INVESTIGATIONS INTO THE EPIDEMIOLOGY AND AETIOLOGY OF CANCERS OF THE SKIN

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<tr>
<td>AA</td>
<td>arachidonic acid</td>
</tr>
<tr>
<td>BCC</td>
<td>basal cell carcinoma</td>
</tr>
<tr>
<td>CRUK</td>
<td>Cancer Research UK</td>
</tr>
<tr>
<td>DALY</td>
<td>disability adjusted life-years</td>
</tr>
<tr>
<td>DHA</td>
<td>docosahexaenoic acid</td>
</tr>
<tr>
<td>EPA</td>
<td>eicosapentaenoic acid</td>
</tr>
<tr>
<td>FFQ</td>
<td>food frequency questionnaire</td>
</tr>
<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>PAF</td>
<td>population attributable fraction</td>
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<tr>
<td>PGE-2</td>
<td>prostaglandin E2</td>
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<tr>
<td>PUFA</td>
<td>polyunsaturated fatty acids</td>
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<tr>
<td>QIMR</td>
<td>Queensland Institute of Medical Research</td>
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<tr>
<td>SCC</td>
<td>squamous cell carcinoma</td>
</tr>
<tr>
<td>TGF-β</td>
<td>transcription growth factor beta</td>
</tr>
<tr>
<td>UK</td>
<td>United Kingdom</td>
</tr>
<tr>
<td>USA</td>
<td>United States of America</td>
</tr>
<tr>
<td>UVR</td>
<td>ultraviolet radiation</td>
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Candidate name: Sarah Cristina Wallingford  
Thesis title: Investigations into the Epidemiology and Aetiology of Cancers of the Skin. 2014

The cancers of the skin, melanoma and the keratinocyte cancers, basal cell and squamous cell carcinomas (BCC and SCC), are among the most common cancers in white populations. While ultraviolet radiation (UVR) is their principal cause, links with non-UVR-related factors have also been noted. Ultimately, the interaction of these elements results in malignancy however, understanding of their specific contributions remains incomplete. This thesis reports findings from six studies aiming to investigate gaps in current knowledge of the role of UVR- and non-UVR-related risk factors on skin cancer. The papers are grouped according to the aspects of skin cancer epidemiology and aetiology they address.

The first two papers address the descriptive epidemiology of melanoma in England, a country with low ambient solar UVR. They arise from ecological studies using national melanoma registration data and document rising trends in melanoma incidence by anatomic site (Paper 1), and by region of residence and socio-economic deprivation (Paper 2). Their findings were consistent with the suggestion that increases in recreational UVR exposure are driving rises in melanoma rates. These results emphasise both the need to closely monitor UVR exposure and melanoma trends and the importance of public health campaigns.

The second group of three papers considers the assessment of associations of nutritional factors with keratinocyte cancer. Two studies use data from a prospective cohort to evaluate the relationship between dietary intake (Paper 3) and blood concentrations (Paper 4) of omega-3 and omega-6 polyunsaturated fatty acids (PUFA) in relation to BCC and SCC risk. Associations with both PUFA types were observed. In addition, Paper 5, a three-way correlational assessment, demonstrated that questionnaire and blood circulating levels of omega-3 PUFA were highly correlated with measures of skin bioavailability. Collectively, these studies give evidence for associations of these nutrients with skin cancer and for the utility of both intake and biomarker measures for assessing the relationships.

The final paper explores the relationship between a widely cited non-UVR risk factor, namely scars and cancers of the skin. It reports a systematic review of all published observational studies quantifying this association. While innumerable case reports were found, quantitative analyses were rare. The review identified a major gap in the literature where knowledge of scar malignancies is not evidence-based, but rather founded mainly on cumulative anecdotal reporting.

Taken together, this body of published work highlights the largely unrecognised complexity of the aetiology of cancers of the skin. Future research must be broad in scope in order to advance understanding of the interaction between UVR and other risk factors and to provide a base for health messages aimed at reducing the burden of these malignancies.
Candidate name: Sarah Cristina Wallingford

Faculty: Medical and Human Sciences

Thesis title: Investigations into the Epidemiology and Aetiology of Cancers of the Skin

Declaration:

i. The nature and extent of the candidate’s contribution and those of the co-authors to each of the publications presented are described here:

Publications 1 and 2: The candidate formulated the study designs and hypotheses, reviewed and summarised the relevant literature, performed all data analyses, interpreted the results of each study and wrote both manuscripts. Alston acquired the data and provided statistical advice and comments on both manuscripts. Birch and Green supported and supervised the design of each study, results interpretations and writing of the manuscripts.

Publications 3 and 4: The candidate formulated the study hypotheses for both papers, reviewed and summarised the relevant literature, performed all data analyses, interpreted the results of each study and wrote both papers. Hughes, Ibiebele and van der Pols provided statistical analysis support and comments on the draft manuscript. Green and van der Pols supported and supervised the study design, results interpretation and writing of the manuscript.
Publication 5: The candidate formulated the study hypotheses, reviewed and summarised the relevant literature, constructed the database, performed all data analyses, interpreted the results and wrote the manuscript. Pilkington commented on the manuscript and collected the data. Massey and Al-Aasswad performed the laboratory analyses. Ibiebele and Hughes supported the statistical analyses and commented on the manuscript. Bennett collected the data. Nicolaou commented on the paper. Rhodes and Green provided support and supervision with study hypotheses, results interpretation and manuscript writing.

Publication 6: The candidate reviewed the literature, collected all relevant studies, interpreted the results and wrote the manuscript. Olsen supported the review methods and commented on the manuscript. Green supervised the study and commented on the manuscript. Plasmeijer wrote the Discussion paragraph on molecular mechanisms.

ii. All of the work presented in this thesis has been completed whilst the candidate has been a member of staff in the post of Research Associate (November 16, 2009 to March 31, 2014) in the Institute of Inflammation and Repair, Faculty of Medical and Human Science at The University of Manchester.

iii. None of the work presented in this thesis has been submitted in support of any successful or pending application for another degree or qualification of this or any other university or of any professional or learned body.
I confirm that this is a true statement and that, subject to any comments above, the submission is my own original work.

Signed: .......................................................... Date: .............................................
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ACKNOWLEDGEMENTS

This thesis represents the conclusion, not only to the last four years of my professional work, but also to a significant chapter in my personal life. My move to the UK from Canada to take on my first research post has proved to be an equally challenging and rewarding decision. Little did I know when this journey first began, that writing my PhD thesis would also become a part of it.

First and foremost, I wish to express my deepest gratitude to Professor Adele Green, my mentor and supervisor, who has been instrumental in the achievement of this thesis. It has truly been a privilege to work alongside such a well-respected and influential researcher. Adele, I am indebted to you for taking me under your wing and enabling me to have so many enriching experiences from which I have been able to hone my research skills. Thank you for your unwavering trust and support and for inspiring within me both confidence and ambition.

It has equally been an honour to collaborate with Professors Jillian Birch and Lesley Rhodes. I am appreciative of the opportunity to have worked within their research groups and for their guidance throughout our respective projects together. I am grateful for the support of my Manchester colleagues both in the CRUK Paediatric and Familial Cancer Research Institute, particularly Dr. Robert Alston for his patience with my myriad of statistical queries, and to those in the Photobiology Unit at the Salford Royal Hospital, especially Dr. Suzanne Pilkington who I am fortunate to call both colleague and friend.
In addition, I extend my gratitude to the Cancer and Population Studies Group at the QIMR Berghofer Medical Research Institute in Brisbane, Australia, who warmly hosted me on two occasions. Specifically, I would like to acknowledge Dr. Jolieke van der Pols to whom I am indebted for sharing her wealth of nutritional epidemiology knowledge and for her direction on our joint projects. Special thanks also to Maricel Hughes, Torukiri Ibiebele and Dr. Catherine Olsen.

I must also recognise my ultimate support system, my family and friends, without whom I would not be where I am today. To my parents, Robert and Joanne, and my siblings, Megan and Ken, thank you for your unconditional love and support and for encouraging me to dream big and chase my goals, even when it meant helping me move “across the pond”. I am grateful to have so many wonderful friends, too numerous to name individually, on both sides of the Atlantic, who have helped motivate me towards this goal. To Gemma Darby especially, I never would have lasted in Manchester without you.

Finally, and most importantly, I thank my best friend and partner in life, who has been unwavering in his belief in my ability to achieve this goal, even when I doubted it would come to fruition. Will, I never anticipated meeting you along this journey, but I could not have made it to the finish line without you.
THE AUTHOR

Degrees and qualifications

Master of Science (MSc) in Epidemiology, Queen’s University, Kingston, Canada (September 2007 - October 2009).

Bachelor of Science (Honours) (BScH) in Biology, Queen’s University, Kingston, Canada (September 2003 - May 2007).

Research experience

Research Associate, Institute of Inflammation and Repair, The University of Manchester, Manchester, UK (November 2009 - March 2014). The various projects I undertook whilst in this post included analyses of melanoma incidence trends in England as well as a comparison of rates among youth in England versus Australia; studies of the relationship between omega-3 and omega-6 fatty acids and skin cancer; reviews of skin cancer arising in scars and of the effects of childhood sun exposure on skin; and a study of actinic keratoses and actinic field change in renal transplant recipients.

Visiting Scientist, Cancer and Population Studies Group, QIMR Berghofer Medical Research Institute, Brisbane, Australia (July 2010 - August 2010 and July 2011 - August 2011). This was a collaborative opportunity as part of the aforementioned post in which I primarily worked with the nutritional epidemiology group on projects related to the association of omega-3 and omega-6 fatty acids and skin cancer. I was also involved in conducting a systematic review and meta-analysis of smoking and skin cancer.
Research Assistant, Department of Community Health and Epidemiology, Queen’s University, Kingston, Canada (July 2007 - January 2008). My principal role involved conducting data collection and telephone interviews for a study of the epidemiology and genetics of breast cancer. Also, I contributed to the development of a media symposium presentation on climate change and public health and to the modifications of the strategic plan for the Queen’s Institute of Population and Public Health.
LIST OF PUBLICATIONS SUBMITTED

Publication 1

Publication 2

Publication 3

Publication 4

Publication 5
Wallingford SC, Pilkington SM, Massey KA, Al-Aasswad N, Ibiebele TI, Hughes MC, Bennett S, Nicolaou A, Rhodes LE, Green AC. Three-way assessment of long-
chain n-3 PUFA nutrition: by questionnaire and matched blood and skin samples.


**Publication 6**

INTRODUCTION

Background context for this thesis

The keratinocyte cancers, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), are the most common cancer types occurring in white populations.\textsuperscript{1} By comparison cutaneous melanoma is somewhat rarer, though its incidence over the last few decades has been increasing much more rapidly than that of BCC or SCC.\textsuperscript{2} Worldwide, the magnitude of incidence rates for these three malignancies ranges widely among predominantly white nations, with peak rates noted in Australia.\textsuperscript{3, 4} A World Health Organisation study estimated that in 2010 the overall global burden of these diseases, measured as the number of years of healthy life lost due to disability or premature death (disability adjusted life-years - DALYs), was 1,169,000 (DALY rate, 17 per 100,000) for melanoma and 798,000 (DALY rate, 12 per 100,000) for keratinocyte cancers.\textsuperscript{5} Further, a systematic review noted that the respective costs attributable to morbidity and mortality from melanoma and keratinocyte cancers collectively ranged from $US28.9 million to $US39.2 million and $US1.0 to $US3.3 billion in studies from predominantly white populations in the period 1990 to 2010.\textsuperscript{6} Thus cutaneous malignancies pose a substantial burden both to public health and to economic costs though the precise magnitude varies between individual populations according to the prevalence of risk factors for skin cancer development.

In terms of primary characteristics, melanomas originate de novo in the melanocytes of the epidermal layer of the skin or arise in existing nevi.\textsuperscript{7} The median age for melanoma diagnosis is approximately 55 years and tumours usually occur with similar overall frequency in both sexes, though variations in
sex-specificity occur by anatomic site: the trunk predominating among males and the lower limb among females.\textsuperscript{8} In contrast, BCC and SCC which originate in keratinocytes of the basal and squamous layers of the epidermis respectively and change following sun damage, typically present as pink, pearly papules or plaques (BCC), or as more scaly nodules or plaques (SCC).\textsuperscript{9} Over 80\% of keratinocyte cancers occur in people over the age of 60 and they predominately afflict males.\textsuperscript{10} The body site distributions of these tumours are consistent with the effects of cumulative solar UVR exposure as the majority occur on the highly exposed head, neck and dorsal hand.\textsuperscript{1, 10}

Exposure to ultraviolet radiation (UVR) is the principal environmental cause of cancers of the skin.\textsuperscript{11} UVR both initiates and promotes skin carcinogenesis by inducing DNA damage in the relevant skin cells and local immunosuppression.\textsuperscript{12} The population attributable fraction (PAF), or proportion of all cases which are thought to be attributable to UVR exposure, ranges from 0.5 to 0.9 for both melanoma and BCC and 0.5 to 0.7 for SCC.\textsuperscript{13} There are both solar and artificial sources of UVR and increases in exposure to both of these are thought to be driving observed increases in rates of cutaneous malignancies to varying extents in different populations. From a global environmental perspective, solar UVR exposure has risen due to measured depletions in the ozone layer since the 1970s, resulting from the accumulation of ozone-destroying chemicals (i.e. chlorofluorocarbons and hydrochlorofluorocarbons) in the atmosphere.\textsuperscript{11, 14} It is thought that as little as a 10\% reduction in stratospheric ozone may increase ambient solar UVR exposure by some 20\%, and may result in up to a 40\% rise in skin cancers after approximately 20 years of increased exposure levels.\textsuperscript{14-16} In addition to possible widespread increases in ambient UVR levels, a clear shift in attitudes and behaviours from sun-avoidance to sun-seeking in white populations
around the world during the last century has resulted in the social desirability of tanned skin;\textsuperscript{17-19} where ambient sun exposure is limited, this has led to a rise in popularity of alternative UVR sources such as sun holidays and sunbeds.\textsuperscript{20-24}

Although estimated PAF values indicate that UVR is responsible for more than half of all cases of cancers of the skin, it is apparent that a proportion of the burden of these diseases occurs independently of UVR. While phenotypic traits can increase susceptibility to the effects of UVR, genetics and family history may affect risk of malignancy separately.\textsuperscript{25-29} Similarly, individual exposures to other environmental agents such as ionizing radiation\textsuperscript{30-32} or certain dietary components\textsuperscript{33-36} may modify risk directly or through effects on UVR-induced carcinogenic pathways. Also linked to increased risk of skin cancers are other diverse aspects of health including tissue injury resulting in scarring\textsuperscript{37-39} and immunosuppressive drugs.\textsuperscript{40-42} However, despite the extensive volume of research conducted on the epidemiology and aetiology of cancers of the skin, our understanding of the specific contributions of UVR- and non-UVR-related risk factors and how they interact to determine risk of cutaneous malignancies, remains incomplete.

**Overview of thesis structure and aims**

The primary objective of this thesis is to further the understanding of the epidemiology and aetiology of cancers of the skin. It comprises six original, peer-reviewed, research articles (including one short communication and one systematic review) each aiming to investigate, directly or indirectly, a specific gap in knowledge of the role of either UVR-related factors or other risk factors for skin cancer. The constituent papers use a variety of epidemiologic designs
and established methodologies to address these gaps. Summary information for each manuscript is provided in Table 1.

In terms of the general layout of the thesis, Chapters 1 to 3 subdivide the publications into three groups according to the specific aspects of skin cancer epidemiology and aetiology that they investigate. Presented in each chapter is a review of the state of knowledge of the topic area, followed by a summary of the aims and methods, a discussion of the findings and the conclusions and implications of the manuscripts. The interrelationship, strengths and limitations and overall impact of the publications as a cohesive body of work are highlighted in the summary discussion following the chapters. The publications themselves appear subsequent to the final discussion.

Regarding the specific chapters, Chapter 1 comprises Publications 1 and 2 which present descriptive epidemiological studies of melanoma in England, a country with relatively low ambient solar UVR levels. Based on national melanoma registration data, they document trends in incidence by anatomic locations of primary melanoma (Publication 1) and by region of residence and level of socio-economic deprivation (Publication 2). The main collective aims of these two papers were to gain insight into, and generate hypotheses about, the specific influence of UVR-related factors (both from ambient sun, as well as alternative UVR sources) through detailed study of the most recently available melanoma incidence trends.
<table>
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<th>Title of Paper</th>
<th>Type of Study</th>
<th>Principal Topic</th>
<th>Source of Data</th>
<th>Time Period</th>
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<td>Intake of omega-3 and omega-6 fatty acids and risk of basal and squamous cell carcinomas of the skin: a longitudinal community-based study in Australian adults</td>
<td>11-year prospective cohort study</td>
<td>Associations of omega-3 and omega-6 PUFA intake and risk of BCC and SCC</td>
<td>Nambour Skin Cancer Study</td>
<td>1997-2007</td>
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<tr>
<td>Plasma omega-3 and omega-6 concentrations and risk of cutaneous basal and squamous cell carcinomas in Australian adults</td>
<td>11-year prospective cohort study</td>
<td>Associations of omega-3 and omega-6 PUFA blood levels and risk of BCC and SCC</td>
<td>Nambour Skin Cancer Study</td>
<td>1997-2007</td>
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<td>Three-way assessment of long-chain n-3 PUFA nutrition: by questionnaire and matched blood and skin samples</td>
<td>Three-way matched comparison</td>
<td>Correlation between EPA intake and circulating blood and skin levels</td>
<td>Double-blind randomized placebo-controlled nutritional study of omega-3 PUFA supplementation</td>
<td>Trial baseline (2010)</td>
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<td>Skin cancer arising in scars: a systematic review</td>
<td>Systematic literature review</td>
<td>Associations of cancers of the skin with scar tissue of any type</td>
<td>Pubmed, Google Scholar</td>
<td>To August 2010</td>
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**Table 1: Summary of individual studies included in thesis.**

*Abbreviations: BCC - basal cell carcinoma, EPA - eicosapentanenoic acid, PUFA - polyunsaturated fatty acids, SCC - squamous cell carcinoma.*
In Chapter 2, a second group of three papers considers the assessment of associations of nutritional factors on keratinocyte cancer risk, in particular that of omega-3 and omega-6 polyunsaturated fatty acids (PUFA) which have been suggested to play opposing roles in skin carcinogenesis. Publications 3 and 4 use data from a prospective cohort study of people living in subtropical Australia with high ambient UVR, evaluating dietary intake (Publication 3) and blood concentrations (Publication 4) of omega-3 and omega-6 PUFA in relation to BCC and SCC risk. In conjunction with these, Publication 5 is a three-way matched assessment of omega-3 PUFA nutrition in a different study population, which compares estimated dietary consumption of these nutrients recorded by food frequency questionnaire (FFQ) with their circulating levels in the bloodstream and with measures of bioavailability in skin. Together these three papers aim to provide evidence for associations of omega-3 and omega-6 PUFA in skin cancer development and to demonstrate the utility of different measures of PUFA in potentially assessing such relationships.

Finally, in Chapter 3 the relationship between a quite different but often invoked non-UVR-linked risk factor, namely scars, and cancer of the skin is explored. Publication 6 reports a systematic review of published observational studies quantifying this association with the aim of shedding light on the magnitude of the risk of skin cancer posed by past tissue injury that has resulted in the presence of substantive scar tissue.
CHAPTER 1:
Descriptive Epidemiology of Melanoma in a Population with Low Ambient Solar UVR Exposure
(Publications 1 and 2)

1.1 Global trends in melanoma incidence

Approximately 200,000 new cases of invasive cutaneous melanoma were diagnosed worldwide in 2008, and while this disease is not exclusive to fair-skinned individuals, some 85% of these cases occurred in predominantly white, developed nations. Solar UVR exposure is the primary environmental cause of melanoma, and variations in ambient levels are linked to differences in incidence rates by country. As ambient sun exposure in Australia and New Zealand is very high, and they have predominantly white-skinned populations, they report the highest incidence rates of melanoma globally. The average lifetime risk of malignancy to age 75 for persons living in these countries was 3.6% in 2000-2002 compared with 1.9% in the United States of America (USA), 1.1% in Canada and 0.3-1.6% across Europe, in the same period. Rates in 2010 among men and women respectively reached 55.9 and 40.3 per 100,000 in Queensland, Australia, and 40.3 and 33.6 per 100,000 in New Zealand. Among European nations melanoma incidence in 2012 was highest in northern countries with low ambient solar UVR but high-risk phenotypic traits: in Switzerland (26.8 and 25.4 per 100,000) and Norway (26.6 and 24.8 per 100,000) respective rates were higher among men than women; in The Netherlands (22.5 and 26.5 per 100,000) and Denmark (22.0 and 26.6 per 100,000) the male:female ratio in incidence was reversed; and in Sweden overall sex-specific rates were equal (both 24.1 per 100,000). Lowest European rates were in more southerly nations, such as Greece (3.8 and 2.6 per 100,000 in men and women
respectively) where the populations are adapted to high ambient sun exposure through easily tanned skin types. Melanoma rates in North American white populations are slightly lower in magnitude compared to those of northern Europe, but are similar to Australia in sex-specificity (2010 rates in males and females respectively: 17.9 and 13.3 per 100,000 in the USA; 11.3 and 9.8 per 100,000 in Canada).

The incidence of melanoma has been rapidly increasing over the last several decades among fair-skinned populations worldwide however recent observations suggest that the increasing trends are now abating in younger generations in some countries. In past decades, the rate of increase in the incidence of melanoma was most pronounced in Nordic countries however, in the 21st century, melanoma is rising most rapidly in other parts of Europe with estimated annual percentage change (APC) reaching over 6% for males in England, Slovakia and Spain and over 5% for females in Denmark, England, Slovenia and Switzerland. On the other hand, trends in melanoma incidence in some of the most high-risk populations such as in Australia, New Zealand, North America and Scandinavia appear to be levelling off (APCs around 1%) and starting to decline particularly among adults 25-44 years old. While the rising trends among some of these countries are thought to be due in part to increases in the diagnosis and reporting of thin melanomas as both clinical surveillance and public awareness of skin cancers have improved, the impact of long-established primary prevention campaigns and supportive public health policies are conversely thought to explain the decline in rates among others.
1.2 UVR-related risk factors for melanoma

Both the intensity and duration of UVR exposure from solar and alternative UVR sources are important to melanoma development, and these are ultimately dictated by both environmental and individual behavioural factors. Ambient UVR levels are highly dependent on latitude as intensity of sun exposure increases with proximity to the equator,\textsuperscript{11, 14, 60} and as a result north-south increasing trends in melanoma incidence have been observed in some countries of the northern hemisphere.\textsuperscript{61-64} While ambient sun exposure has been elevated in recent decades due to environmental changes (i.e. stratospheric ozone depletion),\textsuperscript{11, 14, 60} personal sun exposure has risen even more with societal shifts towards sun-seeking behaviour: increases in outdoor recreational activities, in the desirability of tanned skin and in skin-exposing clothing fashions.\textsuperscript{17-19} This shift in attitude has coincided with greater wealth and leisure time of many white populations affording them increases in sunny holidays abroad,\textsuperscript{20, 21, 64} and has also underpinned the rise in sunbeds use\textsuperscript{22, 23, 65} - all positively linked to melanoma risk. Furthermore, a history of sunburn due to intense or sustained UVR exposure from any or a combination of these sources, particularly during childhood, may double the overall risk of melanoma.\textsuperscript{66} However, the effect of these UVR sources on risk for this malignancy can also vary according to overall timing and patterns of exposure such that a divergent pathway model has been hypothesised whereby high cumulative or chronic sun exposure is associated most closely with tumours on highly exposed body sites (i.e. head and neck) while ‘intermittent’ or recreational exposure patterns are linked to less-exposed sites (i.e. trunk).\textsuperscript{67, 68}

The relationship between UVR and melanoma within white populations is not solely a function of the environment and individual behaviours, but also
dependent on genetics and phenotype. Among the most important phenotypic traits influencing melanoma development is the presence of a large number of melanocytic nevi. People with one or more atypical nevi have over 3.5 times the risk of melanoma compared to those without any (PAF, 0.25) and risk of melanoma increases by approximately 2% with each common nevus counted on an individual. A meta-analysis of the relation between nevi and melanoma estimated that 42% of all melanomas are attributable to the presence of 25 or more nevi on the body. Other high-risk constitutional factors include skin phototypes I/II (always burns, never tans/usually burns, tans with difficulty), red/blond hair and presence of freckling, each of which on average doubles the risk of melanoma in people with these traits compared to those without. Moreover, approximately a quarter of all melanomas are attributable to each of these characteristics. A positive familial history of melanoma is also linked to greater risk for this disease, though a comparatively small proportion of new cases (less than 7%) have been attributed to this factor and perhaps more so in populations with greater UVR exposure.

While the interplay of these environmental, behavioural and genetic risk factors causes melanoma, as previously mentioned their relative roles and specific contributions vary according to the population under examination. Consequently, it is important to study and compare incidence trends of melanoma in different settings in order to identify the key aetiologic factors which could be targeted in prevention strategies in each setting or population.

1.3 Melanoma incidence in England - aims and methods

Given the complexities in the interaction between environment and behaviour in determining individual UVR exposure levels, the first two studies reported in this
thesis were conceptualised in order to obtain an overview of melanoma incidence in England, a country with low ambient solar UVR levels. Publication 1 specifically examined melanoma incidence by anatomic site since the distribution of tumours on the body is thought to reflect the relative levels and patterns of UVR exposure of skin at various sites.\textsuperscript{68, 69} Publication 2 investigated melanoma trends by region of residence and level of socio-economic deprivation given that both decreasing latitude (in the Northern hemisphere) and increasing wealth are linked to melanoma through enabling greater UVR exposure.\textsuperscript{64, 70-72} Ecological studies such as these are often used to gain insight into the magnitude of disease incidence rates in an entire population as well as the emerging patterns of incidence with respect to population-level factors. Though a limitation of this study design is that disease risk cannot be inferred at an individual-level, descriptive studies are basic to understanding the epidemiology of a disease and can lead to more rigorous studies of associations with specific risk factors. In the case of these papers, documenting national trends in melanoma incidence in England resulted in the generation of hypotheses about the influence of specific determinants of UVR exposure on increasing melanoma rates in this country.

Imperative for validity of ecological analyses is the accuracy and completeness of the data available. In England, cancers are registered by eight regional registries which use standard criteria set by the United Kingdom (UK) Association of Cancer Registries to collect information on new primary invasive cases (recurrences and metastases not registered).\textsuperscript{73} Histology reporting is the main source for registration of new melanomas in these registries. Regional data at the time of collection for Papers 1 and 2 were collated into a national database by the Office of National Statistics, UK and then distributed accordingly by the
closest regional registry, in this case the Northern and Yorkshire Cancer Registry and Information Service. Data collected and disseminated via this system are considered to be of a high standard (approximately 97% complete), though some variability in data capture and efficiency of processing remains between regional registries.

1.4 Melanoma incidence in England - discussion of main findings

Investigations into the incidence of melanoma by anatomic site carried out in different populations have proven extremely useful in furthering understanding of melanoma aetiology. An important consideration in the interpretation of these studies is whether adjustments for body surface area have been carried out. Performing such adjustments permits the comparison of the burden of this malignancy per unit area of skin in order to account for large disparities in the amount of surface area of different body sites, though this method assumes an even distribution of tumours on all body sites. As no previous nationwide studies of site-specific melanoma incidence in England had been conducted, Publication 1 was conceived with the specific aim to examine the trends in rates in this country over three decades (1979-2006). Surface area was thus not accounted for here as these adjustments would have no bearing on the interpretation of temporal trends. Previous studies with a similar approach (no adjustments) are used in the subsequent comparative discussion.

