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Omega-3 fatty acid supplement skin cancer prophylaxis in lung transplant recipients: randomized controlled pilot trial

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Abstract

Background: Lung transplant recipients are at very high risk of skin cancer. Omega-3 fatty acids (FAs) are anti-inflammatory and immune-modulating and potentially could reduce this risk. We assessed the feasibility of omega-3 FA supplementation to reduce skin cancer among these patients.

Methods: LTRs aged 18+ years, at least one year post-transplant, were recruited from the outpatient clinic, Prince Charles Hospital, Brisbane. Participants were randomly allocated to daily supplements containing either 4g omega-3 FA (3.36g eicosapentaenoic acid (EPA) + docosahexaenoic acid) or placebo (4g olive oil) for 12 months. Primary outcomes were rates of recruitment, retention, adherence (assessed by plasma omega-3 FA) and safety. Secondary outcomes were incident skin cancers.

Results: Among 106 eligible lung transplant recipients, 49 consented to take part (46%) with 25 allocated to omega-3 FA and 24 to placebo supplements. Of these 22 (88%) and 20 (83%) respectively completed the trial. After 12 months, median plasma EPA increased substantially in the intervention (125.0 to 340.0μmol/L) but not placebo group (98.0 to 134.5μmol/L). In the intervention group, 6 patients developed skin cancers compared with 11 in the placebo group, giving an odds ratio (95% confidence interval) of 0.34 (0.09 to 1.3). There were no serious, intervention-related adverse events.

Conclusions: This pilot trial among lung transplant recipients demonstrated acceptable recruitment and high retention and adherence. We demonstrated a signal for reduction of new skin cancer cases in those taking omega-3 FA supplements supporting the notion that a larger more definitive trial is warranted.

Introduction

Despite the very high risk of skin cancer in lung transplant recipients (LTRs) (1), little prevention research has been conducted and most control strategies have aimed for early detection through skin surveillance of LTRs, ideally in dedicated specialist skin clinics (2). However, primary prevention of skin cancer is achievable through behavior change and requires a much smaller outlay of resources than surveillance, for much greater long-term gain (3). In the general community, solar ultraviolet (UV) radiation is the main cause of skin cancer (4) and thus sun protection is the mainstay of prevention. In LTRs, immunosuppression is also a major driver of skin cancer and therefore other preventive strategies besides sun protection are required.

A large body of evidence suggests that diet can assist in prevention of various types of cancer (5) and the preventive potential of diet has been observed in several studies of skin cancer (6, 7) though evidence is relatively sparse. Diets with anti-inflammatory components like omega-3 FAs appear to hold promise since inflammation plays a major role in the pathogenesis of skin cancer. For example, hairless mice on diets rich in omega-3 FAs showed reduced skin tumor latency and inhibition of tumor multiplicity (8, 9) and decreased UV-induced immunosuppression (10) contributing to the observed chemo-preventive effect (8, 11).

In humans, omega-3 FA (fish oil) intake is associated with reduced cutaneous p53 expression (12, 13) and a reduced inflammatory sunburn response (12). Omega-3 FAs also appeared to reduce UV-induced cutaneous immune suppression in a randomized clinical study (14). In a systematic review of the literature on the association between consumption of omega-3 FAs and keratinocyte skin cancer (basal cell carcinoma (BCC) and squamous cell carcinoma (SCC)) and melanoma, high consumption was inversely associated with SCC development though non-significantly (pooled odds ratio (OR), 0.86) (15). Diets rich in omega-3 FAs have also been associated with decreased prevalence of actinic keratoses, lesions that are strongly predictive of skin cancer (16).

Despite their potential to prevent skin cancer (15), no studies have evaluated omega-3 FA supplementation in LTRs to reduce skin cancer risk. We therefore conducted a randomized controlled pilot trial to assess the feasibility and acceptability, and safety of daily supplementation with omega-3 FAs to prevent skin cancers in these patients. To inform future trials, we also assessed if omega-3 FA supplementation reduced skin cancer incidence.

