

# Dietary supplementation with n-3 polyunsaturated fatty acids in early childhood: effects on blood pressure and arterial structure and function at age 8 y<sup>1-3</sup>

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## ABSTRACT

**Background:** n-3 Fatty acid supplementation in adults results in cardiovascular benefits. However, the cardiovascular effects of n-3 supplementation in early childhood are unknown.

**Objective:** The objective was to evaluate blood pressure (BP) and arterial structure and function in 8-y-old children who had participated in a randomized controlled trial of dietary n-3 and n-6 modification over the first 5 y of life.

**Design:** The children ( $n = 616$ ; 49% girls) were randomly assigned antenatally to active ( $n = 312$ ; increase in n-3 intake and decrease in n-6 intake) or control ( $n = 304$ ) diet interventions implemented from the time of weaning or introduction of solids until 5 y of age. At age  $8.0 \pm 0.1$  y, BP, carotid intima-media thickness, carotid artery distensibility, augmentation index, and brachial pulse wave velocity were measured in 405 of these children. Venous blood was collected for measurement of plasma fatty acids, lipoproteins, high-sensitivity C-reactive protein, and asymmetric dimethylarginine. Plasma fatty acid concentrations were also assessed during the intervention.

**Results:** Plasma concentrations of n-3 fatty acids were higher and of n-6 were lower in the active than in the control diet group at 18 mo and 3 and 5 y ( $P < 0.0001$ ). Concentrations of n-3 and n-6 fatty acids were similar at 8 y. At 8 y of age, no significant differences were found in BP, carotid intima-media thickness, carotid artery distensibility, augmentation index, asymmetric dimethylarginine, high-sensitivity C-reactive protein, or lipoproteins between diet groups.

**Conclusion:** A dietary supplement intervention to increase n-3 and decrease n-6 intakes from infancy until 5 y does not result in significant improvements in arterial structure and function at age 8 y. This trial was registered at the Australian Clinical Trials Registry as ACTRN012605000042640. *Am J Clin Nutr* 2009; 90:438–46.

## INTRODUCTION

Omega-3 (n-3) fatty acids, such as eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), are now widely added to infant formulas and weaning foods as a result of studies that have reported an improvement in cognitive and visual development after supplementation with these nutrients (1–3). Many epidemiologic studies have reported a lower prevalence of cardiovascular disease (CVD) in populations with a high fish consumption (4–6). There is evidence

of a beneficial effect of n-3 fatty acid supplementation on certain CVD risk factors, for example, a reduction in triglycerides and blood pressure (BP) and an elevation in HDL cholesterol (7, 8). Despite this, the effect of higher n-3 fatty acid intakes in adults on the risk of CVD is controversial, and many systematic reviews, prospective cohort studies, and randomized controlled trials have provided different conclusions (9–11).

Recent data suggest that nutritional interventions, when initiated in early childhood, have the potential to alter BP, vascular stiffness, and lipoprotein concentrations in later childhood (12–14). Two randomized controlled trials have reported the effect of n-3 fatty acid supplementation in early childhood on BP and/or lipoprotein concentrations (15, 16). Both studies examined a limited number of markers of vascular health after short durations of supplementation. Methodologic issues, such as incomplete adherence data and, in one study, a factorial design that tested another dietary intervention concomitantly, have also limited the ability to draw conclusions from these studies about the cardiovascular benefits of n-3 fatty acid supplementation in early life. Therefore, we undertook a detailed evaluation of BP and arterial structure and function in a large group of 8-y-old boys and girls enrolled in a randomized controlled trial of polyunsaturated fatty acid modification from early infancy to 5 y of age. Our principal aim was to study the effects of the dietary

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supplement intervention on BP and arterial structure and function. The study design allowed us to adjust the analysis, when necessary, for differences in adherence, lipoprotein concentrations, and current concentrations of n-3 and omega-6 (n-6) plasma fatty acids. We also aimed to examine the association between plasma fatty acid concentrations measured during the intervention period and markers of vascular structure and function measured at age 8 y.

## SUBJECTS AND METHODS

### Subjects

The subjects were healthy 8-y-old children recruited from the Western and southwestern areas of Sydney, Australia, who had been enrolled before birth into the Childhood Asthma Prevention Study (CAPS). CAPS is a randomized controlled trial to test the effectiveness of interventions on the prevention of asthma and allergic disease in children at risk of these conditions. Omega-3 fatty acid supplementation and house dust mite avoidance were tested in a  $2 \times 2$  factorial design. The details of the study including recruitment methods, inclusion criteria, and procedures for data collection were published elsewhere (17, 18). Briefly, pregnant women whose unborn children had at least one parent or sibling with current asthma or wheezing were identified before birth and were randomly assigned to 1 of the 4 study groups. Exclusion criteria included infants from multiple births, gestational age <36 wk, birth weight <2.5 kg, hospitalization for >1 wk, or serious illness. Six hundred and sixteen subjects were enrolled from September 1997 to December 1999.

The children were assessed at 18 mo, 3 y, 5 y (the end of the randomized intervention period), and 8 y with measurement of growth, lung function, atopy, and plasma fatty acids. At 8 y, the families were also invited to participate in a substudy examining arterial structure and function.

