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n-3 index is associated with cardiometabolic risk factors but is not improved by walnut intake in free-living elderly: a single-blind, randomised controlled trial

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Abstract

n-3 index, the erythrocyte proportion of the EPA + DHA fatty acids is a clinical marker of age-related disease risk. It is unclear whether regular intake of α-linolenic acid (ALA), a plant-derived *n*-3 polyunsaturated fatty acid, raises *n*-3 index in older adults. Of the 356 participants at the Loma Linda, CA centre from the original study, a randomly selected subset (*n* 192) was included for this secondary analysis (mostly Caucasian women, mean age 69 years). Participants were assigned to either the walnut (15 % of daily energy from walnuts) or the control group (usual diet, no walnuts) for 2 years. Erythrocyte fatty acids were determined at baseline and 1-year following intervention. No differences were observed for erythrocyte EPA, but erythrocyte DHA decreased albeit modestly in the walnut group (–0·125 %) and slightly improved in the control group (0·17 %). The change in *n*-3 index between the walnut and control groups was significantly different only among fish consumers (those who ate fish ≥ once/month). Longitudinal analyses combining both groups showed significant inverse association between the 1-year changes of the *n*-3 index and fasting plasma TAG ($\beta = -10$), total cholesterol ($\beta = -5{\cdot}59$) and plasma glucose ($\beta = -0{\cdot}27$). Consuming ALA-rich walnuts failed to improve *n*-3 index in elders. A direct source of EPA/DHA may be needed to achieve desirable *n*-3 index, as it is inversely associated with cardiometabolic risk. Nevertheless, incorporating walnuts as part of heart healthy diets is still encouraged.

Keywords: **Cardiometabolic disease: α-Linolenic acid: *n*-3 Index: Walnuts**

With an increase in the global ageing population, there is a concomitant rise in the prevalence of cardiometabolic diseases[1], which may be prevented with a diet abundant in fruits, vegetables, legumes, nuts and fatty fish[2,3]. These foods are rich with nutrients and other bioactive compounds, including polyphenols, phytosterols, fibre, antioxidants, micronutrients and essential fatty acids that independently and synergistically play a role in improving the health of the aging population[4,5]. One of the fundamental nutrients that fosters cardioprotective benefit is *n*-3 PUFA, especially EPA and DHA acids[6]. The *n*-3 index, a composite clinical marker, expressing the combined fraction of the erythrocyte EPA and DHA, is associated with disease risk and improves when a direct source of EPA and DHA is consumed[7–9]. In contrast, the rate of conversion of the most abundant plant *n*-3 PUFA (α-linolenic acid, ALA) to EPA and DHA is not very efficient[10].

A tree nut particularly rich in ALA are walnuts, which is associated with reduced risk of CVD incidence and all-cause mortality[4]. The cardioprotective effects of walnuts are partly attributed to their ability to lower atherogenic lipoproteins including LDL-cholesterol and inflammation[11,12]. These benefits underlie the presence of bioactive phytochemicals (mainly antioxidants such as polyphenols and phytosterols), fibre and PUFA, including the *n*-3 PUFA, ALA. Given that ALA can be converted to the longer chain EPA and to a lesser extent DHA[13–16], there has been much interest in whether consumption of ALA-rich foods

Abbreviations: ALA, α-linolenic acid; TC, total cholesterol.

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truly translate to an increase in the *n*-3 index, and if some of the cardioprotective benefits of walnuts may be exerted partly via increases in *n*-3 index.

We recently concluded a dual-centre, randomised controlled trial in healthy elders on cognition and age-related macular degeneration following consumption of walnuts over 2 years[17]. In a randomly selected subset of the study cohort from one of the centres, we investigated whether dietary supplementation with walnuts for one year would increase the *n*-3 index in the elderly participants. As a secondary aim, we determined the association between changes in *n*-3 index and selected cardiometabolic risk factors within the same population independent of the intervention.