Methods

Study participants

LTRs aged 18 years or more who were at least one year post-transplant and able to attend baseline and follow-up clinical assessments were recruited in the outpatient clinic at The Prince Charles Hospital, Brisbane, Australia between November, 2014 and February, 2015. LTRs unable to give informed consent, who had fish or soy allergy, were unable to take gelatin, had a bleeding disorder, bleeding episode in the last 3 months or were taking anti-thrombotic medication or omega-3 FA supplements, and those who were pregnant or at negligible risk of skin cancer due to innately dark or black skin, were not eligible. The trial was approved by two institutional ethics committees and registered with the ANZ Clinical Trial Registry (12614000873628). The full trial protocol can be obtained from the authors on request. Study protocols were in agreement with the guidelines set forth by Declaration of Helsinki, and all study participants provided written informed consent.

Randomization and intervention

This was a parallel, double blind, placebo-controlled randomized controlled pilot trial conducted over 12 months. The randomization sequence was derived from computer-generated random numbers and concealed from the study team. Treatment allocation was stratified by previous history of skin cancer (yes; no) with random allocation in a 1:1 ratio to either omega-3 FA or placebo supplements. All study participants, study coordinators, and pathology laboratory staff were blinded to treatment allocation. All capsules were identical in appearance and stored in identical containers.

Trial participants were required to take 4 x 1g capsules daily containing 3.36g total omega-3 FAs (46% eicosapentaenoic acid (EPA) ethyl ester, 38% docosahexaenoic acid (DHA) ethyl ester) (Omacor®) or 4 x 1g placebo capsules daily containing 4g of olive oil, as supplied by Pronova BioPharma, Lysaker, Norway.

Data collection

Participants completed questionnaires to provide socio-demographic information, and report their tanning ability and usual sun protection behaviors prior to randomization and at end of the study. A food frequency questionnaire was administered to measure usual dietary intake. Dates of transplantation and immunosuppression regimens were obtained from medical records. Skin color was assessed and categorized by dermatologists at baseline. All participants underwent full skin examination by dermatologically-trained physicians at recruitment and conclusion of the trial. Suspicious lesions were mapped on a body chart and affected participants were referred for further management. Histological confirmation of lesions diagnosed at study clinics or in the interim (as reported by participants during 3-monthly visits) was obtained through patients' referral physicians and/or pathology laboratories. All adverse events during the study period were recorded regardless of relation to the intervention.

Fasting blood samples were collected prior to randomization and at end of the intervention. Samples were centrifuged within 45 minutes of collection, and separated plasma was stored at –11 to –25°C until analysis. Plasma saturated and unsaturated fatty acids were assayed using procedures described previously (17). Briefly, acidic hydrolysis of plasma specimens was followed by basic hydrolysis and re-acidification. Hexane extraction then proceeded to derivatization with pentafluorobenzyl (PFB) bromide. Separation and detection of the corresponding PFB-esters were accomplished by capillary gas chromatography-electron capture negative ion mass spectrometry. Quantification was enabled by plotting the response ratios of samples and quality control against the standard calibration curve.

Data analysis

As this was a pilot study, no formal sample size calculation was carried out. However, we aimed to enroll sufficient numbers to show if the interventions were acceptable, the clinical evaluations were feasible and to provide a basis for calculating the sample size of a future definitive trial.

Feasibility and acceptability of the intervention were measured by rates of participation and retention and by adherence to daily supplementation as indicated by changes in the specific plasma fatty acids assessed, namely EPA, DHA, total omega-3 FA and omega-3: omega-6 FA ratio. Analysis of covariance (ANCOVA) was used to compare plasma FA status between treatment groups at the end of the study, accounting for potential heterogeneity of baseline plasma omega-3 FA status (18). Since plasma omega-3 FAs typically are not normally distributed, all values were log-transformed before undertaking ANCOVA. Safety was indicated by the number of participants who experienced adverse events determined to be possibly, probably, or definitely related to the allocated intervention.

