HAL
open science

Patterns of Omega-3 and Omega-6 Fatty Acid Dietary Intake and Melanoma Thickness at Diagnosis

Yahya Mahamat-Saleh, Maria Celia B. Hughes, Kyoko Miura, Maryrose K. Malt, Lena A. von Schuckmann, Kiarash Khosrotehrani, B. Mark Smithers, Adele C. Green

To cite this version:

Yahya Mahamat-Saleh, Maria Celia B. Hughes, Kyoko Miura, Maryrose K. Malt, Lena A. von Schuckmann, et al.. Patterns of Omega-3 and Omega-6 Fatty Acid Dietary Intake and Melanoma Thickness at Diagnosis. Cancer Epidemiology, Biomarkers and Prevention, 2020, 29 (8), pp.1647-1653. 10.1158/1055-9965.EPI-20-0319 . hal-02938427

HAL Id: hal-02938427

https://hal.science/hal-02938427v1

Submitted on 23 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Patterns of Omega-3 and Omega-6 fatty acid dietary intake and melanoma thickness at diagnosis

Authors: Yahya Mahamat-Saleh[1,2], Maria Celia B. Hughes[3], Kyoko Miura[3,4], Maryrose K. Malt[3], Lena von Schuckmann[3,5], Kiarash Khostotehrani[6,7], B. Mark Smithers[8], Adèle C Green[3,9]

Author Affiliations:

[1]Centre for Research in Epidemiology and Population Health (CESP) - School of Medicine, Universite Paris Sud - School of Medicine,Universite Versailles Saint-Quentin-en-Yvelines (UVSQ); INSERM French National Institute for Health andMedical Research), Universite Paris Saclay, Villejuif, France; [2]Gustave Roussy, Villejuif, France

[3]Population Health Department, QIMR Berghofer Medical Research Institute, Brisbane, Australia

[4]Faculty of Medicine, The University of Queensland, Australia

[5]School of Public Health, The University of Queensland, Australia

[6]Experimental dermatology group, The University of Queensland Diamantina Institute, Translational research institute, Brisbane, Australia

[7]Department of Dermatology, Princess Alexandra Hospital, Brisbane, Australia

[8]Queensland Melanoma Project, Princess Alexandra Hospital, The University of Queensland, Australia

[9]CRUK Manchester and Faculty of Biology, Medicine and Health, University of Manchester, Manchester, UK

Running Title (60 characters or less): Fatty acid dietary patterns and melanoma thickness

Keywords (5): dietary patterns, fatty acids, omega-3 fatty acids, omega-6 fatty acids, melanoma, reduced rank regression

Financial support:

The study is supported by National Health and Medical Research Council, Australia (NHMRC) Program grants 1073898 and 552429. YM-S was supported by research scholarship from the Paris Ile-de-France region; LvS was funded by NHMRC Postgraduate Scholarship 1133317; KK was funded by a NHMRC Career Development Fellowship.

Corresponding author:

Maria Celia B Hughes

Population Health Department

QIMR Berghofer Institute of Medical Research

300 Herston Road, 4006 Herston, Australia

Telephone: +61 7 3362 0255

E-mail: maricel.hughes@qimrberghofer.edu.au

Disclosure of Potential Conflict of interest

The authors declare no potential conflicts of interest.

Word count (up to 4000): 3563

Tables and/or Figures (up to 6)**:** 4 Tables/0 Figures

Supplementary data (up to 8): 5 Tables

Abbreviations:

FA – fatty acid

AA – arachidonic acid

ALA – α-linolenic acid

EPA – eicosapentaenoic acid

DHA – docosahexaenoic acid

LA – linoleic acid

FFQ – food frequency questionnaire

BMI – Body mass index

UVR – ultraviolet radiation

Abstract

Background: Experimental evidence suggests that dietary intakes of omega-3 and omega-6 polyunsaturated fatty acids (FAs) have divergent effects on melanoma growth, but epidemiologic evidence on their combined effect is lacking.

Methods: In 634 Australian primary melanoma patients, we assessed pre-diagnosis consumption of 39 food groups by food frequency questionnaires completed within 2 months of diagnosis. We derived, by reduced rank regression, dietary patterns that explained variability in selected omega-3 and omega-6 FA intakes. Prevalence ratios (PRs) and 95% confidence intervals (CIs) for the association between tertiles of dietary patterns and melanoma thickness >2mm vs ≤ 2mm were estimated using Poisson regression.

Results: Overall omega-3 FA intakes were low. Two major FA dietary patterns were identified: 'meat, fish, fat', positively correlated with intakes of all FAs; and 'fish, low-meat, low-fat', positively correlated with long-chain omega-3 FA intake, and inversely with medium-chain omega-3 and omega-6 FA intakes. Prevalence of thick melanomas was significantly higher in those in the highest compared to lowest tertile of the 'meat, fish, fat' pattern (PR 1.40 95% CI 1.01-1.94), especially those with serious co-morbidity (PR 1.83, 95% CI 1.15-2.92) or a family history (PR 2.32, 95% CI 1.00-5.35). The 'fish, low-meat, low-fat' pattern was not associated with melanoma thickness.

Conclusion: People with high meat, fish, and fat intakes who thus consumed relatively high levels of omega-3 and high omega-6 FA intakes, are more likely to be diagnosed with thick than thin melanomas.

Impact: High omega-3 and omega-6 FA intakes may contribute to patients' presentation with thick melanomas.

