

Whole blood omega-3 fatty acid concentrations are inversely associated with blood pressure in young, healthy adults

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Background: Omega-3 fatty acids ($n-3$ FA) may have blood pressure (BP)-lowering effects in untreated hypertensive and elderly patients. The effect of $n-3$ FA on BP in young, healthy adults remains unknown. The Omega-3 Index reliably reflects an individuals' omega-3 status. We hypothesized that the Omega-3 Index is inversely associated with BP levels in young healthy adults.

Methods: The current study ($n=2036$) is a cross-sectional study investigating the baseline characteristics of a cohort, which includes healthy adults, age 25–41 years. Individuals with cardiovascular disease, known diabetes or a BMI higher than 35 kg/m^2 were excluded. The Omega-3 Index was determined in whole blood using gas chromatography. Association with office and 24-h BP was assessed using multivariable linear regression models adjusted for potential confounders.

Results: Median Omega-3 Index was 4.58% (interquartile range 4.08; 5.25). Compared with individuals in the lowest Omega-3 Index quartile, individuals in the highest had a SBP and DBP that was 4 and 2 mmHg lower, respectively ($P < 0.01$). A significant linear inverse relationship of the Omega-3 Index with 24-h and office BP was observed. Per 1-U increase in log-transformed Omega-3 Index the lowering in BP (given as multivariable adjusted β coefficients; 95% confidence interval) was -2.67 mmHg ($-4.83; -0.51; P=0.02$) and -2.30 mmHg ($-3.92; -0.68; P=0.005$) for 24-h SBP and DBP, respectively.

Conclusion: A higher Omega-3 Index is associated with statistically significant, clinically relevant lower SBP and DBP levels in normotensive young and healthy individuals. Diets rich in $n-3$ FA may be a strategy for primary prevention of hypertension.

Keywords: blood pressure, hypertension, nutrition, omega-3 fatty acids, population, prevention

Abbreviations: ALA, alpha-linolenic acid; BP, blood pressure; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; $n-3$ PUFA, omega-3 polyunsaturated fatty acid

INTRODUCTION

Hypertension is one of the most prevalent health conditions [1] affecting over 1 billion people worldwide [2] and a leading cause of heart disease, stroke and premature death [3]. Despite important preventive efforts, incidence and prevalence of hypertension are not only rising in elderly people [4] but also in younger and otherwise healthy individuals – even in athletes [5]. Hence, simple, well tolerated and cost-effective strategies to prevent hypertension at an early stage are needed.

Omega-3 polyunsaturated fatty acids ($n-3$ PUFAs) consist of the fish-derived fatty acids (FAs) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as well as the plant-derived alpha-linolenic acid (ALA). Although their role in hypertension is still subject to an intense and ongoing scientific debate, they have been shown to have multiple beneficial effects in cardiovascular disease [6]. Of note, they have anti-inflammatory [7] as well as antithrombotic [8] activity, they improve endothelial dysfunction [9] and positively influence resting heart rate (HR), HR variability [10,11], heart rhythm [12], cardiac remodeling [13] and cardiac ion channel functions [14].

Concerning their antihypertensive properties, $n-3$ PUFAs were reported to directly modulate murine ion channel functions in blood vessels, leading to

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vasodilatation [15]. In humans, higher red blood cell membrane omega-3 content was associated with lower brachial artery diameter (an independent predictor of cardiovascular events) and improved vasodilatory function [16]. Overall, omega-3 FAs were shown to reduce pulse wave velocity and improve arterial compliance in humans [17]. Correspondingly, three meta-analyses show that *n* – 3 PUFA administration reduces blood pressure (BP) in untreated hypertensive patients, when supplemented with high dosages of at least 3–4 g/day, with almost no or only very little effect in normotensive individuals [18–20].

Few data are available on the effects of physiologic nutritional *n* – 3 on BP in healthy individuals, in whom prevention measures could be implemented. From an observational point of view, in the subcohort of 2038 normotensive adults without antihypertensive medication, the observational INTERMAP study reported an inverse relationship of total dietary *n* – 3 PUFA consumption with BP, resulting in an estimated difference of –1.01 mmHg SBP and –0.98 mmHg DBP with two SDs higher dietary *n* – 3 PUFA intake [21]. However, *n* – 3 PUFA intake was estimated by nutritional assessments, which are known to be unprecise [22]. Also, the study lacked ambulatory 24-h BP measurements [21], which are better predictors of cardiovascular disease events [23–25] than office BP. Further, the investigated subgroup included individuals already suffering from cardiovascular disease.

Therefore, aim of our study was to assess the association of whole blood *n* – 3 PUFA levels (given as the Omega-3 Index) with BP in a large population-based sample of young and healthy adults.

METHODS

Study population

All participants of the current study are also part of the GAPP study (genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors), a large population-based cohort, which investigates the determinants of BP and other cardiovascular risk factors in young and healthy adults [26]. For this study, cross-sectional data from the baseline measurements were analyzed. Briefly, the cohort is composed of a representative population of 2170 healthy adults aged 25–41 with residence in the Principality of Liechtenstein. All inhabitants in the given age range were invited to participate in this study. Patients were screened and enrolled from 2010 to 2013. Main exclusion criteria include established cardiovascular disease, chronic kidney disease, a BMI higher than 35 kg/m², obstructive sleep apnea, daily intake of non-steroidal anti-inflammatory medication, diabetes and other severe illnesses such as multiple sclerosis or cancer [26].