The potential effect of omega-3 FA supplementation on skin cancer incidence was assessed by the difference in incidence of skin cancer in the intervention period between the active and placebo treatment groups. Skin cancer outcomes were SCC, BCC, total SCC and BCC combined, and total skin malignancies (SCC, BCC, melanoma, other rare skin malignancies). Person-based incidence was calculated as the rate of persons newly affected by skin cancer in each intervention group. ORs with 95% confidence intervals (CIs) were calculated by logistic regression to estimate the risk of developing any new skin cancer by allocation status. Tumor-based incidence, the rate of total new skin cancers was also estimated. Relative risks (RRs) with 95% CIs were calculated using generalized linear models with negative binomial distribution and person-time of follow-up as offset. All models were adjusted for the stratification variable, previous skin cancer and all analyses were performed using SAS version 9.4 (SAS Institute, Inc., Cary, NC, US).

Results

Recruitment and participant characteristics

Of 127 LTRs attending the outpatient clinic, 21 were ineligible or unable to take part, and of the remaining 106, 57 (54 %) declined, leaving 49 LTRs who agreed and consented to participate (Figure 1). Of these, 25 LTRs (median age 55 years) were allocated to FA acid supplements, and 24 (median age 54 years), were allocated to placebo supplements. Other baseline characteristics relevant to skin cancer risk, namely sex, education, skin color, occupational sun exposure, and years since transplantation were broadly similar between the groups, though more participants with skin prone to sunburn were allocated to placebo (17; 71%) than to omega-3 supplements (10; 40%), and fewer in the placebo (2; 8%) than omega-3 FA (8; 36%) group never applied sunscreen to the head/neck in summer (Table 1). Median time since transplantation was 2 years in the omega-3 FA group (range 1 to 21) and 3 years in the placebo group (range 1 to 13).

Retention

During follow-up, one death unrelated to the intervention occurred in the omega-3 FA group, and 6 (12%) participants withdrew, 2 from the omega-3 FA group (one listed for re-transplant, one gave no reason), and 4 from the placebo group (one listed for re-transplant, 2 recruited to another study, one too sick to participate). Thus, 42 (86%) participants completed the trial (88% and 83% of those allocated to omega-3 FA and placebo arms, respectively).

Adherence

Among the 42 LTRs completing the trial (22 omega-3 FA, 20 placebo), 34 (18 active, 16 placebo) provided fasting blood samples at study's end (the remainder provided non-fasting samples that were not informative). At baseline, plasma levels of EPA, DHA, total omega-3 FAs and omega-3: omega-6 ratio were comparable between the two groups; after 12-months' intervention, geometric mean values of all these measures increased substantially, and significantly more in the omega-3 FA than placebo group (Table 2)(Figure 2).

Safety

There were no serious adverse events related to omega-3 FA supplementation though two patients randomized to omega-3 FA supplements complained of mild gastrointestinal discomfort and two had minor bleeding episodes. In the placebo arm, five had gastrointestinal discomfort, three minor bleeding events and one reported renal problems.

Skin cancer

In addition to baseline and end-of-study skin examinations, 3 patients in the omega-3 FA group and 9 in the placebo group had external dermatologic evaluations, resulting in a median (range) of biopsies per patient of 0 (0–4) and 0 (0–21) in the groups, respectively. Incidence of persons developing new skin cancer in the total intervention period was reduced by two-thirds in the omega-3 FA group though not significantly (Table 3). Specifically, 5 (23%) participants developed SCC (median 280 days to first SCC), 4 (18%) developed BCC (median 347 days to first), and 6 (27%) developed skin cancer of any type in the active treatment group, compared with 9 (45%) who developed SCC (median 240 days to first SCC), 7 (35%) who developed BCC (median 148 days to first), and 11 (55%) who developed any skin cancer in the placebo group. Unadjusted ORs were consistently reduced in omega-3 FA vs. placebo supplement group; after adjustment for previous skin cancer, ORs remained substantially reduced in the intervention group (SCC, OR = 0.41 (95% CI 0.09 to 1.79); BCC, OR = 0.47 (95% CI 0.11 to 2.02); all skin cancer, 0.34 (0.09 to 1.32) (Table 3). To account for those developing multiple skin cancers, we compared total number of new tumors in each group and again the risk of developing a new skin cancer was consistently reduced in the omega-3 FA group after adjustment for previous skin cancer (RR = 0.43 (95% CI 0.14 to 1.34) (Supplementary Table 1)).