Of the 410 original children (67%) who consented to participate in the cardiovascular substudy, 5 subjects were excluded [2 with established type 1 diabetes mellitus, 2 who consented to the study but subsequently refused cardiovascular testing, and 1 who was older than the prespecified age range ( $8.0 \pm 0.5$  y) at the time of testing], which left 405 children, who are the subject of this report. Although maternal age and education were higher, the children who returned for vascular assessments were otherwise representative of the initial study population for baseline characteristics. This study was approved by the Human Research Ethics Committees of the University of Sydney, the Children's Hospital at Westmead and Sydney South West Area Health Service. The parent or legal guardian of the children provided written informed consent.

### Dietary supplement intervention

The details of the dietary supplement intervention were previously described (18, 19). The active intervention was intended to increase the proportion of n-3 fatty acids in the diet and to reduce the content of n-6 fatty acids so as to provide an n-3 to n-6 ratio of  $\approx 1:5$ . We provided families in this group with canola-based oils and spreads, which are low in n-6 fatty acids (16% n-6 and 6% n-3; Goodman Fielder Foods, Macquarie Park, New South Wales, Australia), to use from the time of

enrollment. Parents also added tuna oil (500-mg capsules; Nu-Mega Ingredients Pty Ltd, Brisbane, Australia) once daily to their child's formula from the time he or she started bottle feeding or to solid foods beginning at age 6 mo, whichever came first. Children who received the supplement before the age of 6 mo were administered 12–18 drops from the capsule, depending on their age. After 6 mo, the children received the entire contents of the capsule. Tuna oil contains 37% n-3 [including 27% (135 mg/capsule) DHA and 6% (32 mg/capsule) EPA] and 6% n-6 (Nu-Mega Ingredients) fatty acids.

The control diet was designed to maintain n-3 and n-6 fatty acids at intakes found in the general Australian population (range: 1:15–1:20). We supplied families in the control group polyunsaturated oils and spreads containing 40% n-6 fatty acids (Goodman Fielder Foods) to use from enrollment. In this group, the parents also added sunola oil (500mg capsules, Nu-Mega Ingredients), which contains 0.3% n-3 and 7% n-6 fatty acids, once daily to the child's formula from the time he or she started bottle feeding or to solid foods beginning at the age of 6 mo, whichever came first. The dose of sunola oil, before and after 6 months of age, was similar to the protocol outlined for the tuna oil capsules. The dietary intervention was maintained until 5 y of age. As reported previously, the active diet intervention at age 5 y had no effect on the prevalence of asthma or atopy (20).

### Assessment of adherence with oil capsules

Oil capsules were initially given to subjects in blister packs and then, from March 2000, in bottles. From the time that the bottles were introduced, consumption of oil capsules was estimated at 6-mo intervals by measuring the difference between the weights of dispensed and returned bottles and dividing by the capsule weight. Because the subjects were expected to consume 1 capsule/d, the number of capsules consumed was divided by the number of days to estimate the percentage adherence (or compliance) over this period. Adherence was averaged over the duration of the study. Data on adherence was available for all 405 children. An estimate of the daily n-3 and n-6 fatty acid intake, calculated from the average capsule compliance multiplied by the dose of n-3 and n-6 contained in the capsules, was previously reported from this study population ( $93 \pm 57$  and  $15 \pm 9$  mg/d for n-3 and n-6 intakes in the active diet group and  $1.0 \pm 0.4$  and  $20 \pm 10$  mg/d for n-3 and n-6 intakes in the control diet group) (19).

### Breastfeeding duration

Information about breastfeeding practices was collected from participating families at 1, 3, 6, 9, and 12 mo of age by using standardized questionnaires. The duration of any breastfeeding was defined as the age at which the child stopped breastfeeding. Because information on breastfeeding beyond 12 mo was not recorded, the duration of breastfeeding was considered in 3 ordered groups: group 1, 0–6 mo; group 2, 6–12 mo; and group 3, >12 mo. Information on water or juice consumption was also not recorded; therefore, it was not possible to determine the duration of exclusive breastfeeding. As previously reported, breastfeeding duration was not associated with protection from asthma or allergy in this study population (21).

## Measurements at 8 y

### *Anthropometric measurements*

Height was measured to the nearest 0.5 cm with a portable stadiometer, and weight was measured to the nearest 0.1 kg with calibrated electronic scales. Body mass index (BMI) was calculated as weight (kg)/height squared (m). Waist circumference was measured at end expiration, midway between the lower margin of the ribs and the iliac crest. The ratio of waist (cm) to height (cm) was calculated. BMI *z* scores were determined by comparison with US growth charts from the Centers for Disease Control and Prevention (22). All subjects studied had anthropometric measures recorded.

### *Blood pressure*

Brachial BP was measured with the use of a validated automated oscillometric device (Welch Allyn Vital Signs Monitor) (23). Supine BP in the left brachial artery was measured after 10 min of quiet rest and repeated after a further 10 min; a third BP measurement was taken if there was a variance of  $\geq 10$  mm Hg; the average of the 2 closest readings was recorded as the brachial BP. All subjects thus had BP measured and recorded.

### *Carotid intima-media thickness*

Carotid intima-media thickness (CMT) was measured in all subjects by B-mode ultrasound with a portable ultrasound system (Terason 3000; Teratek), a 5–12-MHz linear array transducer, and electrocardiogram gating. The right and left carotid arteries were scanned by one sonographer according to a standardized protocol, and all measurements were made by a single observer who was blinded to subject clinical details using edge detection software that was previously validated as accurate and reproducible (24). Several 5-s moving-image clips of the distal common carotid artery (CCA) were obtained and stored in a digital format for subsequent offline analysis. Three right and 3 left end-diastolic frames were selected and, for each, the mean IMT of the artery far wall was measured over the region of interest beginning 1 cm proximal to the edge of the carotid bulb. The average of the 6 measurements was used in the analysis as mean IMT. To assess interobserver variability, a second observer measured mean IMT in a random selection of 33 subjects, with a 2.3% CV. CMT is an accurate measure of arterial wall thickness, which corresponds to the histologic intima-media complex (25). In adults, CMT correlates with coronary and carotid atherosclerosis and is a significant predictor of future cardiovascular events (26).