From a total of 2170 patients enrolled in the GAPP study, we excluded participants with invalid 24-h BP measurements as defined below (*n* = 90), missing conventional BP measurements (*n* = 2), current BP-lowering treatment for diagnosed hypertension (*n* = 34) and missing blood sampling (*n* = 8), resulting in 2036 individuals for this analysis.

The study protocol was approved by the local ethics committee (Cantonal Ethics Commission of Zurich, Zurich, Switzerland). Informed written consent was obtained from every individual participant.

Blood pressure measurement

Conventional office BP was obtained in a quiet environment after at least 5 min of rest from the sitting participant by use of a validated oscillometric device (Microlife BP3AG1; Microlife AG Swiss Corporation, Widnau, Switzerland) in all participants [26]. Three consecutive measurements were performed, and the mean of the last two readings was used for all analyses.

Ambulatory BP measurements were obtained by a fully automatic, noninvasive device (BR-102 plus; Schiller AG, Baar, Switzerland) which has been validated by international protocols [27]. The device measured BP every 15 min between 0730 and 2200 h, and every 30 min in the remaining time within 24 h. Daytime and night-time BP was identified with a diary, to be kept by all participants during the measurement period. Recordings were repeated if possible in individuals with less than 80% of valid BP measurements. For this analysis, we included only participants with at least 10 valid awake and at least five valid asleep BP measurements in accordance with other large BP-databases [28].

Blood sampling and whole blood fatty acid composition

Venous whole blood samples were collected from every participant at baseline after an overnight fast. Samples were immediately stored at –80 °C [26], which has been proven to have no altering effect on the *n* – 3 levels measured [29]. Whole blood composition was analyzed according to the high sensitivity-Omega-3 Index methodology, initially described for erythrocyte samples [30], by the use of a validated correction factor applicable for whole blood aliquots [31]. FA methyl esters were generated from whole blood by acid transesterification and analyzed by gas chromatography using a GC2010 Gas Chromatograph (Shimadzu, Duisburg, Germany) equipped with a SP2560, 100-m column (Supelco, Bellefonte, Pennsylvania, USA) hydrogen as carrier gas. FAs were identified by comparison with a standard mixture of FAs characteristic of erythrocytes. Results are given as relative amounts of EPA (C20:5*n*3) and DHA (C22:6*n*3), expressed as a percentage of a total of 26 identified FAs, referred to as Omega-3 Index. Where mentioned, ALA (C18:3*n*3) is given as percentage of a total of 26 identified FAs. The coefficient of variation for FA levels was 5%. Analyses were quality controlled according to DIN ISO 15189.

Assessment of other biomarkers

Creatinine and high-sensitivity C-reactive protein (hs-CRP) were assayed from fresh samples immediately after blood draw on a Roche Cobas (F. Hoffmann-La Roche, Basel, Switzerland). Glycated hemoglobin A1c (HbA1c) was measured using HPLC. Estimated glomerular filtration rate (eGFR) was calculated using the creatinine-based chronic kidney disease epidemiology collaboration formula [32].

Other study variables

For all participants, a detailed assessment of personal, medical, lifestyle and nutritional factors was performed. Smoking status was self-assessed and categorized into current, past and never smokers. Moderate and vigorous physical activity was assessed using the validated international physical activity questionnaire [33]. Regular physical activity was defined as moderate or vigorous physical activity of at least 75 or 150 min/week,

respectively. Alcohol consumption and frequency of fruit, vegetable and fish consumption were obtained with the Swiss health survey questionnaire from 2007. Healthy diet was defined as a fruit and vegetable consumption of at least five servings per day. Highest educational status was reported. Weight and height were measured in a standardized way. BMI was calculated by dividing weight in kg by height in m².

Statistical analysis

Data were tested against a predefined hypothesis, assuming an inverse association of Omega-3 Index with BP indices. Baseline characteristics were presented overall as well as stratified according to quartiles of Omega-3 Index. Distribution of continuous variables was checked using visual inspection of the histogram, skewness and kurtosis. Continuous data are presented as mean ± SD or median [interquartile range (IQR)], depending on the distribution. Categorical data were shown as numbers (percentages). Group comparisons across quartiles were performed using analysis of variance, Kruskal–Wallis tests or chi-square tests, as appropriate.

To assess the relationship of *n* – 3 PUFAs with BP, different multivariable linear regression models were constructed using BP variables as the outcome variable. We used quartiles of FAs to assess the shape of the association with BP variables. *P* values for trend were calculated using quartile-specific medians. Afterwards continuous FA variables were included to the model to assess β-coefficients [95% confidence intervals

(CIs)] per 1-U increase in FA. Due to the distribution of the variables, levels of FAs were log-transformed for all analyses. In addition, relationships between specific *n* – 3 PUFAs (DHA, EPA and ALA) and BP indices were calculated from a hypothesis generating perspective.

The first model was adjusted for age and sex. In a second model, the associations were additionally adjusted for BMI, smoking status, HbA1c, educational status, fruit and vegetable consumption, alcohol consumption, physical activity, eGFR and hs-CRP. A prespecified subgroup analysis was performed using multivariable adjusted regression analysis stratified by sex. A multiplicative interaction term was included to the unstratified model to evaluate differences among both strata.

The multivariable adjusted regression model was adjusted for age, sex, educational status, alcohol consumption, hs-CRP and eGFR.

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc, Cary, North Carolina, USA), and a value of *P* value less than 0.05 was prespecified to indicate statistical significance.

