1 Never ☐ 2 Rarely ☐ 3 Sometimes ☐ 4 Usually ☐ 5 Always or almost always

Hats

During all the years when you were at secondary school, about how often did you wear a hat when you were outdoors and exposed to the sun……

31. … on schooldays in summer? *Please tick one box*

☐ 1 Never ☐ 2 Rarely ☐ 3 Sometimes ☐ 4 Usually ☐ 5 Always or almost always

32. … on weekends and holidays in summer? *Please tick one box*

☐ 1 Never ☐ 2 Rarely ☐ 3 Sometimes ☐ 4 Usually ☐ 5 Always or almost always

Hair style

33. During the years when you were at high school, when outdoors were your EARS usually

☐ 1 completely covered by hair ☐ 2 partly covered by hair ☐ 3 uncovered: no hair worn over ears at all

Sunburn

34. During all the years when you were at secondary school (including all weekends and all holidays), how many times were you sunburned so badly that you developed either:

- blistering of the skin,
- soreness for 2 days or more,
- peeling of the skin? *Please tick one box*

☐ 1 More than 50 times ☐ 4 6 to 10 times

☐ 2 21 to 50 times ☐ 5 1 to 5 times

☐ 3 11 to 20 times ☐ 6 Never

Section 6. Time Since Leaving School

Now we would like you to tell us about your time since leaving school. Please complete the table on page 12 opposite, telling us about your sun exposure when 'at work' and when not at work for each main 'job' you have had and place where you have lived. Remember, please feel free to ask if you have any questions.

Complete the table on page 12 and then continue below

35. Since leaving school how many times were you sunburnt so badly that you developed either:

- blistering of the skin,
- soreness for at least 2 days or more,
- or had peeling of the skin? *Please tick one box*

☐ 1 More than 50 times ☐ 3 11 to 20 times ☐ 5 1 to 5 times

☐ 2 21 to 50 times ☐ 4 6 to 10 times ☐ 6 Never

Hair style

36. Since leaving school, when outdoors were your EARS usually

☐ 1 completely covered by hair ☐ 2 partly covered by hair ☐ 3 uncovered: no hair worn over ears at all

Section 6. Time Since Leaving School Table

Complete the following table using a pencil. Circle the number for your answer to each question for work and non-work days.

Job type List in order each main job: eg office, shop assistant, administration, labourer, farmer, builder, teacher etc **include retirement, study periods, homeduties, unemployment** etc., longer than 1 year, you have had since leaving school	**Place** List the town & state, or country if outside Australia	**Age start**	**Age stop**	**Days per week working in this job**	How much time did you spend outdoors in the sun in summer? *(Circle a number for each job using the following key)* 1. Indoors mostly, (outdoors less than 1 hour per day) 2. Outdoors between 1 to 4 hours per day 3. Outdoors mostly, (over 4 hours per day)		How often did you wear clothing that covered your back and chest when outdoors and in the sun? *(Circle a number for each job using the following key)* 1. Never 2. Rarely 3. Sometimes 4. Often 5. Always	
					on work days	**on non-work days** (eg weekends, days off)	**on work days**	**on non-work days** (eg weekends, days off)
1					1 2 3	1 2 3	1 2 3 4 5	1 2 3 4 5
2					1 2 3	1 2 3	1 2 3 4 5	1 2 3 4 5
3					1 2 3	1 2 3	1 2 3 4 5	1 2 3 4 5
4					1 2 3	1 2 3	1 2 3 4 5	1 2 3 4 5
5					1 2 3	1 2 3	1 2 3 4 5	1 2 3 4 5
6					1 2 3	1 2 3	1 2 3 4 5	1 2 3 4 5
7					1 2 3	1 2 3	1 2 3 4 5	1 2 3 4 5
8					1 2 3	1 2 3	1 2 3 4 5	1 2 3 4 5
9					1 2 3	1 2 3	1 2 3 4 5	1 2 3 4 5
10					1 2 3	1 2 3	1 2 3 4 5	1 2 3 4 5

When complete, return to Section 6 on page 11 and continue questionnaire

Section 7. Sun Protection as an Adult

FIVE YEARS AGO

37. Thinking of the time you spent outside in the sun 5 years ago, how often did you ...?

		Never	Less than 50% of time outdoors	More than 50% of time outdoors	All the time outdoors
Apply sunscreen on your face or arms?	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4
Wear sunglasses?	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4
Wear a hat?	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4
Wear long sleeves?	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4
Stay in the shade?	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4

38. During summer, 5 years ago how many days per week did you apply sunscreen (SPF 15+) to your ?:

		None	Occasional	1-2 days	3-4 days	5-6 days	Every day
Face or head or neck	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Hands or forearms	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Other parts of your body	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