### *Augmentation index*

The augmentation index (AIx) was measured at the left carotid and left radial arteries by obtaining pulse waveforms with a high-fidelity applanation tonometer (SPC-301; Millar Instruments). Data were collected directly into a laptop computer and processed with customized waveform analysis software (Sphygmocor version 7.1; Atcor Medical). After 10 s of continuous pulses were recorded, an ensemble-averaged waveform was generated. This waveform was calibrated to the mean and diastolic BP; these values remain relatively constant throughout the arterial tree (27). At both sites, recordings were repeated until representative averaged waveforms were obtained. Waveforms were visually

inspected by a single observer, blinded to subject details, and discarded if movement and respiration artifacts were present. Satisfactory radial pulse waveforms were available for 386 subjects (200 boys and 186 girls), and 19 were excluded as technically unsatisfactory. During the course of the study, carotid applanation tonometry was added to the protocol, and carotid waveforms were thus acquired in the last 269 subjects (135 boys and 134 girls) and 6 of these were excluded with unsatisfactory waveforms. Data from a minimum of 2 averaged waveforms were used to calculate mean values that were used in subsequent analysis. AIx was calculated by using the customized software as follows: pressure at the second systolic peak – pressure at the first systolic peak/pulse pressure, expressed as a percentage. Thus, AIx measures the degree of late systolic augmentation in the pulse waveform and is greatly influenced by arterial stiffness (28).

Because AIx is also influenced by heart rate (HR) (29), the relation between HR (average HR, obtained at the time of pulse wave acquisition) and AIx was determined for both sexes. The slope of the relation ( $\beta$ ) was used to standardize AIx to an arbitrary HR value of 75/min (AIx75):

$$\text{AIx75} = \text{AIx} - \beta \times (\text{HR} - 75) \quad (1)$$

where  $\beta = -0.37$  for boys and  $-0.24$  for girls.

### *Carotid pulse pressure*

Carotid pulse pressure (CPP) was directly measured in those 263 subjects with satisfactory carotid waveforms obtained by applanation tonometry (*see above*). In these subjects there was a linear relation between radial pulse pressure (RPP) and CPP:

$$\text{CPP} = 0.8 \times \text{RPP} + 0.71 (r^2 = 0.52) \quad (2)$$

Using this regression equation, CPP was derived in those subjects for whom only RPP was available.

### *Brachial pulse wave velocity*

Brachial pulse wave velocity (PWVb) represents the velocity of pulse wave transit from the carotid to radial arteries and is greatly influenced by arterial stiffness. PWVb was determined from sequentially measured electrocardiogram-gated left carotid and radial waveforms by using the foot-to-foot method to determine the pulse travel time. The travel distance of the pulse wave was calculated as the difference in the distance between the suprasternal notch and each recording site, which was measured with a tape measure over the body surface. Measurements were made at least in duplicate, and the mean PWVb was used in the analysis. Data on PWVb were available in 133 control and 143 active diet children.

### *Carotid artery distensibility*

To assess carotid artery distensibility (CAD), a measure of the elastic properties of arteries (30), the best quality 5-s B-mode ultrasound clip of the left distal CCA was selected. The internal diameter of the CCA was measured 1 cm from the edge of the carotid bulb, by an observer blinded to all other results, in end-systole and end-diastole (30). Measurements were made for 3 consecutive cardiac cycles, and means of the measurements were used as the end-systolic (Ds) and end-diastolic (Dd) diameters. Satisfactory images were available for CCA diameter





measurements in 390 of the 405 subjects. To assess interobserver variability, a second observer made CCA diameter measurements in 40 subjects chosen at random. The CV was 2.5% for Ds and 2.7% for Dd. B-mode diameters were compared with M-mode diameters in 55 randomly selected subjects, with CVs of 3.1% and 3.4% for Ds and Dd, respectively. The CCA diameters and CPP, either directly obtained or derived, were used to calculate CAD:

$$\text{CAD} = [(D_s - D_d) / D_d] / (\text{CPP}) \quad (3)$$

expressed as a percentage per 10 mm Hg of the change in BP as recently described (30).

### Blood analyses

At age 8 y, 328 (81%) subjects gave consent for the collection of a nonfasting venous blood sample. The subjects who had blood collected were representative of the whole cohort with respect to baseline characteristics. Samples underwent centrifugation at 3000 rpm for 5 min, and the collected serum was stored at  $-20^{\circ}\text{C}$  for  $<2$  wk before total cholesterol, HDL cholesterol, and triglycerides (with standard enzymatic procedures) and high-sensitivity C-reactive protein; with an immunoturbidimetric method) were assayed with a modular autoanalyzer (Roche, Basel, Switzerland).