RESULTS

Study population

Baseline characteristics for the whole population and stratified by quartiles of the Omega-3 Index are shown in

TABLE 1. Baseline characteristics across Omega-3 Index quartiles

	All, <i>n</i> = 2036	Quartile 1, <i>n</i> = 508	Quartile 2, <i>n</i> = 511	Quartile 3, <i>n</i> = 508	Quartile 4, <i>n</i> = 509	<i>P</i> value*
Omega-3 Index range (%)		2.43–4.06	4.08–4.58	4.59–5.25	5.26–9.26	
Omega-3 Index (%)	4.58 (4.08; 5.25)	3.76 (3.54; 3.94)	4.32 (4.19; 4.47)	4.89 (4.71; 5.04)	5.80 (5.49; 6.37)	–
Docosahexaenoic acid (%)	3.09 (2.63; 3.62)	2.38 (2.18; 2.55)	2.87 (2.73; 3.02)	3.34 (3.17; 3.50)	4.02 (3.78; 4.42)	–
Eicosapentaenoic acid (%)	0.61 (0.49; 0.78)	0.50 (0.41; 0.60)	0.57 (0.47; 0.67)	0.64 (0.52; 0.77)	0.83 (0.68; 1.06)	–
Alpha-linolenic acid (%)	0.23 (0.18; 0.29)	0.22 (0.17; 0.28)	0.23 (0.18; 0.28)	0.23 (0.19; 0.29)	0.24 (0.19; 0.30)	–
Age (years)	36.8 (31.2; 40.3)	36 (30; 40)	36 (31; 40)	37 (31; 40)	37 (33; 40)	0.22
Male sex (%)	950 (46.7)	280 (55.1)	245 (48.0)	228 (44.9)	197 (38.7)	<0.0001
BMI (kg/m ²)	24.5 ± 3.7	25.1 ± 4.0	24.7 ± 3.7	24.4 ± 3.5	23.7 ± 3.5	<0.0001
Smoking (%)						<0.0001
Current	477 (22.0)	165 (32.5)	131 (25.7)	96 (18.9)	55 (10.8)	
Past	478 (23.5)	114 (22.5)	117 (22.9)	123 (24.3)	124 (24.4)	
Never	1108 (54.5)	228 (45.0)	262 (51.4)	288 (56.8)	330 (64.8)	
Education (%)						<0.0001
Basic	155 (7.7)	45 (9.0)	37 (7.3)	29 (5.8)	44 (8.7)	
Middle	1091 (54.2)	317 (63.7)	289 (57.2)	274 (54.6)	211 (41.6)	
High	766 (38.1)	136 (27.3)	179 (35.5)	199 (39.6)	252 (49.7)	
LDL (mmol/l)	2.98 ± 0.85	3.05 ± 0.90	2.98 ± 0.83	2.97 ± 0.83	2.92 ± 0.85	0.12
HDL (mmol/l)	1.54 ± 0.42	1.44 ± 0.41	1.51 ± 0.40	1.56 ± 0.43	1.63 ± 0.42	<0.0001
Cholesterol (mmol/l)	4.81 (4.29; 5.48)	4.85 (4.34; 5.51)	4.82 (4.24; 5.40)	4.85 (4.29; 5.56)	4.73 (4.24; 5.40)	0.26
Triglycerides (mmol/l)	0.84 (0.60; 1.20)	0.98 (0.65; 1.46)	0.87 (0.62; 1.22)	0.83 (0.60; 1.14)	0.75 (0.57; 1.02)	<0.0001
Glycated hemoglobin A1c (%)	5.4 (5.2; 5.6)	5.5 (5.2; 5.7)	5.4 (5.2; 5.6)	5.4 (5.2; 5.6)	5.4 (5.2; 5.6)	0.31
Regular physical activity (%)	1635 (80.3)	408 (80.3)	414 (81.0)	404 (79.5)	409 (80.4)	0.95
Fish consumption ≥2 servings/week (%)	264 (13.0)	29 (5.7)	31 (6.1)	67 (13.2)	137 (27.0)	<0.0001
Fruit/vegetable consumption (%)	393 (19.3)	74 (14.6)	90 (17.6)	96 (18.9)	133 (26.1)	<0.0001
hs-CRP (mg/l)	0.90 (0.50; 1.90)	0.9 (0.5; 1.7)	0.9 (0.5; 2.1)	1.0 (0.5; 2.0)	0.8 (0.4; 1.7)	0.05
Estimated glomerular filtration rate (ml/min)	112 (103; 118)	113 (106; 119)	112 (103; 118)	111 (103; 118)	112 (101; 118)	0.06
Blood pressure						
Systolic office BP (mmHg)	120 ± 13	122 ± 13	121 ± 12	119 ± 12	118 ± 13	<0.0001
Diastolic office BP (mmHg)	78 ± 9	79 ± 9	79 ± 8	78 ± 9	77 ± 9	0.0003
24-h SBP (mmHg)	123 ± 11	125 ± 12	123 ± 11	123 ± 11	121 ± 11	<0.0001
24-h DBP (mmHg)	78 ± 8	79 ± 9	79 ± 7	78 ± 8	77 ± 8	0.0002
Systolic daytime BP (mmHg)	127 ± 12	129 ± 13	127 ± 11	126 ± 11	125 ± 12	<0.0001
Diastolic daytime BP (mmHg)	82 ± 8	83 ± 9	82 ± 7	81 ± 8	81 ± 8	0.0005
Systolic night-time BP (mmHg)	109 ± 11	111 ± 12	109 ± 10.9	109 ± 11	107 ± 11	<0.0001
Diastolic night-time BP (mmHg)	66 ± 8	67 ± 8	67 ± 8	66 ± 8	66 ± 8	0.0006

n = 2036; data are means ± SD, medians (interquartile range) or numbers (percentages). Regular physical activity = moderate or vigorous physical activity at least 150 or 75 min/week. Fruit and vegetable consumption = fruit and vegetable consumption at least five servings per day. BP, blood pressure; hs-CRP, high-sensitivity C-reactive protein. **P* value was calculated using analysis of variance, Kruskal–Wallis test or chi-square test, as appropriate, to compare the respective values across the quartiles.