Materials and methods

Study design and population

This study was a secondary analysis using data from the Walnuts and Healthy Aging study, a randomised controlled, clinical trial conducted in Loma Linda, CA, USA, and Barcelona, Spain, to determine the effect of walnut consumption over 2 years on healthy aging including cognition among elderly subjects (clinicaltrials.gov: NCT01634841). The detailed study design, methods and objectives/aims of the Walnuts and Healthy Aging study have been previously published[17]. The original study protocol was approved by the Institutional Review Board at Loma Linda University, and the Hospital Clinic at Barcelona, Spain and conducted in accordance with the Declaration of Helsinki. All participants provided a written informed consent before enrollment into the study.

The original study utilised a parallel design, with cognitively healthy elderly participants, aged 63–79 years, randomly assigned to either a walnut diet (15 % of energy of the usual diet from walnuts) or control diet (usual diet, abstaining from walnuts) for two years. Briefly, a multi-stage recruitment process was followed to screen and select eligible participants. Candidates w were morbidly obese, had uncontrolled chronic diseases and were allergic to walnuts consumed tree nuts more than 2 servings/week and/or other significant dietary sources of *n*-3 PUFA such as flaxseed oil and fish oil were excluded. After obtaining baseline measurements, eligible participants were randomly assigned to either the walnut or the control group. For this secondary data analyses, we utilised only a randomly selected subset (*n* 192) from a total of 356 participants at the Loma Linda, CA centre following one year of intervention. This duration (1 year) is more than adequate to detect changes in the erythrocyte membrane fatty acids. We selected participants from only one centre (Loma Linda, CA), to avoid confounding by EPA/DHA intakes that might differ in the two geographically diverse cohorts. Our primary objective was to determine the *n*-3 index following consumption of walnuts for one year compared with the non-walnut control group. As a secondary aim, we combined both groups and considered the association between the changes in the *n*-3 index and changes in selected cardiometabolic disease risk factors in the entire cohort of the sub study.

Intervention

Subjects in the walnut group continued their habitual diet and in addition were provided with 1–2 ounces/d (30, 45 and 60 g/d) of packaged walnuts equivalent to ≈ 15 % of their estimated daily energy calculated using the WHO formula for energy for adults over 60 years of age and the Harris-Benedict Equations as previously described[17]. Walnuts could be eaten all at once, or spread throughout the day, eaten alone or incorporated into other foods. The control group continued their habitual diet, while abstaining from walnuts and other high ALA foods (such as flaxseed oil) for the duration of the study. To ensure compliance with study protocols, all subjects visited the clinic every 2 months to meet with a trained registered dietitian. Those in the walnut group were provided with two-month supplies of raw, shelled walnuts in packs containing the daily dose for each participant.

Data collection and procedures

The complete data collection protocols of the original study are described elsewhere[17]. During clinic visits, at baseline and then every 2 months body weight (to the nearest 0·1 kg) was measured with a calibrated bio-impendence scale and blood pressure with a Semiautomatic oscillometer (Omron 705-CP, Omron Healthcare Group). All participants provided fasting blood samples at baseline and yearly thereafter. The blood samples were centrifuged to separate plasma and erythrocyte pack. Both fractions were aliquoted and stored at –80°C. At the end of the study, both plasma and erythrocyte samples were shipped as a single batch to the appropriate laboratories for biochemical determination. Fasting plasma glucose and lipids, including LDL-cholesterol, HDL-cholesterol, total cholesterol (TC) and TAG,were assessed according to previously published methods[11].

The fatty acid profiling of erythrocyte was performed in a randomly selected sub-sample (*n* 192), according to the method described in Sala-Vila et al.[7]. In brief, erythrocyte were haemolysed and spun. Then, the pellet containing the erythrocyte membranes were dried and dissolved in 1 mL BF_3 methanol solution. The solution was then heated for the hydrolysis and methylation of glycerophospholipid fatty acids. To isolate the fatty acid methyl esters, N-hexane was used. GC (Agilent HP 7890 Gas Chromatograph equipped with a 30 m × 0·25 µm × 0·25 mm SupraWAX-280 capillary column (Teknokroma), a flame ionisation detector and an autosampler) was used to separate the fatty acids. The amount of each fatty acid was expressed as a percentage of total identified fatty acids in the erythrocyte sample. For the current investigation, the *n*-3 index was computed by expressing the sum of EPA plus DHA as a percent of total erythrocyte fatty acids.