Serum was also stored at  $-80^{\circ}\text{C}$  and analyzed in a single batch after one thaw cycle for apolipoprotein B (apo B, the major protein component of atherogenic lipoproteins) and apolipoprotein A-I (apo A-I, the major protein component of HDL) by immunoturbidimetry, and asymmetric dimethylarginine (ADMA) was measured by enzyme-linked immunosorbent assay (ELISA; Immundiagnostik AG, Bensheim, Germany). ADMA was assessed in duplicate (intraassay CV: 13%), and the average was recorded as the ADMA concentration. Measurement of ADMA by ELISA has been shown to be accurate and reproducible (31). ADMA, an endogenous inhibitor of the enzyme that produces nitric oxide (nitric oxide synthase), has also been shown to be an independent risk factor for CVD (32). ADMA concentrations are associated with CVD risk factors in adults (33, 34), and many studies have suggested a direct role for ADMA in the pathogenesis of endothelial dysfunction.

### Plasma fatty acid analysis

Blood was collected at 18 mo, 3 y, 5 y, and 8 y for the measurement of plasma phospholipids by gas chromatography. The details of this technique, including the sample preparation and settings for the gas chromatograph, were previously described (35). Specific n-3 fatty acids that were measured included  $\alpha$ -linolenic acid (ALA, 18:3n-3), EPA, DHA, docosapentaenoic acid (DPA, 22:5n-3), and other minor n-3 fatty acids (16:2n-3, 18:4n-3, and 20:3n-3). The n-6 fatty acids that were measured included linoleic acid (LA, 18:2n-6), arachidonic acid (AA, 20:4n-6), dihomo- $\gamma$ -linolenic acid (20:3n-6), and other minor n-6 fatty acids (18:3n-6, 20:2n-6, 22:2n-6, and 22:4n-6). Total plasma n-3 and n-6 concentrations were calculated and expressed as a percentage of total fatty acids, and the ratio of n-3 to n-6 fatty acids was then determined. Plasma fatty acid results were available for 299 (74%), 328 (81%), 336 (83%), and 313 (77%) of the children

who participated in the cardiovascular substudy at 18 mo, 3 y, 5 y, and 8 y respectively. Subjects who had plasma fatty acid data available at 8 y were similar to the entire study group in age, sex, BP, BMI z score, and proportion from the active group.

### Statistics

Continuous variables are expressed as means  $\pm$  SDs or medians (interquartile ranges), as appropriate. Categorical variables are presented as percentages. Comparisons between the dietary groups were undertaken with a 2-sample *t* test or a chi-square test, as appropriate. The association between the major plasma fatty acids (DHA, EPA, ALA, LA, and AA) and dependent variables was assessed by linear regression. Statistical significance was inferred at a 2-sided *P* value  $< 0.05$ . Because we were exploring predefined hypotheses about the effects of the dietary intervention and plasma fatty acid concentrations on BP and measures of arterial structure and function, adjustments for multiple comparisons were not performed. This was in line with recent recommendations (36, 37). All statistical analyses were performed by using SAS version 9 software (SAS Institute, Cary, NC).

### RESULTS

Of the 405 subjects included in this analysis, there were no important differences in the basic characteristics at age 8 y between children who had been in the active and control diet groups (Table 1). Within both dietary groups, the proportion of subjects in each category of breastfeeding duration was similar. There was no significant difference in compliance with the oil capsules between the 2 groups (*P* = 0.19). The concentrations of

**TABLE 1**  
Characteristics of active and control diet subjects at 8 y<sup>1</sup>

Characteristic	Active	Control
Subjects [n (%)]	205 (51)	200 (49)
Age (y)	8.0 $\pm$ 0.1 <sup>2</sup>	8.0 $\pm$ 0.1
Sex (% male)	50	52
Birth weight (kg)	3.51 $\pm$ 0.50	3.48 $\pm$ 0.50
Breastfeeding duration [n (%)]		
Group 1, 0–6 mo	121 (59)	115 (58)
Group 2, 6–12 mo	42 (20)	49 (25)
Group 3, >12 mo	42 (20)	36 (18)
Weight (kg)	29.6 $\pm$ 7.3	28.6 $\pm$ 5.9
BMI (kg/m <sup>2</sup> )	17.8 $\pm$ 3.3	17.4 $\pm$ 2.8
Height (cm)	129 $\pm$ 6	128 $\pm$ 6
Waist (cm)	59.8 $\pm$ 8.1	59.1 $\pm$ 7.2
Waist circumference:height ratio	0.46 $\pm$ 0.05	0.46 $\pm$ 0.05
HDL cholesterol (mmol/L)	1.44 $\pm$ 0.38	1.48 $\pm$ 0.36
Apolipoprotein A-I (mmol/L)	1.45 $\pm$ 0.22	1.49 $\pm$ 0.22
Apolipoprotein B (mmol/L)	0.65 $\pm$ 0.15	0.63 $\pm$ 0.16
Non-HDL cholesterol (mmol/L)	3.01 $\pm$ 0.71	2.89 $\pm$ 0.68
Triglycerides (mmol/L)	1.2 (0.9–1.7) <sup>3</sup>	1.2 (0.9–1.6)
hs-CRP (mg/L)	0.40 (0.21–1.46)	0.22 (0.21–0.72)
Average full compliance, oil capsule (%)	53.5	57.3

<sup>1</sup> hs-CRP, high-sensitivity C-reactive protein. There were no significant differences in any characteristic between the groups.

<sup>2</sup> Mean  $\pm$  SD (all such values).

<sup>3</sup> Median; interquartile range in parentheses (all such values).