Discussion

To the best of our knowledge, this is the first randomized, placebo-controlled trial of omega-3 FA supplementation to reduce skin cancer occurrence in LTRs. We have shown that omega-3 FA supplementation among these patients is feasible and safe and moreover that omega-3 FA supplements have the potential to reduce LTRs' risk of SCC and BCC. Specifically, recruitment was reasonable at around 50%, retention was high at 86%, adherence was high indicated by the substantial

increase in plasma omega-3 FAs, and there were no serious adverse outcomes. The incidence of skin cancers decreased by a half to two-thirds in the omega-3 FA group compared with controls.

Regarding adherence in particular, plasma EPA and omega-3:omega-6 fatty acid ratio showed a substantial increase in the group allocated to omega-3 FA supplements, with a negligible increase in EPA in the placebo group and a small decrease in the omega-3:omega-6 ratio. Only a few participants did not adhere to the intervention, and only one reported they found 4 capsules daily excessive in addition to their usual medications. Although adherence to medication among organ transplant recipients is not well described (19, 20), similar increases in biomarkers of omega-3 FA intake have been reported, for example, in a randomized controlled trial (RCT) among kidney transplant recipients allocated to either 6g of total omega-3 FAs or 6g of coconut oil daily for 12-months (21). Adherence was assessed using plasma cholesterol esters and the results showed EPA concentration increased from a median of 0.39 mol% (range 0.18 to 10.31) to 4.7 mol% (0.89 to 10.31) in the omega-3 FA group but no change in controls after 6 months. Similar changes were seen among heart transplant recipients who took omega-3 FA supplementation vs. placebo over a 12-month period (22). In the current study, omega-3 FA status also increased in some of the placebo group, in particular, one participant's DHA level increased noticeably, but no other markers of omega-3 FA levels rose in the placebo group.

Of 106 eligible patients, around half declined to participate in the trial. The most frequently reported reasons were time constraints and lack of interest. However, once taking part, retention of participants was high with 86% retention over the 12-month intervention period. Previous systematic reviews of RCTs among kidney transplant recipients with omega-3 FA supplementation for longer than 3 months reported attrition rates up to 32%, with lower attrition rates over shorter intervention periods (19). Although attrition was low in our study, we failed to obtain plasma FA status from some trial participants mostly due to their non-fasting state when providing blood. Thus, to ensure that blood collected from participants is usable in any future similar study, sending reminders ahead of collection may be helpful. Further, using red blood cells from a non-fasting state and collecting small amounts

of blood via finger-pricks would reduce participants' burden. Concerning adverse events, participants in the placebo group reported more than in the omega-3 FA group. All reported similar complaints (mild gastrointestinal discomfort, episodes of unusual bleeding), consistent with reports in previous studies that used omega-3 fatty acid supplements and olive oil as placebo (19, 23-25).

When we explored the potential effectiveness of omega-3 FA supplementation for skin cancer prevention, we found skin cancer development was reduced by half to two-thirds in the omega-3 FA compared with the placebo group though in this pilot trial the decrease was not significant. This reduction was observed for the number of LTRs affected and the total number of new skin cancers they developed. Previous studies suggest omega-3 FAs, especially EPA, protect against skin cancer through reducing skin inflammation caused by UV exposure which initiates and promotes skin cancer development (12). In addition, omega-3 FAs may have immuno-modulating properties that inhibit tumor growth (14). The changes in plasma EPA and omega-3:omega-6 FA ratio in the omega-3 FA supplement group also indicate their balance shifted in a favorable direction. EPA and DHA are long chain omega-3 fatty acids which exist in different forms in the body, and are further metabolised by several enzymatic and non-enzymatic processes. Major enzymes involved in their metabolism include cyclooxygenases and lipoxygenases, and as partial agonists of the omega-6 fatty acids their competition with the latter for metabolism results in production of a range of mediators including prostaglandins, leukotrienes and hydroxy fatty acids with a generally anti-inflammatory and anti-carcinogenic profile (26). Levels of omega-3 fatty acids vary in the diet, but are generally very low, and correlate with the amounts found in serum and skin (27). Their epidermal lipid content increases on daily omega-3 fatty acid supplementation (14). However, it appears that the omega-3: omega-6 fatty acid ratio is of greater relevance than the level of omega-3 fatty acids in protection of the skin from ultraviolet radiation, as explained by their partial agonist effects (26). Historically, amounts of omega-3 and omega-6 fatty acids in the human diet were more balanced (28). This is now skewed towards omega-6 fatty acids in most populations; supplementation helps address this imbalance. Therefore, achieving a higher ratio meant reduced pro-inflammatory eicosanoid metabolites and reduced inflammatory status (29) perhaps mitigating skin cancer occurrence.