Dietary intake from five telephone-administered dietary recalls was analysed using Nutrition Data System for Research (version 2013, Nutrition Coordinating Center, University of Minnesota, Minneapolis, MN, USA). The recalls were spaced at regular intervals to capture seasonal variations in food intake. The diet recalls were unannounced, included one weekend day, with an average of 2 recalls determined over the first year and 3 recalls over the second year. Since preliminary analysis of diet

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recalls between year one and year two was not different for important diet variables including type of fat and total energies (data not reported), and since five diet recalls would provide a more robust estimate, we chose to use the mean of the five 24-hour diet recalls for the secondary analyses. Fish intake (not distinguished by the type of fish) is presented as a binary variable (those that ate fish at least once a month *v.* non-consumers) for the purpose of adjusting for them in the statistical models. The compliance to the study protocol and intervention were assessed by (a) study dietitian reviewing the diary for any deviations from the study protocol at each clinic visit, (b) assessing nutrient intake from the 24-hour diet recalls and (c) determining the erythrocyte membrane ALA at baseline and end of year one.

Statistical analyses

For the secondary analyses, we included *n* 192 subjects (ninety nine in the walnut and ninety three3 in the control group) of the 356 participants that were randomised in the Loma Linda centre (Fig. 1). Only a subset of participant samples from both centres was analysed for the erythrocyte fatty acids, and thus all 192 at the Loma Linda centre with erythrocyte fatty acid determinations were included in this study. According to sample size calculations using two-sample *t* test comparing the change in *n*-3 index between two treatment groups with α level of 5 %, power of 80 % and effect size of 0·8 %, 24–48 subjects would have been sufficient. Thus, with *n* 192, our study was more than adequately powered. Based on distribution of the variables, data are displayed as either mean ± SD or median (interquartile range). Baseline differences between participants from the walnut group and the control group were assessed by 1-factor ANOVA, Mann–Whitney or the χ^2 test, as appropriate.

To estimate significant differences between the two groups for *n*-3 index changes from baseline to year 1, a general linear model was used. An independent variable of main interest was the group (walnut or control), and the dependent variable was 1-year change of *n*-3 index (1 year minus baseline). The model was adjusted for fish (consumers *v.* non-consumers), *n*-6 PUFA intake (linoleic acid + arachidonic acid) and total energy intake.

To investigate whether the change in *n*-3 index (independent variable) of both groups combined was associated with the changes in selected cardiometabolic risk factors from baseline to year 1 (dependent variables), linear regression models were fitted for LDL-cholesterol, TC, TAG, HDL-cholesterol, systolic blood pressure, diastolic blood pressure, fasting glucose and body weight. The associated beta values, standard errors and *P* values were reported for every association. The final regression model adjusted for age, gender, race, education, baseline weight, total energy, physical activity (metabolic equivalents) hours/week), fish (consumers *v.* non-consumers), saturated fat, *n*-6 PUFA, 1 and the baseline dependent variable. In addition to the above listed confounders, additional dietary variables for plasma TAG (total carbohydrate), plasma LDL-cholesterol, TC and body weight (total fibre intake), fasting blood glucose (sucrose intake) and for systolic blood pressure and diastolic blood pressure (Na intake) were adjusted for. Several assumptions were satisfied before conducting the analysis. All variables and their residuals were normally distributed except for fasting plasma glucose. A natural-log transformation was thus used for the change variable of glucose (between baseline and year 1) which normalised the residual distribution. The linearity assumption was verified by partial regression plots. Statistical analysis was conducted using the SPSS software, version 25 (IBM Corp.).

Results

Table 1 summarises the baseline characteristics of participants. None of the variables were significantly different between the two groups at baseline except for HDL-cholesterol. The mean age of the participants was 69 ± 3 years and mean BMI was 27·4 ± 4 kg/m^2. Participants were mostly non-smokers, Caucasian women, with an average education of 16 years. As reported previously[17], objective compliance to the intervention food (walnuts) was very high, as noted by an increase in erythrocyte membrane ALA levels from baseline to year one in the walnut compared with the control group. The change in the erythrocyte ALA within the LLU cohort mirrored the changes observed when both centre participants were combined (mean change from baseline to one year for walnut group in Loma Linda, CA centre was 0·07 % total fatty acids; control group in Loma Linda centre, CA was –0·06 % total fatty acids, $P < 0·01$ between the two groups).