Overall, the magnitude of incidence rates in England in the most recent 5-year period of analysis (125 and 138 per million person-years in males and females respectively) was somewhat lower in Paper 1 compared to those reported above in other European countries. Site-specific incidence in England for the entire period of study was consistently highest on the lower limb of females (38-60 per
million) followed by the trunk in males (15-56 per million) and these peak sites were identical to those noted in two UK-based region specific studies restricted to Scotland (1979-2003) and to Southeast England (1960-1998), though the magnitude of the rates from these were somewhat lower (2.5-4.0 per 100,000 on male trunk, 3.0-5.0 per 100,000 on female lower limbs).\textsuperscript{50, 81} In other northern countries with similar low ambient solar UVR exposure during similar calendar periods, site-specific rates also favoured the trunk in males and the lower limb in females respectively, for example in Finland (5.8 and 2.9 per 100,000),\textsuperscript{55} Sweden (9.2 and 6.3 per 100,000),\textsuperscript{82} The Netherlands (7.7 and 8.8 per 100,000),\textsuperscript{83} Canada (3.29 and 2.58 per 100,000)\textsuperscript{84} and the USA (8.3 and 4.9 per 100,000).\textsuperscript{85} In the southern hemisphere, the same peak sites for melanoma were observed in New Zealand in an earlier period (1968-1993), but absolute rates were higher (male trunk, 6.4 per 100,000 and female lower limbs, 10.9 per 100,000).\textsuperscript{86} Similarly, a more recent analysis in Australia (1982-2008) demonstrated melanomas were also most common overall on the trunk (17.3 per 100,000), though followed by the upper limbs (11.7 per 100,000).\textsuperscript{87}

From this comparison it is evident that the site predominance patterns for melanoma in England are consistent with those observed both in other northern countries and in the southern hemisphere, irrespective of the considerable difference in ambient solar UVR levels between the two regions.\textsuperscript{43} That the trunk and legs show peak incidence rates reflects both their large surface area for exposure, as well as global trends of increasing occasional or recreational exposure to UVR which has notably been linked to melanoma at these “intermittently” exposed body sites.\textsuperscript{67, 68} As alluded to previously, this pattern of UVR exposure has resulted from the societal shift towards sun-seeking attitudes and behaviours which has permitted greater exposure of usually-covered
skin. However, that the magnitude of rates in Australia and New Zealand are much higher than in England (and other countries of similar latitude) reflects the overall greater ambient solar UVR exposure obtained in these two countries. Also of note is the contrast between England, and other European and North American countries with respect to the absolute peak in site- and sex-specific rates, which was the lower limb of women for the former, but the trunk in men for the latter. While the underlying reason for this difference remains unknown, it is likely that the aforementioned variations between countries in both ambient solar UVR levels as well as the prevalence of related behaviours play a role.

Following on from the analysis of the magnitude of recent melanoma rates by site, the investigation of site-specific trends over time in England as a whole also found that the greatest annual percentage increases in rates over the period 1979-2006 were observed for trunk melanomas (males, 6.8%; females, 5.9%) followed by the upper limbs (males, 6.7%; females 5.3%) and were even higher at these sites for people over the age of 45. These results are some of the highest site-specific rates of increase of melanoma that have been reported in this period in the northern hemisphere. Comparatively, in the USA melanoma incidence in people up to age 80 increased much more slowly per year in the same relative period, by 3.8% for female truncal melanomas (driven mainly by increases at this site among women younger than 40 years) and around 3% for tumours on the limbs and head and neck for males. Canadian APC estimates were highest for the trunk, as in England, in people to age of 60, but overall increases in rates levelled off from 6.9% in 1956-1988 to 1.0% in 1989-2005. Similar to Canada, APC estimates in Finland slowed at all sites from the mid-1980s to 2003 (males, 1.2%; females, 1.4%) compared with earlier periods (1953 to mid-1980s: males, 5.2%; females, 5.1%). In stark contrast to England
however, Australian rates have recently (up to 2008) been in decline (negative APC values) on the trunk and upper limb, particularly in people under age 60, though in older cohorts, melanoma continues to rise especially on highly sun exposed head and neck sites.87

Overall the high APC estimates in England relative to the attenuations and declines in incidence observed in similar predominantly fair-skinned populations, are mostly likely explained by the lag between initiation of public education and prevention strategies and subsequent population-level effects. Australia, for example, has had skin cancer prevention campaigns and policies in place since the 1980s so recent declines in rates are assumed to be partly the result of long-term implementation of interventions and awareness campaigns aimed at reducing UVR exposure in the population.87 Primary prevention activities in the UK have been implemented only very recently in comparison - since 2003 Cancer Research UK has operated a SunSmart campaign to increase public education.90-92 Thus, the continued increases in rates in England were perhaps unsurprising as beneficial effects of such initiatives on melanoma incidence take years to be observed and usually require very concerted effort and simultaneous policy changes. Also, the observation that rates increased most rapidly among men likely reflects their poorer awareness of and compliance with UVR protection measures (e.g. sunscreen use) than women.91, 93

Using the same dataset as for Publication 1, Publication 2 provided the opportunity to report an additional investigation into melanoma incidence - latitudinal trends across England. The anticipation was that this analysis would help further elucidate the general relationship between melanoma trends and solar UVR exposure and related behaviours. As expected, regional melanoma
rates were observed to be highest in the southern parts of the country (South East: 15.44 and 16.81 per 100,000 and South West: 18.40 and 19.35 per 100,000 in men and women respectively). This had been previously documented: the South East and South West regions of England have the highest rates of cutaneous malignancy not only due to greater ambient sun as demonstrated by the distribution of hours of sunlight across the whole of the UK (Figure 1) but also to the higher proportion of white Caucasian people and those of higher socio-economic class in these areas compared to other parts of the country. African and Asian groups have been shown to be at innately low risk of melanoma, while wealth, as indicated by lower socio-economic deprivation, higher education, and professional type occupations, has been previously linked to higher rates of melanoma incidence in England.
Figure 1: Average annual sunshine duration across the UK (1971-2000).

Boundaries indicate designated Government Office Regions. Used and adapted with permission from the Met Office Crown Copyright. Image courtesy of Madeleine Flynn. Cited from Wallingford et al. 2013.\textsuperscript{94}
The most notable finding of the study however, was the difference in latitudinal trends by age as highlighted in the title of the paper. It was among adults over age 30 that melanoma incidence increased significantly with more southerly latitudes, but this was not seen among young people aged 10-29 years. Specifically, there was an absence of any latitude trend in young males, and a strong inverse trend (increasing incidence from south to north) among young females. Prior to this analysis, the north-south latitudinal trend had been described some three decades earlier in a study of melanoma in England and Wales, but since then no others had been conducted. The striking disparity between age groups especially in females was further highlighted in that several other populations of the northern hemisphere have similarly shown north-south gradients in incidence which principally reflected ambient sun exposure. There have been only a few other reports of deviations from the increasing north-south pattern and these have been explained by regional variations in other melanoma risk factors, such as the predominance of certain skin phenotypes or higher income groups.

Given the distinct nature of the age-specific latitudinal findings, the second part of Paper 2 involved examining melanoma latitude trends in young people by socio-economic deprivation in order to explore its possible confounding effect on the observed reversal/lack of association of melanoma incidence with latitude. In most geographical areas, the observed inverse associations between melanoma incidence rates and socio-economic deprivation were consistent with those of previous UK-based studies. Exception was held however, for the northern regions of England where uniquely this inverse trend was not significant among young males and highest rates of all were observed for young females of moderate socio-economic status.
Although it has already been considered that ambient solar UVR levels have increased due to a combination of environmental and behavioural factors, this source is unlikely to explain such drastic trend reversals in England as these changes are widespread and not region-specific. Increasingly relevant contributors to individual UVR exposure which may explain both the reversal of latitudinal trends and peak incidence among moderately deprived young women are the recent surges in the popularity of sun holidays and sunbed use. The growing industry of budget airlines in England and declining airfares have made travel abroad to sunny destinations increasingly affordable and accessible to people of all ages and socio-economic statuses, and the number of hours spent on sun holidays has been positively associated with melanoma risk. In addition, the principal non-solar sources of UVR, sunbeds are highly coveted for the deep and long-lasting tan induced by their UVR lamps, and provide exposure up to 15 times stronger than that obtained in the midday Mediterranean sun. As a result, melanoma risk is elevated by approximately 20% in ever (compared to never) users of sunbeds with risk increasing by 1.8% with each additional session of use per year. In England, a reported 6% of all 11-17 years olds have ever used a sunbed, and use is most common in those living in the northern parts of the country, especially of lower socio-economic grades. Consequently, these alternative sources to local ambient UVR exposure appear to be contributing to the disruption of both the previously described inverse latitude gradient in melanoma incidence across England, as well as the positive relationship with socio-economic status, especially in young women.
1.5 Conclusions and implications

The findings of Publications 1 and 2 are consistent with the belief that increases in recreational UVR exposure, from ambient sun as well as artificial UVR sources, over the last 50 years are driving the recently observed changes in trends in melanoma incidence in England. Since the early 20th century, the western world has witnessed a shift both in attitudes and behaviours towards sun-seeking and sun-tanning, such that having a tan has become a coveted symbol of overall good health and well-being.\(^{17-19}\) This has inspired a movement towards what is now considered an integral part of modern-day lifestyle, which encourages recreational outdoor activities, active sunbathing and revealing clothing fashions (e.g. raised hemlines and bikinis).\(^{2, 57, 107}\) In particular, these lifestyle modifications have permitted increased solar UVR exposure in every-day life, but have also influenced behaviours towards sun obtained on holidays abroad,\(^{20, 21}\) and have encouraged the use of sunbeds as an alternative means of obtaining a tan.\(^{22, 23, 65}\) Consequently, in addition to sun exposure in temperate England, these alternative sources of UVR exposure have also been augmented in the English population with the result that traditional melanoma patterns have been modified, both with respect to the distribution of tumours by anatomic site, as well as the relation of melanoma incidence to socio-economic deprivation and latitude gradients, specifically among young women.

Analyses of incidence trends in melanoma as in Papers 1 and 2 can provide evidence for the presumed impact of public health measures focussed on raising awareness and encouraging safe behaviours in relation to UVR exposure. Public health campaigns in Australia such as Slip! Slop! Slap! in the 1980s and the current and continuing SunSmart skin cancer prevention campaign (advocating sun safety and encouraging practices such as wearing protective clothing and
applying sunscreen before going outdoors) as well as related policies such as shade provision in relevant outdoor areas (e.g. in child-care centres and schools), are likely to have helped achieve the recent downturns in melanoma incidence in the youngest generations. On the other hand, primary prevention activities in many other white populations have been implemented only very recently in comparison. In England, these activities include the SunSmart program in 2003, as well as legislation in 2010 placing a UK-wide ban on sunbed use by youth under the age of 18 years.

Considering the lag-time between initiation of sun-protection efforts and observed decreases in melanoma incidence in places such as Australia, similar effects on rates in young people in England should start to become evident over the next decade, but will take many decades to affect rates in older cohorts. Furthermore, decreases in melanoma incidence will only occur if prevention programs and activities are sustained at a high level, otherwise initiatives such as those mentioned above are unlikely to have any dramatic effect on incidence of this disease. In order to continue to promote the dangers of sunbeds and the importance of practicing sun safe behaviours, mass-media must be utilised to engage all facets of society, including through the use of traditional media such as school programs and ad campaigns, but also by new social media formats to capitalise on the opportunity to engage young people especially, through interactive discussions on these issues. Recent examples of these include the “Dear 16-year old Me” melanoma awareness video campaign, smart phone applications providing real-time UVR levels and sunscreen reapplication notifications, and Facebook and Twitter discussion groups and forums run by organisations such as the Melanoma Research Foundation. Furthermore, while the potential implications of the UK-wide sunbed ban represent a positive
stride towards reducing the harmful effects of this artificial UVR source, unfortunately it is not all-encompassing since it applies only to young people and to commercial outlets so private use remains unregulated. Altogether outlawing of sunbeds, as has recently been achieved in Brazil, remains an important goal for the future.

In summary, Publications 1 and 2 indicate that increases in melanoma incidence in England are likely due to parallel increases in UVR exposure especially through recreational behaviours. Future ecological studies must continue to concurrently monitor melanoma incidence trends at the population-level as well as UVR exposure patterns in order to assess the effectiveness and appropriateness of targeted primary prevention strategies and legislation in place. Other types of observational studies, ideally prospective cohorts, should also be used in the future to establish risk estimates of melanoma in this population in relation to intense UVR exposure obtained on holidays abroad and from sunbeds.

1.6 Impact of publications

Prior to Publication 1, national-level, site-specific trends in melanoma incidence for England had not previously been described. Since it was made available in August 2011, this study has been cited 9 times (ISI Web of Knowledge) or 15 times (Google Scholar) in publications. Collation and analysis of the data for this study also led to the inception of subsequent work - Publication 2.

Publication 2 is the first study in over three decades to examine latitude trends in melanoma incidence in England. As it has only just been released in print (October 2013), this paper has yet to be cited by other scientific work. However, as an indication of its positive reception, upon acceptance for publication this
manuscript was the focus of issued press releases from the British Association of Dermatologists, as well as from The University of Manchester.

Both Publications 1 and 2 are published in the British Journal of Dermatology, impact factor 3.759 and currently ranked 5th out of 59 Dermatology journals for 2013.
CHAPTER 2:
Assessing Associations Between Nutritional Fatty Acids and Keratinocyte Cancers
(Publications 3, 4 and 5)

2.1 Keratinocyte cancer incidence

While melanoma is the most rapidly increasing cutaneous cancer, the absolute incidence rates of the keratinocyte cancers are much higher, making them both a significant health and economic burden in white populations.\(^1\), \(^{107}\), \(^{115}\) Worldwide, rates of BCC and SCC range widely with highest incidence observed in Australia at 884 and 387 per 100,000 for each cancer respectively (2002), \(^{116}\) while in European populations, recent peak rates of approximately 90 and 20 per 100,000 for BCC and SCC respectively, have been noted in northern nations such as The Netherlands and Denmark.\(^{51}\), \(^{117}\) Yet, overall BCC and SCC remain some of the most poorly quantified cancer types because of the scarcity of high quality incidence data, as historically, mandatory reporting of new cases to national cancer registries has rarely been in place.\(^1\), \(^4\) In some countries (e.g. England, Northern Ireland, Finland), nationwide cancer registration systems record only the first case of BCC and SCC each, despite many patients presenting with multiple tumours at once and/or over their lifetime (subsequent BCC/SCC and multiple concurrent malignancies are not recorded),\(^4\), \(^{118}\), \(^{119}\) thereby making it difficult to assess the true magnitude of the problem or accurate incidence trends over time. Many studies using this type of data also admit to limitations regarding the completeness of records on BCC and/or SCC.\(^{51}\), \(^{117}\), \(^{120}\) In countries where such comprehensive registration of these cancers does not exist, incidence rates may be assessed through registries in specific areas of the country,\(^{121-123}\) using population-based surveys\(^{116}\), \(^{124}\) or by longitudinal studies of
primary care or other population-based samples.\textsuperscript{125, 126} Bearing in mind these limitations, knowledge about the impact of these malignancies and their relationship to causal factors are mainly assessed through observational studies, which focus on investigating specific risk factors.

2.2 Environmental and phenotypic risk factors for keratinocyte cancers

There are many risk factors shared by both BCC and SCC as well as by melanoma as discussed in Chapter 2. Generally, both main types of keratinocyte cancers occur with greater frequency in males than in females of the same population (up to 2-fold greater incidence for BCC and up to 4-fold for SCC).\textsuperscript{116, 119, 122, 124} As with melanoma, BCC and SCC are rare in black people and occur mostly in white Caucasians such that their phenotypic risk factors are similar (namely blond or red hair, blue eyes, fair complexion and skin which burns easily and rarely or never tans).\textsuperscript{27, 28} Also, sun exposure is the primary environmental cause of keratinocyte cancers\textsuperscript{11, 13} so UVR-related behaviours (i.e. sun-seeking) and sunbed use have both broadly been associated with increased risk for these tumours, though links to sunbeds especially are more strongly established for SCC than for BCC.\textsuperscript{4, 22, 24}

The main differences in the aetiologies between BCC and SCC pertain to a differential in the amount and timing of UVR exposure linked to each cancer. While lifelong, cumulative and occupational exposures have the greatest influence on SCC risk, a combination of cumulative and occasional (i.e. during recreational activities) sun exposure is thought to cause BCC.\textsuperscript{115, 127} Consistent with this combination of UVR exposure patterns, BCC tumours tend to occur in adults over the age of 60 (mean age at diagnosis approximately 65 years in
predominantly white countries of the northern hemisphere). They are also found not only on highly exposed areas of the body but also on the less exposed trunk, accounting for 15-25% of all tumours. They are also found not only on highly exposed areas of the body but also on the less exposed trunk, accounting for 15-25% of all tumours. SCC, on the other hand, are observed principally on the head, forearms and hands. They are associated with later onset (mean age approximately 75 years in northern populations) and rates that increase more rapidly with older ages, consistent with the greater total lifelong UVR exposure needed for a tumour to develop.

2.3 Nutritional fats and keratinocyte cancers

Separate from the well-described environmental and phenotypic risk factors for keratinocyte cancers, there is a substantial body of research investigating hypotheses around the effects of nutritional factors, in particular fats, on skin cancer development. Dietary fat is a specific nutrient of interest as consumption varies widely with patterns of food intake in different countries and daily intake in Western populations is so high that it is one of the greatest macronutrient exposures in this group. Data from human interventions as well as epidemiologic studies have demonstrated reductions in keratinocyte cancers with low-fat dietary patterns (or the reverse - increases with high-meat/fat), as well as positive links between SCC and total fat consumed. However, upon more detailed examination, it has become apparent that different classes of fatty acids may have distinct contributions to UVR-induced carcinogenesis (photocarcinogenesis) in skin due to their respective compositions and specific metabolic effects. Of particular importance appears to be the ratio between omega-3 and omega-6 PUFA. Nonetheless, much controversy remains over the link between fats and skin cancer given that intake of these nutrients is extremely difficult to accurately capture as diets can vary
greatly between individuals and because dietary habits often lack consistency over time.\textsuperscript{140-144}

Omega-3 and omega-6 PUFA are essential fatty acid groups primarily obtained via dietary intake of fish and plant oils, respectively, as they cannot be synthesised endogenously in humans.\textsuperscript{131, 137} Of the long-chain omega-3 PUFA, eicosapentaneoic acid (EPA) and docosahexaenoic acid (DHA) are the most biologically active and are in constant competition with the long-chain omega-6 PUFA arachidonic acid (AA) for metabolism by cyclooxygenase and lipoxygenase enzymes to produce eicosanoids (e.g. prostaglandins), important mediators of inflammation.\textsuperscript{138, 145-147} As a result of this competitive metabolism, these long-chain PUFA are thought to have modulatory effects on inflammatory processes (omega-3: anti-inflammatory, omega-6: pro-inflammatory) in skin, which may result from various intrinsic or extrinsic triggers including tissue injury, infection, immunosuppression or environmental insult.\textsuperscript{147, 148} UVR-induced inflammation is especially pertinent to the development of skin cancers thus the modulation of its cutaneous effects by PUFA is of primary focus in the remainder of this chapter.

Animal studies have shown that omega-6 PUFA-rich diets enhance the early promotional stages of photocarcinogenesis by shortening the latency to tumour appearance following UVR exposure and increasing the number of tumours.\textsuperscript{137, 149} Elevated omega-6 PUFA blood levels have also been associated with specific deleterious immune responses including increased prostaglandin E2 (PGE-2) levels, a pro-inflammatory immunoregulator linked with aggressive keratinocyte cancer growth in humans.\textsuperscript{150} On the other hand, omega-3 PUFA inhibit photocarcinogenic expression by increasing tumour latency and reducing tumour
multiplicity in animal models.\textsuperscript{137, 151} Omega-3 supplementation studies conducted among healthy volunteers have demonstrated protective effects of EPA especially on UVR-exposed skin in participants randomised to taking 4g daily of oral supplements compared to those taking a placebo. Specifically, EPA has been noted to significantly decrease both basal and UVR-induced PGE-2 levels,\textsuperscript{152, 153} as well as reduce sunburn sensitivity through increasing the skin’s threshold response to erythema (reddening of the skin).\textsuperscript{152-156} This effect of EPA supplementation on minimal erythema dose is thought to be approximately equivalent to a sun protection factor of 1.15,\textsuperscript{156} and if provided over the long-term is hypothesised to have positive cumulative effect potentially resulting in as much as a 30\% reduction in skin cancer incidence.\textsuperscript{137} Furthermore, an inverse dose-response relationship between total omega-3 PUFA and p53 immunoreactivity has been shown, suggesting that these PUFA may prevent DNA damage caused by mutant p53 by influencing the process by which UVR induces mutations in the p53 gene.\textsuperscript{155, 157}

2.4 Associations of PUFA intake and blood PUFA levels with BCC and SCC - aims and methods

Given the above evidence from laboratory and human experimental studies of possible associations of omega-3 and omega-6 PUFA with skin carcinogenesis, the aims of Publications 3 and 4 were to identify and quantify risk of BCC and SCC in relation to these PUFA, whilst controlling for potential confounding factors (e.g. sex, age, UVR exposure). These two papers utilised existing data from a prospective cohort study (Nambour Skin Cancer Study) among white Caucasian adults living in an area of high ambient solar UVR, the town of Nambour in Queensland, Australia.
The prospective cohort study is a more appropriate (less biased) observational epidemiologic approach than the case-control study for investigating the relationship between exposures and diseases such as cancer, as exposure data is collected prior to the outcome thereby ensuring the correct temporality between the two, i.e. that exposure precedes outcome. In the domain of nutritional epidemiology, large-scale prospective cohorts have often used FFQ to assess dietary intake as a primary exposure of interest in relation to cancer (e.g. Nurses’ Health Study, Health Professionals Follow-up Study and European Prospective Investigation into Cancer and Nutrition). FFQ offer a relatively inexpensive and non-invasive means of measuring long-term food intake, capturing both the frequency of consumption and the portion size of foods eaten in order to accurately measure individual nutrient consumption. This was the method of assessing dietary intake in Paper 3 of this thesis, including intakes of individual omega-3 and omega-6 PUFA in relation to BCC and SCC risk.

The strengths of the cohort design in establishing definitive links between dietary elements and disease however, are counterbalanced by the potential limitations of the dietary assessments themselves, for example the self-report nature of estimates of intake via the FFQ, the general inconsistencies in individual diets and the complexities in translating FFQ responses into nutrient quantities from standard nutrient databases since the composition of food items may vary by brand, cooking method, etc. As a result, although more difficult to obtain, more objective measures of nutrient intakes often used in these studies are biomarkers which directly quantify nutrient concentrations in the body, especially for PUFA which are not endogenously synthesised. As such, subsequent to Paper 3, plasma concentrations of omega-3 and omega-6 PUFA from blood samples were examined in relation to BCC and SCC occurrence.
in the same study population for Paper 4. This was done in order to help substantiate any observed associations with the FFQ intake data.

2.5 Associations of intake and blood PUFA levels and BCC and SCC - discussion of main findings

Prior to Publications 3 and 4 of this thesis, epidemiological studies of the effect of omega-3 and omega-6 PUFA on skin cancer risk were scarce. In general, the few existing studies in this field provided some evidence for associations of these nutrients with SCC but had shown little in the way of links between PUFA and BCC development.\textsuperscript{139, 161-163} Intake data measured by FFQ in the Nambour Study (Paper 3) showed no association of omega-3 PUFA and incident BCC in age-and sex-adjusted nor in multivariate models,\textsuperscript{164} results which mirrored those of a cohort study of 40,000 male health professionals,\textsuperscript{162} the only other investigation to examine this tumour in relation to intake of individual types of omega-3 PUFA. Blood concentrations measured in the Nambour population (Paper 4) however, indicated that linolenic acid was inversely associated with BCC occurrence in fully adjusted models, though solely in people with a personal history of keratinocyte cancers.\textsuperscript{165} Also of novel report were the documented reductions in BCC risk with higher total omega-6 PUFA and linoleic acid blood levels, which were again strongest among those with a history of keratinocyte cancers.\textsuperscript{164, 165}

Despite clinical demonstration of photoprotective effects of omega-3 PUFA in skin (as summarised previously)\textsuperscript{152-155, 166} these nutrients have never previously been linked directly to reducing BCC risk. Thus, it seems the consistency of Publication 3 findings in noting this lack of association are more valid than the supposed inverse relationship suggested in Publication 4. Undoubtedly this
discrepancy between the results of the two Nambour Study analyses can be attributed, at least in part, to the use of different measures of intake (FFQ versus blood). The correlation between the FFQ used for measuring PUFA in Paper 3 and plasma phospholipid measures as in Paper 4 have previously been investigated in a small subset of the Nambour study population however, the correlation coefficients indicated that a great deal of variation remains unaccounted for between these measures. It is also possible that the association of plasma linolenic acid levels with BCC may be explained by chance, as a very large number of comparisons were conducted in each analysis, which inherently raises the likelihood of observing an effect. In addition, that inverse associations of omega-6 PUFA with BCC were observed, was somewhat counterintuitive given molecular data have shown the potential for these nutrients in tumour promotion. The most likely explanation for these unexpected finding is confounding by other risk factors (e.g. socioeconomic status, lifestyle factors, etc.). Distilling the independent effects of individual PUFA from these other variables poses a great challenge to nutritional epidemiologic studies. Performing multivariate adjustments to include these factors in regression modelling can help overcome this issue however, their inclusion in the models can complicate the interpretation of the resultant risk estimates and may not always be possible if variables are collinear.

With respect to SCC, the PUFA intake paper (Paper 3) did not find evidence of an association of this malignancy with omega-3 intake measured from FFQ after multivariate adjustments. These results were in contrast to an earlier population-based case-control study which showed a trend towards decreased risk with greater total omega-3 PUFA intake and a reduction in odds of SCC with increasing consumption of diets high in the ratio of omega-3/omega-6 PUFA.
Also, a longitudinal analysis of the Nambour data examining the association of specific foods and dietary patterns with actinic keratoses, precursory lesions to SCC, found acquisition of actinic keratoses was decreased by 28% among participants consuming diets high (on average one serving per 5 days) in oily fish, the principal source of EPA and DHA for humans.\textsuperscript{163} Publication 4 however, confirmed the observations of these other cohorts as indeed higher plasma concentrations of EPA significantly reduced the risk of SCC after controlling for age and sex and especially after multivariate adjustments among those with a prior history of skin cancer. The ratio of omega-3/omega-6 levels was similarly inversely associated with SCC after basic confounder adjustments, but associations became non-significant in multivariate and stratified models.\textsuperscript{165} As for omega-6 PUFA, only higher AA red blood cell concentrations has previously been demonstrated to increase SCC risk.\textsuperscript{161} The sole hint of support for the AA-SCC relationship among Nambour participants was noted with respect to dietary intake in age- and sex-adjust, but not multivariate, models.\textsuperscript{164}

For the most part, the SCC-related results from the two Nambour cohort analyses are consistent with previous research in this field highlighting omega-3 PUFA, especially EPA, as potentially protective agents against this malignancy.\textsuperscript{139, 151, 155, 168} Although no clear linear dose-response relationships were noted, collectively the works indicate that the moderate levels of omega-3 PUFA intake observed, especially of EPA (approximately 100mg/daily in Paper 3), may sustain circulating and skin target tissue concentrations enough to influence photocarcinogenesis to SCC. In regards to the AA-related observations, that earlier epidemiologic studies have noted an inverse association of SCC with this specific PUFA, as well as with related food sources (i.e. diets high in meat/fat with high AA content),\textsuperscript{135, 161} provides support for the Publication 3
results over the lack of association in Publication 4. As mentioned in relation to BCC, the dissimilarities between the study findings are likely explained by a number of limitations of both the FFQ and blood measurement methods. It is also possible that the limited range of PUFA values and relatively small numbers of cancers observed amongst study participant may have limited the statistical power of the analyses to detect some effects. However, again the issue of confounding by other variables presents the biggest challenge to isolating the precise effect of specific PUFA in relation to skin cancer and is likely to explain majority of the variability between these results.

