



Omega-3 fatty acid supplementation improves vascular function and reduces inflammation in obese adolescents

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ABSTRACT

Objective: Compared to normal weight adolescents, obese adolescents have lower serum omega-3 (n-3) polyunsaturated fatty acid (PUFA) concentrations, augmented inflammatory activity and endothelial dysfunction. We wanted to assess whether n-3 supplementation increases the serum n-3 PUFA concentration, improves vascular function and morphology, and lowers inflammation in obese adolescents.

Methods: Twenty-five obese adolescents (14 females, 11 males, age 15.7 ± 1.0 years, BMI 33.8 ± 3.9) were randomized to receive capsules containing either 1.2 g/day n-3 or placebo for 3 months. The study was performed using a double-blind, cross-over design with a 6-week washout period. Anthropometry, blood pressure measurements and fasting blood samples were obtained before and after each treatment period. The vascular structure and function was measured after each treatment period.

Results: The serum n-3 PUFA concentration increased with n-3 treatment. The reactive hyperemia response improved with n-3 treatment compared to placebo ($p < 0.01$). N-3 supplementation also decreased the lymphocyte, monocyte, TNF- α , IL-6 and IL-1 β levels. No difference was found in the total cholesterol, triacylglycerol, HDL cholesterol, anthropometry, blood pressure, pulse wave velocity or vascular structure between the two treatment groups.

Conclusion: Daily supplementation with n-3 capsules increases the serum n-3 PUFA concentration, improves vascular function, and lowers the degree of inflammation in obese adolescents.

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1. Introduction

The prevalence of overweight and obese children and adolescents is increasing worldwide and is likely to have a great impact on their future cardiovascular health. The worldwide development of obesity can be explained, in part, by the “Western diet” that contains small amounts of essential n-3 polyunsaturated fatty acids (PUFAs), which are well known to prevent cardiovascular disease [1].

We recently showed that obese children have lower n-3 concentrations in serum phospholipids than age-matched lean controls [2]. We found correlations between n-3 PUFAs and different markers of metabolic syndrome. Whether low n-3 serum phospholipid concentrations in young obese subjects hamper vascular function and increase inflammatory activity remains to be elucidated.

In obese children and adolescents, radial artery intima thickness may constitute an early sign of vascular wall atherogenic

and inflammatory processes. Using a new ultrasound system with very high-resolution and a discrimination power of $30 \mu\text{m}^3$, obese children have been shown to have increased arterial intimal thickness compared to lean controls, a change that is not detected by traditional measurements of the intima-media complex [4]. Endothelial dysfunction occurs in obese children [5], indicating early atherosclerosis. In addition, positive associations have been found between high serum n-3 levels and endothelial function in young adults with risk factors for cardiovascular disease [6,7].

Given the improved lipid profile and reduced magnitude of inflammation evident in adults with increasing n-3 concentrations, we wanted to examine the effect of n-3 supplementation on vascular structure and function in obese adolescents and its possible relationship to inflammation and oxidative stress.

2. Materials and methods

2.1. Study subjects

A total of 108 patients aged 14–17 years and referred to an outpatient clinic for the treatment of obesity were invited to enroll in the study in 2005. Of these patients, 47 agreed to participate

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in an informational meeting and 31 agreed to participate in the present study. One subject was excluded before the study began due to smoking, one moved abroad, three dropped out during the study, and one was excluded due to poor compliance, which left 25 subjects (14 females and 11 males) who completed the study in 2006. The protocols were approved by the Ethics Committee at the University of Gothenburg and presented to the adolescents and their parents to obtain informed consent. The trial was conducted according to the guidelines of Good Clinical Practice.

2.2. Study design

The study was performed using a double-blind, cross-over design with a 6-week washout period. Subjects were randomized to placebo or n-3 treatment. Both treatments consisted of ingesting 10 capsules daily for 3 months, which provided medium chain triglycerides as placebo or a total of 930 mg eicosapentaenoic acid (EPA; 20:5n-3), 290 mg docosahexaenoic acid (DHA; 22:6n-3), 100 mg gammalinolenic acid (GLA; 18:3n-6), and 18 mg vitamin E per day. The capsules used in the present study are commercially produced (EyeQ, Equazen, UK) and have been used in other studies with children [8]. Fasting venous blood samples, blood pressure measurements and anthropometry were taken before the start of treatment and after each 3-month treatment period. Further blood samples were taken after the washout period to ensure that the n-3 concentrations had decreased to baseline levels before cross-over.