The group differences in *n*-3 index, erythrocyte EPA and DHA between baseline and year 1 are presented in Table 2. At baseline, there were no significant differences between the walnut and control groups for erythrocyte EPA or DHA. While there were no differences between the two groups for erythrocyte EPA from baseline to one year, a small, but statistically significant 1-year changes in erythrocyte DHA were observed. The walnut group had a slightly higher *n*-3 index at baseline (4·86 %) compared with the control group (4·40 %) but was not statistically significant. The change in *n*-3 index from baseline to 1-year was also not significant.

Given that the intake of EPA and DHA of participants of the Loma Linda centre at baseline was low (0·1 % of total energy, ~ 160 mg/d)[11] and did not change over the study period, we considered fish intake as a binary variable. Sensitivity analyses on the *n*-3 index changes from baseline to year 1 and between-group differences stratified by fish intake are shown in Table 3. Among fish consumers (those who consumed fish at least once/month), we found a statistically significant decrease of the *n*-3 index in those allocated into the walnut arm, while the opposite was observed for the control group, translating into a significant 1-year differences between groups ($P = 0·009$). No statistically significant changes were observed in those not consuming fish in either of the two groups.

Table 4 shows the association between the change in the *n*-3 index and the change in various cardiometabolic risk factors between baseline and year 1 for both groups combined after multiple-factor adjustment. Significant inverse associations were observed between the change in *n*-3 index and the change in TC and TAG. With every 1 % increase in the *n*-3 index there was a 5·59 mg/dL decrease in TC ($\beta = -5·59$; $P < 0·01$) and a 10 mg/dl

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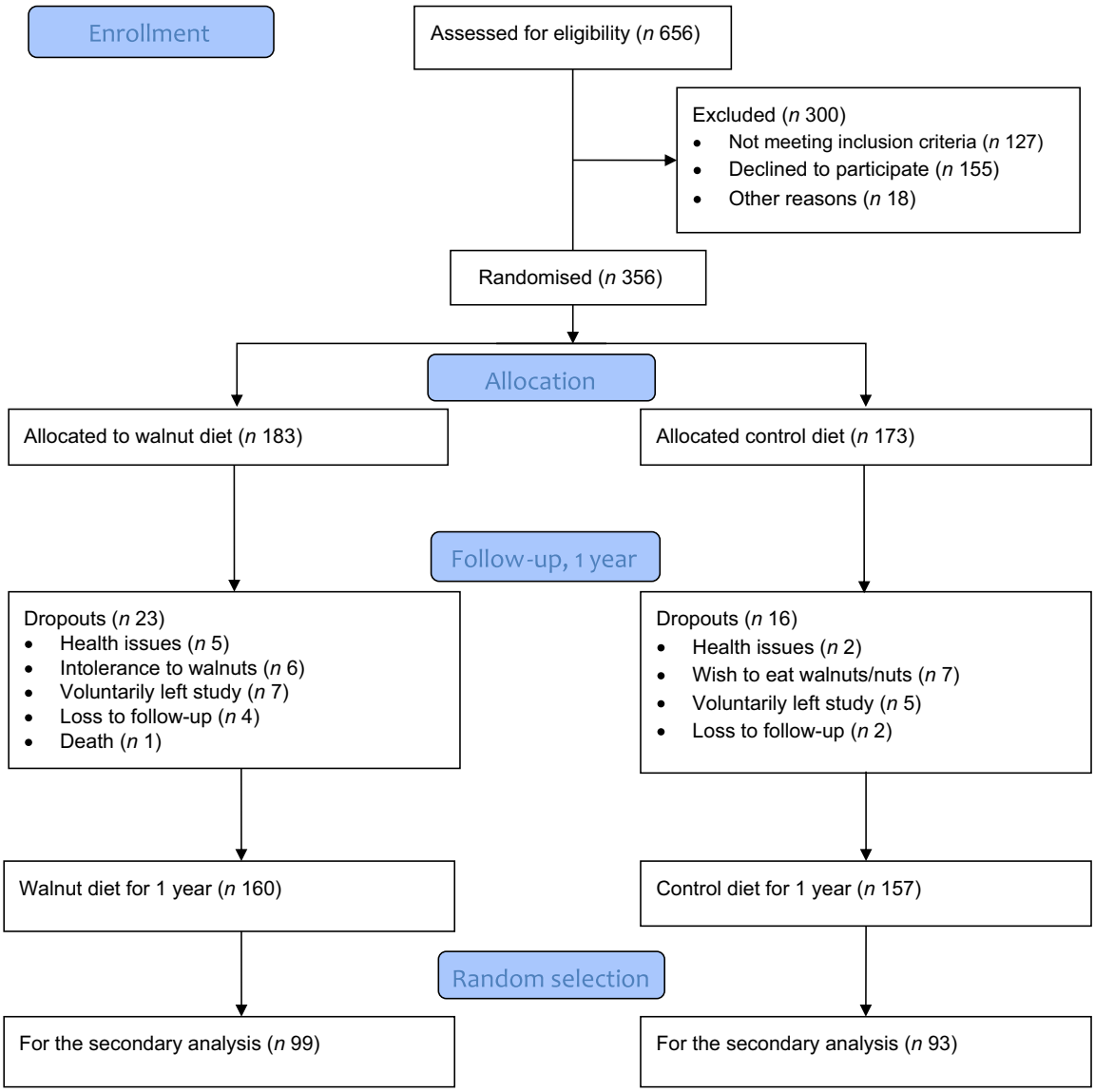