It is not totally unsurprising that the observed associations of specific PUFA with BCC and with SCC differed given that these are distinct diseases for which differentials in associations with other nutritional factors have previously been noted (e.g. retinol/vitamin A, vitamin E, etc.). However, overall, the individual findings of the two Nambour Study analyses with regard to these malignancies and PUFA must be interpreted with caution. An extensive body of research demonstrating consistency in results is needed in order to imply causality, but evidence from these works are still too variable and thus inadequate to establish such a strong relationship of omega-3 and omega-6 PUFA with either type BCC or SCC.

2.6 Correlations of omega-3 PUFA levels measured by FFQ, blood and skin - methods, aims and discussion of main findings

Given that Publications 3 and 4 presented two different approaches to examining PUFA exposure in relation to skin cancer and demonstrated that these methods can produce different results, Publication 5 was conceived as a methodological study that addressed and sought to quantify some of the error potentially
involved in these types of nutritional epidemiology investigations. This assessment was achieved by a triangulation of methods to assess nutritional levels of omega-3 PUFA: by FFQ, and blood and skin target-tissue levels, the latter ultimately being the tissue biomarker most relevant to the development of keratinocyte cancers.

Validation studies are often used to ensure indirect methods of measurement of an exposure reliably reflect the actual levels within an individual (i.e. that FFQ measurement error is low).\textsuperscript{144, 160, 169} As previously mentioned, FFQ are measures of long-term dietary intake from which the specific nutrients that are composite of food consumed can be quantified. They pose an alternative to using invasive blood or tissue sampling procedures and are simpler and less costly to implement in large study populations as they can be self-administered.\textsuperscript{143, 144, 158, 159} Both FFQ and blood are regularly used to estimate intake (as discussed above for Papers 3 and 4) but few other human target tissues have been as easily sampled. Besides blood markers (i.e. erythrocytes, platelets, serum, plasma, cholesterol esters, phospholipids)\textsuperscript{158, 159, 170-173} other methods of assessment of the bioavailability of omega-3 PUFA include adipose tissue and buccal mucosa.\textsuperscript{174-177} Erythrocytes, however, remain the best known blood markers of circulating levels of PUFA as they reflect intake over several weeks.\textsuperscript{131}

Before publication of the fifth paper in this thesis, there were only a few studies documenting the validity of FFQ data for measuring omega-3 PUFA in relation to erythrocyte levels (as opposed to other blood markers). These earlier studies reported correlation coefficients ranging from 0.37-0.40 and 0.16-0.39 for EPA and DHA, respectively.\textsuperscript{158, 171} The coefficients for intake measured by FFQ versus erythrocyte concentrations in Publication 5 were much higher - 0.57 and 0.59 for
these same specific PUFA. In comparison, these correlations were also stronger than those reported in the validation of the Nambour Study FFQ, from which the Paper 5 questionnaire was derived, with plasma phospholipid PUFA levels (correlation coefficients of 0.21 and 0.32 for EPA and DHA respectively). An additional study examining the validity of a new electronic food frequency questionnaire specifically designed for measuring PUFA intakes also found high validity coefficients for EPA, DHA and total long chain omega-3 PUFA (0.87, 0.64 and 0.73 respectively) against erythrocyte measures.

Paper 5 was also novel in its assessment of both FFQ intake and blood levels of omega-3 PUFA in relation to levels directly present in skin and found that EPA had particular good correlations (FFQ, 0.33 and erythrocyte, 0.45). Although there were no validity studies to which we could directly compare these results, omega-3 PUFA content in human skin has been previously quantified. Overall, EPA content reportedly ranged from 0.05% to 1.1% in full-thickness skin biopsies compared with skin-shave biopsies largely comprised of the epidermal skin layer. The 0.07% EPA content of the dermal biopsies from our assessment was within this range and largely consistent these reports.

It is of importance to note however, the possibility that outlying data points, visible particularly at the upper extremes of some of the scatter plots presented in Paper 5, may have been highly influential in determining the correlations between measures of specific omega-3 PUFA, and could have resulted in the overestimation of some of the correlation coefficients. Although not carried out prior to publication of the manuscript, influence analyses could have proven useful for formally identify any such observations and the magnitude of their effects on correlations, including assessment of standardised and studentised
residuals, Cook’s D, DFFITS or DFBETAS statistics. Logarithmic or other transformations to the data could also have helped spread the data more uniformly in the figures, i.e. reduced the skew of data points.

In much the same way that there were variations in the associations of BCC and SCC with intake and plasma PUFA data noted by Publications 3 and 4, no perfect correlations between FFQ, erythrocyte and skin measures of omega-3 PUFA were observed in Paper 5. Discrepancies between intake and biomarker data were expected due to their respective limitations, especially those of FFQ highlighted earlier.\textsuperscript{140, 143, 144} Nevertheless, that Paper 5 results were an improvement on the correlations found between questionnaire and erythrocytes measures of omega-3 PUFA in other studies, may reflect the greater appropriateness of the FFQ used here for quantifying these nutrients. In addition, although they are the benchmark to which FFQ intake data are compared, biomarkers also do not always reflect true intake due both to measurement error in the techniques used for quantification and to the effects of absorption, tissue uptake, metabolism and excretion which are individually regulated within the body.\textsuperscript{131, 144} Thus, no true “gold standard” biomarker exists by which FFQ validity can be assessed as in fact these tools do not measure precisely the same thing.\textsuperscript{144} However, the higher correlations of PUFA data from a similar FFQ observed with erythrocyte levels here compared to with plasma phospholipids levels as in the Nambour Study validation, provides supportive evidence that different types of blood measures may be better than others at reflecting true intake of these nutrients.

That omega-3 PUFA from FFQ, blood and skin were all closely correlated in the Paper 5 assessment suggests that intake estimates from these questionnaires provided a good measure of both circulating and target tissue levels of these
PUFA, though cannot replace them altogether. Consequently, both questionnaires and biomarkers can be reliably used in investigations of cutaneous effects of PUFA such as those from the Nambour Study, but given their respective limitations cannot on their own establish a causal role of these nutrients on skin cancer development. Continued attempts at refining existing tools for measuring dietary intake, such as real-time diet recording using new technologies and applications, are required in order to advance the field. Future epidemiologic studies should ideally utilise these measurement methods in combination to ensure a well-rounded interpretation of results however, the large costs and complex logistics associated with collecting and processing multiple forms of data are limitations to their applicability in large cohorts and to their widespread use.

2.7 Conclusions and implications

The interest in nutritional factors and their associations with different types of cancers continues to rapidly expand as diet represents a major modifiable exposure, which can vary both at the individual and population levels. Keratinocyte cancers are the commonest malignancies in white populations, and UVR is their primary cause. As a result, a good deal of attention has been placed on exploring dietary nutrients which may mitigate the carcinogenic effects of UVR, and animal and clinical studies have demonstrated omega-3 PUFA in particular may offer photoprotection. The collective findings of Publications 3 and 4 give epidemiologic evidence for associations of omega-3 and omega-6 PUFA with BCC and SCC, an aspect of this field which required further substantiation due to inconsistencies in the few published papers. For the most part, the Nambour Study results were in line with previous
research highlighting omega-3 PUFA, and particularly EPA, as potentially protective agents against SCC especially.\textsuperscript{139, 151, 153, 155, 168}

Crucial to the validity of interpretations of such nutritional epidemiology studies with their great potential for confounding of results by secondary socio-economic and other lifestyle risk factors, is the utility of the tools used for quantifying dietary intake, both in relation to estimating the true quantities of nutrients consumed and to circulating levels in the body. While these have become more specific and tailored to the nutrients of interest over time, they still present many limitations which must be heeded.\textsuperscript{131, 140, 143, 144} As discussed in the latter portion of this chapter, the correlational assessment of Paper 5 indicated that both FFQ and erythrocyte measures of long-chain omega-3 PUFA, especially EPA, may be utilised as well-correlated measures of these nutrients in skin,\textsuperscript{138, 155, 166} and for investigating the associations as in Papers 3 and 4. Links between the key aims, conclusions, strengths and limitations of the three papers are summarised in Figure 2.
Figure 2: Flow diagram of main aims, conclusions, strengths and limitations of Papers 3-5.

Abbreviations: BCC - basal cell carcinoma, EPA - eicosapentaneoic acid, FFQ - food frequency questionnaire, PUFA - polyunsaturated fatty acids, SCC - squamous cell carcinoma.
Even in combination however, the three studies presented in this chapter are inadequate to establish a definitive relationship between omega-3 and omega-6 PUFA and cancers of the skin. The full impact of widespread increases in recreational UVR exposure on the incidence of cutaneous malignancies has yet to be felt, as demonstrated by the continued increases in rates of both BCC and SCC in many white populations worldwide\(^4\) as well as by the previous discussions relating to melanoma in Chapter 1. Consequently, the importance of investigating potentially photoprotective agents such as omega-3 PUFA also continues to grow. Further research in this field among diverse populations is required in order to confirm the associations observed and to determine whether a specific threshold of individual or combined totals of omega-3 PUFA is needed to sustain cutaneous effects. Intervention trials may be the most effective means of ultimately establishing a relationship between individual PUFA and skin cancer risk and should take into account other lifestyle factors (e.g. socio-economic status) which may affect dietary patterns and related behaviours.\(^{142}\)

In summary, the three publications discussed in this chapter use different methodological approaches to provide evidence for assessing associations of omega-3 and omega-6 PUFA with skin cancer development. Although inconclusive in defining a role for these nutrients, this work provides an important contribution towards the interpretation of measured intake and biomarker data and the promotion of skin health through nutritional means. As diet is an easily modifiable aspect of individual health, elements for which an evidence-based relationship (either positive or negative) with disease can be established, pose a unique opportunity for targeted primary prevention strategies. As an example, current UK dietary recommendations for total consumption of long-chain omega-3 PUFA among healthy adults have recently
been augmented from 100-200mg\textsuperscript{179} to 450mg\textsuperscript{180} daily as a result of emerging evidence of their potential benefits in reducing the risk of cardiovascular disease. However, with respect to the influence of omega-3 and omega-6 PUFA on cancers of the skin, a great deal more research is required to understand these relationships before any such application to public health recommendations could be considered.

### 2.8 Impact of publications

Publication 3 is published in the journal Nutrition and Cancer, impact factor 2.695 and ranked 26\textsuperscript{th} out of 76 journals in Nutrition and Dietetics and 93\textsuperscript{rd} out of 196 journals in Oncology for 2013. It has been cited once (ISI Web of Knowledge) or twice (Google Scholar) since it was first made available online in September 2012 and is the first longitudinal study describing the associations between intake of individual omega-3 and omega-6 PUFA and BCC and SCC in both sexes.

As a very recent paper first published in October 2013, Publication 4 has only been cited once according to Google Scholar. It has been published in the journal Cancer Epidemiology Biomarkers and Prevention, impact factor 4.559 and currently ranked 39\textsuperscript{th} out of 196 Oncology journals and 10\textsuperscript{th} out of 58 Public Health journals.

Publication 5 has again only recently been made available (January 2013), and is cited once according to Google Scholar. However, it is the first and only study to compare concentrations of omega-3 PUFA with those measured from blood and skin with dietary intake quantified from FFQ. Publication 5 is published the British Journal of Nutrition, impact factor 3.03 and currently ranked 18\textsuperscript{th} out of 76 journals in Nutrition and Dietetics.
CHAPTER 3:
Association of Scars and Cancers of the Skin
(Publication 6)

3.1 Characteristics of skin cancers arising in scar tissue

Among non-UVR causes of cancers of the skin, scars involving skin tissue have been postulated as being potential sites of skin cancer development, especially those scars that are atrophic or unstable. Scars of various origins including from vaccinations, surgery, illness (e.g. chickenpox) and traumas such as animal bites, frostbite, and burns have reportedly been implicated, as have chronically inflamed, non-healing wounds which also generate scar tissue, such as osteomyelitic sinuses, pressure ulcers or ulcers secondary to venous insufficiency. However, despite the almost innumerable case reports in the literature describing individual cases of skin cancer arising at sites of scarring, there has been little in the way of epidemiological research to estimate the incidence of BCC, SCC or melanoma arising in these tissues and to quantify the association of these malignancies in regard to scars and their features.

From the vast collection of clinical case studies, the keratinocyte cancers (SCC primarily, followed by BCC) are notably the most common cutaneous scar malignancies, but melanomas have also been demonstrated to arise in these tissues. In particular, burn-scar-associated skin cancers appear to constitute the vast majority of documented scar neoplasms, and include several varieties associated with cultural practices of self-warming and heating, such as the Kangri, Kang and Kairo cancers. The flagship case series of skin cancer occurring in burn scars.
conducted by Treves and Pack in 1930 estimated that in a group of nearly 2,500 patients in the USA with keratinocyte cancers, 3% of all SCC and 0.03% of all BCC arose in burn scars.\textsuperscript{193}

Overall, judging from case series, there appears to be a male preponderance of the keratinocyte tumours in scars (male:female ratio of 2:1) whereas scar-associated melanomas in cases series appear to occur more frequently in women.\textsuperscript{182, 194, 204, 206} Specifically in regards to burn scars, a review of 412 cases found that the average latency period from burn injury to SCC tumour has been reported at approximately 30 years, with a mean age at diagnosis of 50 years; however, BCC seemed to have an older age at onset at these sites.\textsuperscript{194} In addition, several case reports have noted that the site distribution of these scar-associated skin tumours appears to favour the extremities (60%), in particular joint creases where blood supply is decreased, followed by the head/face (30%) and the trunk (10%).\textsuperscript{38, 193-195}

3.2 Proposed mechanisms of malignant transformation of scars

Despite the fact that the malignant potential of burn scars (and other scar tissue types) is clinically recognised, in general the understanding of the mechanisms behind malignant transformation remains unclear. Prevailing hypotheses are again based primarily on evidence from clinical case studies and suggest that chronic inflammation and tissue irritation; continuous exposure to toxins and co-carcinogens post-injury; and poor blood flow and lymphatic drainage in the scar tissue can all result in a cutaneous region of impaired immunological defence where tumour cells can avoid elimination.\textsuperscript{194, 196, 208, 212} Various cytokines and growth factors, particularly interleukin (IL)-1\textsuperscript{-α} and transforming growth factor beta (TGF-β)\textsubscript{1, -β2 and -β3 respectively, play major roles in epidermal wound
healing as they are known to initiate keratinocyte proliferation and repair.\textsuperscript{148} However, imbalance between these has also been associated with tumourigenesis.\textsuperscript{213} In addition, it has been suggested that rather than increasing the rate of skin cancer initiation, scar tissues may facilitate tumour progression in cells where cancer is already developing.\textsuperscript{214} Genetic factors may also be involved in the pathogenesis of scar tissue skin cancers again based on clinical observations. Somatic mutations in the p53 gene have previously been shown in people with burn-scar carcinomas,\textsuperscript{215, 216} and similar alterations in the region of the \textit{Fas} gene responsible for apoptosis in cells and tissues have also been observed in those with burn-scar SCC.\textsuperscript{217}

3.3 Skin cancer in scars systematic review - aims and methods

Initially, when the concept for Publication 6 was formulated, the aim was to conduct both a systematic literature review and a meta-analysis of observational studies published in this field to August 2010. The data collected would then be used to deduce a summary risk estimate for the association of scars with the BCC, SCC and melanoma. However, upon initiating the literature search, it quickly became apparent that quantitative studies of this relationship were so few that a meta-analysis was not possible. Consequently, this paper comprised only a systematic literature review. Systematic reviews are crucial to identifying, appraising and synthesising sound research on a specific topic.\textsuperscript{218} They are a cornerstone of evidence-based medical practice and when new interventions or prevention strategies are being formulated by policymakers and government.\textsuperscript{219} Despite the fact that an overall estimate for the relationship between scars and skin cancers could not be obtained, the sixth and final study in this thesis was valuable in formally demonstrating the limited state of
knowledge of this association which is nonetheless widely accepted by the medical establishment.\textsuperscript{181}

3.4 Skin cancer in scars systematic review - discussion of main findings and conclusions

Publication 6 identified over 450 case studies in the published literature describing patients with cancers of the skin arising in scars of diverse origins and their associated tumour characteristics observed in clinical settings.\textsuperscript{220} Only two observational studies of this association were identified: both were population-based cohort studies, one in Sweden\textsuperscript{221} and the other in Denmark.\textsuperscript{214} These specifically focussed on patients who had been hospitalised for burn injuries and who were followed up using national cancer registration (mean follow-up time approximately 15 years for both studies). Neither study found any significant elevation in the risk of SCC (Sweden: standardised incidence ratio (SIR), 0.88; 95% confidence interval (CI) 0.70-1.09 and Denmark: SIR, 0.9; 95% CI, 0.6-1.5 and) or of melanoma (Sweden: SIR, 0.88; 95% CI, 0.68-1.12 and Denmark: SIR, 0.4; 95% CI, 0.4-1.1) in people with burn injuries compared to the general population.\textsuperscript{214, 221} Only the Danish registry collected data on BCC, but again did not note any greater risk for this tumour (SIR, 0.7; 95% CI, 0.6-0.9).\textsuperscript{214} This same study also further examined risk of cutaneous malignancies by severity of burn and at the specific site of a previous burn injury; while the SIR for SCC increased to 1.2 (95% CI, 0.4-2.7) for the latter, neither of these sub-analyses demonstrated a significant increase or decrease in risk of any skin cancer type.

Evidence from the two Nordic studies suggests that burn patients are not at greater risk than the general population for skin cancer however, these studies have their limitations. Firstly, the Swedish investigation did not examine the risk
of BCC as this malignancy was not recorded by the national registry at the time of study, thus a definitive conclusion as to the relationship between BCC and burn injuries cannot be established based on the sole estimate provided by the Danish analysis. A second notable limitation of both studies was that the mean duration of follow-up in each was approximately half the 30-year mean latent period between burn injury and skin cancer previously highlighted from case reports,\textsuperscript{194} so it is possible that some malignancies may have been missed in these cohorts due to inadequate follow-up. Furthermore, the populations of Sweden and Denmark are similar with respect to many important risk factors for skin carcinogenesis (e.g. ambient UVR levels, sun habits, genetic and phenotypic traits) so the results of these studies may not be generalisable to other countries. Consequently, even though the systematic review findings contrast with the often-quoted link made between scars, especially from burns, and cancers of the skin established on the basis of cumulative reporting by health professionals, given the limitations of these epidemiologic studies and the weight of anecdotal evidence, associations of scar tissue with cutaneous malignancies cannot be excluded.

Taken as a whole, it is clear from Paper 6 that there is a major gap in the evidence base of the associations of cancers of the skin and scars. The majority of the published work identified in this field mainly pertains to burn scars so there is a definite deficiency in comprehension, both mechanistic and epidemiological, of other types of scars as sites with carcinogenic-potential. Certainly further research is needed in order to overcome some of the shortcomings of previous investigations. Ideally future epidemiologic studies should be prospective in nature and should distinguish between the origins of the scars identified (e.g. burns, surgery, vaccination, bites, etc.). This would permit
the calculation of population-level estimates of the risk for cancers of the skin associated with different scar tissue types and would enable comparison of their relative rates of malignant change. Due to the rarity of scar carcinomas, the best settings for such examinations would be amongst populations with high rates of skin cancer, such as Australia. Further retrospective cohort studies similar to the two discussed here could also be conducted in other countries where linkage systems exist between hospital and general practitioner records and cancer registry data to provide sound population-based relative risk estimates of these associations.

In summary, Publication 6 has made it exceedingly apparent that scientific knowledge is lacking as to the incidence of cutaneous scar neoplasms, despite the plethora of case reports in the published literature. The only way to rectify this is through conducting well-designed epidemiologic studies over sufficient periods of time.

3.5 Impact of publication

Publication 6 has been cited once according to ISI Web of Knowledge and twice according to Google Scholar since June 2011. It is published in Dermatologic Surgery, which has an impact factor of 1.866 and is ranked 20th of 59 Dermatology journals and 67th out of 199 Surgery journals for 2013.
SUMMARY DISCUSSION

Overall conclusions

The six publications presented for this thesis were grouped into three chapters according to the epidemiological approach and class of skin cancer that they each addressed. The first chapter concerned the descriptive epidemiology of melanoma in England and concluded from the two component ecological studies that recent observed increases in melanoma incidence by anatomic site and changes in latitudinal trends in melanoma rates are explicable by increases in recreational UVR exposure both from solar and artificial sources. In Chapter 2, the assessment of associations of nutritional factors, specifically individual omega-3 and omega-6 PUFA, and keratinocyte cancer risk was addressed. The first two publications of this section used prospective cohort data to demonstrate associations of intake estimated by FFQ and by blood levels of these nutrients with risk of BCC and SCC, while the third study was an assessment in a different population of the validity of these exposure measurement methods in relation to levels in skin target tissue. Collectively, these studies demonstrated evidence for associations of PUFA with these skin cancers, and confirmed the utility of the intake and biomarker measures for assessing these relationships. Finally, in Chapter 3 the widely-believed association between scar tissue and cancers of the skin (keratinocytic or melanocytic) was explored in a systematic review. It showed the major gap in the current evidence base regarding the risk for malignancies in scars, amounting to an inability to measure the purported association.
**Strengths and limitations**

Unlike conventional theses, which are usually restricted to more narrow fields of research, presenting these studies in the format of a PhD by published work thesis has allowed for the demonstration of their relationships to each other and their connections to existing studies and recent developments. Although the component subsections are distinct in focus, they share overlapping and linking elements that unite them as a cohesive body of work. Thus, the interrelation between the publications is the foremost strength of this collection and is chiefly illustrated by their common overarching aim to address specific gaps in our understanding of the epidemiology and aetiology of cancers of the skin, as is evident from the conclusions summarised above.

A major methodological strength of this collection of studies is that the component papers span several epidemiologic study designs. These are wide-ranging: from the broad overview of ecological studies such as Papers 1 and 2, which provide the groundwork for understanding the magnitude and patterns of disease incidence in order to generate hypotheses as to the influence of various environmental and behavioural factors. Next, the more in depth longitudinal cohort studies as in Papers 3 and 4 can be used to explore in detail the specific risk factors of interest which have been hypothesised to have associations with disease. Concurrently, metabolic assessments as in Publication 5 provide support for the validity of the tools used in analytical studies to quantify exposures of interest. Lastly, systematic reviews like Publication 6 collate all research on a specific relationship of interest in order to generate evidence-based conclusions as to the implications of the exposure-disease link. As such, even though the published work were grouped according to topic, this arrangement also allowed the papers to be presented in a logical sequence based on the scope of the study.
designs and methods by which the epidemiology and aetiology of any disease is examined. Furthermore, the validity of the achievements of this work as a whole is reinforced by the vast array of tools employed by the studies, including national cancer registration data, questionnaires, biological sampling and online search engines, in addition to various established statistical tests and regression models. The use of such a variety of epidemiologic designs and tools contributes to the overall thoroughness with which the aim of this thesis was explored and provides a well-rounded approach to investigating and interpreting the associations of cutaneous malignancies with various aetiological factors.

That the factors of interest in each chapter, recreational UVR exposure, omega-3 and omega-6 PUFA nutrition and scar tissue respectively, are themselves complementary is a further strength of this collection of papers. For example, the influence of sources of UVR exposure as discussed in Chapter 1 are similarly relevant to the omega-3 and omega-6 PUFA associations explored in Chapter 2 since these nutrients are thought to modify the process by which UVR initiates and promotes skin carcinogenesis. Accordingly, UVR-related factors (like high-risk phenotypic traits and surrogate measures of intensity/duration of UVR exposure) were controlled for in the models of PUFA with BCC and SCC. In addition, even though UVR had little bearing on the Chapter 3 discussion of scars as a determinant of skin cancer, a commonality with Chapter 2 was the assessment of hypothesised risk factors for keratinocyte cancers whose mechanisms of influence involved inflammatory processes in skin. Thus, this thesis provides a network of evidence for the importance of assessing both UVR- and non-UVR-related risk factors in studying the epidemiology and aetiology of cancers of the skin.
In terms of the limitations of this body of work, these mainly result from the constraints of the various epidemiologic methods used in the papers presented. For one, ecological studies as in the first chapter can be subject to ecological fallacy whereby population-level results are inferred at the individual-level. As such, the descriptive results on melanoma incidence in England cannot inform on the magnitude of individual risk associated with various UVR determinants, though they do provide a comparative basis for future investigations of the contributions of these factors to melanoma incidence in this country or others with similar ambient sun exposure. Secondly, although unlikely to explain the full extent of the dearth of epidemiologic evidence on skin cancers arising scars, publication bias, that is the selective acceptance or submission of manuscripts with only positive or significant findings to scientific journals, can restrict the foundation on which conclusions are drawn by systematic reviews, like that in Chapter 3. Moreover, the specificity of the population under study can also present limitations to the application of result to different contexts. As in Chapter 2, the associations of omega-3 and omega-6 PUFA in relation to BCC and SCC were investigated in a cohort from Queensland, Australia, an area noted for the highest global rates of skin cancer due to high ambient UVR levels,\(^2\),\(^4\) thus the findings may not be generalisable to populations at inherently lower risk of these malignancies due to lower ambient UVR.

**Impact and future directions**

The publications in this thesis have mostly been published within the last three years and consequently, their impact in the field as measured by citations in scientific journals and other work is yet to be established. However, within each of the areas investigated the component studies were novel with respect to either their approach or their findings as already stated. In summary,
Publications 1 and 2 were the first studies to present national melanoma incidence rates for England by the specific population-level variables addressed; Publications 3 and 4 were among the only longitudinal studies of the effects PUFA nutrition on keratinocyte cancer risk, Publication 5 was the first study of the bioavailability of omega-3 PUFA in skin in relation to circulating and intake levels; and finally, Publication 6 appears to be the only comprehensive review of skin cancers arising in scars and showed a deficiency in evidence-based research on this topic that seems to have gone largely unrecognised.