Introduction

Cutaneous melanoma is the most serious form of skin cancer, diagnosed in over 350,000 people worldwide in 2015 (1) and incidence is set to continue increasing beyond 2030 (2). Tumor thickness at diagnosis is a measure of depth of invasion of malignant melanocytes and is the main indicator of severity of primary melanoma (3) and risk of death (4,5). Increased tumor thickness can reflect late presentation of the primary tumor, or the intrinsic fast growth rate of a melanoma (6). With growing public awareness about the significance of suspicious pigmented lesions, melanomas are increasingly being detected early. Despite this, there remains a proportion of aggressive, rapidly-growing melanomas that are already thick when first diagnosed (7).

Exposure to ultraviolet radiation (UVR) is the major environmental risk factor for melanoma (8) and sun protection remains the mainstay of prevention. However, if factors associated specifically with thick primary tumors could be identified, it would inform targeted prevention of potentially aggressive melanoma. Family history is associated with thicker melanoma (9), but diet, a modifiable lifestyle factor, may also play a role. Recent reviews have concluded that fish (10) and caffeine (11) may decrease, while citrus fruits and alcohol may increase, melanoma risk. Results for all other nutrients including polyunsaturated fatty acids (FAs) were mostly inconsistent (11). A case-control study noted that frequent consumption of red meat was associated with reduced survival from melanoma among patients with tumors >1mm thick (12). While studies that assessed diet and melanoma thickness specifically are lacking, dietary omega-3 (eicosapentaenoic (EPA), docosahexaenoic acids (DHA), α-linolenic acid (ALA)) and omega-6 (linoleic acid (LA), arachidonic acid (AA)) polyunsaturated FAs (FAs) since they appear to possess both anti-oncogenic and pro-oncogenic properties respectively (13-15) besides being

essential for normal growth and development. LA and ALA cannot be synthesized by humans and synthesis of AA (from LA), EPA and DHA (from ALA) are low, making dietary sources necessary, for example through vegetable and seed oils (LA, ALA), meat and eggs (AA) and oily fish (EPA, DHA) (16).

Levels of consumption of omega-3 and omega-6 FAs determine the FA composition of cell membranes, with EPA and DHA competing with, and partially replacing AA, thus influencing the synthesis of metabolites involved in inflammatory responses to environmental insults (17,18) such as UVR. Specifically, increasing intakes of omega-3 FAs enhance anti-inflammatory metabolites while increasing omega-6 FAs increase pro-inflammatory metabolites (18,19). In regard to melanoma specifically, in vitro studies have also shown that dietary omega-3 and omega-6 FAs have divergent effects on both growth and invasiveness of tumors (20,21) but how this applies to melanoma presentation in clinical settings is unknown.

The study of single nutrients especially in experimental settings is valuable to identify possible mechanisms linking diet and disease. However, nutrients are not eaten in isolation, but rather interact within and across foods, making it difficult to ascribe effects to individual nutrients. Also, individual nutrient may be too small to detect, while cumulative effects may be detectable when multiple nutrients are considered. Hence, the study of dietary patterns has been recommended to capture the joint effects of nutrient and foods as they are normally eaten by free-living individuals (22). Adherence to dietary patterns considered “ideal” are associated with reduced risk of chronic diseases including cancer (23). Currently, there are 3 methods to identify dietary patterns. *A priori* methods such as diet quality indices use evidence-based knowledge of a ‘healthy’ diet; *a posteriori* methods such as principal components or cluster analysis that are purely data-driven resulting in risk estimates that are non-comparable with other studies (24);

and Reduced Rank Regression (RRR) (24,25). RRR combines both approaches where prior knowledge of nutrients specific to the disease of interest is use to derive patterns in an exploratory way (25). RRR-derived dietary patterns have been shown to predict disease outcomes (26,27) and methods to derive reproducible dietary scores from RRR results have been identified (24). This novel approach to dietary pattern derivation has not been applied to studies on omega-3 and omega-6 FAs.

We hypothesised that omega-3 and omega-6 FAs would have synergistic and antagonistic effects respectively on the invasiveness of melanoma. We assessed the associations of dietary patterns derived by RRR with measured thickness of incident primary melanoma in a large cohort of newly diagnosed patients.

Methods

Design and study population

We prospectively recruited people in Queensland, Australia, newly diagnosed with tumor stage 1b to 4 cutaneous melanoma between October 2010 and October 2014 from public specialist hospital clinics, private practices and private pathology laboratories, as fully described elsewhere (28). Patients were eligible if they had no clinical sign of nodal spread at presentation, were aged ≥16 years (deemed age of consent by Queensland health) and capable of completing questionnaires. Of 1,254 eligible patients, 825 agreed to participate (66%). A further 36 were later found to be ineligible (mainly because loco-regional metastases were later revealed) and 89 were excluded after application of the 8th edition of the American Joint Committee on Cancer melanoma staging which took effect in January 2018 (29,30), leaving 700 study participants. Finally, for this analysis, patients were eligible if they completed a diet questionnaire. A comparison of our study cohort showed no significant differences in age or sex distributions

when compared with all cases aged less than 80 years diagnosed in Queensland as recorded by the Cancer Registry within the study period (28). Patients provided written informed consent and the study was approved by the Human Research Ethics Committees of the Metro South Hospital and Health Service and the QIMR Berghofer Medical Research Institute.

Outcome

The outcome is tumor thickness (in mm) of the primary melanoma, extracted from histopathology report of the specimen obtained at presentation or subsequent surgery (if performed), whichever is greater. Other features of the tumor were also extracted, namely, ulceration (yes, no), mitosis (per mm^2), site of melanoma (head or neck, trunk, upper limb, lower limb) and histologic sub-type (SSM, nodular, other, not classified).