TEN YEARS AGO

39. Thinking of the time you spent outside in the sun, 10 years ago, during summer, how often did you?

		Never	Less than 50% of time outdoors	More than 50% of time outdoors	All the time outdoors
Apply sunscreen on your face or arms?	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4
Wear sunglasses?	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4
Wear a hat?	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4
Wear long sleeves?	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4
Stay in the shade?	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4

40. During summer, 10 years ago, how many days per week did you apply sunscreen (SPF 15+) to your:

		None	Occasional	1-2 days	3- 4 days	5-6 days	Everyday
Face or head or neck	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Hands or forearms	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6
Other parts of your body	Tick one ►	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6

Section 8. Skin Checks

Skin screening by a doctor

41. During the 3 years before your melanoma was diagnosed, how many times had a DOCTOR deliberately checked the skin on all (or nearly all) of your whole body for early signs of skin cancer?

☐ 1 Never (or 'never until I was diagnosed with melanoma').

☐ 2 1 time ☐ 3 2 times ☐ 4 3 to 5 times

☐ 5 more than 5 times ☐ 6 don't remember

Skin screening by people who are not doctors

42. During the 3 years before your melanoma was diagnosed, how many times had SOMEONE WHO IS NOT A DOCTOR (eg spouse, partner, friend etc) deliberately checked the skin on all (or nearly all) of your whole body for early signs of skin cancer?

☐ 1 Never (or 'never until I was diagnosed with melanoma').

☐ 2 1 time ☐ 3 2 times ☐ 4 3 to 5 times

☐ 5 more than 5 times ☐ 6 don't remember

Section 9. Some questions about skin cancers and sunspots

*Now we would like to ask about any other problems with your skin that have needed medical treatment. We want to know separately about **SKIN CANCERS** and **SUNSPOTS**. Skin cancers are more serious than sunspots, and generally (but not always) occur one at a time. Sunspots (also called "solar keratoses") are small, scaly, reddish patches that often occur on the face and arms.*

43. Has a doctor EVER told you that you have either SKIN CANCERS or SUNSPOTS? *(not including your **melanoma**) Tick as many as apply.*

- ☐ A No, I have never been told that I have had a skin cancer or sunspot (***go to section 10**, below*)
- ☐ B Yes, I have been told by a doctor that I have a sunspot(s)
- ☐ C Yes, I have been told by a doctor that I have a skin cancer(s)
- ☐ D Yes, but I'm not sure whether I have had skin cancer or sunspots

Let's start with ***sunspots*** *(small, scaly, reddish patches)*

44. How many SUNSPOTS have you EVER had ……..

…. **which have been CUT OUT** (treated surgically)? *Please tick one box*

☐ 1 none ☐ 2 1 ☐ 3 2 to 5 ☐ 4 6 to 10
☐ 5 11 to 20 ☐ 6 21 to 50 ☐ 7 more than 50

…. **which have been FROZEN** or **BURNT OFF?** *Please tick one box*

☐ 1 none ☐ 2 1 ☐ 3 2 to 5 ☐ 4 6 to 10
☐ 5 11 to 20 ☐ 6 21 to 50 ☐ 7 more than 50

… **which have been treated with SPECIAL CREAMS?** *Please tick one box*

☐ 1 none ☐ 2 1 ☐ 3 2 to 5 ☐ 4 6 to 10
☐ 5 11 to 20 ☐ 6 21 to 50 ☐ 7 more than 50

…. **which have been treated in other ways?** *Please tick one box*

☐ 1 none ☐ 2 1 ☐ 3 2 to 5 ☐ 4 6 to 10
☐ 5 11 to 20 ☐ 6 21 to 50 ☐ 7 more than 50

45. Which parts of your body have had sunspots treated by your doctor?, *please tick all that apply*

☐ A none treated ☐ B face, head or neck
☐ C back ☐ D shoulders ☐ E hands ☐ F arms
☐ G legs ☐ H stomach ☐ I chest
☐ J other *(please specify)* ____

46. **How old were you when your first sunspot was treated?** ☐☐ yrs ***OR*** ☐ not applicable

Now, ***skin cancers*** *that have been diagnosed by a doctor.*

47. **How many SKIN CANCERS (not including your melanoma) have you EVER had** .....