Clearly there is a need for more rigorous epidemiologic studies on each of the specific skin cancer determinants of focus, namely recreational UVR exposure, omega-3 and omega-6 PUFA nutrition and scar tissue, in order to further substantiate the findings of this suite of papers. New longitudinal studies in high- and moderate-risk populations could further elucidate the link between cancers of the skin and UVR-related recreational behaviours or with scar tissue respectively. In the case of nutritional factors, intervention studies of fish oil or omega-3 supplements or of Mediterranean diets may be key to overcoming the limitations of previous work. Additionally, some of the findings and methodologies of the studies presented here could be used as a point of departure for future research among the skin cancer types not addressed by the individual papers. For example, as UVR is equally the primary causal factor for keratinocyte cancers, hypotheses from Papers 1 and 2 around the individual contributions of specific UVR determinants on the distribution of melanoma in the population could likewise be relevant to BCC and SCC incidence patterns. In reference to Papers 3 and 4, similar cohort studies could also be used to assess associations of omega-3 and omega-6 PUFA intake and biomarker levels with melanoma, a emerging concept in the literature with the recent publication of a
case-control analysis in this area. By and large, as aimed for this in this collective work, future research needs to be broad in scope and consider as wide a range of determinants, both UVR- and non-UVR related, and as many discrete skin cancer endpoints (among keratinocyte cancers and melanoma) as possible in order to advance comparative understanding of the epidemiology and aetiology of cancers of the skin and thereby provide a base for targeted health messages aimed at reducing the burden of cutaneous malignancies.

In conclusion, the overarching achievement of this thesis is highlighting the often-overlooked complexity: the commonality yet diversity, of the aetiology of cancers of the skin. While causal relationships are difficult to establish, the collective findings of the papers presented have provided new hypotheses and contributed evidence of associations of a range of factors that will fuel the future of research in this field.
THE PUBLISHED WORK

Publication 1

Increases in invasive melanoma in England, 1979–2006, by anatomical site

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Summary

Background National melanoma incidence trends with details of anatomical site have not been previously described for England.

Objectives To describe site-specific trends in cutaneous melanoma for England as a whole during the last three decades.

Methods Anonymized data, 1979–2006, were obtained from national cancer registrations of all patients in England up to age 89 years with incident primary invasive cutaneous melanomas (n = 124 055). Sex-specific age-standardized incidence rates and average annual percentage change in rates were calculated for each broad anatomical site.

Results Overall incidence rates of cutaneous melanoma in England, 1979–2006, were 81 and 100 per million, in males and females, respectively. Site-specific rates were consistently highest on the lower limbs in females followed by the trunk in males. Greatest annual increases occurred on the trunk in both sexes over 45 years (males 9\%–9\%, females 6\%–8\%), then upper limbs (males 8\%–7\%, females 6\%–8\%). Incidence trends in males relative to females varied little across sites apart from a more rapid rise in head/neck melanomas in males than in females after the 1980s.

Conclusions Invasive melanoma rates continue to rise in England, particularly on the trunk and arms, and in males on the head/neck. The steeper increases in melanoma rates among males are consistent with their greater sun exposure and poorer compliance with sun protection measures than females.

Incidence of cutaneous melanoma has been rising worldwide over the past few decades in countries with predominantly white populations. The overall incidence of melanoma in the U.K. has almost doubled in the past two decades to 10.2 and 11.8 per 100 000 in males and females, respectively. As elsewhere, background incidence markedly increases between early adulthood and seniority in the U.K., by 10-fold in males and 3.5-fold in females. There is, however, evidence to suggest that the rising incidence of melanoma is at least partly explained by diagnostic drift, whereby lesions previously reported as benign are now reported as malignant, resulting in the rise in incidence being predominantly early, preinvasive disease.

Little recent information is available about the anatomical sites of occurrence of melanoma in the U.K. Based on a study in Scotland, 1979–2003, and another in Southeast England, 1960–98, trunk melanomas predominated in males (2.5–4.0 per 100 000), while lower limb tumours predominated in females (3.0–5.0 per 100 000). A detailed comparison of anatomical sites of melanoma in Western Scotland and Queensland, Australia, which accounted for relative surface areas of different body sites, showed that rates on the lower limbs of Scottish women under age 60 years were higher than on any other site (17.1 per 100 000 at ages 40–59 years). Beyond the U.K., analyses of site-specific trends have shown steep increases in incidence on the head/neck in both sexes, as well as on the trunks of males and lower limbs of females. Previous incidence studies grouping anatomical sites by the frequency with which they are exposed to the sun (i.e. occasionally exposed, regularly exposed, etc.) have shown significant increases in incident melanoma on occasionally exposed sites in both sexes.

Detailed national data on melanoma incidence trends by anatomical site have not been documented for England. These
data are needed in order to understand the changing patterns and causes of melanoma development and to inform targeted primary prevention strategies. The aim of this study therefore was to describe site-specific trends in cutaneous melanoma for England as a whole during the past three decades. To obviate the effect of diagnostic shift in the reporting of thin noninvasive melanomas, in situ melanomas were excluded a priori from these analyses.

**Methods**

Anonymized, individual-level, national cancer registry data were obtained from the Northern and Yorkshire Cancer Registry and Information Service for all people in England up to 89 years of age with a newly diagnosed, primary, invasive, cutaneous melanoma over the 28-year period, 1979–2006. Cancer registration in England is of a consistently high standard and the majority of cancers, including melanomas, are captured (98%). Histology reporting is the main source (at least 95%) for registration of new melanomas (South West Public Health Observatory, personal communication). Diagnostic criteria per se did not materially alter for invasive melanoma during the study period. Data were coded using the International Classification of Diseases (ICD) 9th revision from 1979 to 1994, while from 1995 onward the ICD 10th revision was used. For site-specific analyses, melanomas were categorized as occurring on the head and neck (ICD-09 and ICD-10 codes: 1720–1724, C43.0–C43.4), trunk (codes 1725, C43.5), upper limb (codes 1726, C43.6), lower limb (codes 1727, C43.7) or unspecified site (codes 1728–1729, and C43.8–C43.9). Annual mid-year national population estimates by sex, single year of age and calendar year were supplied by the Population Estimates Unit, Office of National Statistics (ONS).

Age was categorized as < 25, 25–44, 45–64 and 65–89 years and time periods were defined by 5-year intervals giving five complete quinquennia for study: 1982–1986, 1987–1991, 1992–1996, 1997–2001 and 2002–2006. Sex-specific incidence rates were calculated for each site and expressed per million person-years at risk. All incidence rates were age-standardized to the European standard population. Person-years at risk were calculated for each subgroup from the population data provided. Average annual percentage change in incidence in each subgroup was estimated using the slope of the linear trend line fitted to the logarithm of incidence rates, by year of diagnosis. Thus, the average percentage change per year in incidence rates was estimated by $100\times(e^{b}-1)$, where $b$ = slope of the linear trend line. Significance was set at $P < 0.05$.

**Results**

Between 1979 and 2006 there were a total of 124 055 cases of primary invasive cutaneous melanoma diagnosed in England in persons up to age 89 years, three-quarters (n = 94 470) being diagnosed from 1990 to 2006. The overall age-standardized incidence rates 1982–2006 across all sites for males and females were 81 and 100 per million person-years at risk, respectively. Since 1979 the greatest proportion of all incident invasive melanoma in both sexes combined occurred on the lower limb (33%), followed by the trunk (25%), the upper limb (18%), and the head/neck (16%) with anatomical site unspecified in 8%. Highest site-specific incidence rates consistently occurred on female lower limbs followed by the male trunk.

Overall age-standardized invasive melanoma rates increased between consecutive 5-year intervals (Table 1) and across the whole study period at all specified anatomical sites in both sexes (Figs 1 and 2). Melanoma incidence on unspecified sites rose early in the study period but then plateaued so was not considered further. Comparing rates in 1982–86 to those in 2002–06, the most marked rises in incidence occurred on the trunk where rates rose almost fourfold from 15 to 56 per million in males, and over threefold in females from 9 to 29 per million (Table 1).

In every age group there were significant increases in melanoma rates over time for each anatomical site ($P$-values < 0.01) (Table 2). Greatest annual increases occurred on the trunk (9.9%) and upper limbs (8.7%) in males aged

---

**Table 1** Age-standardized site-specific incidence rates (per 10^6 person-years at risk) of cutaneous melanoma in males and females in England, by 5-year period (1982–2006)

<table>
<thead>
<tr>
<th>Period</th>
<th>Head/neck M</th>
<th>F</th>
<th>Trunk M</th>
<th>F</th>
<th>Upper limb M</th>
<th>F</th>
<th>Lower limb M</th>
<th>F</th>
<th>All sites M</th>
<th>F</th>
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</thead>
<tbody>
<tr>
<td>1982–1986</td>
<td>n</td>
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<td>n</td>
<td>Rate</td>
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<td>Rate</td>
</tr>
<tr>
<td>1987–1991</td>
<td>858</td>
<td>1031</td>
<td>1524</td>
<td>15</td>
<td>1049</td>
<td>9</td>
<td>731</td>
<td>7</td>
<td>1466</td>
<td>12</td>
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<tr>
<td>1992–1996</td>
<td>1334</td>
<td>1422</td>
<td>2484</td>
<td>23</td>
<td>1457</td>
<td>12</td>
<td>1079</td>
<td>10</td>
<td>1876</td>
<td>16</td>
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<tr>
<td>1997–2001</td>
<td>1809</td>
<td>1715</td>
<td>3334</td>
<td>30</td>
<td>1869</td>
<td>16</td>
<td>1460</td>
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<td>1982–2006</td>
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<td>18 842</td>
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<td>10 619</td>
<td>18</td>
<td>8305</td>
<td>14</td>
<td>13 490</td>
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</table>

*All sites includes 4580 male and 4934 female cases with unspecified site (age-standardized rate of 7 per million for both sexes).
> 64 years. In females, the greatest increases were on the upper limb for the oldest age group (6.8%) and on the trunk in 45–64-year-olds (6.8%). Lowest annual increases in melanoma occurred on the lower limb in females < 65 years. Across all sites and age groups, the annual increase in incidence was 5.8% in males and 3.8% in females.

The magnitude of incidence rates in males relative to females (M : F) did not vary greatly over time across sites apart from divergence for head/neck melanoma (Fig. 3). Specifically, rate ratios for upper (~0.6) and lower (~0.3) limbs were consistent over the study period, with incidence remaining higher in females than in males at these sites. Despite M : F rate ratio fluctuations (1.4–2.1), incidence of trunk melanoma was always higher in males. In contrast, head/neck melanoma showed similar rates among men and women in the 1980s (0.9) but from the early 1990s rates in men began to rise noticeably faster than the rise in women (M : F ratio ~1.6).

Discussion

In this study based on national cancer registry data, we have shown that incidence rates of invasive cutaneous melanoma have been increasing on all body sites, in both males and females, across the whole of England since 1979, to 2006. As elsewhere in the U.K., highest incidence rates were observed on the lower limb in females, but during the last three decades, increases in invasive melanoma in England have been most pronounced on the trunk in both sexes.

Several studies of anatomical incidence of invasive cutaneous melanoma have been conducted around the world, over varying time periods, and our results show that the actual site distribution of cutaneous melanomas appears similar to other countries with predominantly white-skinned populations, with trunk melanomas predominating in males and lower limb melanomas in females.6–13 Ours is the first study to characterize site-specific incidence trends of cutaneous melanoma in the English population, however, and to have analysed changes in site-specific rates in males relative to females. Most sites have maintained steady differences in melanoma incidence between men and women, but this was not true of the head/neck where rates in men and women were similar in the 1980s but diverged after the early 1990s, with rates in men rising faster, not only at older ages > 65 years, but also in the youngest men < 25 years.

Given that solar ultraviolet radiation (UVR) is the primary environmental cause of melanoma,19,20 a widespread increase in sun exposure is the likely explanation for rising melanoma trends in England and worldwide. Increases in recreational sun exposure have been facilitated by a growing industry of budget airlines in England and declining airfares21,22 making travel abroad to sunny destinations both accessible and affordable to people of all ages. Such vacations abroad have been linked directly to increasing melanoma incidence.21,22 At the same time, changes in clothing fashions have also resulted in increases in sun exposure on previously little-exposed body sites like the trunk and limbs7,11,13,23,24 and such exposure is specifically associated with melanoma at these sites.20,25

Meanwhile, the sunbed industry has provided a means of artificial UVR exposure in temperate England. Sunbed use, now recognized as a cause of melanoma,26 is particularly prevalent among teens and young adults,27 and thus may be the cause of some of the increase in melanoma in younger adults in England. Several studies have explored the relationship between sunbed use and melanoma and have noted significantly increased risk among sunbed users, particularly at earlier ages of first use.28–31 Further studies specifically among those highly exposed to UVR and among adolescents and young adults have noted significant positive dose–response relationships between sunbed usage and melanoma and have
not sex-specific. With high cumulative UVR exposure leading to the development of melanoma on the head and neck, the steeper increase in males than in females on this site is consistent with ongoing higher levels of exposure of the face to UVR in males than in females at all ages, including youth. This is consistent with the general observation that women tend to be less photodamaged than men and have sustained fewer sunburns. They are also more informed about, and compliant with, health promotion measures than men. Women tend to be more conscious of their appearance and to perceive a suntan as a greater threat to attractiveness and health than their male counterparts. Similarly, it is notable that melanoma mortality rates have stabilized in women although are still rising in men in England suggesting that improved diagnosis has yet to benefit men with more advanced disease to the same degree as women.

The strengths of these findings are their national population base and their coverage of recent trends over almost three decades. While melanoma registration and data capture are currently of high quality, there were some deficiencies in melanoma registration in England at the outset of the study period. Improved registration late in the study period, including the recording of multiple primary melanomas on different sites, could therefore have contributed to the observed increase in incidence rates overall, although this should not have affected anatomical sites differentially. Thus the relative site-specific distributions reported here should be accurate. Lack of information about precise anatomical subsites of melanomas prevented further insights into exact sites of UVR exposure which might be amenable to sun-protection measures (e.g. face vs. ear or leg vs. thigh). Laterality details were not available to us, although a previous report has shown a left-sided excess of cutaneous melanoma in England along with several other mainly white populations around the world. Lack of adjustment for body surface area in the calculation of anatomical site-specific incidence rates may also be seen as a potentially

Table 2 Average annual percentage change (AAPC) and 95% confidence limits (CL) of cutaneous melanoma incidence according to anatomical site, by sex and age group (1979–2006)

<table>
<thead>
<tr>
<th></th>
<th>Head/neck</th>
<th></th>
<th>Trunk</th>
<th></th>
<th>Upper limb</th>
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<th>All sites*</th>
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<tr>
<td></td>
<td>AAPC (%)</td>
<td>95% CL</td>
<td>AAPC (%)</td>
<td>95% CL</td>
<td>AAPC (%)</td>
<td>95% CL</td>
<td>AAPC (%)</td>
<td>95% CL</td>
<td>AAPC (%)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt; 25 years</td>
<td>6.3</td>
<td>5.4, 7.2</td>
<td>4.2</td>
<td>3.5, 5.0</td>
<td>2.9</td>
<td>1.9, 3.8</td>
<td>3.2</td>
<td>2.4, 4.0</td>
<td>3.8</td>
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<tr>
<td>25–44 years</td>
<td>4.0</td>
<td>3.6, 4.4</td>
<td>4.9</td>
<td>4.6, 5.2</td>
<td>5.5</td>
<td>5.0, 6.0</td>
<td>4.1</td>
<td>3.7, 4.5</td>
<td>4.4</td>
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<tr>
<td>45–64 years</td>
<td>5.3</td>
<td>4.9, 5.7</td>
<td>6.7</td>
<td>6.4, 7.0</td>
<td>6.6</td>
<td>6.3, 7.0</td>
<td>4.5</td>
<td>4.2, 4.9</td>
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<td>65–89 years</td>
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<td>9.9</td>
<td>9.6, 10.2</td>
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<td>6.8</td>
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<td>6.7</td>
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<td>4.3</td>
<td>4.0, 4.5</td>
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<tr>
<td>&lt; 25 years</td>
<td>5.1</td>
<td>3.9, 6.3</td>
<td>4.2</td>
<td>3.5, 4.8</td>
<td>5.2</td>
<td>4.4, 5.7</td>
<td>3.1</td>
<td>2.5, 3.6</td>
<td>3.9</td>
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<td>25–44 years</td>
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<td>5.8</td>
<td>5.4, 6.1</td>
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<td>1.3</td>
<td>1.0, 1.6</td>
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<tr>
<td>45–64 years</td>
<td>4.1</td>
<td>3.8, 4.4</td>
<td>6.8</td>
<td>6.5, 7.0</td>
<td>5.3</td>
<td>5.0, 5.7</td>
<td>2.5</td>
<td>2.3, 2.7</td>
<td>3.8</td>
</tr>
<tr>
<td>65–89 years</td>
<td>4.0</td>
<td>3.8, 4.3</td>
<td>5.1</td>
<td>4.7, 5.4</td>
<td>6.8</td>
<td>6.5, 7.0</td>
<td>4.6</td>
<td>4.3, 4.9</td>
<td>4.7</td>
</tr>
<tr>
<td>0–89 years</td>
<td>3.9</td>
<td>3.6, 4.1</td>
<td>5.9</td>
<td>5.7, 6.1</td>
<td>5.3</td>
<td>5.0, 5.6</td>
<td>2.5</td>
<td>2.3, 2.8</td>
<td>3.8</td>
</tr>
</tbody>
</table>

*All sites includes cases with unspecified site.

![Fig 3. Cutaneous melanoma male to female (M : F) incidence rate ratios by anatomical site, in England, 1979–2006.](image)
limiting factor, but as we were assessing only trends in melanoma incidence over time, body surface area would not have influenced the results and indeed our analyses are quite comparable to those of other studies which have adjusted for body surface area.\textsuperscript{8,11}

In conclusion, the incidence of invasive cutaneous melanoma on all body sites continued to rise in England among both sexes over the last three decades, with the greatest increases on the trunk, upper limbs and male head and neck. Melanoma prevention messages emphasizing the dangers of high UV exposure, both outdoors in summer or on holiday abroad, and indoors from sunbeds, should continue to be a focus of public health initiatives in England. Men in particular should be encouraged to protect themselves from harmful sun exposure. Continued monitoring of trends in the incidence of cutaneous melanoma is required to track future changes in incidence rates and to assess the effectiveness of current public health messages.

**What’s already known about this topic?**

- Incidence of cutaneous melanoma is still rising worldwide, particularly in countries with predominantly white populations.
- Diagnostic drift may account for some of the increases observed in rates.
- Rates increase with age and are generally highest on the trunk in males and on the lower limb in females.
- National-level site-specific melanoma incidence trends have not previously been described in England.

**What does this study add?**

- To our knowledge this is the first study to describe trends in invasive melanoma incidence trends by anatomical site, for England as a whole.
- Invasive melanoma rates continue to rise in England across all body sites, but particularly on the trunk and arms, and on the head/neck of males.
- The steeper increases in male rates are consistent with their greater ultraviolet radiation exposure and poorer compliance with health promotion messages compared with females.

**Acknowledgments**

We thank staff at the South West Public Health Observatory for information about quality of melanoma registration. Data used in this study were contributed by the eight regional cancer registries in England. Census output is Crown copyrighted and is reproduced with the permission of the Controller of Her Majesty’s Stationary Office and the Queen’s Printer for Scotland.

**References**


Publication 2


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Summary

Background Melanoma incidence often shows an increasing latitudinal gradient from north to south among white European populations.

Objectives To assess emerging regional melanoma incidence patterns in England.

Methods All primary invasive cutaneous melanomas diagnosed in England in people aged 10–89 years, in 1996–2006, were ascertained. Age-standardized incidence rates by sex, age and Government Office Region were calculated for the entire population and for the white population only. Rates according to socioeconomic deprivation were further calculated among those aged under 30 years. Regional heterogeneity and latitude and deprivation trends were assessed by Poisson regression and tests for trend.

Results Overall, melanoma incidence in England was highest in the South West (overall, 18/175; white, 19/103 per 100 000) and lowest in London (overall, 8/185; white, 11/22 per 100 000). Incidence significantly increased with more southerly latitudes in all white adults aged over 30 years (P < 0.0001), except women aged 30–49 years (1.8%, P = 0.10). However, these north–south latitude trends were reversed in white 10–29 year olds, with sex-specific analyses showing an absence of trend in male subjects (2.7%, P = 0.41) and a strong decreasing trend (−9.8%, P < 0.0001) in female subjects. The highest rates in the young female population occurred in the North West (5.46 per 100 000), and specifically in the second most deprived (5.69 per 100 000) and the second most affluent (6.48 per 100 000) groups.

Conclusions Melanoma incidence is high in young people in northern England, including among the moderately deprived, reversing the expected north–south incidence gradients. Prevalent sunbed use in northern England and holiday sun exposure abroad may explain these emerging trends.

What’s already known about this topic?

- Increasing trends in melanoma incidence from north to south have been noted in England and other northern countries.
- Melanoma predominantly affects white, affluent populations; however, neither ethnic groups nor socioeconomic status are uniformly distributed across England.

What does this study add?

- This study describes a striking reversal in the well-established north–south latitude trend in melanoma incidence among young women in England.
- Incidence was high among young people in northern regions, and particularly among moderately deprived female subjects.
Over the past several decades rates of cutaneous melanoma have risen rapidly in white populations worldwide,1 and sun exposure is widely accepted as the principal environmental cause.2 Ambient solar ultraviolet (UV) levels are highly dependent on latitude of residence. Accordingly, north-to-south gradients of increasing melanoma rates have been described in various countries of the northern hemisphere, including England and Wales,3 Norway,4 Canada and the U.S.A.7,12 The few studies in northern countries reporting deviations from these trends have mostly been explained by the regional distribution of other melanoma risk factors such as phenotypic traits, income and outdoor recreation.9,10

In order to assess recent latitude trends in melanoma incidence in England in light of observed increases in rates across the country,11 the current sizeable and unevenly distributed nonwhite population needs to be taken into account.12 Because cutaneous melanoma is predominantly a disease of the white skinned, its incidence is intimately linked with ethnicity as a determinant of skin pigmentation.13 However, previous studies of melanoma incidence in England have not accounted for the geographical distribution of African and Asian residents who are at innately low risk of melanoma compared with the white population.13,14 Wealth is similarly a predictor of melanoma in England,15–17 and is not uniformly spread across the country or across ethnic groups.12,18 Using currently available national data and accounting for both skin colour and socioeconomic status as potential confounding factors, we explored regional melanoma incidence rates and latitudinal trends across England.

Materials and methods

Data sources and variables

Cancer registration in England is conducted by eight regional registries. Each registry uses standardized registration criteria set out by the United Kingdom Association of Cancer Registries for collecting data on all new primary cancer cases (recurrences and metastases not registered).19 Data collected via this system are considered to be of a high standard. Regional data at the time of collection were collated into a national database by the Office of National Statistics and then distributed accordingly by the regional registries, although national cancer registration has been adopted subsequently (from 1 April 2013) by Public Health England.20

This was an ecological study in which we obtained anonymized, individual-level, national cancer registry data from the Northern and Yorkshire Cancer Registry and Information Service (data were obtained prior to 2013) for all people in England up to 89 years of age with a primary, invasive cutaneous melanoma diagnosed between 1996 and 2006. Histology reporting is the main source (at least 95%) for registration of new melanoma cases, and diagnostic criteria did not materially alter for invasive melanoma during the study period. In situ melanomas were excluded a priori.

Individual data on sex, age at diagnosis, location of residence at diagnosis and socioeconomic deprivation were available for each case, although ethnicity was not. Location of residence was based on postal code of residence at the time of diagnosis and categorized as one of nine Government Office Regions in England. Each region was assigned a rank (1–4) based on increasing sunlight hours – a measure often used as a proxy for ambient UV exposure8 – from northern to southern latitude (Fig. 1), such that regions of similar latitude and sun exposure were grouped together as follows: North East (1); North West, Yorkshire and the Humber (2); East Midlands, West Midlands, East of England (3); London, South East, South West (4). Also based on postal code at diagnosis, each case’s category of socioeconomic deprivation was determined using the Index of Multiple Deprivation.21 This index is a national measure of deprivation for specified geographical areas of England, based on census information, and the Income Deprivation Domain specifically captures the proportion of people in a specified area experiencing income deprivation according to means-tested benefits.21 The index consists of five ranked categories of socioeconomic deprivation ranging from 1 (least deprived) to 5 (most deprived).

Overall population estimates by skin pigmentation/ethnicity and by deprivation rank were available for each sex, age and region for only the 2001 census point in England (Population Estimates Unit, Office of National Statistics). White-only population counts were based on the combined total of those self-reporting as ‘White British’, ‘White Irish’ or ‘other White’ on the 2001 census. Single-year population counts in each region were extended to a 5-year interval on either side of the census point to define the study period (1996–2006).

Statistical methods

Melanoma incidence rates in each region of England were calculated by sex and by age group (10–29, 30–49, 50–69 and 70–89 years) using both the total and white-only population bases. Melanoma cases in children aged 0–9 years were excluded from our analyses (n = 31), as young children have a very low incidence of melanoma and a distinct disease aetiology (largely genetic rather than environmental factors implicated).22 Rates were expressed per 100 000 person-years at risk and were age standardized to the European Standard Population using direct methods.23 Incidence rates by deprivation rank were also calculated for 10–29 year olds by broad geographical grouping of regions according to latitude: northern (North East, North West, Yorkshire and the Humber), central (East Midlands, West Midlands, East of England), southern (South East, South West) and London.

Poisson regression models were used to test for heterogeneity in regional melanoma incidence trends by sex, age group and deprivation level, and to estimate percentage change in incidence per unit increase in latitude rank (north to south, 1–4) and in deprivation rank (least to most, 1–5). Tests for trends with decreasing latitude across sex and age groups, and with increasing deprivation in 10–29 year olds by broad geography, were also performed. Significance was set at $P < 0.05$. Analyses were performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, U.S.A.).
Results

Over the 11-year period of study (1996–2006), 70,632 cases of invasive cutaneous melanoma (45% male) were registered in England among people aged 10–89 years. Nearly all cases (99%) had complete data on all variables (n = 69,788); the 1% of cases with missing data (age, region or deprivation rank) were excluded from the analyses. The age-standardized (European Standard) melanoma incidence rates in England during this period were 13.57 per 100,000 (male, 12.86; female, 14.43) among the entire population, and 14.46 per 100,000 (male, 13.64; female, 15.46) in the white population (Table 1). The highest incidence in both sexes occurred in the South West (male, 18.40; female, 19.35 per 100,000), followed by the South East; the lowest rates were in London (male, 8.77; female, 9.03 per 100,000). When the denominator was restricted to the white population, incidence rates in London rose by 25% in male subjects to 10.62 per 100,000, overall, compared with neighbouring southern regions, the London rates remained incongruously low. Overall and in both sexes, there was a significant increasing trend in melanoma rates from north to south across the country (all \( P < 0.0001 \)), although in the white population men showed a greater percentage increase in incidence per latitude rank than women (male, 18.1%; female, 11.1% – excluding London) (Table 1). Regional rates were statistically significantly different between the sexes (\( P < 0.0001 \)).

Regional patterns of melanoma by age were investigated for the male and female (Table 2) subsets of the total and white-only populations, but only the latter adjusted estimates are detailed here. The percentage change estimates and trends highlighted are excluding London. In both sexes, regional rates significantly differed between the age strata (both \( P < 0.0001 \)). Melanoma incidence rates in white male subjects aged 10–29 years showed some variation across regions, from a low of 1.66 per 100,000 in London to 2.47 per 100,000 in the South West (Table 2). However, among the young male
### Table 1

<table>
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<th>Region</th>
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<th>All White</th>
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<td>2683</td>
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<td>3</td>
</tr>
<tr>
<td>Adjusted rates by sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1034</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>Female</td>
<td>949</td>
<td>23</td>
<td>1</td>
</tr>
<tr>
<td>Regional trends</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P value for latitudinal trend</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Among 10–29-year-old white female subjects a significant trend of increasing melanoma rates with more northerly latitudes emerged, such that rates rose by 9.8% per unit decrease in latitude rank: rates were around 3 per 100 000 in London and the East of England, but 5.46 per 100 000 in the North West (P < 0.0001) (Table 2). Among 30–49 year olds, melanoma rates were similarly high at latitudinal extremes (South West, 17.69; Yorkshire and Humber, 17.25 per 100 000), and no trend was apparent (1.8%, P = 0.10). In contrast, melanoma rates in women aged over 50 years significantly increased with more southerly latitudes, corresponding to the trends seen in older men (all P < 0.0001). The older age groups differed in that incidence was lowest in London (18.18 per 100 000) in 50–69 year olds, but lowest in the North East (24.62 per 100 000) for those aged 70–89 years, while the magnitude of rates was higher in the older group.