Fig. 1. Flow chart of recruitment and final selection of participants from the Loma Linda center. Secondary analysis represents the data set utilised to determine *n*-3 index (*n* 99 in the walnut and *n* 93 in the control group within Loma Linda center).

decrease in TAG ($\beta = -10$; $P < 0{\cdot}01$). A significant inverse association was also observed between the change in *n*-3 index and natural-log transformed change in fasting blood glucose ($P = 0{\cdot}01$). The β coefficient of –0·27 associated with this variable indicates that a 1-unit increment in the *n*-3 index was associated with ~24 % reduction in fasting plasma glucose. No significant associations were observed between the 1-year change in *n*-3 index and other cardiometabolic risk factors (HDL-cholesterol, LDL-cholesterol, systolic blood pressure, diastolic blood pressure and body weight).

Discussion

Consumption of ALA-rich walnut daily for 1 year by older adults was ineffective in increasing their *n*-3 index. It appears that a direct source of EPA and/or DHA may be required to improve *n*-3 status[18,19]. The well-documented cardio- and neuroprotective benefits of walnuts[4,11,12] may be mediated by mechanisms other than increases in the *n*-3 index. It is likely that ALA and other bioactive compounds in walnuts such as polyphenols, phytosterols, antioxidants and fibre, independently and synergistically confer these health benefits[4,20,21].

Since the *n*-3 index is the sum of both EPA and DHA expressed as a percent of the total fatty acids, any increase to either EPA or DHA or both would be expected to increase the *n*-3 index. Studies with ALA supplementation have demonstrated an increase in erythrocyte membrane ALA and EPA levels, but not DHA because conversion of ALA to DHA is much restricted[22–24]. Consistent with our observations, ALA-rich flaxseed oil or milled flaxseed failed to improve *n*-3 index[16], while ALA-rich hempseed oil increased *n*-3 index, but in younger subjects with very low baseline *n*-3 index (2·52 %) compared with what was noted in other studies of older adults with baseline

Table 1. Baseline characteristics of study participants (*n* 192)