Limitations of this study include the allocation of more sunburn-prone participants to the placebo than active treatment group. This imbalance may have influenced observed reduced skin cancer occurrence in the omega-3 FA group. Further, the sample size was small and this study was not designed to formally assess the clinical effectiveness of omega-3 FA supplementation. Thus, interpretation of results requires caution. Another limitation of this pilot study, arising from its small number of participants, was the additional imbalance in duration of immunosuppression between the randomized groups whereby the placebo group had been immunosuppressed for a year longer on average. This discrepancy could also have influenced the observed reduction of new skin cancer cases in the omega-3 fatty acid group compared with the placebo group. As well, the lack of capsule counts in the trial participants was a potential limitation. After baseline, participants were to return their capsules at each 3-monthly visit but a number did not, making it impossible to assess adherence by capsule count. On the other hand, capsule counts are known to be unreliable and may overestimate adherence (30, 31), while we showed high adherence in the active group by the measurement of plasma omega-3 FA levels. Finally, the findings from this study in Australia may not be generalizable to LTRs elsewhere.

In conclusion, this randomized controlled pilot trial of omega-3 FA supplements to prevent skin cancer among LTRs showed it to be feasible and safe, with most participants adhering to the intervention and able to complete the study. We also explored the potential effects of omega-3 FA supplementation on skin cancer incidence and found a potential protective effect in these high-risk immunosuppressed patients. A full-scale RCT is now warranted.

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Figure 2: Changes in plasma eicosapentaenoic acid (EPA) status from baseline to the end of study (12-months) by intervention status

Each line indicates each participant EPA status at each point.

Figure
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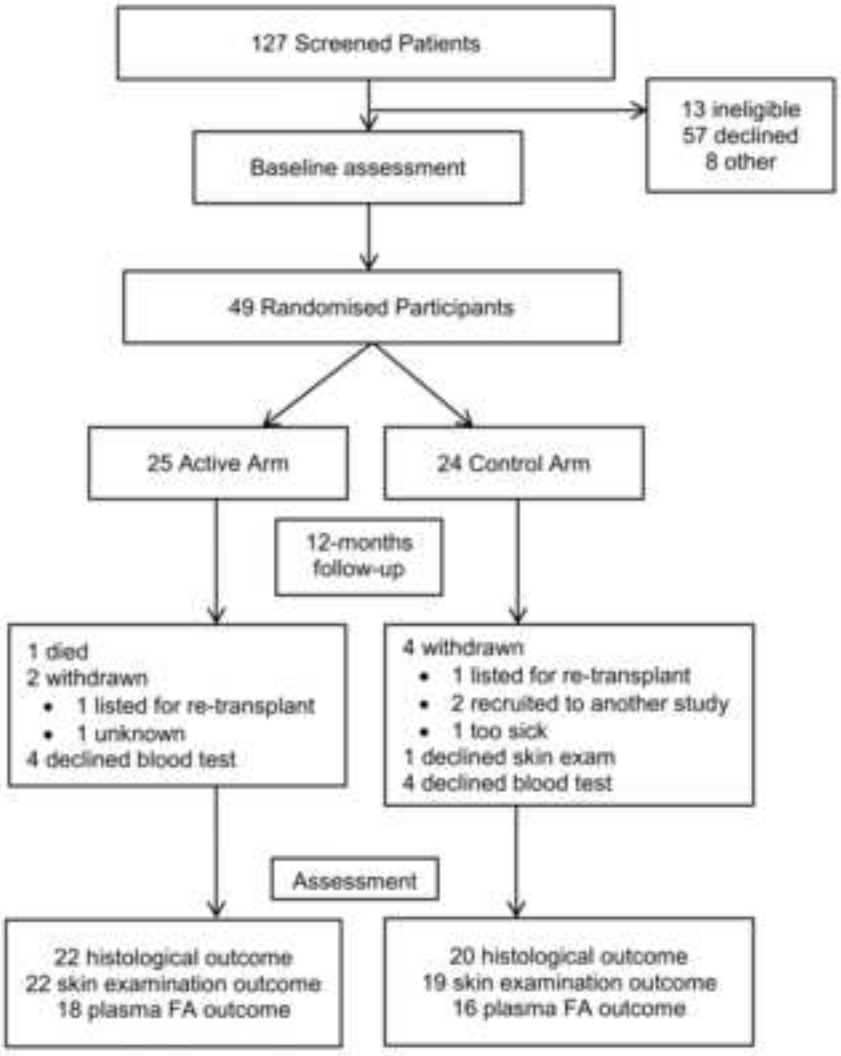