To examine the observed trends more closely in 10–29 year olds, regional melanoma rates in young people in England were examined by level of deprivation. Rates in each deprivation rank were significantly different between latitudes for the female (P = 0.002) and male (P = 0.02) subgroups. With the exception of white male subjects in the north, young people across the country showed the same trend of significantly decreased melanoma incidence with greater deprivation (Table 3). Notably, among the young white female population in the north, the second most affluent group had the highest rates of all (6.48 per 100 000), followed closely by the second most deprived group (5.69 per 100 000).

### Discussion

In this ecological study of emerging trends of regional melanoma incidence in the English population, we observed a striking difference in latitude gradients among young people compared with older adults. The expected north-to-south increase in rates was reversed in those aged 10–29 years, driven mainly by the strong trend reversal among the young female population, particularly those of intermediate socioeconomic status. Across the remaining age groups, the north–south increasing trend was maintained, although melanoma rates were remarkably lower in London compared with other southern regions.

Three decades ago, Swerdlow described latitude trends in melanoma incidence in England,³ but no further national studies have since been conducted. We have previously compared melanoma incidence in the Government Office Regions of England in 13–24 year olds only, and showed that in the period 1990–3, rates ranged from 10.0 (North East) to 22.0

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**Table 1**: Number of cases (n) and age-standardised (European Standard) incidence rates of cutaneous melanoma in people aged 10–89 years (per 100 000 person-years) by Government Office Region of England and by sex, in the whole population and in white individuals only (1996–2006)
Table 2 Number of cases (a) and age-standardized (European Standard) incidence rates of cutaneous melanoma (per 100 000 person-years) by Government Office Region of England and by age group, in the whole population and in white individuals only (1996–2006) – (a) male population and (b) female population

<table>
<thead>
<tr>
<th>Region</th>
<th>Age group</th>
<th>10–29 years</th>
<th>30–49 years</th>
<th>50–69 years</th>
<th>70–89 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n All White</td>
<td>n All White</td>
<td>n All White</td>
<td>n All White</td>
<td>n All White</td>
</tr>
<tr>
<td>(a) Male only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North East</td>
<td>75 2.29  2.39</td>
<td>347 8.93  9.15</td>
<td>523 16.89 17.09</td>
<td>358 2.84  2.93</td>
<td></td>
</tr>
<tr>
<td>North West</td>
<td>174 1.96  2.14</td>
<td>980 9.63 10.16</td>
<td>1627 19.94 20.50</td>
<td>1163 3.63  3.72</td>
<td></td>
</tr>
<tr>
<td>Yorkshire &amp; the Humber</td>
<td>144 2.14  2.38</td>
<td>772 9.54 10.14</td>
<td>1185 19.88 20.49</td>
<td>856 3.67  3.75</td>
<td></td>
</tr>
<tr>
<td>East Midlands</td>
<td>98 1.76  1.93</td>
<td>598 9.25 9.90</td>
<td>1060 20.52 21.28</td>
<td>763 3.90  3.70</td>
<td></td>
</tr>
<tr>
<td>West Midlands</td>
<td>122 1.73  2.05</td>
<td>663 8.34 9.31</td>
<td>1241 19.21 20.38</td>
<td>956 3.93  3.53</td>
<td></td>
</tr>
<tr>
<td>South East</td>
<td>244 2.27  2.45</td>
<td>1400 11.20 11.77</td>
<td>2548 26.53 27.24</td>
<td>2017 5.26  5.08</td>
<td></td>
</tr>
<tr>
<td>South West</td>
<td>148 2.38  2.47</td>
<td>939 12.85 13.15</td>
<td>2050 32.23 32.59</td>
<td>1782 6.16  6.54</td>
<td></td>
</tr>
<tr>
<td>London</td>
<td>157 1.88  1.66</td>
<td>684 5.90 8.14</td>
<td>1041 15.45 19.36</td>
<td>801 2.95  3.14</td>
<td></td>
</tr>
<tr>
<td>All England</td>
<td>1300 1.90 2.17</td>
<td>7145 9.45 10.35</td>
<td>12891 22.15 23.29</td>
<td>9822 4.18  4.34</td>
<td></td>
</tr>
<tr>
<td>Change N to S, %</td>
<td>–5.7</td>
<td>–1.7</td>
<td>1.3</td>
<td>5.3</td>
<td>12.7</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(–11.0–0.00)</td>
<td>(–7.3–4.2)</td>
<td>(–1.3–3.9)</td>
<td>(2.7–8.0)</td>
<td>(10.6–14.9)</td>
</tr>
<tr>
<td>P&lt;sub&gt;trend&lt;/sub&gt;</td>
<td>0.05</td>
<td>0.56</td>
<td>0.33</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Change N to S excluding</td>
<td>–2.1–10.3</td>
<td>–3.7–9.5</td>
<td>7.4–13.4</td>
<td>18.1–123.1</td>
<td>17.8–22.7</td>
</tr>
<tr>
<td>London, % (95% CI)</td>
<td>0.31</td>
<td>0.41</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>P&lt;sub&gt;trend excluding London&lt;/sub&gt;</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>(b) Female only</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North East</td>
<td>157 4.66  4.84</td>
<td>596 14.80 15.13</td>
<td>606 18.85 19.03</td>
<td>459 2.51  2.62</td>
<td></td>
</tr>
<tr>
<td>North West</td>
<td>458 4.99  5.46</td>
<td>1660 15.64 16.46</td>
<td>1917 22.74 23.29</td>
<td>1454 2.57  2.88</td>
<td></td>
</tr>
<tr>
<td>Yorkshire &amp; the Humber</td>
<td>316 4.60  5.12</td>
<td>1258 16.28 17.25</td>
<td>1300 21.09 21.67</td>
<td>999 2.76  2.90</td>
<td></td>
</tr>
<tr>
<td>East Midlands</td>
<td>198 3.58  3.94</td>
<td>901 13.70 14.70</td>
<td>1194 22.78 23.63</td>
<td>865 2.96  2.94</td>
<td></td>
</tr>
<tr>
<td>West Midlands</td>
<td>232 3.24  3.88</td>
<td>1059 13.12 14.69</td>
<td>1356 20.64 21.88</td>
<td>1029 2.74  2.79</td>
<td></td>
</tr>
<tr>
<td>East of England</td>
<td>202 2.86  3.07</td>
<td>1176 13.82 14.66</td>
<td>1631 23.79 24.53</td>
<td>1212 3.03  3.17</td>
<td></td>
</tr>
<tr>
<td>South East</td>
<td>417 3.90  4.22</td>
<td>2040 16.08 16.95</td>
<td>2735 27.55 28.27</td>
<td>2256 3.76  3.79</td>
<td></td>
</tr>
<tr>
<td>South West</td>
<td>244 4.04  4.20</td>
<td>1299 17.28 17.69</td>
<td>2162 32.95 33.40</td>
<td>1941 4.63  4.39</td>
<td></td>
</tr>
<tr>
<td>London</td>
<td>325 2.26  2.35</td>
<td>952 7.69 11.14</td>
<td>1044 14.99 18.18</td>
<td>980 2.47  2.52</td>
<td></td>
</tr>
<tr>
<td>Change N to S, %</td>
<td>–15.9</td>
<td>–11.8</td>
<td>–6.9</td>
<td>–2.4</td>
<td>8.1 10.9 13.6</td>
</tr>
<tr>
<td>(95% CI)</td>
<td>(–19.2 to −12.4)</td>
<td>(–15.3 to −8.1)</td>
<td>(–5.0 to −4.3)</td>
<td>(6.1–10.1)</td>
<td>(8.9–12.9)</td>
</tr>
<tr>
<td>P&lt;sub&gt;trend&lt;/sub&gt;</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Several other countries in the northern hemisphere have examined melanoma latitude trends. Predominantly fair-skinned populations of northern Europe, such as in Norway, have shown north–south increasing trends in incidence. Furthermore, an analysis of melanoma mortality rates in major cities across a range of latitudes in Canada and the U.S.A. demonstrated the same higher rates at more southerly latitudes. In contrast, a study in the Netherlands reported that melanoma rates were highest in the northern and western parts of the country, but the authors explained this by greater hours of sunshine and more recreational activities undertaken in coastal areas, analogous to the proposed reasons for the north–south trends elsewhere. Furthermore, in an Italian study, south-to-north increasing trends were in part explained by the predominance of the fair-hair phenotype and higher income in the north. While our results among older adults are consistent with previous findings, this study now points to a striking deviation from the established trend in young people, including the at-risk white population.

Changes in UV exposure, the single major cause of cutaneous melanoma, are likely to explain the majority of the variation in regional melanoma rates observed in this study. For older generations it has been estimated that approximately 90% of melanomas in men and 82% in women in the U.K. may be attributed to excess solar UV radiation. Widespread increases in recreational sun exposure along with changes in clothing fashions are major contributing factors to the rising rates of melanoma in the late twentieth century in Europe, and can provide intense UV dosages. The prevalence of the fair-hair phenotype and higher income in the north may be attributed to excess solar UV radiation.

More recent relevant changes in UV exposure over the past few decades, which may explain the general tendency for high melanoma rates in the north, include the rise in popularity of sunbeds and the advent of budget holidays. In the last 30 years, sunny holiday destinations abroad have become more affordable, and thus accessible to people of all ages and socioeconomic status, with the result that they are augmenting local sun exposure and influencing traditional patterns of sun-related skin disease. Sunbeds too are widely accessible and can provide intense UV dosages 10–15 times stronger than those obtained from midday sun exposure in a hot climate. They are thought to cause melanoma in up to 76% of young people who ever use them. In England, sunbed use has become particularly prevalent among young people, with a reported 6% of all 11–17 year olds having ever used them.

### Table 2 (Continued)

<table>
<thead>
<tr>
<th>Age group</th>
<th>Region</th>
<th>N</th>
<th>All White</th>
<th>n</th>
<th>Change N to S</th>
<th>Confidence interval (95% CI)</th>
<th>P-value for latitudinal trend in rates</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–19 years</td>
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<td>20–29 years</td>
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<td>70–89 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Note: The table continues with additional data, including percentages and P-values for various comparisons.*
Table 3 Number of cases (n) and age-standardized (European Standard) incidence rates of cutaneous melanoma (per 100 000 person-years) among 10–29 year olds in England by socioeconomic deprivation (rank 1, least deprived to rank 5, most deprived) and regional grouping, in the whole population and in White individuals only (1996–2006)

<table>
<thead>
<tr>
<th>Deprivation</th>
<th>Northern latitude</th>
<th>Intermediate latitude</th>
<th>Southern latitude</th>
<th>London</th>
<th>All England</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
</tr>
<tr>
<td></td>
<td>n All White</td>
<td>n All White</td>
<td>n All White</td>
<td>n All White</td>
<td>n All White</td>
</tr>
<tr>
<td>1</td>
<td>74 2.29 2.39</td>
<td>87 2.37 2.37</td>
<td>125 3.05 2.88</td>
<td>23 1.91 2.26</td>
<td>309 2.49 2.65</td>
</tr>
<tr>
<td>2</td>
<td>82 2.63 2.74</td>
<td>86 2.12 2.24</td>
<td>96 2.49 2.61</td>
<td>29 1.83 2.12</td>
<td>293 2.35 2.49</td>
</tr>
<tr>
<td>3</td>
<td>72 2.19 2.30</td>
<td>82 1.96 2.11</td>
<td>88 2.13 2.25</td>
<td>35 1.53 1.98</td>
<td>277 2.01 2.19</td>
</tr>
<tr>
<td>4</td>
<td>74 1.99 2.11</td>
<td>60 1.46 1.62</td>
<td>60 1.83 1.98</td>
<td>50 1.28 1.82</td>
<td>244 1.65 1.90</td>
</tr>
<tr>
<td>5</td>
<td>91 1.65 1.99</td>
<td>43 1.13 1.55</td>
<td>23 1.72 1.90</td>
<td>20 0.46 0.78</td>
<td>177 1.18 1.58</td>
</tr>
<tr>
<td>Change</td>
<td>−9.1 −6.1</td>
<td>−15.9 −11.9</td>
<td>−26.1 (−33.9) −19.5</td>
<td>−13.6 −12.8</td>
<td>−14.4 −11.2</td>
</tr>
<tr>
<td>per rank</td>
<td>(−15.0 −12.3)</td>
<td>(−22.1 (−18.5) to −17.4)</td>
<td>(−28.1 (−20.3) to −19.6)</td>
<td>(−17.8 (−14.7) to −14.0) to −7.5)</td>
<td></td>
</tr>
<tr>
<td>1–5,b % (95% CI)</td>
<td>to −2.8) to 0.5)</td>
<td>to −9.3) to −4.8)</td>
<td>to −9.8) to −6.4)</td>
<td>to −5.5) to −11.0) to −7.5)</td>
<td></td>
</tr>
<tr>
<td>P&lt;sub&gt; trend&lt;/sub&gt;</td>
<td>0.005 0.07</td>
<td>&lt;0.0001 0.001</td>
<td>0.0003 0.0002</td>
<td>&lt;0.0001 0.0001</td>
<td>&lt;0.0001 0.0001</td>
</tr>
</tbody>
</table>

CI: confidence interval. aGrouping of regions by similar latitude and mean hours of sunshine – Northern: North East, North West, Yorkshire & the Humber, Intermediate: East Midlands, West Midlands, East of England; Southern: South East, South West; London region alone. bPercentage change in rates per unit increase in deprivation rank (least to most deprived, 1–5). cP-value for trend in rates with increasing deprivation.
and within the country young women in the north are the most prevalent users of all (up to 50%). The observed reversal of the north–south latitude gradient and peak incidence in the North West in young people are consistent with these sunbed and holiday trends, and thus appear to be the most likely explanation for our findings.

Aside from UV exposure variables, it is possible that other factors may explain some of the variation observed (i.e. phenotypic traits – hair, eye and skin colour, immunosuppression and family history). We were unable to assess these risk factors, as individual case data were unavailable, although the confounding effects of phenotypic variables, in particular, would mainly have been controlled for when the analyses were restricted to the white population.

The reason for the observed deficit of melanoma in the London region is unknown. While lack of access to sunny holidays cannot explain this observation, London’s highly urban environment perhaps affords its residents less opportunity for exposure to ambient sunlight than those of neighbouring southern regions. The heterogeneity and mobility of the white population resident in London may also contribute to the unexpectedly low incidence. A further possible explanation is the low registration rate of melanomas in the Thames Registry, possibly as a result of greater use in London of private healthcare facilities where cancer data may not be readily available to registration. While cancer registration in England is highly regulated and of a high standard, some variability in data capture and efficiency of processing still remains between the regional registries.

The particular strengths of this study are its relative recentness and the high quality of the data from the eight cancer registries in England. Novel strengths include accounting for both the uneven geographical distribution of low-risk nonwhite populations, and the influence of socioeconomic status in England, when considering the geographical distribution of melanoma. As this was an ecological study, the issue of ‘ecological fallacy’ is a possibility, as our findings are based on regional populations and cannot be inferred to individuals within these populations. We were limited in the calculation of incidence rates among white individuals as we did not have data on individual case ethnicity and thus could not restrict the numerator. The effect on our results was that some cases occurring in nonwhite subjects may have been misclassified, thereby overestimating white incidence rates. However, this should be a nearly negligible effect on our rates, as melanomas are extremely rare in nonwhite populations, and acri lentiginous melanoma, the subtype occurring most frequently in nonwhite individuals, occurs in the white population at the same relative frequency, so there is no differential with respect to skin colour. We also inferred the size and ethnic composition of each Government Office Region, and deprivation rank, from a single census point in 2001, a method that assumes little or consistent change in England’s population structure across the study period. Notwithstanding their uneven geographical distribution, the overall proportion of nonwhites in the total population of England is small, and this inference method has been used previously. Finally, as deprivation rank is not an individual measure of income deprivation, some misclassification was possible, although it should not have differentially affected the regions.

In conclusion, although maintained in older adults, the well-established north–south increasing gradient in melanoma incidence has been reversed among young people under the age of 30 years. This significant finding was driven primarily by the reversal of latitude trends in young women, particularly in those of intermediate socioeconomic status. The affordability of sun holidays and the high prevalence of sunbed use among young adults, especially young women living in the north of England, may explain these trends. Recent banning of sunbed use in those under 18 years of age in the U.K. should eventually bring a reduction in harmful exposure to artificial UV in the future; however, this regulation will not completely resolve the issue as it applies only to commercial outlets, so private use remains unregulated and its effects may continue to be seen. It is important to monitor both UV exposure patterns and melanoma incidence closely in the wake of these trends and the recently implemented legislation.

Acknowledgments

We extend our gratitude to Madeleine Flynn at the Queensland Institute of Medical Research for her work on Figure 1, and to the Met Office for permission to adapt this figure.

References


Publication 3

Wallingford SC, van As JA, Hughes MC, Ibiebele TI, Green AC, van der Pols JC.
Intake of omega-3 and omega-6 fatty acids and risk of basal and squamous cell
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Nutrition and Cancer

Publication details, including instructions for authors and subscription information:
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Intake of Omega-3 and Omega-6 Fatty Acids and Risk of Basal and Squamous Cell Carcinomas of the Skin: A Longitudinal Community-Based Study in Australian Adults

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Intake of Omega-3 and Omega-6 Fatty Acids and Risk of Basal and Squamous Cell Carcinomas of the Skin: A Longitudinal Community-Based Study in Australian Adults

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Intake of omega-3 and omega-6 fatty acids may modify the risk of basal and squamous cell carcinoma of the skin (BCC and SCC), but population-based evidence is limited and inconsistent. We examined prospectively associations between intake of omega-3 and omega-6 fatty acids estimated from food frequency questionnaires and BCC and SCC incidence among 1322 randomly selected adults in Nambour, Australia. Relative risks (RR) and 95% confidence intervals (CI) were estimated based on histologically confirmed tumors diagnosed between 1997 and 2007. Incidence of BCC was lowest in the middle third of both total omega-6 intake (RR_{mv.adj} = 0.74, 95% CI = 0.56–0.97) and linoleic acid intake (RR_{mv.adj} = 0.75, 95% CI = 0.57–0.99) compared with the lowest third of intake. Evidence for associations with SCC was weak, though persons with arachidonic acid intake in the middle third had a marginally increased risk of SCC (RR_{mv.adj} = 1.42, 95% CI = 1.00–2.02). Consumption of omega-3 fatty acids was not associated with subsequent skin cancer risk. Suggestion that intake of arachidonic acid may be associated with increased SCC incidence and total omega-6 with reduced BCC from our study is still highly uncertain and may be due to chance. These data do not support an association between these fatty acids and risk of BCC or SCC.

INTRODUCTION

The keratinocytic skin cancers basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) are the most commonly occurring cancers in white-skinned populations worldwide. Approximately 90% of all keratinocytic skin cancers in humans are attributed to excessive exposure to solar ultraviolet radiation (UVR) (1,2). UVR is a complete carcinogen capable of initiation and promotion of skin cancer and acts in several ways such as by inducing DNA damage and modulating immunosuppression (3).

There is increasing evidence that dietary factors modify carcinogenic processes in the skin and that UVR-induced skin carcinogenesis may be sensitive to the quantity and type of dietary fat ingested. Animal studies have indicated that diets high in omega-6 polyunsaturated fatty acids can reduce the latent period before tumor appearance after UVR exposure, increase the number of tumors, and affect the promotional stage of UV carcinogenesis (4,5). High levels of omega-6 fatty acids are also associated with specific deleterious immune responses such as elevation of prostaglandin E2 levels, an immunoregulator
known to exacerbate UVR-carcinogenesis and to be associated with aggressive keratinocytic skin cancer growth in humans (1,6,7). Conversely, omega-3 fatty acids including α-linolenic acid, docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), appear to reduce inflammatory effects on skin cells following UVR exposure thereby inhibiting UVR carcinogenesis (1,3,8–11). Further, an inverse dose-response relationship between omega-3 intake and p53 immunoreactivity has been shown such that omega-3 fatty acids appear to prevent DNA damage caused by mutant p53 by influencing the process by which UVR induces mutations in the p53 gene (3,12). Because of the opposing effects of omega-3 and omega-6 fatty acids, the ratio of their intakes may determine the overall effect on skin carcinogenesis (13,14).

Results from previous epidemiological studies of omega-3 and omega-6 fatty acids have been mixed. A decrease in risk of SCC with increased intake of diets with a high omega-3/omega-6 ratio was observed in a population-based case-control study of 792 subjects from southeastern Arizona (14); however a prospective study of more than 40,000 American male health professionals failed to demonstrate a reduction in BCC risk with low dietary fat or high omega-3 fatty acid intake over the 8 yr of follow-up (15).

In the present community-based study we investigated prospectively the association between estimates of dietary intake of omega-3 and omega-6 fatty acids, and the risk of BCC and SCC.

**MATERIALS AND METHODS**

**The Nambour Trial and Follow-Up Study**

We conducted an 11-yr prospective cohort study from 1997 to 2007 among White Caucasian adults who were originally randomly selected using the electoral roll (enrollment is compulsory by law in Australia) from the community in 1986 and who participated in a skin cancer prevention field trial between 1992 and 1996. Detailed descriptions of the community sample, the field trial, and its outcomes have been reported previously (16,17). Briefly, some 1600 residents of Nambour, a township in Queensland, Australia, took part in a trial that evaluated the prevention of skin cancer using β-carotene supplements and/or daily application of sunscreen. Original study participants from 1986 were eligible for the trial if they attended the 1992 survey, underwent a complete skin examination by a dermatologist with removal of all diagnosed skin cancers, and gave written consent to participate in the trial until 1996 (16,17). Persons with Gorlin’s syndrome or porokeratosis were ineligible to participate. Trial participants were eligible for the present study if they had completed a food frequency questionnaire (FFQ). The study was approved by the ethics committee of the Queensland Institute of Medical Research and all participants provided informed written consent.

**Dietary Intake Estimates**

Habitual diet during the past 6 mo was assessed using a self-administered, semiquantitative FFQ consisting of 129 food or food group items. These were completed in 1992, 1994, and 1996. The FFQ, originally developed for the U.S. Nurses’ Health Study, was adapted for the Australian setting and validated against weighed food records (18). For each food item, a commonly used unit or portion size was specified and participants were asked to estimate how often on average they had eaten the given amount of food over the past 6 mo. The 9 response options ranged from “never” to “4+ times per day.” Information on cooking methods; specific types of fats, oils, and margarines used; and frequency of eating breakfast cereals and fried takeaway foods was also collected, as well as detailed information regarding consumption of nutritional supplements. Average daily intake was calculated by expressing the response to the food item as a proportion of daily use, which was then multiplied by the amount, in grams, of specified portion sizes. Daily intakes of linoleic acid, arachidonic acid, α-linolenic acid, EPA, docosapentaenoic acid (DPA), and DHA were calculated using a comprehensive fatty acid database for Australian foods (19). Total omega-3 fatty acid intake was calculated by summing the intake of α-linolenic acid, EPA, DPA, and DHA (total long chain omega-3’s excluded α-linolenic acid), and total omega-6 fatty acid intake by the sum of linoleic acid and arachidonic acid intake. The ratio of omega-3/omega-6 fatty acids was also calculated. Intake of dietary supplements containing fatty acids or oils was estimated using a supplement database (20). These calculations were compiled for each of the 1992, 1994, and 1996 FFQs and mean nutrient values across the 3 were used in the analysis. Validity of fatty acid intake estimates from the FFQ was evaluated relative to plasma phospholipid measurements and weighed food records. FFQ validity coefficients ranged from 0.45 (arachidonic acid) to 0.63 (total omega-3). Diet-plasma correlations were moderate for total omega-3, α-linolenic acid, EPA, DPA, and DHA (21).

**Other Variables**

Information on demographic variables, eye color, and hair color was obtained via an interviewer-administered questionnaire (22). Additional standard self-administered questionnaires provided information on education, smoking habits, compliance with the trial treatment supplement, presence of certain medical conditions, and standard skin cancer risk factors such as natural skin color, tanning ability of the skin, and occupational and leisure-time sun exposure (17). During a physical examination in 1996, height and weight were measured and elastosis of the neck was recorded as a measure of long-term sun exposure history.

**Determination of Skin Cancer**

An intensive surveillance system of incident skin cancers in the study population that was set up during the Nambour trial continued during the complete posttrial follow-up period.
(1997–2007). Questionnaires were mailed twice-yearly to participants and any reported skin cancers were confirmed through histological reports. In addition in 2000, a full-body skin examination for skin cancer was conducted by a dermatology specialist trainee among an unselected proportion of ongoing study participants, and in 2007 all remaining active participants underwent full-body skin examination. Finally, independent pathology laboratories throughout Queensland provided histology reports for all skin cancers diagnosed among study participants throughout the follow-up period. These methods ensured virtually 100% ascertainment of histologically confirmed skin cancers in the study population (23).

Outcomes

The two outcomes of analyses were (a) incidence of persons affected by a new BCC or SCC calculated as the number of patients diagnosed from January 1, 1997, to December 31, 2007, divided by the person-yr of follow-up accumulated between these dates and expressed per 100 000 person-yr; and (b) incidence of BCC or SCC tumors during the same person-yr follow-up time as calculated for the person-based analysis. Tumors diagnosed in 1996 were not included in the analyses to exclude disease that already existed during the baseline nutritional assessment. Tumors and person-yr of follow-up were counted until date of withdrawal from the study, date of death, or December 31, 2007, whichever occurred first.

Data Analysis

For the linear modeling, intake estimates of dietary fatty acids were adjusted for total energy intake using the nutrient residual method as described by Willet (24). Distributions of dietary intake were identified as skewed and variables were log-transformed to improve normality prior to calculation of the residuals. Dietary intakes were classified into ranked thirds. For tumor-based analyses, relative risks (RR) and 95% confidence intervals (CI) for increasing levels of dietary intake compared to the lowest third were derived using generalized linear models with negative binomial distribution and person-years of follow-up as offset (25). RR and 95% CI for person-based analysis were estimated by generalized linear models specifying Poisson distribution with a robust error variance (26).

We first applied models controlling for age and sex ("basic" models). We also assessed the multivariate adjusted model, which considered the confounding effects of tanning ability (always burn, burn then tan, only tan), skin color (fair, medium/olive), eye color (blue/grey, hazel/green, light brown, dark brown), hair color (blonde, light brown, ginger/auburn, dark brown/black), mean daily energy intake, supplement use (yes/no), freckling of the back (none, few, many), elastoses of the neck (none, mild, moderate, severe), total number of solar keratoses (0, 1–10, 11–50, >50), pack-yr of smoking (nonsmoker, 0 to 7, >7 to 20 pack-yr), body mass index (BMI), randomized treatment allocation, lifetime number of painful sunburns (0, 1–10, >10), weekday and weekend hours spent outdoors (<1, 1–4, 5–8, 9–12), habitual sunscreen use on face, hands, and other body parts independent from treatment allocation (less than or more than 50% of the time), use of nonsteroidal antiinflammatory drugs (yes/no), and previous history of skin cancer before 1997 (yes/no). Among these, the factors that changed the relative risk estimates of the basic model by ≥10% were considered significant confounders of the model (27) in the context of findings of previous studies on diet and skin cancer. Adjustment variables included in the final multivariate models were age, sex, freckling on the back, elastosis of the neck, and trial treatment allocation for BCC models, and age, sex, elastosis of the neck, total number solar keratoses, and trial treatment allocation for SCC models.