Table 1. Median age of our population was 37 years (IQR 32; 40), and 53% were women. Median Omega-3 Index was 4.58% (IQR 4.08; 5.25). Mean systolic and diastolic office BP was 120 ± 13 and 78 ± 9 mmHg, respectively.

A higher Omega-3 Index was associated with a higher prevalence of women, a lower BMI and less smoking (*P* value for all <0.0001). In addition, individuals with a higher Omega-3 Index had higher HDL levels (*P* < 0.0001), lower triglycerides (*P* < 0.0001) and consumed significantly more fruits and vegetables (*P* < 0.0001) as well as fish (*P* < 0.0001). Groups with a higher Omega-3 Index also had a higher educational status (*P* < 0.0001). With increasing Omega-3 Index, all BP indices decreased linearly (Table 1). Compared with individuals in the lowest Omega-3 Index quartile, those in the highest had a 4 and 2-mmHg lower 24-h SBP and DBP, respectively (*P* < 0.01).

Relationship between omega-3 fatty acids and blood pressure

On a continuous scale, systolic and diastolic office and 24-h BP remained inversely associated with the Omega-3 Index after correction for a multitude of potential confounders. Results of multivariable regression models for the relationship of Omega-3 Index and individual FAs with BP are shown in Table 2. Per 1-U increase in log-transformed Omega-3 Index multivariable adjusted β coefficients (95% CI) were –2.66 (–4.83; –0.51; *P* = 0.02) for 24-h SBP, –2.30 (–3.92; –0.68; *P* = 0.005) for 24-h DBP, –2.81 (–5.22; –0.40; *P* = 0.02) for systolic office and –1.86 (–3.68; –0.04; *P* = 0.05) for diastolic office BP. A subgroup analysis stratified by sex showed no significant differences (Table S1, <http://links.lww.com/HJH/A919>).

DHA was inversely associated with virtually all BP indices except for systolic night-time and diastolic conventional BP (Table 2). However, no significant results could be found for the associations between BP and EPA or BP and ALA (Table 2). The relationships of BP indices across

quartiles of FAs are presented in Tables S2–S5, <http://links.lww.com/HJH/A919>.

DISCUSSION

In the current study, we found a linear inverse association of *n* – 3 PUFAs with SBP and DBP in young, healthy individuals aged 25–41 years – no specific sex differences being observed. Importantly, the results were consistent throughout all measured indices, namely not only in-office but also 24-h BP as a much more reliable assessment of true BP levels [25], and after comprehensive adjustments.

Our findings support the hypothesis of BP-lowering effects of *n* – 3 PUFAs, as indicated in a variety of intervention studies [34]. Even more importantly, our study specifically shows that, in a young and healthy population, whole blood *n* – 3 PUFAs are associated with lower BP. Significantly, previous studies mainly administered large amounts of more than 3-g *n* – 3 PUFAs, which are largely impossible to obtain through usual diets [35] and included mostly hypertensive and middle-aged individuals [36]. Data on cardiovascular effects of physiological, ‘every day’ dietary *n* – 3 FA consumption in healthy individuals have been scarce [18,37]. Yet early, long-term and rather low-dose dietary exposure to *n* – 3 PUFAs may be particularly attractive as a well tolerated intervention to lower BP and prevent the development of hypertension with little expected side effects, if any [38].

In the current study population, we observed a significantly higher prevalence of traditional cardiovascular risk factors like age, male sex, higher BMI, smoking, lower educational status and higher LDL and triglyceride profile with lower Omega-3 Index [39]. However, after correcting for these risk factors and a broad variety of other possible confounders, the inverse association between the Omega-3 Index and all BP indices, except for isolated nocturnal 24-h SBP, remained significant, attesting to the robustness and independence of our findings.