Variables	Walnut group *n* 99	Variance	Control group *n* 93	Variance	*P* value
Age, years	69·4	3·4	68·7	3·0	0·212
Men, *n* (%)	34	34	28	30	0·493
White, *n* (%)	78	79	71	76	0·408
Education, years	16·1	2·4	15·8	2·6	0·421
Non-smoker, *n* (%)	96	9·7	93	10	0·201
BMI, kg/m^2	27·8	5·2	27·1	4·8	0·396
Physical Activity, METs h/week	2·61	2·63	3·6	3·12	0·105
Systolic BP, mmHg	128·1	14·8	127·8	16·0	0·873
Diastolic BP, mmHg	78·4	9·0	78·2	9·5	0·841
TC, mg/dl	187·7	38·3	196·2	39·4	0·133
LDL-cholesterol, mg/dl	112·1	30·0	117·5	32·4	0·244
HDL-cholesterol, mg/dl	54·0	15·6	59·1	14·6	0·020*
TAG, mg/dl	108·0	52·3	98	40·5	0·142
FBG, mg/dl	97·1	12·9	98·2	15·0	0·811

MET, metabolic equivalent; Systolic BP, systolic blood pressure; Diastolic BP, diastolic blood pressure; TC, total cholesterol; FBG, fasting blood glucose.
Data displayed as mean and standard deviation for age, years of education, BMI, systolic, diastolic BP, TC, LDL, HDL and TAG; data displayed as median and interquartile range for physical activity and FBG; gender, ethnicity and smoking status are denoted as mean and %.
P value generated through χ^2 for categorical variables and independent *t* test (or Mann–Whitney) for continuous variables.
* Denotes statistically significant *P* value.

Table 2. Changes in the *n*-3 index from baseline to year 1 between the walnut and control groups

		Walnut group *n* 99	95 % CI	Control group *n* 93	95 % CI	*P*
n-3 index (%)	Baseline	4·861	4·532, 5·201	4·404	4·011, 4·701	
	Final	4·763	4·441, 5·101	4·513	4·200, 4·840	
	Change	–0·112	–0·352, 0·143	0·162	–0·102, 0·413	0·231
Erythrocyte EPA (%)	Baseline	0·728	0·665, 0·792	0·677	0·612, 0·742	
	Final	0·737	0·676, 0·798	0·667	0·604, 0·730	
	Change	0·009	–0·052, 0·069	–0·010	–0·073, 0·052	0·674
Erythrocyte DHA (%)	Baseline	4·009	3·807, 4·212	3·813	3·605, 4·021	
	Final	3·884	3·693, 4·076	3·986	3·789, 4·183	
	Change	–0·125	0·263, 0·013	0·174	0·032, 0·315	0·004*

Data, expressed as means and 95 % confidence interval), after adjusting for *n*-3 supplement intake (yes *v.* no), total energy intake, dietary fish consumption (consumers *v.* non-consumers) and dietary *n*-6 fatty acid intake.
* Denotes statistically significant *P* value.

Table 3. Baseline and 1-year changes in *n*-3 index by intervention group and self-reported fish consumption

Fish consumer	*n*-3 index	Walnut group *n* 99	95 % CI	Control group *n* 93	95 % CI	*P*
Yes†	Baseline	5·401	4·750, 6·042	4·504	3·862, 5·053	
	Final	4·864	4·230, 5·506	5·121	4·530, 5·712	
	Change	–0·544	–1·043, –0·033	0·674	0·202, 1·141	0·009*
No‡	Baseline	4·477	4·101, 4·862	4·172	3·746, 4·617	
	Final	4·473	4·121, 4·820	4·103	3·702, 4·502	
	Change	–0·000	–0·261, 0·261	–0·121	–0·401, 0·122	0·651

Fish consumers defined as consuming fish once or more per month.
Data, expressed as means (95 % confidence interval), after adjusting for total energy intake, and dietary *n*-6 fatty acid intake.
* Denotes statistically significant *P* value.
† *n* 46 (walnut group) and 49 (control group).
‡ *n* 53 (walnut group) and 44 (control group).

n-3 index of ~ 4–5 %.[14]. Even though our study participants complied with the walnut intake as reflected by an increase in the erythrocyte ALA[17], the intervention failed to increase erythrocyte membrane EPA and even slightly decreased DHA. It has been noted that DHA is more effective than EPA in raising the *n*-3 index,[23] which explains partly the lack of improvements in *n*-3 index following walnut ingestion. Others have observed that even with a dose as high as 8 g of ALA/d/2400 kcal[25], there is an increase in erythrocyte ALA, EPA and docosapentaenoic acid, but not DHA[16,22]. Since the duration of the interventions in many of the trials with ALA-rich foods was between 8 and 12 weeks[16,22,25], we considered that perhaps a longer duration (more than 6 months) may increase both erythrocyte EPA and DHA, and thus the *n*-3 index. However, it appears that duration is of no consequence in this metabolic process.