**TABLE 2**Cardiovascular variables by dietary group assignment<sup>1</sup>

Variable	Active	Control	<i>P</i> value <sup>2</sup>
Systolic BP (mm Hg)	101 ± 7 (205)	100 ± 7 (200)	0.66
Diastolic BP (mm Hg)	59 ± 6 (205)	59 ± 5 (200)	0.93
Mean BP (mm Hg)	73 ± 5 (205)	73 ± 5 (200)	0.79
Heart rate (beats/min)	81 ± 9 (200)	81 ± 11 (192)	0.91
CIMT (mm)	0.59 ± 0.06 (205)	0.59 ± 0.06 (200)	0.80
CAD (% per 10 mm Hg)	6.0 ± 1.7 (195)	6.0 ± 1.7 (195)	0.93
PWVb (m/s)	6.7 ± 1.1 (143)	6.7 ± 1.1 (133)	0.95
Radial AIx75 (%)	-39 ± 12 (195)	-38 ± 11 (189)	0.38
Carotid AIx75 (%)	-11 ± 9 (140)	-12 ± 9 (128)	0.44
ADMA (μg/mL)	0.92 ± 0.23 (162)	0.90 ± 0.22 (151)	0.46

<sup>1</sup> All values are means ± SDs; *n* in parentheses. BP, blood pressure; CIMT, carotid intima media thickness; CAD, carotid artery distensibility; PWVb, brachial pulse wave velocity; AIx75, augmentation index standardized for heart rate at 75 min; ADMA, asymmetric dimethylarginine.

<sup>2</sup> Comparison between active and control diet groups by 2-sample *t* test.

lipoproteins and high-sensitivity C-reactive protein at age 8 y were similar in subjects who had been in the active and control diet groups.

BMI, BP, heart rate, and arterial structure and function were similar at age 8 y in the children who had been in the active and control diet groups (**Table 2**). In particular, no significant differences were seen in CIMT, HR-standardized radial and carotid AIx, or PWVb. CAD was similar in the active and control diet groups, and this remained the case when the analysis was restricted to only those individuals with directly measured CPP (5.9 ± 1.5 compared with 5.8 ± 1.3% per 10 mm Hg for the active and control groups, respectively; *P* = 0.49). ADMA concentrations were also similar in the children from the 2 dietary groups. The BMI *z* score was significantly correlated with systolic (*r* = 0.42, *P* < 0.0001) and diastolic (*r* = 0.21, *P* < 0.0001)

BP. However, the interaction between BMI *z* score and diet group on BP was not significant (*P* > 0.6).

Plasma n-3 concentrations were higher and n-6 concentrations were lower in the active diet group than in the control diet group at 18 mo, 3 y, and 5 y (**Table 3**; *P* < 0.0001), as described previously (19). The ratio of n-3 to n-6 fatty acids was also significantly higher in the active diet group at each time point. However, at age 8 y, similar plasma n-3 and n-6 fatty acid concentrations and similar ratios of n-3 to n-6 fatty acids were observed in children who had been in the active and control diet groups. During the intervention period, plasma n-3 and n-6 concentrations were correlated over time within subjects [intra-class correlation coefficients: 0.53 (95% CI: 0.47, 0.60) and 0.36 (95% CI: 0.29, 0.43), respectively]. During the intervention period, DHA, EPA, and ALA concentrations were higher and LA and AA concentrations were lower in the active than in the control intervention diet group (**Table 4**). At age 8 y, there was no significant difference in concentrations of the specific polyunsaturated fatty acids between the 2 intervention groups.

Plasma concentrations of the specific n-3 and n-6 fatty acids at 18 mo, 3 y, and 5 y were not significantly associated with BP or with most of the measures of arterial structure and function at age 8 y (**Table 5**). For plasma concentrations at 5 y, weak but significant associations existed between EPA and PWVb (*r* = -0.15, *P* = 0.04), LA and radial AIx75 (*r* = -0.14, *P* = 0.01), and AA and CIMT (*r* = 0.13, *P* = 0.02). The total ratio of n-3 to n-6 fatty acids at 18 mo, 3 y, and 5 y was not significantly associated with any measure of arterial structure or function at 8 y (data not shown). Plasma concentrations of EPA at 8 y were associated with radial AIx75 (*r* = -0.16, *P* = 0.01). Concentrations of the n-3 fatty acids (**Table 6**) and the ratios of n-3 to n-6 (data not shown) were otherwise not significantly associated with measures of vascular structure and function. LA was associated with CIMT (*r* = 0.16, *P* = 0.005) and AA with radial AIx75 (*r* = -0.14, *P* = 0.02) at 8 y (Table 6).

**TABLE 3**Polyunsaturated fatty acid concentrations by dietary group at 18 mo and 3, 5, and 8 y<sup>1</sup>

Fatty acid	Total <i>n</i>	Diet intervention group <sup>2</sup>		Difference		<i>P</i> value <sup>3</sup>
		Active	Control	Mean	95% CI	
Plasma n-3 (% of total fatty acids)						
18 mo	299	6.94 ± 1.97 (155)	4.96 ± 1.23 (144)	1.98	1.60, 2.36	<0.0001
3 y	328	6.11 ± 1.44 (165)	4.75 ± 1.20 (163)	1.36	1.08, 1.65	<0.0001
5 y	336	5.93 ± 1.36 (169)	5.06 ± 1.11 (167)	0.86	1.13, 0.60	<0.0001
8 y	313	4.72 ± 1.31 (161)	4.61 ± 1.16 (152)	0.11	−0.17, 0.38	0.452
Plasma n-6 (% of total fatty acids)						
18 mo	299	32.43 ± 2.8 (155)	35.13 ± 2.84 (144)	−2.70	−3.34, −2.06	<0.0001
3 y	328	33.22 ± 2.40 (165)	35.65 ± 2.28 (163)	−2.43	−2.94, −1.92	<0.0001
5 y	336	33.63 ± 2.02 (169)	35.40 ± 1.79 (167)	−1.77	−2.18, −1.36	<0.0001
8 y	313	31.75 ± 2.34 (161)	32.17 ± 2.19 (152)	−0.42	−0.93, 0.08	0.099
Plasma n-3:n-6 ratio						
18 mo	299	0.22 ± 0.07 (155)	0.14 ± 0.04 (144)	−0.07	−0.09, −0.06	<0.0001
3 y	328	0.19 ± 0.05 (165)	0.13 ± 0.04 (163)	−0.05	−0.06, −0.04	<0.0001
5 y	336	0.18 ± 0.05 (169)	0.14 ± 0.04 (167)	−0.03	−0.04, −0.02	<0.0001
8 y	313	0.15 ± 0.05 (161)	0.15 ± 0.04 (152)	0	−0.02, 0.01	0.332