People with a history of skin cancer have an increased risk of developing subsequent skin cancers (28,29) and may be more prone to risk modification by dietary factors. Thus the above analyses were repeated using stratification for previous history of skin cancers prior to 1997. Information on skin cancer history was based on skin cancers identified during skin examinations and surveys conducted in the participants prior to 1996 (16,22,30,31). Analyses stratified by sex were also performed. Statistical significance was set at P value <0.05 (2-tailed). The statistical analyses of the data were carried out using SAS version 9.2 (SAS, Cary, NC).

RESULTS

Of the 1621 original Nambour trial participants, 1334 completed at least one FFQ and consented to be followed-up. Two persons died soon after and 4 participants were excluded because they did not indicate consumption frequencies for 10% or more of the FFQ food items or they reported energy intakes outside the normal ranges (24). A further 6 persons were excluded because they were not of white Caucasian ethnicity, leaving 1322 people for study. No significant difference was found between the subjects in the present study and the 1621 trial participants in terms of randomized treatment allocation, age, sex, education, occupation, smoking status, use of dietary supplements, and skin cancer factors. Participants who had any incident skin cancer during follow-up were more likely to be male, older, have mainly outdoors occupations, have elastosis of the neck, have freckling on the back, and have a previous history of skin cancer (Table 1). In addition, persons affected by SCC were also more likely to have fair skin color and to always sunburn on acute sun exposure.

In the 10-yr follow-up period a total of 325 participants with 746 histologically confirmed new BCC tumors were diagnosed in during 13 903 person-yr of follow-up. This gave a BCC person-based incidence rate of 2,338/100,000 and a tumor-based incidence rate of 5,366/100,000 person-yr. One hundred ninety-six participants with 368 histologically confirmed new SCC tumors were diagnosed during the same follow-up giving an SCC person-based incidence rate of 1,409/100,000 and a tumor-based incidence rate of 2,646/100,000. Among the 1322
**TABLE 1**
Baseline characteristics by skin cancer status of 1322 participants of the Nambour Skin Cancer Study, 1997–2007

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Basal cell carcinoma</th>
<th>Squamous cell carcinoma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present (n = 318)</td>
<td>Absent (n = 1004)</td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>159 (50)</td>
<td>421 (42)</td>
</tr>
<tr>
<td>Female</td>
<td>159 (50)</td>
<td>583 (58)</td>
</tr>
<tr>
<td><strong>Age (mean ± SD)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mainly outdoors</td>
<td>73 (23)</td>
<td>173 (17)</td>
</tr>
<tr>
<td>Both</td>
<td>130 (41)</td>
<td>359 (36)</td>
</tr>
<tr>
<td>Mainly indoors</td>
<td>115 (36)</td>
<td>471 (47)</td>
</tr>
<tr>
<td><strong>Pack-years smoked</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Life-long non-smoker</td>
<td>178 (56)</td>
<td>562 (56)</td>
</tr>
<tr>
<td>≤7 pack-years smoked</td>
<td>52 (16)</td>
<td>168 (17)</td>
</tr>
<tr>
<td>7–20 pack-years smoked</td>
<td>32 (10)</td>
<td>117 (12)</td>
</tr>
<tr>
<td>≥20 pack-years smoked</td>
<td>56 (18)</td>
<td>157 (16)</td>
</tr>
<tr>
<td><strong>Skin color</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fair</td>
<td>191 (60)</td>
<td>544 (54)</td>
</tr>
<tr>
<td>Medium</td>
<td>114 (36)</td>
<td>385 (38)</td>
</tr>
<tr>
<td>Olive/black/brown</td>
<td>13 (4)</td>
<td>74 (8)</td>
</tr>
<tr>
<td><strong>Skin type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Always burn</td>
<td>78 (25)</td>
<td>195 (19)</td>
</tr>
<tr>
<td>Burn/tan</td>
<td>216 (68)</td>
<td>701 (70)</td>
</tr>
<tr>
<td>Only tan</td>
<td>24 (8)</td>
<td>107 (11)</td>
</tr>
<tr>
<td><strong>Painful sunburns</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>48 (16)</td>
<td>144 (15)</td>
</tr>
<tr>
<td>Once</td>
<td>181 (60)</td>
<td>592 (63)</td>
</tr>
<tr>
<td>2–5 Burns</td>
<td>43 (14)</td>
<td>144 (15)</td>
</tr>
<tr>
<td>&gt;5 Burns</td>
<td>31 (10)</td>
<td>64 (7)</td>
</tr>
<tr>
<td><strong>Elastosis of the neck</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>17 (6)</td>
<td>191 (20)</td>
</tr>
<tr>
<td>Mild/moderate</td>
<td>151 (51)</td>
<td>539 (58)</td>
</tr>
<tr>
<td>Severe</td>
<td>127 (43)</td>
<td>207 (22)</td>
</tr>
<tr>
<td><strong>Freckling on the back</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>39 (13)</td>
<td>199 (21)</td>
</tr>
<tr>
<td>Mild</td>
<td>115 (40)</td>
<td>417 (44)</td>
</tr>
<tr>
<td>Moderate</td>
<td>86 (30)</td>
<td>228 (24)</td>
</tr>
<tr>
<td>Severe</td>
<td>51 (18)</td>
<td>95 (10)</td>
</tr>
<tr>
<td><strong>History of skin cancer</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>198 (62)</td>
<td>231 (23)</td>
</tr>
<tr>
<td>No</td>
<td>120 (38)</td>
<td>773 (77)</td>
</tr>
</tbody>
</table>

*P value from chi-square test (categorical data) or ANOVA (continuous data); significance p < 0.05.

Participants, 429 (32%) had a prior history of skin cancer before 1997.

From the person-based analyses after multi-variable adjustments, incidence of BCC was lower in persons in the middle thirds of both total omega-6 intake (RR_{mv,adj} = 0.74, 95% CI = 0.56–0.97) and linoleic acid intake (RR_{mv,adj} = 0.75, 95% CI = 0.57–0.99) compared to those in the lowest but not the highest third of intake (Table 2). BCC incidence was lowest in participants with intake of arachidonic acid in the highest third (RR_{mv,adj} = 0.78, 95% CI = 0.59–1.03) but this was not
TABLE 2
Relative risks (RR) and 95% confidence intervals (95% CI) for basal cell carcinoma by ranked thirds of dietary intake of omega-3 and omega-6 fatty acids from food frequency questionnaire, person-based analysis in 1322 participants of the Nambour Skin Cancer Study, 1997–2007

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Ranked thirds of intake</th>
<th></th>
<th></th>
<th>$P$ for trend$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low ($n = 440$)</td>
<td>Middle ($n = 441$)</td>
<td>High ($n = 441$)</td>
<td></td>
</tr>
<tr>
<td>Sum of omega-3, g/day$^b$</td>
<td>&lt;0.90</td>
<td>0.90–1.10</td>
<td>&gt;1.10</td>
<td></td>
</tr>
<tr>
<td>Number of cases, $n$</td>
<td>109</td>
<td>99</td>
<td>117</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)$^c$</td>
<td>1.00</td>
<td>0.89 (0.68–1.17)</td>
<td>0.94 (0.72–1.23)</td>
<td>0.67</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)$^d$</td>
<td>1.00</td>
<td>0.89 (0.68–1.18)</td>
<td>0.91 (0.70–1.19)</td>
<td>0.49</td>
</tr>
<tr>
<td>Sum of long-chain omega-3, g/day$^e$</td>
<td>&lt;0.16</td>
<td>0.16–0.26</td>
<td>&gt;0.26</td>
<td></td>
</tr>
<tr>
<td>Number of cases, $n$</td>
<td>10.4</td>
<td>110</td>
<td>104</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>1.01 (0.77–1.32)</td>
<td>0.89 (0.67–1.17)</td>
<td>0.38</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>1.02 (0.77–1.33)</td>
<td>0.88 (0.67–1.16)</td>
<td>0.35</td>
</tr>
<tr>
<td>$\alpha$-Linolenic acid (18:3), g/day</td>
<td>&lt;0.68</td>
<td>0.68–0.83</td>
<td>&gt;0.83</td>
<td></td>
</tr>
<tr>
<td>Number of cases, $n$</td>
<td>112</td>
<td>99</td>
<td>114</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.87 (0.67–1.14)</td>
<td>0.96 (0.74–1.25)</td>
<td>0.79</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.86 (0.66–1.14)</td>
<td>0.93 (0.71–1.21)</td>
<td>0.59</td>
</tr>
<tr>
<td>EPA (20:5), g/day</td>
<td>&lt;0.05</td>
<td>0.05–0.09</td>
<td>&gt;0.09</td>
<td></td>
</tr>
<tr>
<td>Number of cases, $n$</td>
<td>103</td>
<td>115</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>1.07 (0.82–1.40)</td>
<td>0.92 (0.70–1.21)</td>
<td>0.54</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>1.07 (0.82–1.40)</td>
<td>0.91 (0.69–1.20)</td>
<td>0.50</td>
</tr>
<tr>
<td>DPA (22:5), g/day</td>
<td>&lt;0.03</td>
<td>0.03–0.05</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Number of cases, $n$</td>
<td>102</td>
<td>122</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>1.12 (0.86–1.45)</td>
<td>0.81 (0.61–1.07)</td>
<td>0.13</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>1.11 (0.85–1.44)</td>
<td>0.79 (0.59–1.04)</td>
<td>0.10</td>
</tr>
<tr>
<td>DHA (C22:6), g/day</td>
<td>&lt;0.07</td>
<td>0.07–0.13</td>
<td>&gt;0.13</td>
<td></td>
</tr>
<tr>
<td>Number of cases, $n$</td>
<td>100</td>
<td>119</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>1.18 (0.90–1.54)</td>
<td>0.97 (0.73–1.28)</td>
<td>0.78</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>1.17 (0.90–1.53)</td>
<td>0.96 (0.72–1.26)</td>
<td>0.73</td>
</tr>
<tr>
<td>Sum of omega-6, g/day$^f$</td>
<td>&lt;7.17</td>
<td>7.17–8.91</td>
<td>&gt;8.91</td>
<td></td>
</tr>
<tr>
<td>Number of cases, $n$</td>
<td>118</td>
<td>92</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.74 (0.56–0.97)$^*$</td>
<td>0.88 (0.68–1.15)</td>
<td>0.37</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.74 (0.56–0.97)$^*$</td>
<td>0.86 (0.66–1.12)</td>
<td>0.28</td>
</tr>
<tr>
<td>Linoleic acid (C18:2), g/day</td>
<td>&lt;7.06</td>
<td>7.06–8.80</td>
<td>&gt;8.80</td>
<td></td>
</tr>
<tr>
<td>Number of cases, $n$</td>
<td>117</td>
<td>93</td>
<td>115</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.76 (0.58–1.00)</td>
<td>0.89 (0.69–1.16)</td>
<td>0.41</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.75 (0.57–0.99)$^*$</td>
<td>0.86 (0.67–1.14)</td>
<td>0.32</td>
</tr>
<tr>
<td>AA (C20:4), g/day</td>
<td>&lt;0.07</td>
<td>0.07–0.10</td>
<td>&gt;0.10</td>
<td></td>
</tr>
<tr>
<td>Number of cases, $n$</td>
<td>113</td>
<td>122</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>1.10 (0.85–1.43)</td>
<td>0.90 (0.60–1.05)</td>
<td>0.12</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>1.08 (0.84–1.40)</td>
<td>0.78 (0.59–1.03)</td>
<td>0.09</td>
</tr>
<tr>
<td>Omega-3/omega-6 ratio</td>
<td>&lt;0.11</td>
<td>0.11–0.14</td>
<td>&gt;0.14</td>
<td></td>
</tr>
<tr>
<td>Number of cases, $n$</td>
<td>115</td>
<td>98</td>
<td>112</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.88 (0.67–1.16)</td>
<td>0.94 (0.72–1.22)</td>
<td>0.65</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.91 (0.69–1.19)</td>
<td>0.94 (0.72–1.22)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

$^a$All $P$ values from 2-sided tests.

$^b$Sum of omega-3: $\alpha$-linolenic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA).

$^c$RR$_{basic}$ adjusted for age and sex.

$^d$RR$_{mv.adj}$ adjusted for age, sex, freckling on back, elastosis of neck, treatment allocation.

$^e$Sum of long-chain omega-3: EPA, DPA, DHA.

$^f$Sum of omega-6: linoleic acid, arachidonic acid (AA).

$^*P < 0.05$. 
findings for BCC contrast with our hypothesis, which was based on the mostly animal evidence available. There is also a lack of consistency between these results and those of van Dam and colleagues, which failed to find an association between polyunsaturated fatty acids and BCC risk (15). Though theirs too was a prospective study with energy- and sun exposure-adjusted results, the study was limited by the restriction to male participants only, and by the small number of BCC cases observed (15). Interestingly, in the same Nambour study participants we have previously noted that a meat and fat diet pattern was associated with an increased risk of SCC tumors (34). As red meats such as beef and lamb are the predominant source of arachidonic acid in this population, our results, although marginally significant, are in agreement with these earlier findings. It remains unclear, however, why omega-6 fatty acids might affect BCC and SCC carcinogenesis differently. A different role for polyunsaturated fatty acids in these cancers would be plausible given that BCC and SCC are 2 quite separate entities with different aetiologies. Dietary factors may influence these skin cancers in diverse ways, as we have previously observed for food groups (35,36).

A possible explanation for the associations seen in middle thirds of intake may be the existence of a U-shaped (linoleic acid and total omega-6 fatty acids and BCC) or inverse U-shaped (arachidonic acid and SCC) relationship. U-shaped relationships where disease risk differs at high and low doses of exposure (37) have been identified in nutritional epidemiology studies; for example, between intake of alcohol and cardiovascular disease (38). Only 1 study has reported such a relation in fatty acid nutrition, for the association between fish and omega-3 consumption and heart failure (39). Though theoretically U-shaped associations are possible in the context of our study, they are difficult to prove given the low-to-moderate fatty acid intake levels we observed and, because of the number of observations, we were restricted to an analysis of 3 intake categories only. Further, there has been no previous indication of U-shaped relations between omega-3 and -6 fatty acids and cancer.

To the best of our knowledge this is the first longitudinal study to describe associations between dietary intake of individual omega-3 and omega-6 fatty acids and subsequent keratinocytic skin cancer risk in both sexes. A major strength of this study is its prospective nature, and our ability to fully assess potential confounding given the extensive and rigorous data collections in this study population. Estimations of intakes of omega-3 and omega-6 fatty acids by FFQ were previously evaluated in a validation study of our study population using weighed food records and plasma phospholipid fatty acids, which had shown adequate estimation of the fatty acids considered in this study (21). As a result, the degree of misclassification in fatty acid intake levels is expected to be low, though if present may have diluted risk estimates to some extent. Our study is based on analyses of histologically confirmed BCC and SCC identified through a comprehensive surveillance system, thus it is unlikely that study participants were misclassified through missed cases or misdiagnosis. Given the large number of group comparisons, chance findings may have occurred in our study and the study
TABLE 3
Relative risks (RR) and 95% confidence intervals (95% CI) for squamous cell carcinoma by ranked thirds of dietary intake of omega-3 and omega-6 fatty acids from FFQ, person-based analysis in 1322 participants of the Nambour Skin Cancer Study, 1997–2007

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Low n = 440</th>
<th>Middle n = 441</th>
<th>High n = 441</th>
<th>P for trenda</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of omega-3, g/dayb</td>
<td>&lt;0.90</td>
<td>0.90–1.10</td>
<td>&gt;1.10</td>
<td></td>
</tr>
<tr>
<td>Number of cases, n</td>
<td>60</td>
<td>58</td>
<td>78</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)c</td>
<td>1.00</td>
<td>0.93 (0.65–1.34)</td>
<td>1.00 (0.71–1.40)</td>
<td>0.98</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)d</td>
<td>1.00</td>
<td>0.94 (0.65–1.36)</td>
<td>1.03 (0.72–1.46)</td>
<td>0.88</td>
</tr>
<tr>
<td>Sum of long-chain omega-3, g/daye</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of cases, n</td>
<td>67</td>
<td>62</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.69 (0.48–0.99)*</td>
<td>0.86 (0.61–1.20)</td>
<td>0.41</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.77 (0.53–1.13)</td>
<td>0.97 (0.69–1.38)</td>
<td>0.90</td>
</tr>
<tr>
<td>α-Linolenic acid (18:3), g/day</td>
<td>&lt;0.68</td>
<td>0.68–0.83</td>
<td>&gt;0.83</td>
<td></td>
</tr>
<tr>
<td>Number of cases, n</td>
<td>65</td>
<td>65</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.93 (0.65–1.31)</td>
<td>0.94 (0.67–1.32)</td>
<td>0.73</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.95 (0.66–1.36)</td>
<td>0.87 (0.61–1.24)</td>
<td>0.44</td>
</tr>
<tr>
<td>EPA (20:5), g/day</td>
<td>&lt;0.05</td>
<td>0.05–0.09</td>
<td>&gt;0.09</td>
<td></td>
</tr>
<tr>
<td>Number of cases, n</td>
<td>67</td>
<td>67</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.73 (0.51–1.05)</td>
<td>0.83 (0.59–1.15)</td>
<td>0.29</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.77 (0.53–1.11)</td>
<td>0.93 (0.66–1.32)</td>
<td>0.72</td>
</tr>
<tr>
<td>DPA (22:5), g/day</td>
<td>&lt;0.03</td>
<td>0.03–0.05</td>
<td>&gt;0.05</td>
<td></td>
</tr>
<tr>
<td>Number of cases, n</td>
<td>59</td>
<td>59</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.92 (0.64–1.32)</td>
<td>0.96 (0.68–1.36)</td>
<td>0.83</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.91 (0.62–1.31)</td>
<td>1.03 (0.72–1.47)</td>
<td>0.86</td>
</tr>
<tr>
<td>DHA (22:6), g/day</td>
<td>&lt;0.07</td>
<td>0.07–0.13</td>
<td>&gt;0.13</td>
<td></td>
</tr>
<tr>
<td>Number of cases, n</td>
<td>67</td>
<td>67</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.77 (0.54–1.10)</td>
<td>0.89 (0.63–1.24)</td>
<td>0.51</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.83 (0.57–1.20)</td>
<td>0.95 (0.67–1.34)</td>
<td>0.80</td>
</tr>
<tr>
<td>Sum of omega-6, g/dayf</td>
<td>&lt;7.17</td>
<td>7.17–8.91</td>
<td>&gt;8.91</td>
<td></td>
</tr>
<tr>
<td>Number of cases, n</td>
<td>63</td>
<td>64</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.92 (0.65–1.31)</td>
<td>0.91 (0.65–1.28)</td>
<td>0.60</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.89 (0.62–1.27)</td>
<td>0.92 (0.64–1.31)</td>
<td>0.65</td>
</tr>
<tr>
<td>Linoleic acid (C18:2), g/day</td>
<td>&lt;7.06</td>
<td>7.06–8.80</td>
<td>&gt;8.80</td>
<td></td>
</tr>
<tr>
<td>Number of cases, n</td>
<td>63</td>
<td>63</td>
<td>70</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.91 (0.64–1.29)</td>
<td>0.92 (0.66–1.30)</td>
<td>0.66</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.89 (0.62–1.27)</td>
<td>0.94 (0.66–1.35)</td>
<td>0.76</td>
</tr>
<tr>
<td>AA (C20:4), g/day</td>
<td>&lt;0.07</td>
<td>0.07–0.10</td>
<td>&gt;0.10</td>
<td></td>
</tr>
<tr>
<td>Number of cases, n</td>
<td>56</td>
<td>79</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>1.47 (1.04–2.07)*</td>
<td>1.08 (0.75–1.56)</td>
<td>0.69</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>1.42 (1.00–2.02)</td>
<td>1.18 (0.81–1.72)</td>
<td>0.39</td>
</tr>
<tr>
<td>Omega-3/omega-6 ratio</td>
<td>&lt;0.11</td>
<td>0.11–0.14</td>
<td>&gt;0.14</td>
<td></td>
</tr>
<tr>
<td>Number of cases, n</td>
<td>60</td>
<td>57</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.99 (0.69–1.43)</td>
<td>1.20 (0.86–1.68)</td>
<td>0.28</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>1.05 (0.72–1.53)</td>
<td>1.22 (0.86–1.73)</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*aAll P values from 2-sided tests.

*bSum of omega-3: α-linolenic acid, eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA).

*cRRbasic adjusted for age and sex.

dRRmv.adj adjusted for age, sex, elastosis of neck, total solar keratoses, treatment allocation.

*eSum of long-chain omega-3: EPA, DPA, DHA.

*fSum of omega-6: linoleic acid, arachidonic acid (AA).

*P value < 0.05.
may have lacked power to detect associations or dose-response relationships if they did indeed exist due to relatively small numbers of participants with a BCC or SCC in each level of intake of a specific fatty acid.

In summary, though our results suggest that moderate intakes of arachidonic acid may be associated with increased SCC and omega-6 fatty acids with reduced BCC, this study did not provide strong support for associations of omega-3 and omega-6 fatty acid intake with human skin carcinogenesis. Replicated analyses in larger study populations are needed to support or refute our findings, in particular with regard to the generally weak associations observed.

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REFERENCES


Publication 4

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**Short Communication**

**Plasma Omega-3 and Omega-6 Concentrations and Risk of Cutaneous Basal and Squamous Cell Carcinomas in Australian Adults**

Sarah C. Wallingford1, Maria Celia Hughes2, Adèle C. Green1,2, and Jolieke C. van der Pols2,3

**Abstract**

Laboratory-based evidence suggests that omega-3 and omega-6 polyunsaturated fatty acids may affect skin photocarcinogenesis, but epidemiologic evidence is inconsistent. In 1,191 White Australian adults, we prospectively investigated associations between baseline plasma concentrations of omega-3 and omega-6 fatty acids and cutaneous basal cell carcinomas (BCC) and squamous cell carcinomas (SCC). Relative risks (RR) and 95% confidence intervals (CI) were estimated on the basis of number of histologically confirmed tumors diagnosed during follow-up (1997–2007). Plasma eicosapentaenoic acid (EPA) concentrations and omega-3/−6 ratio showed significant inverse associations with SCC tumors, comparing higher tertiles with the lowest, in age- and sex-adjusted models (P trend = 0.02 and 0.03, respectively) which weakened after adjustment for past sun exposure. Associations between EPA and SCC were stronger among participants with a history of skin cancer at baseline (n = 378; highest vs. lowest tertile: RR = 0.50; 95% CI, 0.28–0.92; P trend = 0.01). Total omega-6 was inversely associated with BCC tumors in multivariate models (P = 0.04; highest vs. lowest tertile: RR = 0.71; 95% CI, 0.51–0.99), and more strongly in the subgroup with past skin cancer. Linoleic and linolenic acids were also inversely associated with BCC occurrence in this subgroup. When fatty acids were analyzed as continuous variables, however, there was no evidence of any linear or nonlinear associations. This study provides some support for reduced skin cancer risk with high plasma concentrations of omega-3 and omega-6 fatty acids, but results depended on how fatty acid data were modeled. Further investigation of these associations in larger datasets is needed. *Cancer Epidemiol Biomarkers Prev; 22(10); 1900–5.* © 2013 AACR.

**Introduction**

Most keratinocytic skin cancers, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC), are attributable to solar ultraviolet radiation (UVR) exposure (1, 2). UVR-induced carcinogenesis (photocarcinogenesis) occurs by initiation and promotion of skin cancer through inducing DNA damage and modulating immunosuppression (3). Evidence suggests that this process may be modified by dietary factors including polyunsaturated fatty acids (PUFA). In particular, omega-3 and omega-6 PUFA, obtained primarily through dietary intake of fish and plant oils, respectively, are thought to play opposing roles. Experimental studies have shown that although omega-3 PUFA affects early promotional stages by increasing tumor latency and decreasing tumor numbers, omega-6 PUFA has reverse effects (1, 4, 5). Elevated blood concentrations of omega-6 PUFA increase the levels of prostaglandin E2 (PGE-2), an immunoregulator associated with aggressive keratinocytic skin cancer growth (6, 7). Conversely, omega-3 PUFA, particularly eicosapentaenoic acid (EPA), has anti-inflammatory effects in skin post-UVR exposure, reducing sunburn sensitivity and PGE-2 levels (3, 8) and preventing DNA damage (9). Because EPA is in constant competition for metabolism with the omega-6 arachidonic acid, the ratio of omega-3/−6 in circulation plays a key role in determining the overall effect on skin photocarcinogenesis (5).

To date, epidemiologic evidence has mainly focused on dietary intake and has been inconsistent in showing a role of individual PUFA in skin cancer development. An Arizona-based case–control study showed an inverse association of omega-3 intake and odds of SCC (10), and an increased SCC risk with higher serum levels of arachidonic acid (11). We have also previously reported a marginal increase in SCC risk with greater arachidonic acid intake and a decrease in BCC risk with increasing total omega-6 intake (12). However, a study of over 40,000 American male health professionals failed to find a link between omega-3 intake and BCC risk (13), whereas an Italian case–control study found a positive association of
serum docosapentaenoic acid with melanoma risk only in men (14). The present study aimed to investigate associations between plasma phospholipid levels of omega-3 and omega-6 PUFA, and the development of BCC and SCC.

Materials and Methods

Study population
This was an 11-year prospective cohort study (1997–2007) among White Caucasian adults originally randomly selected using the electoral roll (enrolment is compulsory by law in Australia) from the community in 1986 who participated in a skin cancer prevention field trial, 1992–1996. Details of the sample, trial, and outcomes have been reported elsewhere (15). Briefly, some 1,600 residents of Nambour, Queensland, Australia, took part in a trial evaluating skin cancer prevention using ß-carotene supplements and/or daily application of sunscreen. Participants who provided a blood sample at trial completion in 1996 were included in the present study (n = 1,191, 73%). Persons with Gorlin syndrome or porokeratosis were ineligible. The study was approved by the Queensland Institute of Medical Research (Brisbane, Australia) ethics committee.

Data collection
Consenting participants provided a 30-mL nonfasting venous blood sample. Samples were processed at collection and stored in 1 mL aliquots at −70°C until analysis. Measurements of plasma phospholipid PUFA were conducted by Flinders Medical Centre, Adelaide, Australia using procedures detailed elsewhere (16). Briefly, plasma was extracted in chloroform: methanol, thin-layer chromatography was used to separate phospholipid fractions, and fatty acid methyl esters were quantified using gas chromatography (Hewlett-Packard 6890, 50-m capillary column). Fatty acid methyl esters were identified on the basis of retention time to authentic lipid standards (GLC-463, Nuchek Prep Inc.) and quantified by comparison with the internal standard using ChemStation software (Agilent).