.... **which have been CUT OUT** (treated surgically)? *Please tick one box*

☐ 1 none ☐ 2 1 ☐ 3 2 ☐ 4 3
☐ 5 4 ☐ 6 5 ☐ 7 6 ☐ 8 more than 6

.... **which have been FROZEN or BURNT OFF?** *Please tick one box*

☐ 1 none ☐ 2 1 ☐ 3 2 ☐ 4 3
☐ 5 4 ☐ 6 5 ☐ 7 6 ☐ 8 more than 6

... **which have been treated with RADIUM?** *Please tick one box*

☐ 1 none ☐ 2 1 ☐ 3 2 ☐ 4 3
☐ 5 4 ☐ 6 5 ☐ 7 6 ☐ 8 more than 6

.... **which have been treated in other ways?** *Please tick one box*

☐ 1 none ☐ 2 1 ☐ 3 2 ☐ 4 3
☐ 5 4 ☐ 6 5 ☐ 7 6 ☐ 8 more than 6

48. **Which parts of your body have had skin cancers treated by your doctor?**

please tick all that apply

☐ A none treated ☐ B face, head or neck
☐ C back ☐ D shoulders ☐ E hands ☐ F arms
☐ G legs ☐ H stomach ☐ I chest
☐ J other *(please specify)* [ ]

49. **How old were you when you had your first skin cancer treated** (not including your melanoma)?

☐☐ years ***OR*** ☐ 1 don't remember ***OR*** ☐ 2 not applicable

Section 10. Family Medical History

Now we would like to ask some questions about melanoma in your family.

50. Have any members of your family ever been told that they have melanoma? *Think about close relatives; that is, your parents, brothers and sisters and your children, and then aunts, uncles, and grandparents.*

☐ 1 Yes ☐ 2 No ☐ 3 Don't know

If **NO** then please **go to Section 11,**

If **YES** then please complete the table below for each relative who has had melanoma**;**

51. Let's start with the closest relation to you who has had melanoma. This person doesn't have to be alive today. The closest relatives to you are full brothers or sisters, mother and father and your children, then grandparents, aunts and uncles.

Name of relative with melanoma	How is this person related to you? (*eg son, father)*	Which **town** and **state** do they live?	How old were they when they got melanoma?	Where on their body was the melanoma?
1				
2				
3				
4				

Section 11. A few final details

52. What is the highest level of education that you have completed? *Please tick one box*

- ☐ 1 primary school
- ☐ 2 secondary school
- ☐ 3 trade certificate
- ☐ 4 technical/college diploma
- ☐ 5 university degree
- ☐ 6 other (please specify)……

53. Your marital status at the time you were diagnosed with melanoma? *Please tick one box*

- ☐ 1 Married / defacto / partner
- ☐ 2 Separated / divorced
- ☐ 3 Single
- ☐ 4 Widowed
- ☐ 5 Other (please specify)……

54. Have you ever smoked cigarettes, cigars or a pipe at least once a day for as long as six months?

☐ 1 Yes ☐ 2 No

If **NO**, then please go to Section 12, If **YES**, then please continue;

55. How old were you when you started smoking? ☐☐ years

56. Do you smoke now? ☐ 1 Yes , go to Q 58 ☐ 2 No

57. If not currently smoking, at what age did you stop? ☐☐ years

58. How many cigarettes do / did you usually smoke on a typical day? ☐☐☐ cigarettes

Section 12. Comments

Having completed the survey, we would value your opinions and comments:

59. Did you have difficulty completing this survey form? ☐ 1 No ☐ 2 Yes

IF YES, please tell us why

……

……

(for example, if English is your second language)

60. Are there any comments that you would like to make about the questionnaire. For example, did you have difficulty with any questions? *(please tell us which ones).*

……

……

……

……

……

……

……

……

……

……

You have now finished the end of the survey. Thank you for taking part.

Appendix F

QUT Melanoma Study Clinical Examination

Clinical Examination

Date: / /

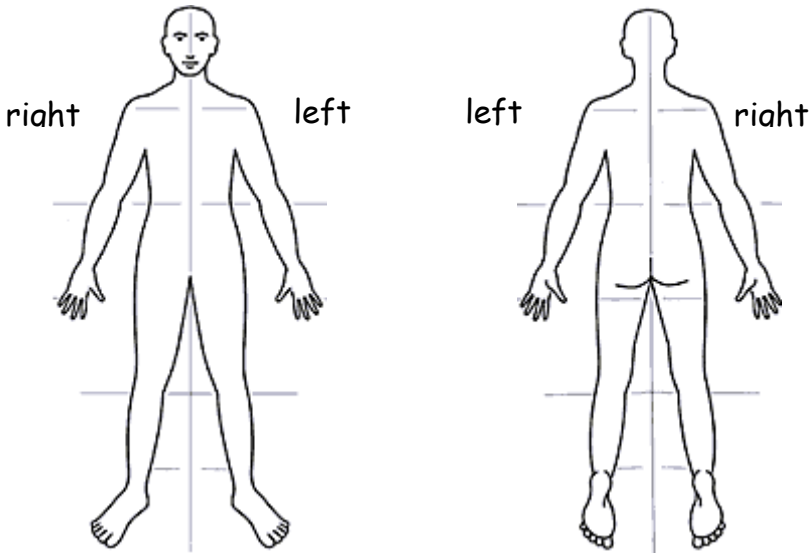
I.D: _____

Date of Birth: / /
dd mm yy

1. Melanoma site

a. Where on the body was the INDEX melanoma found?