<sup>1</sup> Data from 18 mo, 3 y, and 5 y were previously reported (19).

<sup>2</sup> All values are means ± SDs; *n* in parentheses.

<sup>3</sup> Comparison between groups at a particular age by 2-sample *t* test.



TABLE 4

Specific polyunsaturated fatty acid concentrations by dietary group at 18 mo and 3, 5, and 8 y<sup>1</sup>

Fatty acid	Diet intervention group		Difference		<i>P</i> value <sup>2</sup>
	Active	Control	Mean	95% CI	
18 mo					
<i>n</i>	155	144			
DHA (% of total fatty acids)	4.38 ± 1.79 <sup>3</sup>	2.82 ± 1.03	1.57	1.23, 1.90	<0.0001
EPA (% of total fatty acids)	1.43 ± 0.36	1.38 ± 0.33	0.04	−0.04, 0.12	0.285
ALA (% of total fatty acids)	0.20 ± 0.08	0.14 ± 0.07	0.06	0.04, 0.08	<0.0001
LA (% of total fatty acids)	18.57 ± 2.83	20.56 ± 3.08	−1.99	−2.67, −1.32	<0.0001
AA (% of total fatty acids)	9.31 ± 1.49	9.78 ± 1.63	−0.46	−0.82, −0.11	0.01
3 y					
<i>n</i>	165	163			
DHA (% of total fatty acids)	3.76 ± 1.34	2.79 ± 0.92	0.97	0.72, 1.22	<0.0001
EPA (% of total fatty acids)	1.27 ± 0.27	1.20 ± 0.20	0.08	0.03, 0.13	0.004
ALA (% of total fatty acids)	0.23 ± 0.09	0.15 ± 0.05	0.08	0.06, 0.09	<0.0001
LA (% of total fatty acids)	19.37 ± 2.33	21.30 ± 2.77	−1.92	−2.48, −1.37	<0.0001
AA (% of total fatty acids)	9.22 ± 1.40	9.54 ± 1.49	−0.32	−0.64, −0.01	0.04
5 y					
<i>n</i>	169	167			
DHA (% of total fatty acids)	3.63 ± 1.15	3.10 ± 0.92	0.53	0.31, 0.76	<0.0001
EPA (% of total fatty acids)	1.21 ± 0.25	1.15 ± 0.18	0.07	0.02, 0.12	0.005
ALA (% of total fatty acids)	0.21 ± 0.07	0.15 ± 0.06	0.06	0.05, 0.07	<0.0001
LA (% of total fatty acids)	19.66 ± 2.46	20.72 ± 2.28	−1.06	−1.57, −0.55	<0.0001
AA (% of total fatty acids)	9.31 ± 1.44	9.71 ± 1.66	−0.41	−0.74, −0.07	0.02
8 y					
<i>n</i>	161	152			
DHA (% of total fatty acids)	2.60 ± 0.82	2.60 ± 0.81	0	−0.18, 0.19	0.965
EPA (% of total fatty acids)	0.71 ± 0.50	0.66 ± 0.38	0.05	−0.05, 0.15	0.344
ALA (% of total fatty acids)	0.19 ± 0.07	0.19 ± 0.09	0	−0.02, 0.02	0.778
LA (% of total fatty acids)	19.18 ± 2.51	19.47 ± 2.54	−0.29	−0.85, 0.27	0.312
AA (% of total fatty acids)	8.26 ± 1.29	8.45 ± 1.29	−0.19	−0.47, 0.10	0.202

<sup>1</sup> DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ALA,  $\alpha$ -linolenic acid; LA, linoleic acid; AA, arachidonic acid.<sup>2</sup> Comparison between groups for the particular plasma fatty acid at a particular age by 2-sample *t* test.<sup>3</sup> Mean ± SD (all such values).

## DISCUSSION

In this study, we found no significant vascular benefits of a dietary supplement intervention that raised n-3 and lowered n-6 fatty acid levels from infancy to 5 y of age on BP and comprehensive measures of arterial structure and function at age 8 y. Moreover, no consistent associations were observed between these vascular variables and the plasma concentrations of specific n-3 and n-6 fatty acids during the first 5 y of life. However, we did observe that, at age 8 y, subjects with higher plasma concentrations of LA had greater CIMT values.