In the body, ALA is mostly used to produce energy with a small amount utilised in the production of long-chain *n*-3 PUFA[23,24]. Ageing may also cause a decline in the desaturase

Table 4. Association between 1-year changes in *n*-3 index and 1-year changes in selected cardiometabolic risk factors for both groups combined, independent of the intervention* (*n* 192)

Cardiometabolic risk factors (1 year-Baseline)	*β* Coefficients	Std. Error	*P* value
TC, mg/dl	–5·591	2·202	0·010
TAG, mg/dl	–10·000	3·021	0·010
LDL-cholesterol, mg/dl	–3·000	1·695	0·082
HDL-cholesterol, mg/dl	–0·652	0·566	0·253
SBP, mmHg	–0·571	1·132	0·611
DBP, mmHg	–1·000	0·807	0·214
Glucose, mg/dl	–0·274	0·102	0·013
Body weight, kg	–0·203	0·273	0·472

TC, total cholesterol; SBP, systolic blood pressure; DBP, diastolic blood pressure.
TAG was additionally adjusted for total baseline carbohydrate intake.
LDL-cholesterol, TC and body weight also adjusted for total baseline dietary fibre.
Glucose was additionally adjusted for baseline sucrose intake.
Systolic and diastolic blood pressures were also adjusted for baseline Na intake.
* Data adjusted for age, gender, race, education (years), baseline weight, total energy, treatment effect (group type), physical activity, dietary fish intake, saturated fat and the baseline-dependent variable.

activity[24] and may explain in part the poor conversion of ALA to longer chain *n*-3 PUFA in our study participants. High intake of EPA can impede the activity of the delta 6 desaturases needed to convert ALA into its longer-chain derivatives[24]. This may explain our finding of a decline in the *n*-3 index only among fish eaters (at least 1 serving/month) in the walnut compared with the control group participants. We have previously observed that even when the background diet has a 2:1 ratio of *n*-6 PUFA to *n*-3 PUFA with the inclusion of ALA-rich food sources such as walnuts and flaxseed, there is poor conversion of the ALA to DHA, but not to EPA[25]. Our findings are congruent with those of other studies conducted in vegetarians and non-vegetarians[18,24]. Thus, many factors may have played a role in the lack of increase in *n*-3 index with the intake of ALA-rich walnuts including the age of the participants, noted poor conversion of ALA to DHA and perhaps an interaction of the background diet EPA and DHA and the conversion of ALA to these long-chain *n*-3 PUFA.

An *n*-3 index of 8 % or higher is associated with the lowest risk for CVD[9]. However, our participants were at the intermediate risk category (*n*-3 index of > 4 % to < 8 %) at baseline, and the changes observed were small (~1 %) after one-year supplementation with walnuts. Thus, we attribute the opposing direction of the change observed in *n*-3 index among fish eaters between the walnut and control group participants to chance finding, as overall the dietary intake of EPA and DHA at baseline of our study participants was low (~160 mg/d) and did not change much over the study duration. At the least, future studies should further explore the interaction between background EPA/DHA and the efficiency of conversion of ALA to these long chain *n*-3 PUFA. In contrast, when a direct source of EPA/DHA either through diet or supplement is consumed, for a dose of 900 mg/d, only 5 months may be required to achieve *n*-3 index values of 8 % or higher[7,26]. Overall, long-term consumption of dietary ALA is not sufficient to achieve an adequate *n*-3 status indicated by the *n*-3 index and may require a direct source of EPA and DHA to accomplish the same.