Dietary assessment

Dietary intake was assessed at recruitment, within an average of 36 days (±26) after diagnosis of the study melanoma, using a validated self-administered, semi-quantitative food-frequency questionnaire (FFQ) consisting of 142 food items. The questionnaire was based on the FFQ developed for the US Nurses' Health Study (31,32), adapted for the Australian setting. Participants estimated how often on average they had consumed a given amount of food or beverage over the past 12 months (amounts were estimated with commonly used units or portion sizes). We measured frequency of intake in nine categories ranging from 'Never' to '4+ times per day '. Respondents also provided additional information on types of fats and oils used for cooking, types of butter, margarine and breakfast cereals regularly consumed, frequency of eating food fried at home, frequency of eating fried take-away or restaurant foods, consumption of visible fat on meat and use of dietary supplements. We calculated average daily intake of each food item using the information on portion sizes and reported frequency of food intake. Average

daily intakes of the nutrients including LA, ALA, AA, EPA and DHA were estimated using the Australian food composition database NUTTAB 2010 (33). Details on the validity and the reproducibility of the dietary questionnaire have been published previously (34-36). Using the method of triads, the validity coefficients of the FFQ relative to plasma phospholipid FAs and weighed food records were 0.45 for AA and 0.62 for both EPA and DHA (37).

Potential confounders

Personal details were also collected at recruitment using standard self-administered questionnaires. These included sex; age; skin phototype (burn only, burn then tan, tan only); height and weight; level of education (up to grade 10, grade 12 to diploma, university or college degree); smoking history (current, ex-smoker, never); average number of sun protection measures when outdoors (none to maximum 4); frequency of skin checks (less than once a year, at least once a year by self/non-doctor, at least once a year by a doctor); first-degree relative with history of melanoma (yes, no); and serious co-morbidities (ever diagnosis of heart or vascular disease, diabetes, non-cutaneous cancer). Body mass index (BMI) was calculated by dividing weight (kilograms) by the square of height (meters) (<18.5, 18.5-24.9, 25-30,> 30 kg/m^2). Patients' past history of melanoma (yes, no) was confirmed through histological reports.

Data analysis

Dietary patterns were derived using reduced rank regression (RRR), a method that determines the linear combinations of food groups as predictors that explain as much variation as possible in nutrients of interest, the 'response variables' (25,38,39). The PROC PLS procedure with the RRR method option in SAS was used to derive dietary patterns predictive of melanoma thickness with LA, ALA, AA and sum of EPA and DHA as the nutrient response variables of interest (18,20,21,40). We defined 39 foods groups (g/day) as predictor variables in the RRR

(**Supplementary Table 1**) based on previous salient studies. Number of patterns generated by RRR depends on number of response variables: we derived 4 patterns from 39 predictor food groups and 4 FAs. We used factor loadings ≥|0.20| to identify food groups that contributed the most to each dietary pattern.

Statistical analysis

RRR-derived dietary pattern scores were classified into tertiles: tertile 1 (reference category) comprised people with the lowest score and therefore lowest adherence to the dietary pattern of interest and tertiles 2 and 3, those with medium and highest adherence respectively. We categorised primary melanoma thickness as >2 mm ('thick' melanomas) and ≤2mm (thin to moderately thick). We described patient characteristics in relation to melanoma thickness and tertiles of dietary pattern using χ^2 tests of homogeneity for categorical or nominal variables and ANOVA for continuous. Prevalence ratios (PRs) and 95% confidence intervals (CIs) for the association between dietary patterns and melanoma thickness were estimated using Poisson regression model with robust error variance (41). The PR has been demonstrated to provide a better estimate of risk ratios compared to odds ratios (42). Linear trends across dietary pattern tertiles were assessed by modelling tertile categories as ordinal variables. Two models were generated: unadjusted, and then adjusted for age at diagnosis (<50, 50-69, ≥70 years), sex, frequency of skin checks and energy intake (in tertiles). Skin checking practices has been linked to melanoma thickness (6), education and health related behaviour such as smoking and sun protection practices (43) known to be associated with dietary habits (44). We evaluated effect modification by sex, age, patients' personal history of melanoma, family history of melanoma and co-morbidities by including interaction terms between them and dietary patterns. Analyses were repeated separately adding skin phototype, education, location of residence, smoking status,

use of omega-3 dietary supplement, BMI and time (in months) from diagnosis to questionnaire completion to adjusted models. We also explored, in multivariate analyses, the association between individual FAs, total omega-3 and omega-6 FAs, omega-6: omega-3 ratio and melanoma thickness.

Finally, to examine the robustness of our findings and to reduce the dependency of the dietary pattern on the study population, we derived simplified dietary pattern scores and used these in sensitivity analyses. Simplified scores were generated by summing the standardised food intakes of only the food groups with factor loadings |≥0.20| for each dietary pattern; a negative algebraic sign was assigned to foods with negative factor loadings (24). We performed analyses using SAS (version 9.4, SAS Institute, Cary, NC).

Results

From a sample of 700 patients, we excluded those who did not complete a dietary questionnaire (n=37), those with extreme energy intake values (31) (n=18) and those who answered less than 90% of the food items on the FFQ (n=11), leaving a final sample of 634 patients for this analysis (38% with >2mm melanoma). Average daily intakes of omega-6 and omega-3 FAs were 8.8g and 1.2g, respectively. Patients with thicker (>2mm) melanomas were older, more likely to be male, to have nodular melanomas that are ulcerated and have >3 mitotic figures per mm^2, and less likely to report having a family history of melanoma compared to those with thin melanomas (**Table 1**).

We obtained four RRR-derived dietary patterns. The first pattern explained 43% variation in intake of LA, ALA, EPA+DHA and AA, but only 5% of the variation in intake of food groups. The second dietary pattern explained 21% of the variation in FA intakes and 4% of the variation

in intake of foods. As the third and fourth dietary patterns combined explained <20% variation in intake of FAs, they were not considered further (**Table 2**).