TABLE 2. Relationship between fatty acids and blood pressure indices

		Omega-3 Index	Docosahexaenoic acid	Eicosapentaenoic acid	Alpha-linolenic acid
SBP 24-h	Model 1	–3.97 (–6.11; –1.82), <i>P</i> < 0.001	–3.71 (–5.49; –1.92), <i>P</i> < 0.0001	–0.25 (–1.41; 0.90), <i>P</i> = 0.66	–0.21 (–1.38; 0.97), <i>P</i> = 0.73
	Model 2	–2.67 (–4.83; –0.51), <i>P</i> = 0.02	–2.49 (–4.28; –0.69), <i>P</i> = 0.007	–0.25 (–1.41; 0.91), <i>P</i> = 0.67	0.18 (–0.98; 1.34), <i>P</i> = 0.76
DBP 24-h	Model 1	–3.31 (–4.92; –1.70), <i>P</i> < 0.0001	–2.75 (–4.09; –1.41), <i>P</i> < 0.0001	–1.00 (–1.86; –0.13), <i>P</i> = 0.02	–0.18 (–1.06; 0.71), <i>P</i> = 0.70
	Model 2	–2.30 (–3.92; –0.68), <i>P</i> = 0.005	–1.90 (–3.25; –0.56), <i>P</i> = 0.006	–0.76 (–1.63; 0.11), <i>P</i> = 0.09	0.24 (–0.62; 1.11), <i>P</i> = 0.58
SBP day	Model 1	–4.02 (–6.25; –1.80), <i>P</i> < 0.0001	–3.87 (–5.72; –2.02), <i>P</i> < 0.0001	0.07 (–1.27; 1.13), <i>P</i> = 0.91	–0.01 (–1.24; 1.21), <i>P</i> = 0.98
	Model 2	–2.66 (–4.91; –0.41), <i>P</i> = 0.02	–2.57 (–4.44; –0.70), <i>P</i> = 0.007	–0.09 (–1.29; 1.12), <i>P</i> = 0.89	0.37 (–0.84; 1.57), <i>P</i> = 0.55
DBP day	Model 1	–3.38 (–5.09; –1.66), <i>P</i> < 0.0001	–2.84 (–4.27; –1.41), <i>P</i> < 0.0001	–0.97 (–1.89; –0.05), <i>P</i> = 0.04	–0.10 (–1.05; 0.84), <i>P</i> = 0.83
	Model 2	–2.22 (–3.95; –0.49), <i>P</i> = 0.01	–1.87 (–3.31; –0.43), <i>P</i> = 0.01	–0.70 (–1.63; 0.23), <i>P</i> = 0.14	0.35 (–0.58; 1.27), <i>P</i> = 0.46
SBP night	Model 1	–3.26 (–5.56; –0.96), <i>P</i> = 0.005	–2.69 (–4.61; –0.78), <i>P</i> = 0.006	–0.97 (–2.20; 0.27), <i>P</i> = 0.12	–0.64 (–1.90; 0.62), <i>P</i> = 0.32
	Model 2	–2.02 (–4.35; 0.31), <i>P</i> = 0.09	–1.64 (–3.58; 0.30), <i>P</i> = 0.10	–0.68 (–1.93; 0.57), <i>P</i> = 0.29	–0.23 (–1.47; 1.01), <i>P</i> = 0.72
DBP night	Model 1	–2.70 (–4.36; –1.04), <i>P</i> = 0.001	–2.12 (–3.50; –0.74), <i>P</i> = 0.003	–1.07 (–1.96; –0.18), <i>P</i> = 0.02	–0.28 (–1.19; 0.63), <i>P</i> = 0.55
	Model 2	–2.14 (–3.84; –0.45), <i>P</i> = 0.01	–1.71 (–3.11; –0.30), <i>P</i> = 0.02	–0.84 (–1.74; 0.07), <i>P</i> = 0.07	0.04 (–0.87; 0.94), <i>P</i> = 0.93
Systolic conventional BP	Model 1	–3.98 (–6.38; –1.58), <i>P</i> = 0.001	–3.47 (–5.47; –1.48), <i>P</i> = 0.0007	–0.81 (–2.10; 0.48), <i>P</i> = 0.22	0.63 (–0.69; 1.94), <i>P</i> = 0.35
	Model 2	–2.81 (–5.22; –0.40), <i>P</i> = 0.02	–2.46 (–4.46; –0.46), <i>P</i> = 0.02	–0.62 (–1.91; 0.67), <i>P</i> = 0.34	0.98 (–0.31; 2.26), <i>P</i> = 0.14
Diastolic conventional BP	Model 1	–2.78 (–4.58; –0.98), <i>P</i> = 0.003	–2.12 (–3.63; –0.62), <i>P</i> = 0.006	–1.07 (–2.04; –0.10), <i>P</i> = 0.03	0.26 (–0.72; 1.25), <i>P</i> = 0.60
	Model 2	–1.86 (–3.68; –0.04), <i>P</i> = 0.05	–1.41 (–2.93; 0.11), <i>P</i> = 0.07	–0.72 (–1.70; 0.25), <i>P</i> = 0.15	0.67 (–0.31; 1.64), <i>P</i> = 0.18

n = 2036; data are β-coefficients (95% confidence intervals). Fatty acid variables were used as a log-transformed variable. Model 1 was adjusted for age and sex; Model 2 was additionally adjusted for BMI, smoking status, glycated hemoglobin A1c, educational status, fruit/vegetable consumption, physical activity, estimated glomerular filtration rate, high-sensitivity C-reactive protein, alcohol consumption. BP, blood pressure.

Of note, our population had a median Omega-3 Index of 4.6% and was therefore highly comparable with data and recently published studies from the United States, with an average Omega-3 Index of 4–5% [40]. Significantly, the Omega-3 Index in Japan, a country with one of the highest fish consumption, highest life expectancies and lowest rates of coronary artery disease, is between 9 and 11% [41,42]. An Omega-3 Index more than 8% has been proposed to provide for optimal cardioprotection [31], whereas no ceiling effect was observed for major coronary events in the large JELIS study ($n = 18\,645$) [43], and a significant raise in Omega-3 Index is achievable through increased dietary intake and/or by long-term $n - 3$ supplementation [44,45]. This conforms with the findings in the present population in which a statically relevant increase in fish consumption across quartiles can be observed – but overall regular fish consumption remains very low in all quartiles and is thus probably responsible for the low median and range of Omega-3 Index.