b. Draw on the figure below the position where the melanoma occurred: *(if more than one melanoma, please draw these on the figure as well)*



2. Spectrophotometry

Site	L*	A*	B*
Dorsal forearm			
Upper inner arm			
Forehead			

3. <u>BMI</u>

Weight ..... kgs Height ..... cms

4. <u>Skin Examination</u>

a. Naevi

- brown to black pigmented macules or papules of any size which are darker than the surrounding skin

Major Site	Sub-Site	**Left-side** **Total** mm	**2-** 5 mm	> 5	**Right-side** **Total** mm	2- 5 mm	> 5
Back and shoulders	Lower back – subscapular						
	Upper back – suprascapular						
	Posterior neck						
Upper limbs	Hand						
	Palmar						
	Forearm						
	Upper arm						
Head and neck	Face						
	Ear						
Anterior trunk	Abdomen sub-costal						
	Chest						
	Anterior neck						

Iris naevi:

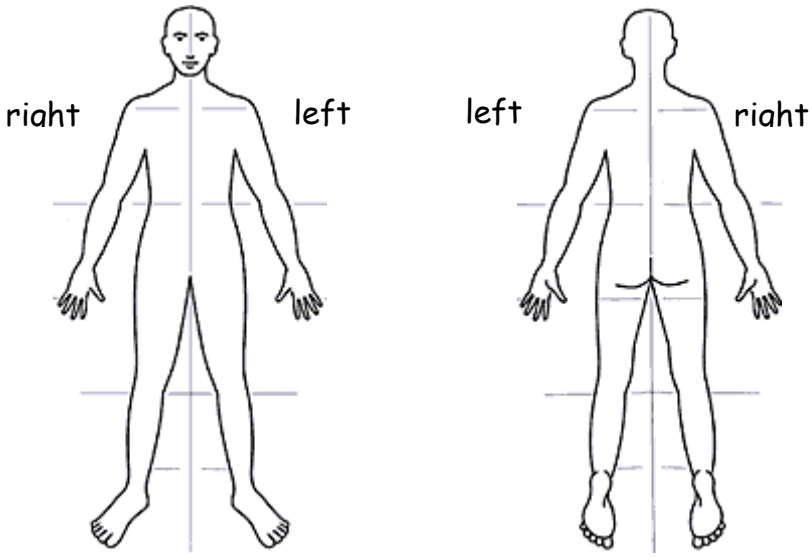
Left iris	**Right iris**

b. Has the patient had any naevi previously excised? $_1$ Yes / $_2$ No

c. How many naevi have been excised? .

.....

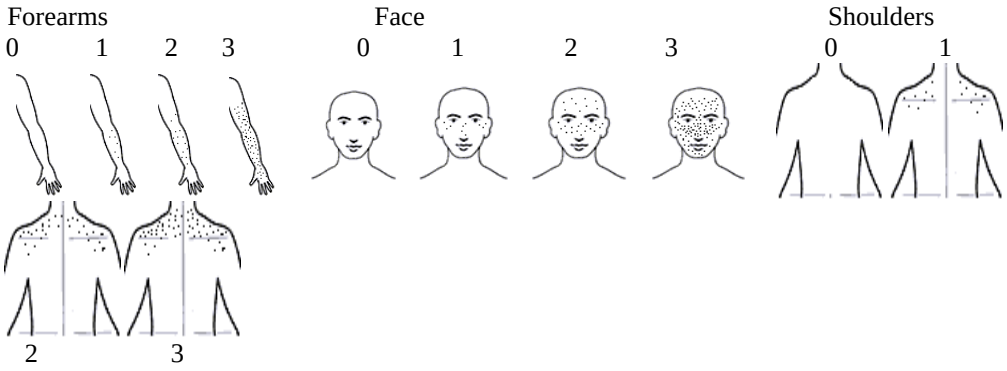
d. Draw the positions of the excised naevi on the figure below:



e. Freckles & lentigines

- Freckles: irregular but sharply demarcated, usually small (<3/4 mm), uniformly pigmented macules (tan/light brown), occurring in clusters, usually on exposed body sites
- Lentigo: irregular but sharply demarcated, size 4-10 mm, usually light brown to dark brown, tendency for confluence on sun damaged / sun exposed skin, sparse or scattered

Site	Freckles	Lentigines
Forearms		
Face		
Shoulders		



f. Solar keratoses

- superficial, rough scaly areas with erythematous background and ill defined margins.
- if more than 50 at one subsite write "50+"

Site	***Left-side***	***Right-side***
Dorsum Hand		
Dorsum Forearm		
Forehead		
Cheek		
Nose		
Chin		

g. Hair colour Natural? $_1$ Yes / $_2$ No

□ $_1$ white/blonde □ $_2$ grey □ $_3$ light brown □ $_4$ dark brown
□ $_5$ black □ $_6$ red/blonde □$_7$ red
□other……

h. Eye colour *Please tick one box*

□ $_1$ Blue □ $_2$ Grey □ $_3$ Hazel □ $_4$ Green □ $_5$ Brown
□ $_6$ Other…….