We labelled the first dietary pattern as 'meat, fish, fat' as it was characterized by high consumption of meat, fish and seafood, processed meat, eggs and peas and beans, and solid fats (**Table 3**) and was moderately correlated (0.40<r<0.60) with intakes of all 4 FAs (**Table 2**). We labelled the second dietary pattern as 'fish, low-meat, low-fat' being characterized by consumption of dark-meat fish and other fish and seafood and lower intakes of meat, processed meat, solid fats and oils (**Table 3**); it was positively correlated (r=0.89) with the sum of EPA and DHA and negatively correlated (all r<–0.30) with LA, ALA and AA (**Table 2**). Melanoma patients with higher intakes of the 'meat-fish-fat' dietary pattern were more likely to be smokers while those in the 'fish, low-meat, low-fat' dietary pattern were more likely to be female and use sun protection. Total intakes of omega-6 FAs were consistently higher than omega-3 intakes with increasing intakes of both dietary patterns (omega-6/omega-3 ratios were up to 8:1) (**Supplementary Table 2)**.

After adjustment for age, sex, skin examinations and energy intake, patients with increasing tertiles of intake of the 'meat-fish-fat' dietary pattern had significantly higher prevalence of thick melanomas compared with those with lowest intakes by 34% (PR=1.34, 95%CI=1.01-1.78) and 40% (PR=1.40, 95%CI=1.01-1.94) respectively (P-trend=0.04, **Table 4**). We found no significant association between the 'fish, low-meat, low-fat' dietary pattern and melanoma thickness, although the trend was inverse (PR=0.89, 95%CI=0.68-1.15, tertile-3 vs. tertile-1, P-trend=0.40). Results were unchanged after additionally adjusting for skin type, education, location of residence, smoking status, use of any omega-3 dietary supplement, BMI and time between diagnosis and dietary assessment.

Repeating the analysis using simplified dietary pattern scores, we observed similar trends. There was a significantly higher prevalence of thick melanomas among those in the highest tertile of the simplified 'meat, fish, fat' dietary pattern scores (PR=1.39, 95%CI=1.04-1.85, P-trend=0.02) compared to the lowest (**Supplementary Table 3**). The simplified 'fish, low-meat, low-fat' dietary pattern was significantly inversely associated with thick melanomas in the unadjusted model (PR=0.76, 95%CI=0.60-0.97, tertile-3 vs. tertile-1, P-trend=0.02) but not after adjustment (P-trend=0.13).

We found no evidence of effect modification by sex, age and history of melanoma, but the positive and significant association between the 'meat, fish, fat' dietary pattern and melanoma thickness was observed more clearly among patients who reported serious co-morbidities at the time of melanoma diagnosis (PR=1.83, 95%CI=1.15-2.92, tertile-3 vs. tertile-1, P-trend=0.01, $P_{interaction}$ = 0.04), than those without. Similarly, for the subgroup of patients with a family history of melanoma, these findings were even more striking with a strong prevalence ratio of thick melanoma (PR=2.32, 95%CI=1.00-5.35, tertile-3 vs. tertile-1, P-trend=0.02, $P_{interaction}$ = 0.04), but no association among those with no family history. There was no effect modification by the above factors ($P_{interaction}$>0.05) with the 'fish, low-meat, low-fat' dietary pattern (**Supplementary Table 4**).

In secondary analyses, when we explored whether any of the individual FAs, total omega-3, omega-6 intakes or ratio of omega-6 to omega-3 FAs played a dominant role in associations, none were significantly associated with melanoma thickness (**Supplementary Table** 5).

Discussion

In this novel study among 634 primary melanoma patients, we defined dietary patterns based on intakes of omega-3 and omega-6 FAs and then investigated their combined effect on melanoma

thickness, the major indicator of melanoma severity. We identified a dietary pattern associated with thick melanoma, namely a 'meat, fish, fat' pattern characterised by high intake of all omega-6 FAs included in the study and relatively high intake of long-chain omega-3 fatty acids within the overall low intake levels in our study population. A second 'fish, low-meat, low-fat' pattern characterised by intake of long-chain omega-3 FAs (EPA and DHA) and low intake of all other FAs showed a non-significant inverse association with melanoma thickness after adjustment for other factors. Our findings are consistent with our hypothesis that a dietary pattern high in pro-inflammatory omega-6 FAs would be associated with thick melanoma while, within a background of generally low omega-3 intakes, we found no evidence to support the hypothesis that anti-inflammatory omega-3 FAs ameliorate growth rate of primary melanoma.

The 'meat, fish, fat' dietary pattern associated with thick melanoma was characterized by increased intakes of foods from mainly animal sources, increased total fat and energy intakes, and a preponderance of omega-6 FAs which lead to an imbalance in omega-6/omega-3 ratio which remained at 7:1 at the highest tertile intake, all typical of Western diets (45). Reduction to a 1:1 ratio similar to diets before industrialization and introduction of processed foods and oils has been advocated (19). Although we are not able to compare our results to other studies, numerous experimental studies have shown that increasing levels of omega-6 FAs especially AA, result in concomitant rises in their intermediates that promote inflammation in chronic inflammatory conditions (14,19,46) including skin cancer (18) and are linked to growth and invasiveness of melanomas (20). We found the association between the 'meat, fish, fat' dietary pattern and thick melanomas most evident among patients with a family history of melanoma (9) and those with concurrent diabetes, heart disease or other cancer (apart from keratinocyte skin

cancer). These patients most likely consumed high levels of the Western-style dietary patterns with high omega-6 but low omega-3 intakes implicated in the development of these inflammation-related chronic diseases (14,19,45,47) in preference to the 'fish, low-meat, low-fat' diet known to demonstrate a protective effect on these conditions (23,48) as well as melanoma (10,49).