Studies in adults have reported changes in certain CVD risk factors at the time of supplementation with n-3 fatty acids (7, 8, 38). However, nutritional interventions, when applied at critical periods in early life, have the potential to remotely influence or "program" future CVD risk, as reported in many animal studies (39, 40). With regard to n-3 fatty acids, such a potential had been suggested by a randomized controlled trial reported by Forsyth et al (15). Bottle-fed infants received either formula supplemented with DHA and AA or standard formula, for the first 4 mo of life. At 6 y of age, diastolic BP was significantly lower (mean difference -3.6 mm Hg, *P* = 0.018) in the 65 children who had received supplemented formula than in the 71 who had received standard formula. However, the effect of the dietary intervention on plasma fatty acid concentrations was not reported. Therefore, the relative contribution of n-3 and n-6

supplementation to the observed reduction in diastolic BP remains unclear. Also unreported (and hence potentially residually confounding) was the exposure to n-3 and n-6 fatty acids between the end of the intervention at 4 mo and the measurement of BP at 6 y.

The potential for n-3 fatty acid supplementation in infancy to reduce BP was also suggested by Damsgaard et al (16). Thirty-nine infants who received fish-oil supplements from 9 to 12 mo of age showed a trend toward a lower systolic BP than did 44 infants who were not supplemented (difference of 4.7 mm Hg; 95% CI: 0, 9.4; *P* = 0.05), when BP was measured at the end of the intervention. However, BP was unrelated to red blood cell concentrations of EPA and only weakly inversely correlated with DHA concentrations.

The study by Damsgaard et al (16) suggests that effect attenuation is one possibility for the principle finding of our study; namely, no significant difference in arterial structure and function between those who had been on the active and control diets when measured 3 y after cessation of the intervention. An effect on BP or arterial structure and function that was present during the intervention may have waned by the time of measurement, when exposure to fatty acids was similar. However we were unable to show consistent and significant associations between the vascular markers and concentrations of plasma fatty acids during the intervention period. Thus, although a time-dependent waning of

**TABLE 5**

Pearson correlation coefficients between vascular measures and polyunsaturated fatty acid concentrations at 18 mo and 3 and 5 y<sup>1</sup>

Fatty acid	SBP	DBP	CIMT	CAD	PWVb	Radial AIx75	Carotid AIx75
5 y							
DHA	-0.01	-0.01	0.01	-0.04	-0.07	-0.02	0.01
EPA	-0.02	-0.02	-0.01	0.03	-0.15 <sup>2</sup>	-0.07	0.01
ALA	-0.01	-0.08	0.07	-0.04	-0.11	0.003	0.001
LA	-0.03	-0.01	-0.10	0.02	0.08	0.14 <sup>3</sup>	0.08
AA	0.04	-0.01	0.13 <sup>4</sup>	0.05	0.07	-0.09	-0.10
3 y							
DHA	-0.05	-0.02	-0.05	-0.03	-0.02	-0.04	-0.02
EPA	-0.04	-0.05	-0.01	-0.01	-0.07	-0.02	-0.004
ALA	-0.03	-0.07	-0.08	-0.01	-0.07	0.01	-0.06
LA	-0.02	0.06	0.02	0.08	-0.04	0.08	0.11
AA	0.02	0.04	0.10	0.01	-0.04	-0.04	-0.11
18 mo							
DHA	-0.05	-0.07	-0.04	-0.03	-0.06	-0.02	-0.04
EPA	-0.08	-0.04	-0.08	0.04	-0.03	-0.01	-0.08
ALA	-0.05	-0.05	0.03	0.003	0.004	-0.05	-0.04
LA	0.02	0.02	0.06	-0.02	-0.07	0.02	-0.01
AA	-0.02	-0.06	-0.08	0.04	0.11	-0.06	-0.07

<sup>1</sup> SBP, systolic blood pressure; DBP, diastolic blood pressure; CIMT, carotid intima-media thickness; CAD, carotid artery distensibility; PWVb, brachial pulse wave velocity; AIx75, augmentation index standardized for heart rate at 75 min; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ALA,  $\alpha$ -linolenic acid; LA, linoleic acid; AA, arachidonic acid. At 5 y,  $n = 336$  (SBP, DBP, and CIMT), 324 (CAD), 221 (PWVb), 318 (radial AIx75), and 214 (carotid AIx75). At 3 y,  $n = 328$  (SBP, DBP, and CIMT), 319 (CAD), 226 (PWVb), 313 (radial AIx75), and 221 (carotid AIx75). At 18 mo,  $n = 299$  (SBP, DBP, and CIMT), 286 (CAD), 195 (PWVb), 282 (radial AIx75), and 189 (carotid AIx75).

<sup>2</sup>  $P = 0.04$ .

<sup>3</sup>  $P = 0.01$ .

<sup>4</sup>  $P = 0.02$ .

a beneficial effect of the active diet remains possible, an absence of even a trend toward an association with plasma n-3 fatty acid concentrations makes this unlikely. Importantly, our data suggest that n-3 fatty acid supplementation in infancy and early childhood does not have residual beneficial effects on vascular structure and function.

Another potential explanation for the apparent lack of benefit from the dietary intervention is that an insufficient dose of n-3 fatty acids was used. Although we prescribed a dose that significantly and consistently altered n-3 and n-6 plasma fatty acid concentrations through to age 5 y, the ratio of n-3 to n-6 fatty acids in the active diet group was still substantially lower than that found in populations with high fish consumption (41). However, the n-3 intake that was achieved in this study, as we previously reported (19), was several times above the adequate intake recommended for Australian children (42). Higher doses in childhood are unlikely to be practically achievable.