In consequence, there is a high likelihood that in future long-term interventional studies, based on the observed association between $n - 3$ PUFAs and BP, early low-dose dietary or $n - 3$ PUFAs supplementation might lower BP and/or prevent the onset of hypertension in healthy younger individuals at risk. Beyond that, the present data suggest that in the measured range of our population, no specific lower $n - 3$ threshold exists. Indeed, $n - 3$ PUFA is likely to be a continuous beneficial variable, as the Omega-3 Index is known to be an independent risk factor for cardiovascular disease [46]. Hence, even a low-dose dietary or pharmacological intervention of longer duration should be effective.

The association of $n - 3$ PUFAs with lower BP may be explained by a variety of direct antihypertensive as well as secondary anti-inflammatory and antiatherosclerotic mechanisms [36]. A significant reduction of endothelial activation, inflammation and eventually atherogenesis has been shown by our group in various experimental models [8,47]. Although statistically NS and with potential residual confounding, we observed lower levels of hs-CRP in the high $n - 3$ PUFA quartile, indicating that anti-inflammatory mechanisms may at least play a role in BP modulation. Further mechanisms for BP reduction by $n - 3$ PUFA include an interaction with the nitric oxide pathway, blockade of the angiotensin pathway and a reduction of vasoconstrictor prostanoids [48]. A direct effect of $n - 3$ PUFA application was reported by Hoshi *et al.* [15] after intravenous administration of a $n - 3$ PUFA bolus, which in rats led to a dose-dependent BP decrease through ion-channel-mediated vasodilatation. Finally, two intervention studies have shown a decrease in BP through Omega-3 Index increase [49,50].

Individual subanalyses of EPA, DHA and ALA in our study suggest that the observed association of $n - 3$ PUFAs with BP is mainly driven by DHA, which has also been observed in a number of experimental and clinical trials [51]. In line with this interpretation, a small randomized controlled trial by Mori *et al.* [52] found that DHA, but not EPA supplementation, resulted in BP reduction in overweight men. The precise mechanism and clinical significance of this observation remains unknown. Possibly, the

BP-lowering effect of $n - 3$ PUFAs is more chain length dependent than other antiatherosclerotic properties which we were able to induce with isolated ALA [8,47].

The strengths of our study include the definition and homogeneity of our large and well characterized, truly healthy population with easy access to preventive and therapeutic medical services in Liechtenstein and careful exclusion of any individuals with treated hypertension, diabetes or any major illness, allowing to minimize confounding. In addition, thanks to the use of 24-h BP recordings, we were able to assess a variety of BP indices. Importantly, our observation was consistent in both in-office and 24-h BP. Also, the implementation of the Omega-3 Index as a marker for $n - 3$ PUFA intake provided good comparability with other studies and a well established measurement of $n - 3$ PUFA levels with a reduced biologic and analytic variability. Compared with plasma phospholipid levels, which may differ up to a factor of 3.5 depending on sample, analyzing laboratory and method used [53] or often unreliable nutritional questionnaires [22], the Omega-3 Index is very robust to short-term changes of $n - 3$ PUFA intake and reliably reflects an individual's long-term omega-3 status and tissue omega-3 PUFA content [29]. Therefore, the Omega-3 Index has the potential to become a cardiovascular risk factor as much as the HbA1c in people with diabetes, which similarly takes advantage of the red cell life span exposure of the past 120 days [29].

Limitations of our study include the cross-sectional design preventing to infer causal relationship or to determine the directionality of the observed effects. Second, despite extensive adjustment, we cannot exclude the possibility that the association between $n - 3$ PUFA levels and outcomes may be due to residual confounding – despite excellent pathophysiological mechanistic evidence. Third, the findings of our study are limited to the population studied (white, young and healthy individuals) and the nutritional habits, which are also influenced by geography and culture. Fourth, none of the results were adjusted for multiple testing, given a clear, predefined hypothesis and to avoid potential overadjustment because of significant correlations between individual FAs. Further, data on estimated sodium intake from dietary questionnaires or urinary excretion were not available, the inclusion of which might have added to the exact definition of the observed association. Lastly, we do not have data on the source of $n - 3$ PUFAs or the number of individuals taking supplements. The latter is likely to be very low given the relatively low levels of the $n - 3$ concentrations measured and are probably not of relevance to the association, with the Omega-3 Index representing an active biologic analogue (independent of data on the form and frequency of intake).

In conclusion, our study shows that even differences in omega-3 levels achieved through standard nutrition are associated with a significant and clinically relevant difference in BP among young and healthy adults. Thus, an $n - 3$ FA rich diet might become a well tolerable strategy for primary prevention of hypertension and its detrimental consequences – provided these observations will be confirmed in future randomized controlled trials. Nevertheless, given the increasing prevalence of hypertension with age, it will be interesting and a great opportunity to observe the

development of this cohort prospectively, with respect to the changes in the Omega-3 Index, the incidence of hypertension and associated cardiovascular diseases.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

- Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, *et al.* Executive summary: heart disease and stroke statistics – 2010 update: a report from the American Heart Association. *Circulation* 2010; 121:948–954.
- Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet* 2005; 365:217–223.
- Kung HC, Xu JQ. *Hypertension-related mortality in the United States, 2000–2013*, NCHS data brief, no 193. Hyattsville, MD: National Center for Health Statistics; 2015.
- Pleis JR, Ward BW, Lucas JW. Summary health statistics for U.S. adults: National Health Interview Survey, 2009. National Center for Health Statistics. *Vital Health Stat* 2010; 10.
- Venecia T de, Lu M, Figueredo VM. Hypertension in young adults. *Postgrad Med* 2016; 128:201–207.
- Mozaffarian D, Wu JHY. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *J Am Coll Cardiol* 2011; 58:2047–2067.
- Zampelas A, Panagiotakos DB, Pitsavos C, Das UN, Chrysoshoou C, Skoumas Y, Stefanadis C. Fish consumption among healthy adults is associated with decreased levels of inflammatory markers related to cardiovascular disease: the ATTICA study. *J Am Coll Cardiol* 2005; 46:120–124.
- Holy EW, Forestier M, Richter EK, Akhmedov A, Leiber F, Camici GG, *et al.* Dietary α -linolenic acid inhibits arterial thrombus formation, tissue factor expression, and platelet activation. *Arterioscler Thromb Vasc Biol* 2011; 31:1772–1780.
- Wang Q, Liang X, Wang L, Lu X, Huang J, Cao J, *et al.* Effect of omega-3 fatty acids supplementation on endothelial function: a meta-analysis of randomized controlled trials. *Atherosclerosis* 2012; 221:536–543.
- Christensen JH. Omega-3 polyunsaturated fatty acids and heart rate variability. *Front Physiol* 2011; 2:84.
- Xin W, Wei W, Li X-Y. Short-term effects of fish-oil supplementation on heart rate variability in humans: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2013; 97:926–935.
- Calò L, Martino A, Tota C. The anti-arrhythmic effects of n – 3 PUFAs. *Int J Cardiol* 2013; 170 (2 Suppl 1):S21–S27.
- da Cunha DN, Hamlin RL, Billman GE, Carnes CA. n – 3 (omega-3) polyunsaturated fatty acids prevent acute atrial electrophysiological remodeling. *Br J Pharmacol* 2007; 150:281–285.
- McLennan PL. Cardiac physiology and clinical efficacy of dietary fish oil clarified through cellular mechanisms of omega-3 polyunsaturated fatty acids. *Eur J Appl Physiol* 2014; 114:1333–1356.
- Hoshi T, Wissuwa B, Tian Y, Tajima N, Xu R, Bauer M, *et al.* Omega-3 fatty acids lower blood pressure by directly activating large-conductance Ca^{2+} -dependent K^{+} channels. *Proc Natl Acad Sci U S A* 2013; 110:4816–4821.
- Colussi G, Catena C, Dialti V, Mos L, Sechi LA. The vascular response to vasodilators is related to the membrane content of polyunsaturated fatty acids in hypertensive patients. *J Hypertens* 2015; 33:993–1000.
- Pase MP, Grima NA, Sarris J. Do long-chain n – 3 fatty acids reduce arterial stiffness? A meta-analysis of randomised controlled trials. *Br J Nutr* 2011; 106:974–980.
- Geleijnse JM, Johanna M, Giltay EJ, Grobbee DE, Donders AR, Adrianus RT, Kok FJ. Blood pressure response to fish oil supplementation: meta-regression analysis of randomized trials. *J Hypertens* 2002; 80:1493–1499.
- Morris MC, Sacks F, Rosner B. Does fish oil lower blood pressure? A meta-analysis of controlled trials. *Circulation* 1993; 88:523–533.
- Appel LJ, Miller ER 3rd, Seidler AJ, Whelton PK. Does supplementation of diet with 'fish oil' reduce blood pressure? A meta-analysis of controlled clinical trials. *Arch Intern Med* 1993; 153:1429–1438.
- Ueshima H, Stamler J, Elliott P, Chan Q, Brown IJ, Carnethon MR, *et al.* Food omega-3 fatty acid intake of individuals (total, linolenic acid, long-chain) and their blood pressure: INTERMAP study. *Hypertension* 2007; 50:313–319.
- Archer E, Blair SN. Implausible data, false memories, and the status quo in dietary assessment. *Adv Nutr* 2015; 6:229–230.
- Staessen JA, Thijs L, Fagard R, O'Brien ET, Clement D, de Leeuw PW, *et al.* Predicting cardiovascular risk using conventional vs ambulatory blood pressure in older patients with systolic hypertension. *JAMA* 1999; 282:539–546.
- Clement DL, De Buyzere ML, De Bacquer DA. Prognostic value of ambulatory blood-pressure recordings in patients with treated hypertension. *ACC Curr J Rev* 2003; 12:33–34.
- Dolan E, Stanton A, Thijs L, Hinedi K, Atkins N, McClory S, *et al.* Superiority of ambulatory over clinic blood pressure measurement in predicting mortality: the Dublin outcome study. *Hypertension* 2005; 46:156–161.
- Conen D, Schön T, Aeschbacher S, Paré G, Frehner W, Risch M, Risch L. Genetic and phenotypic determinants of blood pressure and other cardiovascular risk factors (GAPP). *Swiss Med Wkly* 2013; 143:w13728.
- Denchev SV, Simova II, Matveev MG. Evaluation of the SCHILLER BR-102 plus noninvasive ambulatory blood pressure monitor according to the International Protocol introduced by the Working Group on Blood Pressure Monitoring of the European Society of Hypertension. *Blood Press Monit* 2007; 12:329–333.
- Thijs L, Hansen TW, Kikuya M, Björklund-Bodegård K, Li Y, Dolan E, *et al.* The International Database of Ambulatory Blood Pressure in relation to Cardiovascular Outcome (IDACO): protocol and research perspectives. *Blood Press Monit* 2007; 12:255–262.
- von Schacky C. The Omega-3 Index as a risk factor for cardiovascular diseases. *Prostaglandins Other Lipid Mediat* 2011; 96:94–98.
- Harris WS, Schacky C von. The Omega-3 Index: a new risk factor for death from coronary heart disease? *Prev Med* 2004; 39:212–220.
- Harris WS. The Omega-3 Index: clinical utility for therapeutic intervention. *Curr Cardiol Rep* 2010; 12:503–508.
- Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009; 150:604–612.
- Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, *et al.* International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003; 35:1381–1395.
- Cabo J, Alonso R, Mata P. Omega-3 fatty acids and blood pressure. *Br J Nutr* 2012; 107 (Suppl 2):S195–S200.
- Liu JC, Conklin SM, Manuck SB, Yao JK, Muldoon MF. Long-chain omega-3 fatty acids and blood pressure. *Am J Hypertens* 2011; 24:1121–1126.
- Colussi G, Catena C, Novello M, Bertin N, Sechi LA. Impact of omega-3 polyunsaturated fatty acids on vascular function and blood pressure:

- relevance for cardiovascular outcomes. *Nutr Metab Cardiovasc Dis* 2017; 27:191–200.
37. Siscovick DS, Barringer TA, Fretts AM, Wu JH, Lichtenstein AH, Costello RB, *et al.* Omega-3 polyunsaturated fatty acid (fish oil) supplementation and the prevention of clinical cardiovascular disease: a science advisory from the American Heart Association. *Circulation* 2017; 135:e867–e884.
 38. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. *Circulation* 2002; 106:2747–2757.
 39. Mendis S, Puska P, Norrving B. *Global atlas on cardiovascular disease prevention and control*. Geneva: World Health Organization in collaboration with the World Heart Federation and the World Stroke Organization; 2011.
 40. Harris WS, Pottala JV, Varvel SA, Borowski JJ, Ward JN, McConnell JP. Erythrocyte omega-3 fatty acids increase and linoleic acid decreases with age: observations from 160,000 patients. *Prostaglandins Leukot Essent Fatty Acids* 2013; 88:257–263.
 41. Ito-mura M, Fujioka S, Hamazaki K, Kobayashi K, Nagasawa T, Sawazaki S, *et al.* Factors influencing EPA+DHA levels in red blood cells in Japan. *In Vivo* 2008; 22:131–135.
 42. Ogura T, Takada H, Okuno M, Kitade H, Matsuura T, Kwon M, *et al.* Fatty acid composition of plasma, erythrocytes and adipose: their correlations and effects of age and sex. *Lipids* 2010; 45: 137–144.
 43. Yokoyama M, Origasa H, Matsuzaki M, Matsuzawa Y, Saito Y, Ishikawa Y, *et al.* Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet* 2007; 369:1090–1098.
 44. Harris WS, Pottala JV, Vasan RS, Larson MG, Robins SJ. Changes in erythrocyte membrane trans and marine fatty acids between 1999 and 2006 in older Americans. *J Nutr* 2012; 142:1297–1303.
 45. Udani JK, Ritz BW. High potency fish oil supplement improves omega-3 fatty acid status in healthy adults: an open-label study using a web-based, virtual platform. *Nutr J* 2013; 12:112.
 46. von Schacky C. Omega-3 index and cardiovascular health. *Nutrients* 2014; 6:799–814.
 47. Winnik S, Lohmann C, Richter EK, Schäfer N, Song WL, Leiber F, *et al.* Dietary α -linolenic acid diminishes experimental atherogenesis and restricts T cell-driven inflammation. *Eur Heart J* 2011; 32:2573–2584.
 48. Mori TA. Omega-3 fatty acids and cardiovascular disease: epidemiology and effects on cardiometabolic risk factors. *Food Funct* 2014; 5:2004–2019.
 49. Skulas-Ray AC, Kris-Etherton PM, Harris WS, West SG. Effects of marine-derived omega-3 fatty acids on systemic hemodynamics at rest and during stress: a dose–response study. *Ann Behav Med* 2012; 44:301–308.
 50. Dewell A, Marvasti FF, Harris WS, Tsao P, Gardner CD. Low- and high-dose plant and marine ($n-3$) fatty acids do not affect plasma inflammatory markers in adults with metabolic syndrome. *J Nutr* 2011; 141:2166–2171.
 51. Mori TA. Dietary $n-3$ PUFA and CVD: a review of the evidence. *Proc Nutr Soc* 2014; 73:57–64.
 52. Mori TA, Bao DQ, Burke V, Puddey IB, Beilin LJ. Docosahexaenoic acid but not eicosapentaenoic acid lowers ambulatory blood pressure and heart rate in humans. *Hypertension* 1999; 34:253–260.
 53. von Schacky C. Omega-3 fatty acids in cardiovascular disease – an uphill battle. *Prostaglandins Leukot Essent Fatty Acids* 2015; 92:41–47.

Reviewers' Summary Evaluations

Reviewer 1

Whilst it is known that fish oil supplementation can lower blood pressure, especially in established hypertension, this study by Filipovic *et al.* highlights the relationship between the omega-3 index, a reliable measure of omega-3 status, and blood pressure in a young, predominantly normotensive population. Although omega-3 intakes were not assessed, the low levels of fish consumption are consistent with the low omega-3 index range observed in this population. The strength of the cross-sectional associations

highlights the importance of monitoring this robust biomarker of omega-3 status in individuals, particularly in intervention trials where many factors including background intake can influence the efficacy of omega-3 supplementation.

Reviewer 2

An inverse relation between blood levels of Omega-3 PUFA's and blood pressure is found in a nonhypertensive, young adult population. This may be entirely related to diet, but alternate pathways should be explored to explain the finding.