While high adherence to the 'fish and low meat-fat' diet, characterized by intakes of fish and seafood and lower intakes of other animal sources of protein and fat and lower levels of total and saturated fats and energy, was inversely associated with melanoma thickness, this was significant only in unadjusted models. While this dietary pattern had increasing intakes of the long-chain omega-3 FAs, intakes of the medium-chain omega-3 ALA decreased; thus, the absolute amount of omega-3 FAs consumed may not have been sufficient to produce a clear effect. Only 27% patients reported consuming oil sources of ALA (eg, flaxseed, canola oil); in addition, only 12% reported eating at least 20g of nuts daily, and in Australia, half of this would be peanuts (50) which do not contain ALA (33). Intake of dark meat fish, the major source of long-chain omega-3 FAs is also low in Australia (50) and in our study population; 34% reported not consuming dark meat fish, and, among fish eaters, the proportion of participants who consumed the recommended 2-3 serves of fish intake per week (51) was low (34%). Consequently, even in the highest intake group, only 10% of melanoma patients (4% of all patients) consumed both ALA and long-chain omega-3 FAs at the recommended levels (52). Finally, despite the decrease in intakes of omega-6 FAs with increasing 'fish, low-meat, low-fat' dietary pattern intakes, virtually all patients consumed the minimum recommended intake of LA (omega-6 FA) (52). Higher levels of LA compared to ALA have been found to exacerbate the already inefficient conversion of ALA to EPA and DHA (45).

We found no association between intakes of individual FAs, total FAs and their ratio, suggesting that the increased melanoma thickness associated with the 'meat, fish, fat' dietary pattern may be the result of the collective, synergistic and antagonistic effects of the fatty acids that characterize this dietary pattern.

Strengths of the present study include the large sample of well-characterized melanoma patients including detailed information on skin checking behavior prior to diagnosis which is linked to melanoma thickness (6). Our study was also a novel use of RRR methods to define dietary patterns based on omega-3 relative to omega-6 FAs and evaluate their association with melanoma severity. In addition, we tested our hypothesis with the simplified dietary pattern score, suggesting robustness of the findings. This study was limited by its design whereby dietary data were collected at the time of diagnosis so that we are unable to infer causality, although patients were unlikely to have changed their usual diet in the lead-up to a localised skin melanoma. Dietary questionnaires and potential confounders were self-reported, which may have led to their misclassification, though non-differential, and thus could have reduced study power rather than introducing bias. While one in five patients had a melanoma prior to the index melanoma, there was no significant effect modification by history of melanoma and inclusion of this in adjusted models did not change results. Finally, late disease presentation among people who consumed a mainly 'meat-fish-fat' dietary pattern may partly explain the observed association with thick melanomas.

In conclusion, in this Western study population with generally low intakes of food sources of omega-3 FAs and high intakes of omega-6 foods, we found that a dietary pattern high in 'meat-fish-fat' was associated with thick melanoma but consuming a dietary pattern high in 'fish, low-meat, low-fat' diet was not. Studies that would apply our simplified dietary pattern scores in

populations with higher intake levels of omega-3 foods would help round out this investigation of the combined effects of omega-3 and omega-6 FAs on melanoma severity and potentially provide evidence for a role for diet to complement standard sun protection measures.

Acknowledgements

We are grateful to the study subjects for their continued participation. We also thank Professor Sarah McNaughton, PhD for advice on dietary pattern methodology.

Authors' contributions:

Concept and design: K. Khosrotehrani, B-M. Smithers, A. Green

Development of methodology: K. Khosrotehrani, B-M. Smithers, A. Green

Acquisition of data: M. Malt, M. Hughes

Analysis and interpretation of data: Y. Mahamat-Saleh, K. Miura, M. Hughes

Writing, review and/or revision of the manuscript: Y. Mahamat-Saleh, M. Hughes, K. Miura, L. von Schuckmann, K. Khosrotehrani, B-M. Smithers, A. Green

Administrative, technical, or material support: M. Malt, M. Hughes, L. von Schuckmann