n-3 index is a biomarker for *n*-3 status, but it is also associated with cardiometabolic risk factors. Since the *n*-3 index did not change with walnut consumption in our cohort, we combined all participants and determined the association between changes in the *n*-3 index over one year with changes in the cardiometabolic disease risk factors. Our findings of an inverse association between the *n*-3 index and TC, TAG and blood glucose are consistent with some of the other studies on relatively younger and overweight populations[27–29]. *n*-3 PUFA downregulate hepatic lipogenesis, increase fatty acid oxidation in the liver and skeletal muscle and improves the flux of glucose to glycogen resulting in a reduced production of VLDL,[30] a TAG-carrying lipoprotein. It appears that men have a more pronounced lipid-lowering effect than women[11,31]. Evidence is somewhat contradictory when it comes to the association between *n*-3 PUFA and total and LDL-cholesterol.[32,33] Pooled meta-analyses point to a direct association between intake of *n*-3 fatty acids and LDL-cholesterol, specifically the larger and less atherogenic LDL particle,[34] while 12-week supplementation of a marine (fish oil) or plant-based (soybean oil) *n*-3 PUFA in older adults with hypertension and/or hypercholesterolaemia led to a significant reduction in LDL-cholesterol in both groups after 3 months[32]. Among our elderly cohort, we previously reported that walnut consumption led to significant shift of the lipoprotein subclass phenotype to a less atherogenic profile.[11] In the absence of an increase in *n*-3 index following walnut consumption, this may be attributed in part to improved membrane fluidity and increased affinity of LDL apo B-100 to LDL receptors[35] with enhanced LDL clearance. Additionally, the synergistic interaction of bioactives such as polyphenols, fibre, antioxidants and the essential fatty acids in walnuts[21] also seems to favourably modify cardiovascular risk factors.

The role of *n*-3 PUFA on glucose metabolism and type-2 diabetes risk is less consistent. Correlational studies and meta-analyses of randomised clinical trials either show a weak positive, null, or inverse association between fish or *n*-3 PUFA intake and type-2 diabetes risk, insulin sensitivity and plasma glucose[36–38]. Consistent with our observations in our cohort of elders, an inverse association between the *n*-3 index and plasma glucose and a favourable correlation with insulin sensitivity were noted in overweight men[27]. While it is not completely clear how the *n*-3 index would influence blood glucose levels, animal studies[39] suggest that EPA/DHA via their role as agonists of PPAR-α may lead to a suppression of glucose production and improve insulin sensitivity.

Our study had several strengths. These include the randomised controlled trial design, a large sample size and a long duration of treatment (1 year). Moreover, our study subjects were free-living individuals who had varying dietary and lifestyle habits, which improves external validity. Limitations of this study were that it was a secondary data analysis that was limited by the exclusion and selection criteria of the original study, and the overall healthy baseline status of the participants. In addition, we did not capture fish intake in g/d but instead reported it as binary variable (fish consumers *v.* non-consumers). This would have helped to throw more light on the differential results in *n*-3 index observed among those who ate fish only. Future studies should explore this potential interaction.

Conclusion

Consuming 1–2 oz of walnuts daily for 1 year as part of the habitual diet was ineffective in improving the *n*-3 index in

healthy elders. Thus, it may be prudent for the elderly population to include a direct source of EPA and DHA in their diet to increase the *n*-3 index to 8 % or higher, a level considered to provide the greatest cardio-protection[7,8]. High *n*-3 index seems desirable as it is inversely related to cardiometabolic risk. However, the cardiometabolic benefits documented for walnuts do not seem to be essentially driven by conversion of ALA to longer-chain *n*-3 PUFA such as EPA and DHA. Instead, it may be an independent function of ALA, and a synergistic action of the multiple bioactives in walnuts including polyphenols, phytosterols, fibre, micronutrients, antioxidants and essential fatty acids, thus validating the inclusion of walnuts as part of a heart healthy diet pattern.

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S. R., J. S. and T. J. Designed the secondary analyses study, and J. S., E. R., S. R. and A. S-V. were involved in the design of the original Walnuts and Healthy Aging Study; A. S-V., S. R., J. S., E. R. and M. C. acquired the data or supervised data collection and analyses; AS-V-provided oversight for all lab analyses; T. J., A. S-V., M. C. and K. O. contributed to data quality assurance and data quality analyses; T. J. and S. R. drafted the manuscript; all authors provided feedback and revisions. All authors read and approved the final submission.

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