Our dietary supplement intervention was specifically aimed to increase the n-3 fatty acid intake and lower the n-6 fatty acid intake. This related, at least in part, to prior studies that suggested lower n-3 concentrations with higher n-6 intakes (43, 44). However, many case-control and prospective cohort studies have reported an association between a greater intake or a higher blood and tissue concentration of n-6 fatty acids and a lower risk of coronary heart disease (45-47). Although we also recently reported lower plasma n-3 concentrations with higher n-6 intakes in this group of children (48), it is possible that a dietary strategy that increased n-3 and maintained normal

n-6 plasma fatty acid concentrations may have resulted in a beneficial effect on arterial structure and function when measured at 8 y. However, we were unable to show a significant association between BP or most of the other vascular measures and the 8-y concentrations of AA or LA. Moreover, our finding of a significant but weakly positive association between LA at 8 y and CIMT might imply an adverse effect from higher LA concentrations. Thus, it is unlikely that in our active dietary supplement intervention a beneficial effect of raising n-3 fatty acid concentrations would have been masked by a detrimental effect resulting from reducing n-6 plasma fatty acid concentrations.

The lack of a significant association between plasma n-3 fatty acid concentrations in early childhood and measures of vascular function at age 8 y likely reflect a truly absent biological effect. Indeed, our findings are consistent with those of Leeson et al (49) in young adults. In this study of 326 subjects aged 20-28 y, plasma and red blood cell concentrations of DHA and EPA were not associated with brachial artery flow-mediated dilatation (a marker of endothelial function), except in smokers or those with high insulin, glucose, or triglyceride concentrations.

Our study had several potential limitations. First, the single point-in-time measurements of plasma fatty acids may not have reflected the long-term dietary and supplement exposure as accurately as tissue or red blood cell concentrations, which were not available in our study (50). However, during the intervention period, measurements were made on multiple occasions, with at least moderate correlations between n-6 and n-3 plasma fatty



**TABLE 6**Pearson's correlation coefficient between vascular measures and polyunsaturated fatty acid concentrations at 8 y<sup>1</sup>

Fatty acid variable	DHA	EPA	ALA	LA	AA
SBP	0.003	0.02	0.04	-0.04	0.002
DBP	-0.04	-0.08	0.09	-0.04	-0.001
CIMT	0.01	0.03	-0.05	0.16 <sup>2</sup>	0.03
CAD	-0.07	-0.03	-0.08	-0.04	0.05
PWVb	0.09	-0.04	-0.06	0.03	-0.06
Radial AIx75	-0.06	-0.16 <sup>3</sup>	0.06	0.08	-0.14 <sup>4</sup>
Carotid AIx75	-0.02	-0.05	-0.03	-0.02	-0.11
HDL-cholesterol	-0.004	0.11	0.11	0.05	0.08
Apolipoprotein A-I	-0.06	0.12 <sup>5</sup>	0.12 <sup>5</sup>	0.04	0.02
Apolipoprotein B	0.10	-0.001	-0.08	-0.04	0.01
Non-HDL cholesterol	0.07	0.03	-0.07	-0.11	-0.04
Triglycerides	0.02	0.07	0.05	-0.15 <sup>3</sup>	-0.10
hs-CRP	0.11	0.05	-0.02	-0.14 <sup>3</sup>	0.05
ADMA	-0.07	-0.02	-0.05	0.07	-0.04

<sup>1</sup> DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ALA,  $\alpha$ -linolenic acid; LA, linoleic acid; AA, arachidonic acid; SBP, systolic blood pressure; DBP, diastolic blood pressure; CIMT, carotid intima-media thickness; CAD, carotid artery distensibility; PWVb, brachial pulse wave velocity; AIx75, augmentation index standardized for heart rate at 75 min; hs-CRP, high-sensitivity C-reactive protein; ADMA, asymmetric dimethylarginine.  $n = 313$  (SBP, DBP, and CIMT), 300 (CAD), 208 (PWVb), 299 (radial AIx75), 202 (carotid AIx75), and 303 (for metabolic variables).

<sup>2</sup>  $P = 0.005$ .

<sup>3</sup>  $P = 0.01$ .

<sup>4</sup>  $P = 0.02$ .

<sup>5</sup>  $P = 0.04$ .

acids at different time points. Thus, we believe that these multiple plasma measurements, combined with other data on adherence, provide an accurate measure of overall exposure to the polyunsaturated fatty acids. Second, nonfasting venous blood samples were collected throughout the study. The concentrations of EPA, but not of DHA, have been shown to rise in the phospholipid fraction of plasma after acute ingestion of high doses of fish oil (51). It is unlikely, however, that the much smaller variations in plasma fatty acids related to normal food consumption would have systematically altered our findings. At age 8 y we assessed non-HDL cholesterol, HDL-cholesterol, apo B and apo A-1, concentrations of which differ little between the fasting and nonfasted states (52). Last, although our study was considerably larger than that described by Forsyth et al (15), it involved only 67% of the original study population. Selection bias was unlikely because those who returned for vascular assessment were representative of the original population, as assessed by baseline characteristics. It is possible, although unlikely, that a larger sample size would have resulted in findings that were different from ours. Importantly, a post hoc power calculation suggests that our study would have a power of >99% ( $\alpha = 0.05$ ) to detect a difference in BP between the dietary groups, similar in magnitude to that shown by Forsyth et al (3.6 mm Hg).

In summary, we found no significant cardiovascular benefits from a dietary supplement intervention that increased n-3 fatty acid and lowered n-6 fatty acid intakes in infancy and early childhood. In an era in which n-3 fatty acids are being widely added to infant formulas and weaning foods, these data provide potentially important information for those planning public health interventions to reduce the future burden of CVD.

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