Demographic variables, phenotypic characteristics, lifestyle habits, sun exposure variables, and presence of medical conditions were obtained via standard interviewer- and self-administered questionnaires. During a physical examination in 1996, height and weight and elastosis of the neck (a measure of long-term sun exposure) were recorded, the latter by dermatologists. A detailed list of variables considered has been described previously (12). Skin cancer history was based on personal history of skin cancer, as these individuals are at increased risk of developing subsequent skin cancers (19) and may be more prone to risk modification by dietary factors (12). Skin cancer history was based on tumors identified during examinations and surveys conducted before 1997 (17). Statistical significance was set at P < 0.05 (two-tailed). Analyses were conducted using SAS version 9.3. Nonlinear trends were tested by analyzing PUFA variables as continuous variables in natural cubic spline regression using R version 3.0.1.

Results
Average age (SD) of the 1,191 study participants was 54.0 (12.8) years and 55% were female. Over the 11-year study period, 337 histologically confirmed new SCC tumors were diagnosed in 176 participants during 12,535 person-years of follow-up giving person- and tumor-based incidence rates of 1,404/100,000 and 2,688/100,000, respectively. During the same follow-up, 300 people developed 700 histologically confirmed new BCC tumors giving a person-based rate of 2,393/100,000, and a tumor-based rate of 5,584/100,000. Three hundred and ninety-eight participants had a personal history of skin cancer.

In age- and sex-adjusted models, there was a significant linear decrease in SCC tumor risk with increasing tertiles of plasma EPA concentrations (middle tertile: RR = 0.64;
95% CI = 0.42–0.97; highest tertile: RR = 0.58; 95% CI, 0.38–0.88; \( P_{\text{trend}} = 0.02 \); Table 1). Associations with omega-3/ω-6 ratio were similar: participants in the highest tertile had significantly reduced SCC tumor risk compared with those in the lowest (RR = 0.61; 95% CI, 0.39–0.95; \( P_{\text{trend}} = 0.03 \)). In the multivariate models, these inverse trends were maintained though did not reach statistical significance. Linolenic acid, docosahexaenoic acid (DHA), linoleic acid, arachidonic acid, and the individual sums of omega-3 and omega-6 PUFA were not associated with SCC tumors. Among participants with a history of skin cancer, EPA was more strongly associated with reduced occurrence of SCC tumors after full confounder adjustment (Table 1), but based on the point estimates of EPA tertiles, there was no clear dose–response relationship (middle vs. lowest tertile: RR = 0.41; 95% CI, 0.19–0.91; highest vs. lowest tertile: RR = 0.50; 95% CI, 0.28–0.92; \( P_{\text{trend}} = 0.01 \)). Omega-3/ω-6 ratio showed similar, though

### Table 1. RR and 95% CIs for SCC by tertiles of plasma omega-3 and omega-6 concentrations, tumor-based analysis in all participants of the Nambour Skin Cancer Study, and in those with a personal history of skin cancer, 1997–2007

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Sum of omega-3, ( \mu g/mL^a )</th>
<th>Sum of omega-6, ( \mu g/mL^a )</th>
<th>All participants ( n = 1,191 )</th>
<th>Participants with previous skin cancers ( n = 398 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of omega-3, ( \mu g/mL^a )</td>
<td>&gt;48.56</td>
<td>&gt;48.56</td>
<td>&gt;63.20</td>
<td>&gt;63.20</td>
</tr>
<tr>
<td>Sum of tumors, ( n )</td>
<td>86</td>
<td>131</td>
<td>120</td>
<td>103</td>
</tr>
<tr>
<td>Basic RR (95% CI)^b</td>
<td>1.00</td>
<td>0.92 (0.59–1.42)</td>
<td>0.71 (0.45–1.11)</td>
<td>0.11</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)^d</td>
<td>1.00</td>
<td>1.01 (0.67–1.54)</td>
<td>0.82 (0.53–1.27)</td>
<td>0.31</td>
</tr>
<tr>
<td>Linolenic acid (18:3), ( \mu g/mL )</td>
<td>&lt;1.67</td>
<td>1.67–2.53</td>
<td>&gt;2.53</td>
<td>&lt;1.67</td>
</tr>
<tr>
<td>Sum of tumors, ( n )</td>
<td>106</td>
<td>117</td>
<td>114</td>
<td>82</td>
</tr>
<tr>
<td>Basic RR (95% CI)^b</td>
<td>1.00</td>
<td>1.06 (0.70–1.61)</td>
<td>1.00 (0.66–1.51)</td>
<td>0.95</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)^d</td>
<td>1.00</td>
<td>0.96 (0.64–1.43)</td>
<td>0.94 (0.62–1.40)</td>
<td>0.76</td>
</tr>
<tr>
<td>EPA (20:5), ( \mu g/mL )</td>
<td>&lt;3.24</td>
<td>9.24–13.28</td>
<td>&gt;13.28</td>
<td>&lt;3.24</td>
</tr>
<tr>
<td>Sum of tumors, ( n )</td>
<td>124</td>
<td>90</td>
<td>123</td>
<td>101</td>
</tr>
<tr>
<td>Basic RR (95% CI)^b</td>
<td>1.00</td>
<td>0.64 (0.42–0.97)</td>
<td>0.58 (0.38–0.88)</td>
<td>0.02</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)^d</td>
<td>1.00</td>
<td>0.70 (0.47–1.06)</td>
<td>0.67 (0.45–1.01)</td>
<td>0.08</td>
</tr>
<tr>
<td>DHA (22:6), ( \mu g/mL )</td>
<td>&lt;36.42</td>
<td>36.42–47.68</td>
<td>&gt;47.68</td>
<td>&lt;36.42</td>
</tr>
<tr>
<td>Sum of tumors, ( n )</td>
<td>99</td>
<td>103</td>
<td>135</td>
<td>76</td>
</tr>
<tr>
<td>Basic RR (95% CI)^b</td>
<td>1.00</td>
<td>0.71 (0.46–1.11)</td>
<td>0.75 (0.49–1.16)</td>
<td>0.27</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)^d</td>
<td>1.00</td>
<td>0.73 (0.48–1.11)</td>
<td>0.83 (0.55–1.28)</td>
<td>0.51</td>
</tr>
<tr>
<td>Sum of omega-6, ( \mu g/mL^a )</td>
<td>&lt;348.9</td>
<td>348.90–408.01</td>
<td>&gt;408.03</td>
<td>&lt;348.9</td>
</tr>
<tr>
<td>Sum of tumors, ( n )</td>
<td>146</td>
<td>86</td>
<td>105</td>
<td>126</td>
</tr>
<tr>
<td>Basic RR (95% CI)^b</td>
<td>1.00</td>
<td>0.83 (0.54–1.27)</td>
<td>0.94 (0.62–1.42)</td>
<td>0.81</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)^d</td>
<td>1.00</td>
<td>0.81 (0.54–1.22)</td>
<td>0.96 (0.65–1.42)</td>
<td>0.89</td>
</tr>
<tr>
<td>Linoleic acid (18:2), ( \mu g/mL )</td>
<td>&lt;228.23</td>
<td>228.23–273.44</td>
<td>&gt;273.44</td>
<td>&lt;228.23</td>
</tr>
<tr>
<td>Sum of tumors, ( n )</td>
<td>143</td>
<td>103</td>
<td>115</td>
<td>115</td>
</tr>
<tr>
<td>Basic RR (95% CI)^b</td>
<td>1.00</td>
<td>1.03 (0.68–1.56)</td>
<td>0.85 (0.56–1.29)</td>
<td>0.44</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)^d</td>
<td>1.00</td>
<td>0.95 (0.64–1.41)</td>
<td>0.91 (0.61–1.36)</td>
<td>0.65</td>
</tr>
<tr>
<td>AA (20:4), ( \mu g/mL )</td>
<td>&lt;113.45</td>
<td>113.45–139.72</td>
<td>&gt;139.72</td>
<td>&lt;113.45</td>
</tr>
<tr>
<td>Sum of tumors, ( n )</td>
<td>126</td>
<td>98</td>
<td>113</td>
<td>109</td>
</tr>
<tr>
<td>Basic RR (95% CI)^b</td>
<td>1.00</td>
<td>0.89 (0.59–1.36)</td>
<td>1.05 (0.70–1.58)</td>
<td>0.76</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)^d</td>
<td>1.00</td>
<td>1.17 (0.78–1.76)</td>
<td>1.15 (0.77–1.71)</td>
<td>0.52</td>
</tr>
<tr>
<td>Omega-3/ω-6 ratio</td>
<td>&lt;0.13</td>
<td>0.13–0.16</td>
<td>&gt;0.16</td>
<td>&lt;0.13</td>
</tr>
<tr>
<td>Sum of tumors, ( n )</td>
<td>94</td>
<td>114</td>
<td>129</td>
<td>75</td>
</tr>
<tr>
<td>Basic RR (95% CI)^b</td>
<td>1.00</td>
<td>0.76 (0.49–1.18)</td>
<td>0.61 (0.39–0.95)</td>
<td>0.03</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)^d</td>
<td>1.00</td>
<td>0.81 (0.54–1.22)</td>
<td>0.67 (0.44–1.03)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

\(^a\) All \( P \) values from two-sided tests.

\(^b\) Sum of omega-3: linolenic acid, EPA, DHA.

\(^c\) Basic RR adjusted for age, sex.

\(^d\) Multivariate RR adjusted for age, sex, elastosis of neck, total solar keratoses, treatment allocation.

\(^e\) Sum of omega-6: linoleic acid, AA.

Abbreviation: AA, arachidonic acid.
nonsignificant inverse associations. For both overall and subgroup analyses, there was no evidence of linear or nonlinear associations between EPA and SCC when EPA was considered as a continuous variable.

After multivariable adjustments, tumor-based incidence of BCC was lower in the highest compared with the lowest tertile of total omega-6 concentrations (RR = 0.71; 95% CI, 0.51–0.99; Table 2), and the linear trend across tertiles was significant ($P_{\text{trend}} = 0.04$). Linoleic acid showed a similar though not statistically significant inverse association with BCC. Among those with a history of skin cancer, BCC tumor incidence was significantly lower after full confounder adjustment for total omega-6, linoleic acid, and linolenic acid, though no dose–response relationships were apparent. There was no evidence of linear or nonlinear associations with BCC occurrence when these fatty acids were considered as continuous variables.

Table 2. RR and 95% CI for BCC by tertiles of plasma omega-3 and omega-6 concentrations, tumor-based analysis in all participants of the Nambour Skin Cancer Study, and in those with a personal history of skin cancer, 1997–2007

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
<th>$P_{\text{trend}}$</th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
<th>$P_{\text{trend}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sum of omega-3, μg/mL</td>
<td>106.85</td>
<td>99.56–114.20</td>
<td>87.20</td>
<td>&lt;0.01</td>
<td>106.85</td>
<td>99.56–114.20</td>
<td>87.20</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sum of tumors, n</td>
<td>200</td>
<td>214</td>
<td>198</td>
<td>0.03</td>
<td>200</td>
<td>214</td>
<td>198</td>
<td>0.03</td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Linoleic acid (18:3), μg/mL</td>
<td>1.67</td>
<td>1.67–2.53</td>
<td>2.53</td>
<td>&lt;0.01</td>
<td>1.67</td>
<td>1.67–2.53</td>
<td>2.53</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sum of tumors, n</td>
<td>242</td>
<td>274</td>
<td>188</td>
<td>&lt;0.01</td>
<td>242</td>
<td>274</td>
<td>188</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.99</td>
<td>1.00</td>
<td>0.05</td>
<td>1.00</td>
<td>0.99</td>
<td>1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.99</td>
<td>1.00</td>
<td>0.05</td>
<td>1.00</td>
<td>0.99</td>
<td>1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>EPA (20:5), μg/mL</td>
<td>9.24</td>
<td>9.24–13.28</td>
<td>13.28</td>
<td>&lt;0.01</td>
<td>9.24</td>
<td>9.24–13.28</td>
<td>13.28</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sum of tumors, n</td>
<td>197</td>
<td>285</td>
<td>218</td>
<td>&lt;0.01</td>
<td>197</td>
<td>285</td>
<td>218</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>0.99</td>
<td>1.00</td>
<td>0.05</td>
<td>1.00</td>
<td>0.99</td>
<td>1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>0.99</td>
<td>1.00</td>
<td>0.05</td>
<td>1.00</td>
<td>0.99</td>
<td>1.00</td>
<td>0.05</td>
</tr>
<tr>
<td>DHA (22:6), μg/mL</td>
<td>36.42</td>
<td>36.42–47.68</td>
<td>47.68</td>
<td>&lt;0.01</td>
<td>36.42</td>
<td>36.42–47.68</td>
<td>47.68</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sum of tumors, n</td>
<td>210</td>
<td>242</td>
<td>248</td>
<td>&lt;0.01</td>
<td>210</td>
<td>242</td>
<td>248</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Linoleic acid (18:2), μg/mL</td>
<td>228.23</td>
<td>228.23–273.44</td>
<td>273.44</td>
<td>&lt;0.01</td>
<td>228.23</td>
<td>228.23–273.44</td>
<td>273.44</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sum of tumors, n</td>
<td>263</td>
<td>263</td>
<td>174</td>
<td>&lt;0.01</td>
<td>263</td>
<td>263</td>
<td>174</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>AA (20:4), μg/mL</td>
<td>113.45</td>
<td>113.45–139.72</td>
<td>139.72</td>
<td>&lt;0.01</td>
<td>113.45</td>
<td>113.45–139.72</td>
<td>139.72</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Sum of tumors, n</td>
<td>211</td>
<td>219</td>
<td>270</td>
<td>&lt;0.01</td>
<td>211</td>
<td>219</td>
<td>270</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Basic RR (95% CI)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Multivariate RR (95% CI)</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*All P values from two-sided tests.

Sum of omega-3: linolenic acid, EPA, DHA.

Basic RR adjusted for age, sex.

Multivariate RR adjusted for age, sex, freckling on back, treatment allocation.

Sum of omega-6: linoleic acid, AA.

Abbreviation: AA, arachidonic acid.
Person-based analyses showed similar patterns to the tumor-based analyses, though risk estimates and trends were generally not statistically significant (results not shown).

Discussion

In this prospective study of associations between plasma phospholipid PUFA and keratinocytic skin cancer risk, there was a reduction in risk of SCC tumors in persons with relatively high EPA concentrations and omega-3/-6 ratio, and a decrease in BCC tumor risk with greater total omega-6, linoleic acid, and linolenic acid concentrations. These inverse associations were particularly evident in people with a history of skin cancer, and were independent of other risk factors. However, they were only apparent when participants were grouped into tertiles of plasma PUFA levels, and not when PUFA were modeled as continuous variables.

Our findings with regards to SCC are consistent with those of Hakim and colleagues who noted an inverse trend between the ratio of omega-3/-6 intake and SCC risk (10), but are at odds with our past observations that intake of omega-3 PUFA was not associated with developing SCC (12). Previous analyses in the Nambour Study population showed a lower rate of acquisition of actinic keratoses (precancerous cutaneous lesions) among the highest consumers of oily fish (high in EPA content; ref. 20). Evidence of EPA’s protective potential in skin has also been shown in human supplementation studies (3, 8). Although our findings did not show a clear dose–response relationship, collectively the evidence suggests that moderate omega-3 intake may sustain circulating and target tissue levels to influence early stages of photocarcinogenesis. With respect to omega-6 PUFA, our study failed to confirm earlier findings that higher serum arachidonic acid levels increase SCC risk (11).

The observed inverse associations of linoleic acid and total omega-6 concentrations with BCC in our study are consistent with our previous findings on dietary intake of PUFA in relation to skin cancer (12), but they are contrary to hypotheses generated from animal studies which indicate omega-6 PUFA increase carcinogenesis (1, 5, 7). Furthermore, the lower BCC tumor incidence with greater linolenic acid levels among persons with a history of skin cancer is a novel finding not previously reported (12, 13). BCC and SCC each have a distinct biology and epidemiology (21) so it is not unexpected that dietary factors have different associations with these 2 different skin cancer types, yet it remains unclear why the direction of associations for BCC and omega-6 were opposite to that expected.

The lack of linear or nonlinear associations when PUFA was considered as continuous variables means that caution is needed in interpreting the associations reported from the tertile comparisons. It suggests that no simple dose–response relationship exists for any of the PUFA explored and that further analyses across a wide range of plasma PUFA levels are needed in larger datasets to confirm optimal circulating concentrations. PUFA levels in our study were relatively low compared with other populations (22), consequently the range of individual PUFA values in the first 2 tertiles was narrow, thus limiting the distribution of PUFA levels being compared.

To our knowledge, this is the first prospective epidemiologic study to report inverse associations between plasma omega-3 and omega-6 PUFA and BCC and SCC incidence. Study strengths include the prospective design and rigorous data collection on potential confounders. Our analyses are based on histologically confirmed BCC and SCC data identified through a comprehensive surveillance system, thus participant misclassification was unlikely. However, the study may have lacked statistical power to detect associations due to a relatively small numbers of cases/tumors in some PUFA tertiles.

In conclusion, our findings generally agree with the notion that omega-3 and omega-6 PUFA may reduce incidence of keratinocytic skin cancers, particularly in high-risk groups. Further prospective studies among larger and diverse populations are warranted to substantiate our findings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors’ Contributions

Conception and design: J.C. van der Pols
Development of methodology: A.C. Green, J.C. van der Pols
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.C. Hughes, A.C. Green
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): S.C. Wallingford, M.C. Hughes, J.C. van der Pols
Writing, review, and/or revision of the manuscript: S.C. Wallingford, M.C. Hughes, A.C. Green, J.C. van der Pols
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.C. Hughes, A.C. Green
Study supervision: J.C. van der Pols

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References


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Publication 5

Three-way assessment of long-chain n-3 PUFA nutrition: by questionnaire and matched blood and skin samples

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2School of Pharmacy and Centre for Skin Sciences, School of Life Sciences, University of Bradford, Bradford, UK
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Abstract

The long-chain n-3 PUFA, EPA, is believed to be important for skin health, including roles in the modulation of inflammation and protection from photodamage. FFQ and blood levels are used as non-invasive proxies for assessing skin PUFA levels, but studies examining how well these proxies reflect target organ content are lacking. In seventy-eight healthy women (mean age 42.8, range 21–60 years) residing in Greater Manchester, we performed a quantitative analysis of long-chain n-3 PUFA nutrition estimated from a self-reported FFQ (n 75) and correlated this with n-3 PUFA concentrations in erythrocytes (n 72) and dermis (n 39). Linear associations between the three n-3 PUFA measurements were assessed by Spearman correlation coefficients and agreement between these measurements was estimated. Average total dietary content of the principal long-chain n-3 PUFA EPA and DHA was 171 (sd 168) and 236 (sd 248) mg/d, respectively. EPA showed significant correlations between FFQ assessments and both erythrocyte (r 0.57, P < 0.0001) and dermal (r 0.33, P = 0.05) levels, as well as between erythrocytes and dermis (r 0.45, P = 0.008). FFQ intake of DHA and the sum of n-3 PUFA also correlated well with erythrocyte concentrations (r 0.50, P < 0.0001; r 0.27, P = 0.03). Agreement between ranked thirds of dietary intake, blood and dermis approached 50% for EPA and DHA, though gross misclassification was lower for EPA. Thus, FFQ estimates and circulating levels of the dietary long-chain n-3 PUFA, EPA, may be utilised as well-correlated measures of its dermal bioavailability.

Key words: n-3 PUFA: FFQ: Erythrocytes: Skin: Correlations

A wide range of health benefits have been related to the consumption of long-chain n-3 PUFA including improvements in cardiovascular and several inflammatory disorders(1). Evidence also suggests that these n-3 PUFA, notably EPA (20 : 5n-3), can benefit skin health, by tempering inflammation and reducing the acute effects of UV radiation such as sunburn, and potentially protecting against longer-term photodamage and photocarcinogenesis(2–6). In the UK, as of 2004, the recommended daily consumption of long-chain n-3 PUFA for a healthy adult was increased from 100–200(7) to 450 mg(8) as a result of the emergence of new evidence as to the diversity of potential benefits of n-3 PUFA(1,8).

EPA and DHA (22 : 6n-3) are believed to be the most biologically active n-3 PUFA, their activities including modulation of lipid messengers and transcriptional activation(9,10). These long-chain n-3 PUFA compete with the long-chain n-6 PUFA arachidonic acid (AA, 20 : 4n-6) for metabolism by cyclooxygenases and lipoxigenases, resulting in the production of less pro-inflammatory eicosanoids(11). The respective short-chain n-3 and n-6 PUFA α-linolenic acid (ALA, 18 : 3n-3) and linoleic acid (LA, 18 : 2n-6) are regarded as essential fatty acids, as they cannot be synthesised endogenously by humans. Ingested ALA is obtained largely from plant sources and can be metabolised endogenously to the longer-chain n-3 PUFA(12); however, the conversion of ALA to its long-chain metabolites is inefficient: up to 21 and 9% for EPA and DHA, respectively(13). Thus, the most effective way of naturally obtaining long-chain n-3 PUFA is through the consumption of oily fish and seafood(14). In recent years, food products fortified with n-3 PUFA, such as milk, margarine, eggs and bread, have also provided other sources of these bioactive fats(15,16).

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; BHT, butylated hydroxytoluene; LA, linoleic acid.

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Several methods of estimating n-3 PUFA nutrition have been described\(^\text{17}\). Erythrocytes and plasma are often used to assess n-3 PUFA levels, but few other human tissue targets are as easily sampled. FFQ have become useful tools for quantifying nutrient intakes from diet as they are non-invasive and easily implemented into studies of large populations. Studies examining the relationship between n-3 PUFA intake estimated from matched FFQ data and blood samples have mostly reported good correlations between these assessments (correlation coefficients between 0.4 and 0.6)\(^\text{17}\). To date, however, there have not been any comparisons of these assessments with concentrations measured in the skin despite the reported relevance of skin n-3 PUFA content to photoprotection and potentially other aspects of skin health, as invasive skin assessment (biopsy) is not generally feasible in population studies.

The principal aim of the present study was to perform a three-way matched assessment of EPA nutrition, in order to assess the value of FFQ and erythrocyte measurements as indicators of the skin ‘target organ’ content. Using a multidisciplinary approach, we quantified n-3 PUFA intake from a self-reported FFQ, and correlated this with concentrations measured in erythrocytes and directly in dermal samples from the same adult volunteers in the UK.

**Methods**

**Study subjects and design**

This analysis used baseline nutritional data collected from participants in a double-blind, randomised, placebo-controlled nutritional study of n-3 PUFA supplementation. Women who consumed more than two fish meals per week were excluded *a priori* in order to reduce undue baseline variation in n-3 PUFA intake among study participants. Eligible participants were healthy white Caucasian women aged 18–60 years with sun-sensitive skin (sun-reactive skin type II, i.e. skin susceptible to sunburn, with minimal tanning) and confirmed Ni-sensitivity (cutaneous allergy, evident most usually to Ni-plated jewellery). Women were excluded if they were pregnant, unable to eat fish or gelatin, taking photoactive medication (e.g. non-steroidal anti-inflammatory drugs), consumed n-3 PUFA supplements or more than two meals per week of oily fish, had sunbathed or used a sunbed in the past 3 months, or reported a history of atopy, skin cancer, photosensitivity disorder or cardiac disease. Participants were recruited through the Contact Dermatitis Investigation Unit of Salford Royal NHS Foundation Hospital, Manchester, UK or by advertisements placed in the hospital, the University of Manchester and local newspapers. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the North Manchester Research Ethics Committee. Written informed consent was obtained from all subjects.

**Dietary assessment**

Dietary intake was assessed using a self-administered, semi-quantitative FFQ modified from the validated Nurses’ Health Study questionnaire (USA)\(^{18}\) and the Nambour Skin Cancer Study questionnaire (Australia)\(^{19,20}\). Only minor modifications were made to the Australian version (validated for PUFA intake using the method of triads\(^{20}\)) in order to ensure that the FFQ used in our present study reflected UK dietary components. There were 147 food items on the questionnaire and participants were asked to report the frequency with which a specified serving of each food item was consumed. Serving sizes were expressed either as standard measurements (i.e. one cup, one teaspoon, etc.) or in natural units (i.e. one egg, one slice, etc.). Frequency options ranged from ‘never’ to ‘4+ times per d’. Additional questions collected information on trimming of visible fat from meat, types of fats and oils used on foods and in cooking, as well as on specific foods consumed that were fortified with n-3 PUFA. Questions regarding self-administered dietary supplements were also included.

Participants reported the average frequency with which they consumed food items in the past 6 months. Average daily intake was calculated by expressing the frequency response of a food item as a proportion of daily use, multiplied by the serving size and by the nutrient content of the food. The McCance and Widdowson’s Composition of Foods Integrated Dataset was used to obtain nutrient compositions for UK food items\(^{21}\). When nutrients could not be obtained from the McCance 2002 database, items were selected from Australian databases\(^{22–24}\). n-3 PUFA contents of additional foods including margarine/spreads, bread and eggs were obtained from manufacturers’ information.

In addition to the FFQ, women were asked regarding their height and weight for estimation of BMI and about their current or former smoking status.

**Biological sampling**

Blood samples were taken from the antecubital fossa of participants and collected in EDTA monovette tubes (Sarstedt) and centrifuged for 15 min at 1500 rpm; the erythrocyte fractions were collected and stored at −70°C until analysis. A random half of the study subjects had 5 mm punch skin biopsies taken from an unexposed site of upper buttock skin under local anaesthesia (2% lignocaine without adrenaline). Skin biopsies were snap-frozen in liquid N\(_2\) and stored at −70°C for analysis.

**Erythrocyte and skin biopsy analysis**

Skin samples were defrosted on ice. Only dermal tissue samples were available for this analysis and these were incubated in chlorform–methanol (4 ml) (2:1, v/v) containing butylated hydroxytoluene (BHT) (0.01% w/v) overnight at 4°C and homogenised using a blade homogeniser. Lipids were extracted using a further two volumes of chlorform–methanol–BHT as described before\(^{25}\). Erythrocyte samples (1 ml) were defrosted on ice and lipids were also extracted with chlorform–methanol–BHT (4 ml). The solvent was removed under N\(_2\) and the lipid extract was stored at −20°C before analysis.
Fatty acid methyl esters were prepared using BF₃ in methanol, and analysed by GC (GC-flame ionisation detector) on a BPX70 GC capillary column (SGE Europe Limited), using He as the carrier gas. Heneicosanoic acid (C21:0) was used as the internal standard (ACS reagent, Sigma-Aldrich) with a thirty-seven fatty acid methyl esters mixed standard (Supelco), the reference for identification of fatty acids. The detection limit for this method was 5 µg per sample, and all measured n-3 PUFA levels were well above this lower boundary. Results for both erythrocytes and dermis were expressed as percentage of total fatty acids in each sample. The PUFA examined included the principal long-chain n-3 PUFA EPA and DHA, the principal short-chain n-3 PUFA ALA, and the principal long- and short-chain n-6 PUFA, AA and LA, respectively.

**Statistical analyses**

Energy intakes from the FFQ were assessed to identify any women with extreme or implausible values (>14,700 and <2100 kJ/d) using Willett’s criteria. Fatty acid intakes were also adjusted for energy intake using the residual method. Spearman correlation coefficients for each PUFA were used to assess the linear relationship between each of the parameters (FFQ and erythrocytes, FFQ and dermis, erythrocytes and dermis). Significance was set at P<0.05. Correlations were also performed stratified by age (21–40; 41–60 years), and, when available, by BMI (<25, ≥25 kg/m²) and by smoking status (never or ever smoked) to assess possible effect modification by these factors. Agreement between the PUFA measurements, in ranked thirds, from FFQ, erythrocytes and dermis was assessed by comparing percentage exact agreement and gross misclassification (disagreement across two tertiles) to random expected values. All statistical analyses were performed using Statistical Analysis Systems software package version 9.2 (SAS Institute).

