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

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ABSTRACT

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

Methods: In 634 Australian patients with primary melanoma, we assessed prediagnosis 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 fatty acid intakes. Prevalence ratios (PR) and 95% confidence intervals (CI) for the association between tertiles of dietary patterns and melanoma thickness >2 mm versus ≤2 mm were estimated using Poisson regression.

Results: Overall omega-3 fatty acid intakes were low. Two major fatty acid dietary patterns were identified: "meat, fish, and fat," positively correlated with intakes of all fatty acids; and "fish, low-meat, and low-fat," positively correlated with long-chain omega-3 fatty acid intake, and inversely with medium-chain omega-3 and omega-6 fatty acid intakes. Prevalence of thick melanomas was significantly higher in those in the highest compared with lowest tertile of the "meat, fish, and fat" pattern (PR, 1.40; 95% CI, 1.01–1.94), especially those with serious comorbidity (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, and low-fat" pattern was not associated with melanoma thickness.

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

Impact: High omega-3 and omega-6 fatty acid 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 UV 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 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 >1 mm thick (12). While studies that assessed diet and melanoma thickness specifically are lacking, dietary omega-3 [eicosapentaenoic acid (EPA), docosahexaenoic acids (DHA), and α-linolenic acid (ALA)] and omega-6 [linoleic acid (LA) and arachidonic acid (AA)] polyunsaturated fatty acids may play a role because they appear to possess both antioncogenic and prooncogenic 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) and EPA and DHA (from ALA) are low, making dietary sources necessary, for example, through vegetable and seed oils (LA and ALA), meat and eggs (AA), and oily fish (EPA and DHA; ref. 16).

Levels of consumption of omega-3 and omega-6 fatty acids determine the fatty acid 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 fatty acids enhance anti-inflammatory metabolites, while

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increasing omega-6 fatty acids increase proinflammatory metabolites (18, 19). In regard to melanoma specifically, *in vitro* studies have also shown that dietary omega-3 and omega-6 fatty acids 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 effects 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 three 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 noncomparable with other studies (24); and reduced rank regression (RRR; refs. 24, 25). RRR combines both approaches where prior knowledge of nutrients specific to the disease of interest is used 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 fatty acids.

We hypothesized that omega-3 and omega-6 fatty acids 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.

Materials and Methods

Design and study population

We prospectively recruited people in Queensland, Australia, newly diagnosed with tumor stage Ib to IV 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 (Brisbane, Queensland, Australia).

Outcome

The outcome was 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 and no), mitosis (per mm^2), site of melanoma (head or neck, trunk, upper limb, and lower limb) and histologic subtype [superficial spreading melanoma (SSM), nodular, other, and 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, semiquantitative food frequency questionnaire (FFQ) consisting of 142 food items. The questionnaire was based on the FFQ developed for the U.S. 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 fatty acids 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, and tan only), height and weight, level of education (up to grade 10, grade 12 to diploma, and university or college degree), smoking history (current, ex-smoker, and 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/nondoctor, and at least once a year by a doctor), first-degree relative with history of melanoma (yes and no), and serious comorbidities (ever diagnosis of heart or vascular disease, diabetes, and noncutaneous 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, and >30 kg/m^2). Patients' past history of melanoma (yes and no) was confirmed through histologic reports.

Data analysis

Dietary patterns were derived using 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, and 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 S1) based on previous salient studies. Number of patterns generated by RRR depends on number of response variables: we derived four patterns from 39 predictor food groups and four fatty acids. 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 categorized primary melanoma thickness as >2 mm ("thick" melanomas) and ≤2 mm (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 (PR) and 95% confidence intervals (CI) 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 with ORs (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, and ≥70 years), sex, frequency of skin checks, and energy intake (in tertiles). Skin checking practices have been linked to melanoma thickness (6), education and health-related behavior such as smoking and sun protection practices (43) are 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 comorbidities 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 fatty acids, total omega-3 and omega-6 fatty acids, 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 standardized 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).

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 (ref. 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 >2 mm melanoma). Average daily intakes of omega-6 and omega-3 fatty acids were 8.8 and 1.2 g, respectively. Patients with thicker (>2 mm) 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 with 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 fatty acid intakes and 4% of the variation in intake of foods. As the third and fourth dietary patterns combined explained <20% variation in intake of fatty acids, they were not considered further (**Table 2**).

We labeled the first dietary pattern as "meat, fish, and fat" as it was characterized by high consumption of meat, fish and seafood, processed meat, eggs, peas and beans, and solid fats (**Table 3**) and was moderately correlated ($0.40 < r < 0.60$) with intakes of all four fatty acids (**Table 2**). We labeled the second dietary pattern as "fish, low-meat, and 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**). Patients with melanoma with higher intakes of the "meat-fish-fat" dietary pattern were more likely to be smokers, while those in the "fish, low-meat, and low-fat" dietary pattern were more likely to be female and use sun protection. Total intakes of omega-6 fatty acids 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 S2).