Study supervision: A. Green

Reference list

1. Karimkhani C, Green AC, Nijsten T, Weinstock MA, Dellavalle RP, Naghavi M*, et al.* The global burden of melanoma: results from the Global Burden of Disease Study 2015. Br J Dermatol **2017**;177(1):134-40 doi 10.1111/bjd.15510.
2. Whiteman DC, Green AC, Olsen CM. The Growing Burden of Invasive Melanoma: Projections of Incidence Rates and Numbers of New Cases in Six Susceptible Populations through 2031. J Invest Dermatol **2016**;136(6):1161-71 doi 10.1016/j.jid.2016.01.035.
3. Wisco OJ, Sober AJ. Prognostic factors for melanoma. Dermatol Clin **2012**;30(3):469-85 doi 10.1016/j.det.2012.04.008.
4. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR*, et al.* Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol **2009**;27(36):6199-206 doi 10.1200/JCO.2009.23.4799.
5. Green AC, Baade P, Coory M, Aitken JF, Smithers M. Population-based 20-year survival among people diagnosed with thin melanomas in Queensland, Australia. J Clin Oncol **2012**;30(13):1462-7 doi 10.1200/JCO.2011.38.8561.
6. Baade PD, English DR, Youl PH, McPherson M, Elwood JM, Aitken JF. The relationship between melanoma thickness and time to diagnosis in a large population-based study. Arch Dermatol **2006**;142(11):1422-7 doi 10.1001/archderm.142.11.1422.
7. Sacchetto L, Zanetti R, Comber H, Bouchardy C, Brewster DH, Broganelli P*, et al.* Trends in incidence of thick, thin and in situ melanoma in Europe. Eur J Cancer **2018**;92:108-18 doi 10.1016/j.ejca.2017.12.024.
8. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P*, et al.* Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer **2005**;41(1):45-60 doi 10.1016/j.ejca.2004.10.016.
9. Li WQ, Cho E, Wu S, Li S, Matthews NH, Qureshi AA. Host Characteristics and Risk of Incident Melanoma by Breslow Thickness. Cancer Epidemiol Biomarkers Prev **2019**;28(1):217-24 doi 10.1158/1055-9965.EPI-18-0607.
10. Noel SE, Stoneham AC, Olsen CM, Rhodes LE, Green AC. Consumption of omega-3 fatty acids and the risk of skin cancers: a systematic review and meta-analysis. Int J Cancer **2014**;135(1):149-56 doi 10.1002/ijc.28630.
11. Yang K, Fung TT, Nan H. An Epidemiological Review of Diet and Cutaneous Malignant Melanoma. Cancer Epidemiol Biomarkers Prev **2018**;27(10):1115-22 doi 10.1158/1055-9965.EPI-18-0243.
12. Gould Rothberg BE, Bulloch KJ, Fine JA, Barnhill RL, Berwick M. Red meat and fruit intake is prognostic among patients with localized cutaneous melanomas more than 1 mm thick. Cancer epidemiology **2014**;38(5):599-607 doi 10.1016/j.canep.2014.08.005.
13. Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem Soc Trans **2017**;45(5):1105-15 doi 10.1042/BST20160474.
14. Huerta-Yepez S, Tirado-Rodriguez AB, Hankinson O. Role of diets rich in omega-3 and omega-6 in the development of cancer. Bol Med Hosp Infant Mex **2016**;73(6):446-56 doi 10.1016/j.bmhimx.2016.11.001.
15. Serini S, Fasano E, Celleno L, Cittadini A, Calviello G. Potential of long-chain n-3 polyunsaturated fatty acids in melanoma prevention. Nutr Rev **2014**;72(4):255-66 doi 10.1111/nure.12093.
16. Lunn J, Theobald HE. The health effects of dietary unsaturated fatty acids. Nutr Bull **2006**;31(3):178-224 doi 10.1111/j.1467-3010.2006.00571.x.
17. Calder PC. Mechanisms of action of (n-3) fatty acids. J Nutr **2012**;142(3):592S-9S doi 10.3945/jn.111.155259.

18. Black HS, Rhodes LE. Potential Benefits of Omega-3 Fatty Acids in Non-Melanoma Skin Cancer. J Clin Med **2016**;5(2) doi 10.3390/jcm5020023.
19. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother **2002**;56(8):365-79 doi 10.1016/s0753-3322(02)00253-6.
20. Denkins Y, Kempf D, Ferniz M, Nileshwar S, Marchetti D. Role of omega-3 polyunsaturated fatty acids on cyclooxygenase-2 metabolism in brain-metastatic melanoma. J Lipid Res **2005**;46(6):1278-84 doi 10.1194/jlr.M400474-JLR200.
21. Reich R, Royce L, Martin GR. Eicosapentaenoic acid reduces the invasive and metastatic activities of malignant tumor cells. Biochem Biophys Res Commun **1989**;160(2):559-64 doi 10.1016/0006-291X(89)92469-8.
22. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol **2002**;13(1):3-9.
23. Schulze MB, Martinez-Gonzalez MA, Fung TT, Lichtenstein AH, Forouhi NG. Food based dietary patterns and chronic disease prevention. Br Med J **2018**;361:k2396 doi 10.1136/bmj.k2396.
24. Schulze MB, Hoffmann K, Kroke A, Boeing H. An approach to construct simplified measures of dietary patterns from exploratory factor analysis. Br J Nutr **2003**;89(3):409-19 doi 10.1079/BJN2002778.
25. Weikert C, Schulze MB. Evaluating dietary patterns: the role of reduced rank regression. Curr Opin Clin Nutr Metab Care **2016**;19(5):341-6 doi 10.1097/MCO.0000000000000308.
26. Hosseinzadeh M, Vafa MR, Esmaillzadeh A, Feizi A, Majdzadeh R, Afshar H*, et al.* Psychological disorders and dietary patterns by reduced-rank regression. Eur J Clin Nutr **2019**;73(3):408-15 doi 10.1038/s41430-019-0399-8.
27. Livingstone KM, McNaughton SA. Dietary patterns by reduced rank regression are associated with obesity and hypertension in Australian adults. Br J Nutr **2017**;117(2):248-59 doi 10.1017/S0007114516004505.
28. Smithers BM, Hughes MCB, Beesley VL, Barbour AP, Malt MK, Weedon D*, et al.* Prospective study of patterns of surgical management in adults with primary cutaneous melanoma at high risk of spread, in Queensland, Australia. J Surg Oncol **2015**;112(4):359-65 doi 10.1002/jso.24013.
29. von Schuckmann LA, Hughes MCB, Lee R, Lorigan P, Khosrotehrani K, Smithers BM*, et al.* Survival of patients with early invasive melanoma down-staged under the new eighth edition of the American Joint Committee on Cancer staging system. J Am Acad Dermatol **2019**;80(1):272-4 doi 10.1016/j.jaad.2018.04.017.
30. Gershenwald JE, Scolyer RA. Melanoma Staging: American Joint Committee on Cancer (AJCC) 8th Edition and Beyond. Ann Surg Oncol **2018**;25(8):2105-10 doi 10.1245/s10434-018-6513-7.
31. Willett W. Nutritional Epidemiology. Oxford, New York: Oxford University Press; 2012. 552 p.
32. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J*, et al.* Reproducibility and validity of a semiquantitative food frequency questionnaire. Am J Epidemiol **1985**;122(1):51-65 doi 10.1093/oxfordjournals.aje.a114086.
33. Food Standards Australia New Zealand. NUTTAB 2010 Electronic Database Files - Australian Food Composition Tables. www.foodstandards.gov.au2011.
34. Ibiebele TI, Parekh S, Mallitt K-A, Hughes MC, O'Rourke PK, Webb PM*, et al.* Reproducibility of food and nutrient intake estimates using a semi-quantitative FFQ in Australian adults. Public Health Nutr **2009**;12(12):2359-65 doi 10.1017/S1368980009005023.
35. Marks GC, Hughes MC, van der Pols JC. Relative validity of food intake estimates using a food frequency questionnaire is associated with sex, age, and other personal characteristics. J Nutr **2006**;136(2):459-65 doi 10.1093/jn/136.2.459.