5. <u>Family history of melanoma</u>

Do you have any relatives who have had a melanoma? $_1$ Yes / $_2$ No

6. <u>Solarium Use</u>

Did you ever use a solarium before 1980? $_1$ Yes/ $_2$ No

Have you ever used a solarium since 1980? $_1$ Yes/ $_2$ No

7. <u>Previous melanoma</u>

Is this the first melanoma you have had? $_1$ Yes / $_2$ No

Sites of previous melanoma:

.....

8. <u>Other internal malignancies</u> $_1$ Yes / $_2$ No

Sites of other malignancies?

.....

9. <u>Vitamin D supplementation</u>

Do you take any vitamin D/calcium supplements? $_1$ Yes / $_2$ No

Describe .....

10. <u>CT scan</u>

Did you have a CT scan or a PET scan prior to your surgery? $_1$ Yes / $_2$ No

11. <u>Week-end activities</u>

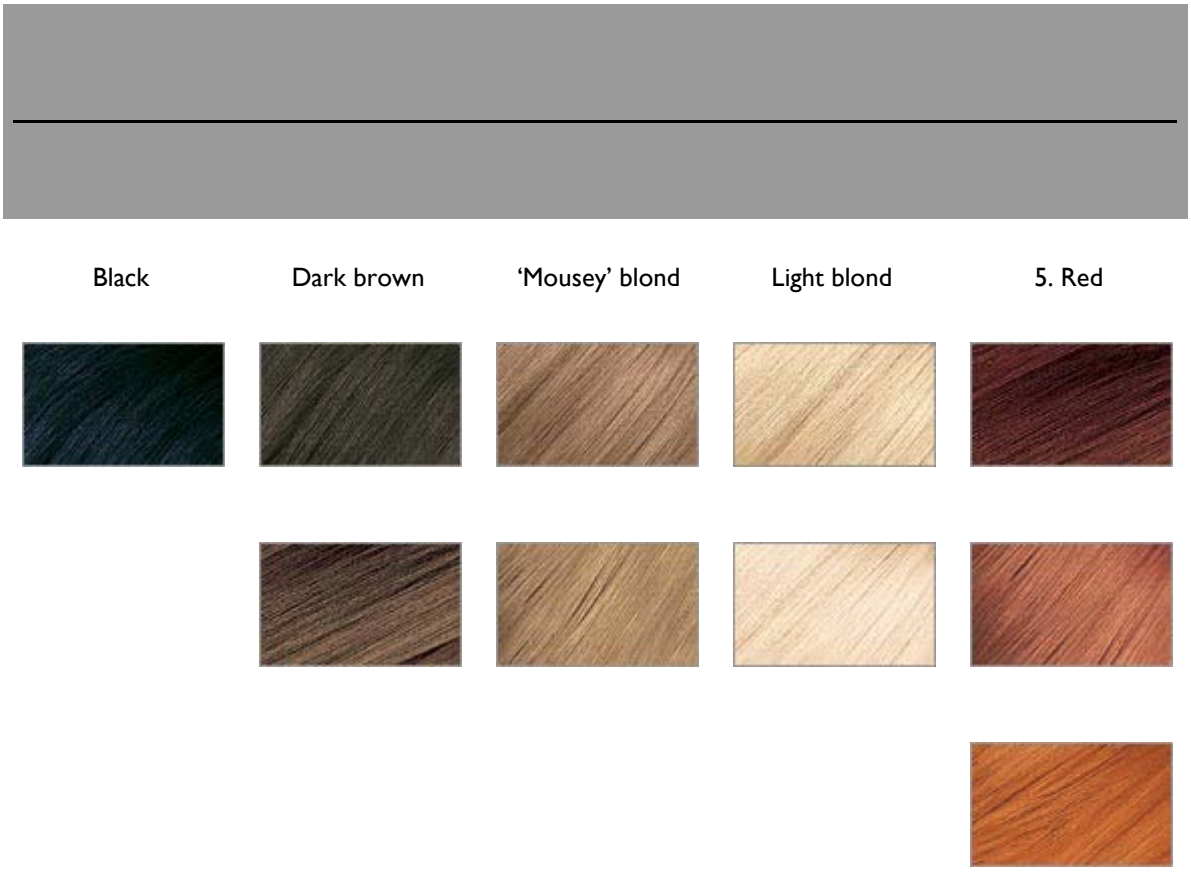
Thinking back to your survey, did you/ do you play/supervise weekend sporting activities?

$_1$ Yes / $_2$ No

Describe .....