**Results**

A total of seventy-nine eligible volunteers were enrolled for the study, one woman withdrew, and baseline information for analysis was thus obtained from seventy-eight volunteers, with a mean age of 42.8 (sd 10.0) years (range 21–60 years) and an average BMI (available for sixty-one women) of 26.7 (sd 5.0) kg/m² (range 19.4–44.2 kg/m²) (Table 1). Of the seventy-eight participants, seventy-five completed and returned their FFQ, and, of these, four people were omitted from further FFQ-related analyses as they had implausibly high energy intakes. The mean daily energy intake of the remaining participants (n 71) was 7889 (sd 2390) kJ. Overall, seventy-two participants provided blood samples, and all thirty-nine volunteers randomised to providing skin samples did so. Mean values and standard deviations were calculated for total and specific n-3 PUFA intakes using FFQ data (energy adjusted), blood samples and skin biopsies (Table 2). Average total EPA, DHA and ALA intake from FFQ was 171 (sd 168), 236 (sd 243) and 850 (sd 260) mg/d. Energy-unadjusted means from FFQ were similar (data not shown). From the tissue measures, mean EPA and DHA (percentage of total fatty acids) were highest in erythrocytes, while mean ALA was highest in dermis.

Several significant correlations were observed between the amount of long-chain n-3 PUFA in FFQ, erythrocytes and dermis. FFQ data for EPA showed the most substantial significant correlation coefficients with erythrocytes and dermal data (r 0.57, P<0.0001 and r 0.33, P=0.05, respectively) (Table 3; Fig. 1(a–c)). We observed a significant correlation between FFQ and erythrocytes for DHA (r 0.50, P<0.0001) and for the sum total of EPA + DHA + ALA (r 0.27, P=0.03) (Table 3; Fig. 2(a–c)). None of the three-way assessments showed significant correlations for ALA. The only significant inverse correlation observed was between erythrocytes and dermal

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Mean</th>
<th>sd</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)*</td>
<td>42.8</td>
<td>10.0</td>
<td>21–60</td>
</tr>
<tr>
<td>BMI (kg/m²)†</td>
<td>26.7</td>
<td>5.0</td>
<td>19.4–44.2</td>
</tr>
<tr>
<td>Smoking status†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>n 30%</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>n 18%</td>
<td>23%</td>
<td></td>
</tr>
<tr>
<td>Current smoker</td>
<td>n 13%</td>
<td>17%</td>
<td></td>
</tr>
<tr>
<td>Pack years (ex/current smokers)†</td>
<td>6.9</td>
<td>11.3</td>
<td>0.25–56</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diet</th>
<th>Nutrient intakes (g/d)‡§</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>84.6</td>
<td>12.9</td>
</tr>
<tr>
<td>Fat</td>
<td>65.8</td>
<td>12.6</td>
</tr>
<tr>
<td>SFA</td>
<td>27.0</td>
<td>6.2</td>
</tr>
<tr>
<td>MUFA</td>
<td>22.5</td>
<td>4.8</td>
</tr>
<tr>
<td>PUFA</td>
<td>9.5</td>
<td>3.3</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>211.5</td>
<td>31.7</td>
</tr>
<tr>
<td>Alcohol (g/d)</td>
<td>10.8</td>
<td>18.9</td>
</tr>
<tr>
<td>Use supplements‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Do not eat meat‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Use n-3 PUFA fortified‡</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Margarine</td>
<td>n 17</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>23</td>
<td></td>
</tr>
<tr>
<td>Bread</td>
<td>n 5</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>n 9</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Milk</td>
<td>n 2</td>
<td></td>
</tr>
<tr>
<td>%</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

* Missing data n 5 (6%)
† Missing data n 17 (22%)
‡ Missing data n 3 (4%)
§ Mean daily nutrient intake values energy-adjusted and exclude people with values >14700kJ (n 4)
EPA + DHA + ALA ($r = -0.39, P=0.02$). When the influence of age, body weight and smoking status on FFQ and erythrocyte correlations (there was an insufficient number of dermal samples to permit stratified analyses) was assessed, correlations remained relatively consistent across categories for EPA and DHA. FFQ and erythrocyte correlations for the sum of the n-3 PUFA fluctuated according to age (<40 years or >40 years), BMI (<25 or $\geq 25$ kg/m$^2$) and smoking habit (ever or never), but only changed by age group for ALA (not shown).

When agreement between ranked thirds of n-3 PUFA nutrition by FFQ, erythrocytes and dermis was assessed (Table 4), EPA had the best agreement between all measures, with over half of subjects classified into the exact third of EPA intake and low gross misclassification. Agreement between ranked thirds of DHA also approached 50%, though a higher proportion of women were misclassified. Overall, assignment to the same or adjacent ranked third category of EPA and DHA was 79–94%. Exact agreement was lower than expected for the sum of the n-3 PUFA and ALA.

All analyses were repeated for LA, AA, the sum of LA + AA and the ratio of total n-6:n-3. There were no significant correlations between the n-6 PUFA measures, and only the FFQ and erythrocyte correlation was significant for n-6:n-3 ($r=0.42$, $P=0.0004$). Exact agreement of ranked thirds and gross misclassification were highly variable (not shown).

**Discussion**

In the present study, we have demonstrated that the assessment of dietary EPA intake using an FFQ, and corresponding circulating levels, are in turn significantly and well correlated with the measurement of dermal content. These findings are consistent with evidence of the functional significance of EPA in skin (14,6,27). This is important new knowledge for those concerned with promotion of skin health through improved nutrition, since previous studies have not examined these parameters synchronously, and confirms the importance of dietary intake of EPA and DHA on their bioavailability in a clinically relevant target tissue.

Among female study populations similar to ours, average daily intakes of EPA and DHA have been reported as 61–280 and 109–460 mg/d, respectively, from FFQ (28–31); the FFQ intakes measured in our present study fell within these ranges. Our mean ALA intakes were only slightly lower than those reported previously (1030–1200 mg/d) (28,30). Current recommended daily intake of total long-chain n-3 PUFA for healthy adults is 450 mg in the UK (8). The relatively low intake of EPA and DHA by the women in this study (half the UK recommendation) might be partly explained by the eligibility criterion of consuming less than two serves of fatty fish a week. In addition, consumption of fatty fish in general has declined markedly in the past century and, although a comparatively minor source of n-3 PUFA, lamb and beef consumption in the UK has also declined over the past 20 years (32); thus these trends may also have contributed to the women’s lower-than-recommended n-3 PUFA consumption.

Various methods have been used for the assessment of bioavailable n-3 PUFA including sampling of erythrocytes, platelets, plasma, serum, cholesterol esters, phospholipids (28,35,54), adipose tissue (31,35,56) and buccal mucosa (37). Erythrocyte measurements are considered better markers of circulating ACCUMULATION because they are more readily accessible than other tissues (28–30).

### Table 2. Principal long- and short-chain n-3 PUFA assessed from FFQ, erythrocytes and dermal tissue

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>FFQ* (n 71)</th>
<th>Erythrocytes (n 72)</th>
<th>Dermis (n 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (mg/d)</td>
<td>Mean (percentage of total FA) SD</td>
<td>Mean (percentage of total FA) SD</td>
</tr>
<tr>
<td>EPA (20:5n-3)</td>
<td>171 168</td>
<td>0.93 0.43</td>
<td>0.07 0.03</td>
</tr>
<tr>
<td>DHA (22:6n-3)</td>
<td>236 243</td>
<td>3.56 0.95</td>
<td>0.18 0.09</td>
</tr>
<tr>
<td>ALA (18:3n-3)</td>
<td>850 260</td>
<td>0.40 0.31</td>
<td>0.74 0.24</td>
</tr>
<tr>
<td>EPA + DHA + ALA†</td>
<td>1256 501</td>
<td>4.90 1.31</td>
<td>0.99 0.29</td>
</tr>
</tbody>
</table>

FA, fatty acid; ALA, $\alpha$-linolenic acid.
* Intakes from FFQ energy-adjusted.
† Sum of EPA, DHA and ALA.

### Table 3. Spearman correlation coefficients for principal long- and short-chain n-3 PUFA assessed from FFQ, erythrocytes and dermal tissue

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>FFQ v. erythrocytes (n 65)</th>
<th>FFQ v. dermis (n 35)</th>
<th>Erythrocytes v. dermis (n 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$P$</td>
<td>$r$</td>
</tr>
<tr>
<td>EPA (20:5n-3)</td>
<td>0.57</td>
<td>$&lt;0.0001^*$</td>
<td>0.33</td>
</tr>
<tr>
<td>DHA (22:6n-3)</td>
<td>0.50</td>
<td>$&lt;0.0001^*$</td>
<td>0.18</td>
</tr>
<tr>
<td>ALA (18:3n-3)</td>
<td>0.04</td>
<td>0.78</td>
<td>$-0.13$</td>
</tr>
<tr>
<td>EPA + DHA + ALA†</td>
<td>0.27</td>
<td>0.03*</td>
<td>$-0.18$</td>
</tr>
</tbody>
</table>

ALA, $\alpha$-linolenic acid.
* Significant correlation ($P<0.05$).
† Sum of EPA, DHA and ALA.
blood PUFA levels than serum or plasma measurements, as they reflect an intake period of several weeks and contain a high proportion of PUFA. Of the studies reporting on the validity of FFQ in reference to blood concentrations, only a few reported erythrocyte data as opposed to serum or plasma phospholipid fatty acids. Our correlation coefficients for intake from FFQ compared with erythrocyte concentrations were slightly higher than those previously reported (0.37–0.40 and 0.16–0.39) for EPA and DHA, respectively, and consistent with the high validity coefficients for FFQ and erythrocyte measures of long-chain n-3 PUFA observed in the analysis of Swierk et al. The apparent tighter fit of the correlations at lower levels and dispersion at higher levels in the correlation figures was probably due to the study exclusion criteria recruiting a population of relatively low n-3 PUFA consumers. Few participants consumed high levels of n-3 PUFA such that these data points appear more as relative outliers. That a correlation was not observed in the present study between FFQ and blood ALA content is probably due to the estimated 30% lost in metabolic oxidation of dietary ALA into carbon dioxide for energy.

Detailed descriptions of n-3 PUFA content of human skin are sparse in the literature, and the currently reported series appears by far the largest to date. Previous smaller human volunteer studies have reported a low skin content of EPA, ranging from 0.05% mol in whole-skin biopsies (n 14) to 1.1% mol in skin-shave biopsies comprising largely epidermis (n 6). The present findings are consistent with these reports, the dermis exhibiting an EPA content within this range. The lead-time for dietary n-3 PUFA to affect skin content is

![Fig. 1. Bivariate plots of EPA levels from (a) FFQ (mg/d) v. erythrocytes (percentage of total fatty acid (FA)), (b) FFQ v. dermis (percentage of total FA) and (c) erythrocytes v. dermis, with Spearman correlation coefficients and P values. (a) r 0.57, P < 0.0001; (b) r 0.33, P < 0.05 and (c) r 0.45, P < 0.008.](image1)

![Fig. 2. Bivariate plots of DHA levels from (a) FFQ (mg/d) v. erythrocytes (percentage of total fatty acid (FA)), (b) FFQ v. dermis (percentage of total FA) and (c) erythrocytes v. dermis, with Spearman correlation coefficients and P values. (a) r 0.50, P < 0.0001; (b) r 0.18, P < 0.29 and (c) r 0.09, P < 0.62.](image2)
uncertain, but it has been observed that daily supplementation with 4 g EPA capsules resulted in an 8-fold increase in skin content of EPA after 3 months\(^{(27)}\). Our findings complement these results by demonstrating that even low dietary EPA intake appears to be reflected in the skin.

Human \(n\)-3 PUFA supplementation studies have additionally demonstrated the role of EPA in decreasing the skin’s UV-induced inflammatory response. Specifically, EPA has been shown to increase the skin’s threshold to sunburn erythema, and to reduce both basal and UV-induced cutaneous levels of the eicosanoid PGE\(_2\)^{(2–6,27,40)}, a key mediator of the erythema and also involved in skin cancer promotion\(^{(41)}\). This is consistent with the known competition with AA for metabolism by cyclo-oxygenase, leading to reduced PGE\(_2\) production\(^{(39)}\). A range of human studies also supports that \(n\)-3 PUFA may protect against the longer-term photodamage of skin carcinogenesis and ageing\(^{(4,27,42)}\). The recent revision of dietary guidelines to intake of 450 mg long-chain \(n\)-3 PUFA per d was done to align with existing recommendations of two weekly servings of fatty fish\(^{(8)}\); however, the adequacy of these new dietary recommendations for skin health remains unknown. Little is known about the threshold \(n\)-3 PUFA intake required for protection against adverse skin health outcomes. This requires examination in future studies, particularly in view of the continued escalation in skin cancer incidence in many white-skinned populations\(^{(43)}\); a dietary approach to prevention could have widespread impact at a population level\(^{(5)}\).

The present study’s limitations include potential for error in the accuracy of PUFA intake measurements from self-reported FFQ due to issues of recall by the participants. Further, although a similar version of the FFQ was validated in an Australian population\(^{(20)}\), the updated FFQ may not be fully valid when used in our UK-based population. We also acknowledge that, as a dietary measurement tool, any FFQ may not be fully valid and reliable\(^{(44)}\). On the other hand, the McCance database\(^{(21)}\), used to identify the nutrient composition of food items in this study, is the database most widely accepted and used in the UK and as such provides the most accurate PUFA data available for the intake frequencies provided from the FFQ. A limitation of our human PUFA data was that a large amount of variation remained unaccounted for when comparing these data with FFQ intake and erythrocyte content. Metabolic differences between individuals in both uptake and transport of \(n\)-3 PUFA to the skin may explain some of this variation, but future investigative studies are needed in order to address this issue. Furthermore, the variability of \(n\)-3 PUFA levels in skin from different anatomic body sites remains unknown, although our EPA data were consistent with previous reports\(^{(2–27)}\). The number of skin samples was also insufficient to precisely evaluate how age, BMI and smoking habits may influence dermal \(n\)-3 PUFA content in relation to dietary content. Finally, our present study participants were all females; so it is not known how our results apply to males, and since study participants were volunteers, their representativeness of similarly aged healthy UK women is unknown.

In summary, concentrations of EPA in dermal and erythrocyte samples from these healthy women showed significant correlations with EPA consumption, demonstrating that FFQ intake estimates provided a good measure of both the circulating and skin bioavailability of this long-chain \(n\)-3 PUFA. Further research in a more diverse population is required to extend these findings and to determine the threshold of \(n\)-3 PUFA intake required to sustain skin benefit.

Acknowledgements

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References

n-3 PUFA assessed by FFQ, blood and skin


Publication 6

Skin Cancer Arising in Scars: A Systematic Review

SARAH C. WALLINGFORD, BSc (Hons) MSc,* CATHERINE M. OLSEN, BSc (Hons 1), PhD, MPH,† ELSEMIEKE PLASMEIJER, MD, PhD,‡ AND ADELE C. GREEN, MBBS (Hons 1), PhD, MSc, FAFPHM*†

BACKGROUND Despite numerous case reports, epidemiologic evidence regarding true rate of skin cancer in scars of any etiology is sparse.

METHODS Systematic literature review of all published epidemiologic studies on skin cancer in scar tissue from surgery, ulcers, or burns using citation databases and manual review.

RESULTS There were no epidemiologic data to quantify risk of skin cancer in surgical scars or chronic ulcers. Two eligible cohort studies were identified, from Denmark and Sweden, in which skin cancers in 16,903 and 37,095 burn patients, respectively, were ascertained through cancer registry follow-up. Each reported standardized incidence ratios (SIRs) for skin cancer types on any site that were uniformly less than unity compared with the general population. Only the Danish cohort assessed skin cancers specifically on past burn injury sites and found a burn-site-specific SIR of 1.2 (95% confidence interval (CI) = 0.4–2.7) for squamous cell carcinoma (SCC), 0.7 (95% CI = 0.4–1.1) for basal cell carcinoma, and 0.3 (95% CI = 0.0–1.2) for melanoma.

CONCLUSIONS Available epidemiologic data suggest that burn patients are not at higher risk of skin cancers in general, although a modest excess of SCC in burn scars cannot be excluded, nor can excess risk with longer follow-up. Risk of skin cancer in scars other than burn scars has not been investigated epidemiologically.

Renovo, Ltd., UK provided funding for this study. A. Green has been a consultant for Renovo Ltd.

Skin cancers are the most common malignancies worldwide, with approximately 2 to 3 million cases of keratinocytic skin cancers and 132,000 cases of melanoma occurring annually.1 Ultraviolet radiation (UVR) exposure from the sun is the most important environmental cause of skin cancer, but cutaneous malignancies have also been reported to result from scars caused by vaccinations, burns, and other injuries.2,3 In nonwhite ethnic groups, in whom UVR exposure plays a minor role in skin carcinogenesis, scar tissue is thought to have greater influence.4 Although proportions in the general population can vary according to country, the most frequently occurring types of skin cancers are basal cell carcinoma (BCC) (~70%), followed by squamous cell carcinoma (SCC) (~15%) and cutaneous melanomas (~10%).5 Of malignancies arising in scars, SCCs are reportedly the most common6 and are typically found in chronic ulcerating scar tissue from burns known as Marjolin’s ulcers.7 Burn scar carcinomas appear to constitute the majority of all recorded scar neoplasms, including several types of burn scar carcinomas, such as Kangri, Kang, and Kairo cancers, associated with various cultural practices related to self-warming and heating methods.7–9 One of the earliest case series estimated that 2% of SCC and 0.03% of BCC arise from burn scars,7 but there appear to have been no population-based estimates of the incidence of skin cancer arising in scars.

Between 1923 and 2004, some 1,078 cases of skin cancer occurring in scar tissue were detailed in case reports;6 412 of these, mostly from Europe, Australia, and the United States, were captured in a major review conducted by Kowal-Vern and Criswell in

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2005. They noted that 71% of the reported cases were SCC, 12% BCC, and 6% cutaneous melanoma. Whereas SCC was reported equally in men and women, BCC was more common in men and melanoma in women. The majority of the case reports concerned skin cancer arising in burns sustained during childhood, with BCC having the shortest latency period to malignancy in patients who were significantly older when burned than patients with other skin cancers.

The etiology of cancers occurring in scars is not fully understood, although the prevailing hypotheses include prolonged proliferation due to chronic inflammation and irritation of tissue, ongoing exposure of tissues to toxins and co-carcinogens after the injury, and poor vascularization of the scar tissue resulting in impaired immunological defence. It has also been suggested that genetic factors might play a role, including for example, mutations in the p53 and Fas genes in patients with burn scar carcinoma.

In view of the paradox of a large volume of case reports but an apparent dearth of epidemiologic evidence associating skin cancer with scars, we sought to review the existing literature systematically to quantify the risk of the major types of skin cancers arising in scar tissue of any etiology.

Materials and Methods

Eligibility Criteria

We included observational studies of all designs in the systematic review provided that they permitted quantitative assessment of the association between skin cancer (including BCC, SCC, and cutaneous melanoma) and scar tissue of any kind.

Literature Search

Eligible studies to August 2010 were identified by searching Medline 1950 (U.S. National Library of Medicine, Bethesda, MD; using PubMed software as the search interface) and Embase 1966 (Elsevier Science, Amsterdam, Holland; using the Embase search interface) and hand-searching the reference lists of the retrieved articles.

For computer searches, we used the following medical subject headings, terms, or text words (using both U.K. and U.S. spellings): “scar,” “burn scar,” “surgical scar,” “burn scar carcinoma,” “skin cancer,” “malignancies” “incidence,” “neoplasms,” “basal cell carcinoma,” “squamous cell carcinoma” and/or “Marjolin’s ulcer.” The same terms and phrases were used to cross-check Google Scholar for additional literature that the PubMed search may not have captured. Only studies of adult populations (≥18) were included. The search was limited to studies published in English. We read the abstracts of all identified studies to exclude those that were clearly not relevant. The full texts of the remaining articles were read to determine whether they met the study inclusion criteria. No attempt was made to identify unpublished literature.

Results

Although some 457 case studies were identified, only two population-based cohort studies emerged. Both were cohort studies that considered only skin cancer in people with scarring as a result of burn injuries. No epidemiologic studies of skin cancer arising in other types of scars were found (Table 1).

The first analytical study examined the occurrence of skin cancer in a cohort study of 16,903 patients admitted to hospital in Denmark with thermal or chemical burn injuries between 1978 and 1993. Two-thirds of the burn cohort were male, and 42% were younger than 20 when they sustained their injury. Thermal burns accounted for 80% of burns, the remaining being chemical burns, and the extremities were the most commonly affected sites. Mean follow-up time was 15.6 years (Table 1).

This cohort was followed up until 2002 for the occurrence of skin cancer using the Danish Cancer Registry and 94 BCCs, 18 SCCs, and 21 melanomas.
were recorded in people with burn injuries. There was no difference in the rates of skin cancer (in general, on any site) compared with the general Danish population. Standardized incidence ratios (SIRs) were 0.9 (95% CI = 0.6–1.5) for SCC, 0.7 (95% CI = 0.6–0.9) for BCC, and 0.7 (95% CI = 0.4–1.1) for melanoma. Risks of different types of skin malignancy did not differ according to sex or age at injury. When analyses of skin cancers were restricted to the anatomic site of the previous burn injury, there was again no significant increase or decrease in skin cancer occurrence, the SIR was unchanged for BCC at 0.7 (95% CI = 0.4–1.1) but increased to 1.2 (95% CI = 0.4–2.7) for SCC and decreased for melanoma (0.3, 95% CI = 0.0–1.2). Further analysis according to severity of burn showed no significant difference in risk of any skin cancer type.

A more recently published cohort study conducted in Sweden identified 37,095 people with a hospital discharge diagnosis of thermal or chemical burn injury between 1964 and 1996 and followed them until 2003 (mean follow-up 16.4 years). The mean age of patients discharged after burn injury was 29; 71% were male. Subsequent SCCs and melanomas were identified through linkage with the Swedish Cancer Registry (BCCs were not registered in the database at the time of study). There were 86 SCCs and 68 melanomas in those with burn injuries. SIRs for both types of skin cancer occurring in burn patients compared with the general Swedish population were less than 1.0, although nonsignificantly (SIR = 0.88, 95% CI = 0.70–1.09 for SCC; SIR = 0.88, 95% CI = 0.68–1.12 for melanoma). Subgroup analyses examining the risks within different age groups and for different follow-up times did not show any appreciable change in the risk estimates. No site-specific estimates of skin cancer incidence were made according to site of burn injury.

Discussion

The malignant potential of scar tissue has been extensively described on a case-by-case basis but has not been well researched at the population level.
Because our literature search found that only two analytic studies have reported quantitative data on this topic, pooling these studies as an assessment of overall risk for skin cancer in scars was of little value. Neither cohort study found any greater occurrence of skin cancer in general in those with burn injuries than in the general population; both found skin cancer occurrence rates overall to be lower than expected, although only the overall lower prevalence of BCC in the Danish burn cohort was significant. Only the Danish study reported the estimate of skin cancer incidence most appropriate to evaluating the question of scar tissue etiology, namely the estimates of skin cancer incidence in relation to anatomic site of past burn injury. There was no difference between incidence estimates for BCC in general and on burn sites, and there was a large deficit of melanoma on burn sites, consistent with a lack of increase in burn tissue. There was a reversal of the overall deficit of SCC in burns patients in general, to a small (20%) excess of incident SCC observed versus the rates expected on the corresponding sites in the Danish population at large. Because the reversal in the direction of association was seen only for SCC, congruent with the bulk of case reports, and because scars are sites of chronic inflammation long known as an underlying factor in the development of malignant tumors, it is unlikely that the observed SCC excess in burn scars in the Danish cohort, albeit modest, was due to chance, and a truly greater risk of SCC arising in burn scars cannot be excluded based on this evidence. The failure of the difference in SCC in burn scars to achieve significance was likely to reflect, to some degree, the young age of the cohort (almost half <20) at the time the burn was sustained. Also, although the duration of the Danish study was 25 years, it is possible that this period was insufficient to cover the full latent period between exposure (time of burn injury) and disease (skin cancer development) in patients with burn scars, because clinical studies have reported mean latent periods ranging from 23 to 48 years.

The two population-based studies identified in this systematic review were well-defined cohorts accrued through national hospital inpatient registration. Because cancer registration is mandatory and almost 100% for all cancers in Denmark and Sweden, including keratinocytic skin cancers, it is likely that case ascertainment was close to complete, particularly for skin cancers occurring at the site of a previous burn injury. It may be relevant to interpreting results that both studies were conducted in Scandinavian populations living at high latitudes where ambient UVR is low. It is unknown whether the incidence of skin cancer in burn scars might be greater in other populations where ambient UVR and skin cancer incidence are high. Furthermore, these results relate only to burn scars, and thus the potential carcinogenicity of scars of other etiology remains unquantified.

Although the malignant potential of burn scars is widely recognized, the possible mechanisms are unclear. It has been suggested that, rather than increasing the rate at which skin cancer is initiated, burn scar tissue may increase tumor progression in cells in which cancer is already initiated, although in temperate climates, this would apply only to adults. Clinical and epidemiologic evidence linking inflammation and skin cancer derives from studies of Marjolin’s ulcer and other nonhealing wounds, including those associated with lupus erythematosus and osteomyelitis, in which malignant transformation occurs in association with the prolonged cell proliferation of chronic inflammation. Multiple SCCs arising in areas of scarring from discoid lupus erythematosus have been reported and SCC can arise in the sinus tracts of patients with chronic osteomyelitis and in the acral skin of patients with epidermolysis bullosa. Cytokines and growth factors play a major role in epidermal wound healing, of which interleukin (IL)-1-α, transforming growth factor beta (TGF-β)1, -β2, and -β3 are the most important to initiate keratinocyte proliferation and tissue repair. Apart from normal wound healing, IL-6 and TGF-β1, -β2, and -β3 are also associated in vitro with tumorigenesis and it is hypothesized that this is due to an imbalance of cytokines and growth factors. TGF-β1 and -β2 are observed in
adult wound-healing processes with scarring, and TGF-β3 is also associated with scar-free embryonic wound healing. The main difference between adult and embryonic wound healing is the lack of scarring in the embryo, probably because of a lack of inflammation. Investigations have been inconclusive regarding the possible role of cytokines in tumor progression related to wound healing, in contrast to tumor initiation.

To address some of the limitations of previously conducted studies and to investigate the malignant potential of scars of all etiologies at the population level, further epidemiologic studies are required. For example, a population-based prospective cohort study following patients with any scar—surgical, burn, or otherwise—over an extended period of time would be an ideal method of obtaining definitive, population-level estimates of the incidence of skin cancers arising in scar tissue. This type of study would be conducted most easily in countries with compulsory reporting of keratinocytic skin cancers (e.g., Sweden) but should also be conducted in populations with high sun exposure and skin cancer rates, such as Australia.

In conclusion, this review has identified a major gap in scientific knowledge regarding the incidence of scar neoplasms, despite a plenitude of case reports. Although burn patients are not at higher risk of skin cancers in general, a modest excess of SCC at sites of past burn injuries cannot be excluded nor can excess risk in longer study follow-up periods. Risk of skin cancer arising in scars other than burn scars has not been investigated epidemiologically. To answer these continuing questions and quantify the risk, well-designed epidemiologic studies in defined populations followed over sufficiently long time periods are required.

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