36. Marks GC, Hughes MC, van der Pols JC. The effect of personal characteristics on the validity of nutrient intake estimates using a food-frequency questionnaire. Public Health Nutr **2006**;9(3):394-402 doi 10.1079/phn2006839.
37. McNaughton SA, Hughes MC, Marks GC. Validation of a FFQ to estimate the intake of PUFA using plasma phospholipid fatty acids and weighed foods records. Br J Nutr **2007**;97(3):561-8 doi 10.1017/S0007114507381385.
38. Hoffmann K, Schulze MB, Schienkiewitz A, Nöthlings U, Boeing H. Application of a new statistical method to derive dietary patterns in nutritional epidemiology. Am J Epidemiol **2004**;159(10):935-44 doi 10.1093/aje/kwh134.
39. Pot GK, Stephen AM, Dahm CC, Key TJ, Cairns BJ, Burley VJ*, et al.* Dietary patterns derived with multiple methods from food diaries and breast cancer risk in the UK Dietary Cohort Consortium. Eur J Clin Nutr **2014**;68(12):1353-8 doi 10.1038/ejcn.2014.135.
40. Serini S, Zinzi A, Ottes Vasconcelos R, Fasano E, Riillo MG, Celleno L*, et al.* Role of β-catenin signaling in the anti-invasive effect of the omega-3 fatty acid DHA in human melanoma cells. J Dermatol Sci **2016**;84(2):149-59 doi 10.1016/j.jdermsci.2016.06.010.
41. Zou G. A modified poisson regression approach to prospective studies with binary data. Am J Epidemiol **2004**;159(7):702-6 doi 10.1093/aje/kwh090.
42. Thompson ML, Myers JE, Kriebel D. Prevalence odds ratio or prevalence ratio in the analysis of cross sectional data: what is to be done? Occup Environ Med **1998**;55(4):272-7 doi 10.1136/oem.55.4.272.
43. Green AC, Hughes MCB, von Schuckmann LA, Khosrotehrani K, Smithers BM. Clustering of prevention behaviours in patients with high-risk primary melanoma. Psycho-Oncol **2018**;27(5):1442-9 doi 10.1002/pon.4565.
44. McNaughton SA, Mishra GD, Paul AA, Prynne CJ, Wadsworth ME. Supplement use is associated with health status and health-related behaviors in the 1946 British birth cohort. J Nutr **2005**;135(7):1782-9 doi 10.1093/jn/135.7.1782.
45. Simopoulos AP. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. Nutrients **2016**;8(3):128 doi 10.3390/nu8030128.
46. Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. Health implications of high dietary omega-6 polyunsaturated Fatty acids. J Nutr Metab **2012**;2012:539426 doi 10.1155/2012/539426.
47. DiNicolantonio JJ, O'Keefe JH. Importance of maintaining a low omega-6/omega-3 ratio for reducing inflammation. Open Heart **2018**;5(2):e000946 doi 10.1136/openhrt-2018-000946.
48. GBD 2017 Diet Collaborators. Health effects of dietary risks in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet **2019**;393(10184):1958-72 doi 10.1016/S0140-6736(19)30041-8.
49. Mahamat-Saleh Y, Cervenka I, Al Rahmoun M, Savoye I, Mancini FR, Trichopoulou A*, et al.* Mediterranean dietary pattern and skin cancer risk: A prospective cohort study in French women. Am J Clin Nutr **2019**;110(4):993-1002 doi 10.1093/ajcn/nqz173.
50. Meyer BJ. Australians are not Meeting the Recommended Intakes for Omega-3 Long Chain Polyunsaturated Fatty Acids: Results of an Analysis from the 2011-2012 National Nutrition and Physical Activity Survey. Nutrients **2016**;8(3):111 doi 10.3390/nu8030111.
51. Heart Foundation Australia. Omega-3 fatty acid: the importance of fat in a healthy diet. https://www.heartfoundation.org.au/news/omega-3-fatty-acid-the-importance-of-fat-in-a-healthy-diet2015.
52. International Society for the Study of Fatty Acids and Lipids. Recommendations for dietary intake of polyunsaturated fatty acids in healthy adults, June 2004. https://www.issfal.org/statement-32004.