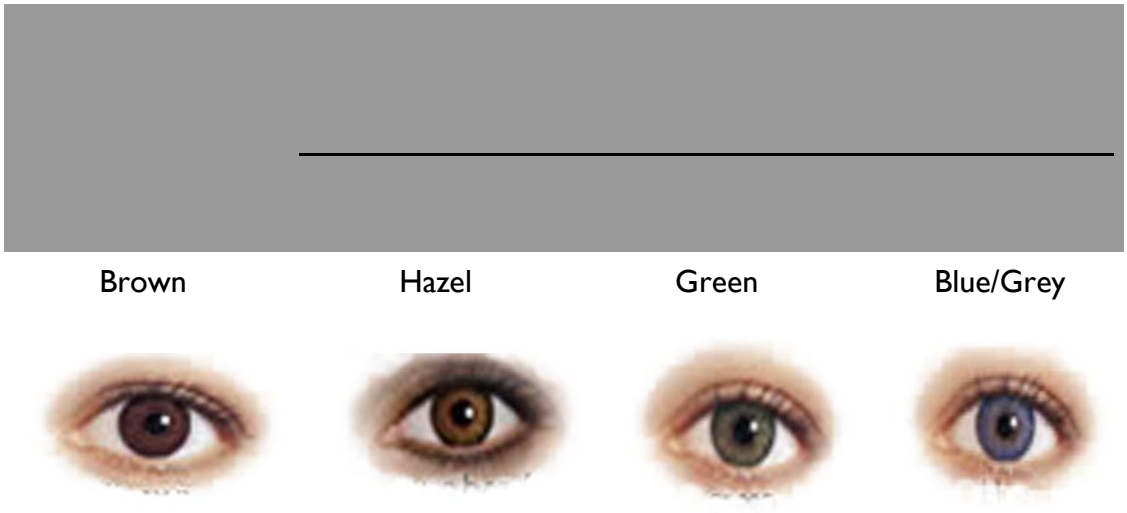
Appendix G

Hair colour guide



Appendix H

Eye colour guide



Appendix I

Protocol for use of spectrophotometer

SPECTROPHOTOMETRY

N.B.

- **HANDLE WITH CARE AT ALL TIMES**
- **DO NOT TOUCH WHITE DOT**
- **REPLACE BLACK CAP AFTER CALIBRATION.**
- **IF MACHINE IS TURNED OFF IT MUST BE RE-CALLIBRATED BEFORE USING AGAIN**
- **WIPE WHITE DISC BETWEEN PATIENTS WITH ALCO WIPE**
- **PLACE "BLUEY" UNDER ARM**

1) Place on flat surface & plug in at power point
2) Turn on at rear of machine
3) Take black cover off base of cradle
4) Highlight "Condition 2" and Enter
5) Highlight "Calibrate" and Enter
6) Highlight "Zero"
7) Hold up to natural light and press button once
8) Wait for 3 clicks
9) Menu automatically goes to "White"
10) Press button again while in cradle
11) For this study we are using "SCI"
12) When measuring, seal but do not press against the skin
13) Record L, A & B on clinical examination form
14) Ensure numbers are recorded correctly
15) Black cap on at end
16) NEVER TOUCH WHITE

Appendix J

Ethics approval

University Human Research Ethics Committee

HUMAN ETHICS APPROVAL CERTIFICATE

NHMRC Registered Committee Number EC00171

Date of Issue: 1/9/09 (supersedes all previously issued certificates)

Dear Prof Michael Kimlin

A UHREC should clearly communicate its decisions about a research proposal to the researcher and the final decision to approve or reject a proposal should be communicated to the researcher in writing. This Approval Certificate serves as your written notice that the proposal has met the requirements of the *National Statement on Research involving Human Participation* and has been approved on that basis. You are therefore authorised to commence activities as outlined in your proposal application, subject to any specific and standard conditions detailed in this document.

Within this Approval Certificate are:

* Project Details
* Participant Details
* Conditions of Approval (Specific and Standard)

Researchers should report to the UHREC, via the Research Ethics Coordinator, events that might affect continued ethical acceptability of the project, including, but not limited to:

(a) serious or unexpected adverse effects on participants; and
(b) proposed significant changes in the conduct, the participant profile or the risks of the proposed research.

Further information regarding your ongoing obligations regarding human based research can be found via the Research Ethics website http://www.research.qut.edu.au/ethics/ or by contacting the Research Ethics Coordinator on 07 3138 2091 or ethicscontact@qut.edu.au

If any details within this Approval Certificate are incorrect please advise the Research Ethics Unit within 10 days of receipt of this certificate.