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, and 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, and fat" dietary pattern scores (PR, 1.39; 95% CI, 1.04–1.85; $P_{trend} = 0.02$) compared with the lowest (Supplementary Table S3). The simplified "fish, low-meat, and 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, and fat" dietary pattern and melanoma thickness was observed more clearly among patients who reported serious comorbidities 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 PR 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, and low-fat" dietary pattern (Supplementary Table S4).

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 >2 mm $n = 238$	P
Age at diagnosis, years, mean (SD)	62.2 (13.4)	60.9 (13.2)	64.3 (13.4)	0.002
BMI, 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
Use 3–4 sun protection measures more than 50% of the time (%)	29.2	29.3	29.0	0.98
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
Comorbidity[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 subtype (%)				
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)				
LA (g)	8.70 (4.76)	8.64 (4.77)	8.92 (4.70)	0.26
ALA (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/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

Abbreviation: IQR, interquartile range.
[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%), and 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 LA (g) and AA (converted to grams) and omega-3 as sum of ALA (g), EPA (converted to grams), and DHA (converted to grams).

In secondary analyses, when we explored whether any of the individual fatty acids, total omega-3 and omega-6 intakes, or ratio of omega-6 to omega-3 fatty acids played a dominant role in associations, none were significantly associated with melanoma thickness (Supplementary Table S5).

Discussion

In this novel study among 634 patients with primary melanoma, we defined dietary patterns based on intakes of omega-3 and omega-6 fatty acids and then investigated their combined effect on melanoma thickness, the major indicator of melanoma severity. We identified a

Table 2. Explained variation (%) in intakes of fatty acids and food groups for each dietary pattern obtained using RRR and correlation coefficients between dietary patterns and fatty acids.

	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

Abbreviation: FA, fatty acid.

Table 3. Food groups associated[a] with dietary patterns identified by RRR.

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|.

dietary pattern associated with thick melanoma, namely a "meat, fish, and fat" pattern characterized by high intake of all omega-6 fatty acids 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, and low-fat" pattern characterized by intake of long-chain omega-3 fatty acids (EPA and DHA) and low intake of all other fatty acids showed a nonsignificant inverse association with melanoma thickness after adjustment for other factors. Our findings are consistent with our hypothesis that a dietary pattern high in proinflammatory omega-6 fatty acids 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 fatty acids ameliorate growth rate of primary melanoma.

The "meat, fish, and 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 fatty acids leading 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 were not able to compare our results with other studies, numerous experimental studies have shown that increasing levels of omega-6 fatty acids 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, and 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, and low-fat" diet known to demonstrate a protective effect on these conditions (23, 48) as well as melanoma (10, 49).

Table 4. PR (95% CI) for tertiles of dietary patterns obtained by RRR and melanoma thickness >2 mm.

	Tertile 1 Reference	Tertile 2 PR (95% CI)	Tertile 3 PR (95% CI)	P_{trend}
DP1: meat, fish, fat DP				
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[a]	1.00	1.34 (1.01–1.78)	1.40 (1.01–1.94)	0.04
DP2: fish, low-meat, low-fat DP				
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[a]	1.00	0.87 (0.67–1.12)	0.89 (0.68–1.15)	0.40

Abbreviation: DP, dietary pattern.
[a]Adjusted for age, sex, skin examination habits, and energy intake.

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 fatty acids, intakes of the medium-chain omega-3 ALA decreased; thus, the absolute amount of omega-3 fatty acids consumed may not have been sufficient to produce a clear effect. Only 27% patients reported consuming oil sources of ALA (e.g., flaxseed and canola oil); in addition, only 12% reported eating at least 20 g 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 fatty acids 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 patients with melanoma (4% of all patients) consumed both ALA and long-chain omega-3 fatty acids at the recommended levels (52). Finally, despite the decrease in intakes of omega-6 fatty acids with increasing "fish, low-meat, and low-fat" dietary pattern intakes, virtually all patients consumed the minimum recommended intake of LA (omega-6 fatty acid; ref. 52). Higher levels of LA compared with 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 fatty acids, total fatty acids, and their ratio, suggesting that the increased melanoma thickness associated with the "meat, fish, and 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 this study include the large sample of well-characterized patients with melanoma 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 fatty acids 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 localized skin melanoma. Dietary questionnaires and potential confounders were self-reported, which may have led to their

misclassification, although nondifferential, and thus could have reduced study power rather than introducing bias. While 1 in 5 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 fatty acids 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, and 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 fatty acids on melanoma severity and potentially provide evidence for a role for diet to complement standard sun protection measures.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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