Table 1. Patient and tumor characteristics by thickness of the primary melanoma

Patient and tumor characteristics	Total N=634	Thickness ≤2 mm N=396	Thickness >2mm N=238	P-value
Age at diagnosis, years, mean (SD)	62.2 (13.4)	60.9 (13.2)	64.3 (13.4)	0.002
Body mass index, mean (SD)	28.3 (5.4)	28.4 (5.5)	28.1 (5.2)	0.65
Male sex (%)	58.8	53.8	67.2	0.0009
Current smoker (%)	7.4	7.3	7.6	0.99
Attained university or college degree (%)	20.3	20.7	19.7	0.27
Number of sun protection measures (%)				
Never	14.8	14.6	15.1	
1 to 2 more than 50 % of the time	56.0	56.1	55.8	
3 to 4 more than 50 % of the time	29.2	29.3	29.0	0.98
Skin examination habits (%)				
skin checks <1/year	41.0	40.1	42.4	
skin checks by self/non-doctor ≥ 1/year	9.9	10.9	8.4	
skin checks by doctor ≥ 1/year	49.1	49.0	49.2	0.58
Personal history of melanoma (%)	19.9	20.4	18.9	0.63
First-degree relative with melanoma (%)	27.6	31.3	21.4	0.008
Co-morbidity[a] (%)	47.5	44.2	52.9	0.03
Ulceration (%)	28.1	20.9	39.9	<0.0001
Mitotic rate >3 per mm^2 (%)	42.4	30.1	63.0	<0.0001
Site (%)				
Head and neck	21.1	18.2	26.1	
Trunk	35.8	37.1	33.6	
Upper limb	20.8	23.0	17.2	
Lower limb	22.3	21.7	23.1	0.06
Histologic sub-type (%)				
SSM	39.9	48.2	62 (26.1)	
Nodular	24.0	14.9	93 (39.1)	
Other[b]	16.7	16.9	39 (16.4)	
Not classified[c]	19.4	20.0	44 (18.5)	<0.0001
Daily dietary intake, median (IQR)				
Linoleic acid (g)	8.70 (4.76)	8.64 (4.77)	8.92 (4.70)	0.26
α-linolenic acid (g)	0.89 (0.54)	0.87 (0.57)	0.94 (0.49)	0.11
AA (mg)	121.4 (70.9)	117.2 (67.7)	130.1 (75.7)	0.06
EPA (mg)	94.7 (129.3)	90.0 (129.4)	99.3 (128.0)	0.29
DHA (mg)	135.6 (185.3)	131.6 (183.2)	142.1 (185.9)	0.46
Omega-6 to Omega-3 ratio[d]	7.6 (3.4)	7.7 (3.3)	7.3 (3.5)	0.06
Total Fat (g)	73.4 (34.3)	72.2 (34.4)	75.6 (34.4)	0.01
Total energy intake (MJ)	8.9 (3.7)	8.8 (3.4)	9.2 (4.0)	0.009

Abbreviations: AA - Arachidonic acid; EPA - Eicosapentaenoic acid; DHA - Docosahexaenoic acid; IQR - inter-quartile range; SSM - superficial spreading melanoma.

[a]Defined as ever diagnosis of heart disease, diabetes, hypertension/stroke or cancers other than skin

[b]Other: lentigo maligna (17.8%), desmoplastic (34.0%) nevoid (22.6%), spitzoid (4.7%), lentiginous (1.9%), acral lentiginous (7.6%), mixed (11.3%)
[c]Not classified: unable to classify (12.2%), not stated (87.0%), and other (0.8%)
[d]Omega-6 was calculated as sum of Linoleic (g) and AA (converted to grams) and omega-3 as sum of α-linolenic acid (g), EPA (converted to grams) and DHA (converted to grams).

Table 2. Explained variation (%) in intakes of FAs and food groups for each dietary pattern obtained using reduced rank regression and correlation coefficients between dietary patterns and FAs

	Explained variation in intakes (%)						Correlation coefficient			
	LA	**ALA**	**AA**	**EPA + DHA**	**All FAs (total)**	**Food intake Total**	**LA**	**ALA**	**AA**	**EPA+DHA**
Dietary pattern 1	48.7	33.9	53.9	34.6	42.8	5.0	0.53	0.45	0.56	0.45
Dietary pattern 2	53.9	40.7	58.9	99.7	20.5	4.2	-0.25	-0.29	-0.25	0.89
Dietary pattern 3	73.6	44.6	94.5	99.8	14.8	3.4	0.58	0.26	-0.77	0.03
Dietary pattern 4	77.9	53.4	94.8	99.8	3.4	3.7	-0.57	0.81	-0.15	0.06

Abbreviations: FA - fatty acid; LA - Linoleic acid; ALA - α-Linolenic acid; AA - Arachidonic acid; EPA - Eicosapentaenoic acid; DHA - Docosahexaenoic acid

Table 3. Food groups associated[a] with dietary patterns identified by reduced rank regression

Dietary pattern 1: Meat, fish, fat dietary pattern	Factor loadings
Positive association	
Meat	0.41
Dark meat fish	0.36
Other fish and seafood	0.31
Solid fats	0.27
Eggs	0.25
Peas and beans	0.22
Processed meat	0.21
Dietary Pattern 2: Fish, low meat, low-fat dietary pattern	
Positive association	
Dark meat fish	0.65
Other fish and seafood	0.28
Inverse association	
Solid fats	-0.29
Meat	-0.28
Processed meat	-0.22
Cooking oils	-0.20

[a]defined as food groups with factor loadings ≥|0.20|

Table 4. Prevalence Ratio (95% CI) for tertiles of dietary patterns obtained by reduced rank regression and melanoma thickness >2mm

	Tertile 1 reference	Tertile 2 PR (95% CI)	Tertile 3 PR (95% CI)	P-trend
DP1: Meat, fish, fat dietary pattern				
Number (%)	209 (33.0)	209 (33.0)	216 (34.0)	
Unadjusted	1.00	1.28 (0.98, 1.67)	1.39 (1.08, 1.80)	0.01
Adjusted[1]	1.00	1.34 (1.01, 1.78)	1.40 (1.01, 1.94)	0.04
DP2: Fish, low-meat, low-fat dietary pattern				
Number (%)	209 (33.0)	210 (33.0)	215 (33.9)	
Unadjusted	1.00	0.80 (0.62, 1.02)	0.82 (0.65, 1.04)	0.11
Adjusted[1]	1.00	0.87 (0.67, 1.12)	0.89 (0.68, 1.15)	0.40

Abbreviations: DP - dietary pattern; PR - prevalence ratio; CI - confidence interval
[a]Adjusted for age, sex, skin examination habits and energy intake