Project Details

Category of Approval: Human - Committee

Approved From: 24/08/2009 **Approved Until:** 24/08/2012 (subject to annual reports)

Approval Number: 0900000681

Project Title: The influence of 25(OH)D status, serum S100BB status and seasonality on the progression of malignant melanoma

Chief Investigator: Prof Michael Kimlin

Other Staff/Students: Dr Robyn Lucas , Mrs Candy Wyatt

Experiment Summary:
Investigate the relationship between sun exposure, vitamin D and melanoma.

Participant Details

Participants:
Approximately 100

Location/s of the Work:
IHBI

Conditions of Approval

Specific Conditions of Approval:
No special conditions placed on approval by the UHREC. Standard conditions apply.

Appendix K

Serum 25(OH)D and Clark level (ordinal) modelling

Ordinal logistic regression modelling for serum 25(OH)D concentration and Clark level

Bivariate analysis

Clark level was analysed as an ordinal categorical variable with three categories: Level 1, Level 2 and Levels 3–5, using ordinal logistic regression (proportional odds model). Results, reported with the coefficients, 95% confidence intervals and *P*-values are shown in Table 1.1.

Table 1.1

Bivariate ordinal logistic regression for Clark level (Level 1, 2 and 3–5) and all covariates

Variable	Coefficient.	95% *CI*	*P*-value
25(OH)D (nmol/L)	-.02	-.04, 0.00	0.12
Vitamin D status			
≥ 50 nmol/L		referent	
< 50 nmol/L)	0.67	-.10, 1.44	0.09
Age	-.00	-.03,0.02	0.76
Age group			
< 60 years		referent	
≥ 60 years	-.40	-1.15, 0.36	0.30
Sex			
Male		referent	t
Female	0.34	-.40, 1.08	0.37
Melanoma type			0.00
Melanoma site			
Head & neck		referent	
Trunk	0.04	-.91, 0.99	0.93
Upper extremities	0.72	-.55, 1.99	0.27
Lower extremities	0.72	-.31, 1.76	0.17
Season of diagnosis			
Winter		referent	
Spring	1.14	0.14 2.15	0.03
Summer	-.06	-1.20, 1.08	0.92
Autumn	0.38	-.64, 1..40	0.46
S-100β	10.78	0.38, 21.17	0.04
Inner arm L*	0.07	-.07, 0.21	0.34
Inner arm a*	-.24	-.51, 0.03	0.09
Inner arm b*	-.25	-.42, 0.07	0.01
Forearm L*	0.01	-.05, 0.08	0.69
Forearm a*	-.02	-.21, 0.17	0.84
Forearm b*	0.03	-.12, 0.18	0.70
Forehead L*	-.01	-.12, 0.10	0.87
Forehead a*	-.06	-.19, 0.07	0.35
Forehead b*	0.14	-.05, 0.33	0.15
Inner arm chroma	-.24	-.41, -.07	0.01
Inner arm hue	-.00	-.09, 0.09	0.98
Forearm chroma	0.01	-.15, 0.17	0.90
Forearm hue	0.01	-.05, 0.07	0.69
Forehead chroma	0.01	-.14, 0.17	0.86
Forehead hue	0.04	-.01, 0.09	0.14
ITA	0.07	0.01, 0.13	0.02
ITA group			
Intermediate/medium		referent	
Light/fair	0.73	-.72, 2.18	0.32
Very light/very fair	1.80	0.17, 3.44	0.03
BMI	0.05	-.03, 0.13	0.26
BMI categories			
< 24.9		referent	
25–29.9	0.47	-.42, 1.35	0.30
30 +	0.51	-.43, 1.46	0.29
Weight	0.00	-.02, 0.02	0.94
Height	-.03	-.07, 0.01	0.16
Naevi burden at diagnosis	-.00	-.00, 0.01	0.82
Naevi burden at diagnosis			
0–9		referent	
10–39	-.20	-1.11, 0.71	0.67
40+	0.22	-.76, 1.20	0.65