Figure 1: Flow diagram of O3 Pilot Study

Figure

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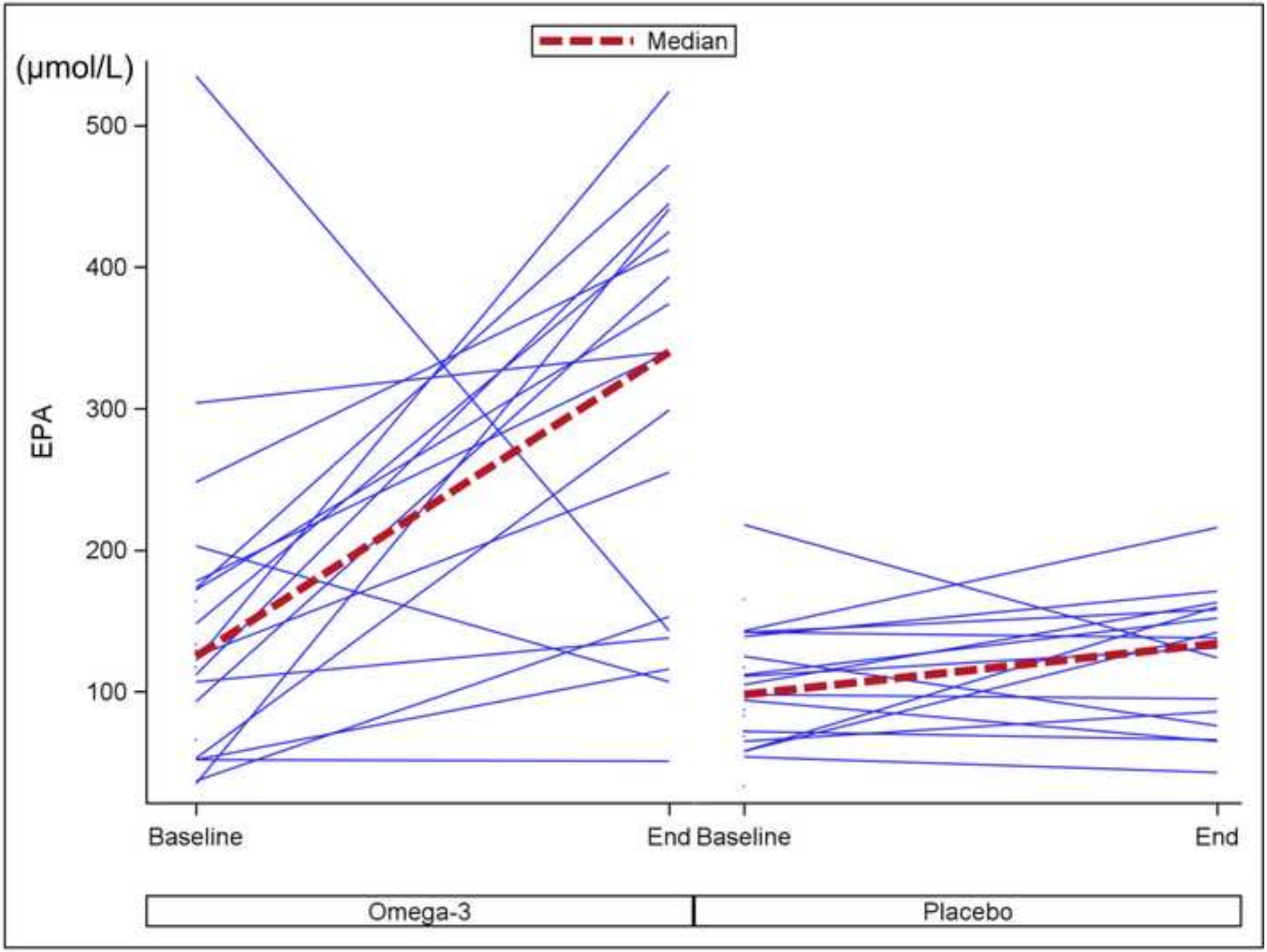