Freckle density at diagnosis			
Low		referent	
Medium	-.03	-.97, 0.90	0.95
High	0.31	-.80, 1.42	0.59
Solar lentigo density at diagnosis			
Low		referent	
Medium	-1.41	-2.68, -.14	0.03
High	-1.10	-2.32, 0.11	0.07
Eye colour			
Brown		referent	
Green/hazel	0.72	-.11, 1.54	0.09
Blue/grey	0.74	-.46, 1.94	0.23
Burning tendency			
Rarely/never burns		referent	
Sometimes burns	-.09	-1.22, 1.04	0.88
Mostly/always burns	-.06	-1.11, 0.99	0.91
Tanning tendency			
Tans deeply/moderately		referent	
Tans slightly/does not tan	0.65	-.19, 1.5	0.13
Hair colour as teenager			
Black/dark brown		referent	
Light brown	0.88	0.02, 1.75	0.05
Blonde, red, auburn, s/b*, blonde	-.32	-1.31, 0.68	0.53
Naevus density as teenager			
Nil		referent	
Few/some	0.06	-.77, 0.89	0.88
Many	-.40	-2.31, 1.52	0.68
Freckle density as teenager			
Nil		referent	
Few/some	-.43	-1.20, 0.35	0.28
Many	1.13	-.90, 3.16	0.27
School-years sun exposure			
Low		referent	
High	0.21	-.64, 1.07	0.62
Adult Reported Sun Exposure (continuous)	0.00	-.00, 0.00	0.59
Adult Reported Sun Exposure			
Low		referent	
High	-.08	-.83, 0.67	0.84
Primary school sunburns			
0–5 burns		referent	
6–20 burns	0.35	-.44, 1.15	0.39
> 20 burns	-.32	-1.50, 0.87	0.60
Secondary school sunburns			
0–5 burns		referent	
6–20 burns	0.83	-.01, 1.67	0.05
> 20 burns	0.41	-.79, 1.61	0.50
Adult sunburns			
0–5 burns		referent	
6–20 burns	0.02	-.77, 0.82	0.96
> 20 burns	-.07	-1.23, 1.08	0.90
Solar elastosis			
Absent		referent	
Present	-.03	-1.20, 1.14	0.96
Sunscreen use 10 years ago			
Wore sunscreen most of time		referent	
Wore sunscreen sometimes	0.85	-1.58, 3.29	0.49
Rarely/never wore sunscreen	1.09	-1.28, 3.46	0.47
Sunscreen use 5 years			
Wore sunscreen most of time		referent	

Wore sunscreen sometimes	0.31	-1.30, 1.92	0.70
Rarely/never wore sunscreen	0.55	-.99, 2.08	0.49
When was spot noticed?			
Less than 3 months		referent	
3–12 months	0.61	-.24, 1.46	0.16
> 12 months	-1.19	-2.43, 0.05	0.06
Family history of melanoma			
No		referent	
Yes	-.58	-1.38, 0.22	0.16
History of previous melanoma			
No		referent	
Yes	-1.16	-2.40, 0.08	0.07
History of other internal cancer			
No		referent	
Yes	0.76	-.26, 1.77	0.14
Take supplements			
No		referent	
Yes	0.67	-.36, 1.79	0.21
Ever smoked			
No		referent	
Yes	-.08	-.83, 0.67	0.84
Education			
Primary/Secondary school		referent	
Trade/ Technical certificate	0.52	-.44, 1.48	0.29
Tertiary education	1.08	0.21, 1.96	0.02

*strawberry blond

Selection of covariates for preliminary multivariable model

Covariates whose *p*-value did not exceed $\alpha = 0.2$ level in either bivariate model (Clark level and serum 25(OH)D concentration), were identified and are summarised below in Table 1.2. As explained previously, age (in years) and sex were also retained. Although serum 25(OH)D concentration was not significantly associated with Clark level at the bivariate level, there was moderate association ($P = 0.12$) and modelling continued to examine if negative confounding may be masking any relationships.

Table 1.2
Age, sex and all covariates flagged at $\alpha < 0.2$ level in the Clark (Level 1, Level 2 and Levels 3–5) and serum 25(OH)D concentration bivariate analyses

Covariates	25(OH)D *P*	Clark *P*
Age		
Sex		
Season at diagnosis	0.06	0.04
Inner arm a*	0.03	0.09
Inner arm b*	0.04	0.15
Inner arm chroma	0.02	0.01
ITA value	0.02	0.02

Final model

Missing values for skin phenotype statistics (2) were taken into account resulting in a model with 98 participants. As described above, age and sex were retained in all steps of the modelling process. The modelling followed the same method as was undertaken to examine the association between serum 25(OH)D concentration and Breslow thickness (≥ 0.75 nmol/L vs. < 0.75 nmol/L), Clark level (Levels 2–5 vs. Level 1) and mitosis (present vs. absent). At the completion of the process the final model (n = 98) contained age, sex, ITA value and primary school sunburns and is presented below in Table 1.3.

Table 1.3
Final models (crude and adjusted) of vitamin D status at three different thresholds for "sufficiency" at time of diagnosis and Clark level (Level 1, Level 2, Levels 3–5)

Vitamin D status	Crude OR	95% CI	*P*	Adjusted OR[a]	95% CI	*P*
≥ 45.25 nmol/L	Reference			Reference		
< 45.25 nmol/L	2.36	0.97, 5.70	0.06	2.45	0.95, 6.30	0.06
≥ 50 nmol/L	Reference			Reference		
< 50 nmol/L	2.01	0.92, 4.39	0.08	2.17	0.96, 4.94	0.06
≥ 69.8nmol/L	Reference			Reference		
< 69.8 nmol/L	1.63	0.65, 4.06	0.30	1.51	0.59, 3.89	0.39

[a] adjusted for age, sex, ITA value, primary school sunburns