Table 1: Baseline characteristics of participants (N=49)

	n (%)	
	Active (n=25)	**Placebo (n=24)**
Males	17 (68)	16 (67)
Age [median (min max)] (years)	55 (32, 66)	54 (29, 75)
Highest education		
Completed high school or less	13 (52)	11 (46)
Trade/technical/diploma/University/college	12 (48)	13 (54)
Previous skin cancer	13 (52)	14 (58)
Skin reaction to acute sun		
Not burn	7 (28)	4 (17)
Burn a little	8 (32)	3 (13)
Burn moderately/burn badly	10 (40)	17 (71)
Skin color		
Medium/Olive	9 (36)	7 (29)
Fair	16 (64)	17 (71)
In summer, used sunscreen to face/head/neck		
None	8 (36)	2 (2)
1–4 days/week	6 (27)	15 (63)
≥5 days/week	8 (36)	7 (29)
Main occupation type		
Mainly indoors	12 (48)	12 (50)
Mainly outdoors	4 (16)	3 (13)
Both indoors and outdoors	9 (36)	9 (38)
Years since transplant [median (min, max)]	2 (1, 21)	3 (1, 13)
1 to < 2	7 (28)	5 (21)
2 to < 5	10 (40)	9 (38)

5 to < 10	3 (12)	6 (25)
≥10	5 (20)	4 (17)

Due to the missing information, not all total add to N=49

Table 2: Plasma fatty acid status at the baseline and 12-months follow-up [Median (min, max)]

	Baseline		**12-months follow-up**		p-value on group difference[a]
	Active (n=23)	Placebo (n=23)	Active (n=18)	Placebo (n=16)	
EPA (µmol/L)	125.0 (35.0, 535.0)	98.0 (33.0, 218.0)	340.0 (51.0, 524.0)	134.5 (43.0, 216.0)	<0.001
DHA (µmol/L)	203.0 (99.0, 337.0)	164.0 (85.0, 302.0)	218.5 (128.0, 342.0)	156.0 (69.0, 277.0)	0.006
Total omega-3 (mmol/L)	0.6 (0.3, 1.2)	0.4 (0.2, 0.8)	0.7 (0.4, 1.2)	0.5 (0.2, 0.6)	0.004
Omega-3:omega-6 ratio	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)	0.2 (0.1, 0.4)	0.1 (0.1, 0.2)	<0.001

[a] p-values from ANCOVA

EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

Table 3: Number of persons affected by incident skin cancers according to randomized status, and odds ratios (OR) of skin cancer risk (n=42)

	n (%)		OR (95% CI)[a]	
	Active	Placebo	Unadjusted	Adjusted[b]
SCC	5 (23)	9 (45)	0.36 (0.10, 1.36)	0.41 (0.09, 1.79)
BCC	4 (18)	7 (35)	0.41 (0.10, 1.71)	0.47 (0.11, 2.02)
SCC+BCC	6 (27)	11 (55)	0.31 (0.09, 1.11)	0.34 (0.09, 1.32)
SCC+BCC+other[c]	6 (27)	11 (55)	0.31 (0.09, 1.11)	0.34 (0.09, 1.32)

[a] Reference=Placebo group

[b] Adjusted for previous skin cancer.

[c] Other skin cancer: n=1 keratoacanthoma, n=1 other rare skin cancer. Both occurred in the placebo group, in people who were already affected by BCC/ SCC.

BCC: basal cell carcinoma; CI: confidence interval; SCC: squamous cell carcinoma

Supplementary Material for online publication only

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