Weight was measured using an electronic balance, and height was measured using a stadiometer, allowing calculations of body mass index (BMI; kg/m²). Resting blood pressure levels were measured in the right arm using an electronic sphygmomanometer (Welch Allyn, Inc., NY, USA). After 15 min of rest in a supine position, three separate readings were taken 2 min apart, and the average of the second and third readings was used for analysis.

To control adherence to the treatment, the participants were contacted monthly. Remaining capsules and a record of capsule intake were presented to an investigator, and subjects with a compliance level below 75% were excluded from the study. All subjects were instructed to maintain their normal dietary and exercise patterns throughout the study. To control dietary changes during the study, subjects completed a food-frequency questionnaire before each treatment period. Dietary habits remained unchanged between the two periods.

Measures of endothelial function, arterial stiffness, and arterial wall morphology were performed by a trained investigator at the end of each 3-month treatment period in a standardized laboratory setting, which ensured calm surroundings with minimal disturbance and controlled temperature.

2.3. Biochemical analysis

White blood cells, red blood cells and platelets were analyzed by fluorescence-activated cell sorting. Fasting insulin, total cholesterol, HDL cholesterol and triacylglycerol levels were analyzed using enzymatic methods (Roche Diagnostics, Mannheim, Germany). LDL cholesterol concentrations were calculated using Friedewald's equation. Free fatty acids were analyzed using enzymatic colorimetry (Diagnostic Systems GmbH, Germany). Apolipoproteins A1, B, and CIII were analyzed using immunoturbidimetric assays (Thermo Scientific and Kamiya Biomedical Company, USA). Inflammation and adhesion molecules were analyzed using sandwich immunoassays (Meso Scale Discovery, MD, USA). Sera were kept frozen (−70 °C) until the fatty acid composition of phospholipids was analyzed. Lipids were extracted and fractionated, and the serum phospholipid fatty acids were analyzed as previously described in Ref. [7].

2.4. Ascorbyl radical measurements

Samples were collected at the end of each treatment period and analyzed by electron spin resonance as previously described in Ref. [9]. The intensity of the ascorbyl radical in each plasma sample was measured using a Bruker ECS 106 EPR spectrometer (Bruker Biospin GmbH, Rheinstetten, Germany).

2.5. Vascular function measurements

Vascular function was assessed using reactive hyperemia peripheral arterial tonometry (RH-PAT) by the Endo-PAT[®] device (Itamar Medical Ltd., Caesarea, Israel). In short, RH-PAT testing is an operator independent, non-invasive technique that measures the arterial pulse wave amplitude using pneumatic fingertip probes during RH [10,11], allowing a RH-PAT index (RHI) to be calculated. The hyperemia vs. baseline responses were not entirely linear; therefore, a natural logarithmic transformation of the RHI was performed [12]. In a separate group of healthy children ($n = 15$), each subject was tested twice by RH-PAT with a 10-week interval. The coefficient of variation was 14%.

The ratio between the average baseline amplitude and post-occlusion amplitude was calculated as an average of each 30 s interval from 0 to 5 min post-occlusion. The post-occlusion period was also analyzed in terms of the area under curve (AUC) for 0–1, 0–3 and 0–5 min post-occlusion. The maximum reactive hyperemic response was extracted manually and analyzed. These measures have commonly been used in earlier studies of endothelial function in children and adults [11].

2.6. Pulse wave velocity measurements

A pressure tonometer (SphygmoCor[®] system, AtCor Medical, Australia) was used to transcutaneously record the pressure pulse waveform in the underlying artery at two different arterial sites, the carotid and radial arteries. Recordings were made simultaneously with an ECG signal, which provided an *R*-timing reference, and the pulse wave velocity (PWV) was then calculated using the mean time difference and distance between the two recording points [13]. In a separate group of 10 healthy children and adolescents, the reproducibility, which was calculated as a coefficient of variation for the PWV measurements, was 9%.

2.7. Ultrasound measurements

We used high-resolution ultrasound of 55 MHz (Vevo 770[™], VisualSonics Inc., Toronto, Ontario, Canada), which was recently validated for use in human peripheral arteries [3]. The right-side radial artery (RA) was scanned in resting subjects in a supine position to obtain 2-D images, which were subsequently analyzed off-line, measuring RA intima thickness (IT), RA intima-media thickness (RA IMT), and RA diameter as previously described in Refs. [3,4].

2.8. Statistical analysis

Statistical analyses were performed by the statistical software SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). All results are expressed as mean ± SD. Statistically significant differences between variables were tested both at baseline and after treatment, and determined by paired *t* test of the difference between these values for each treatment arm, respectively. All of these differences were normally distributed. No statistically significant differences were found between the means at the beginning of the two treatment periods for any tested variable. For variables measured solely at the end of each treatment period, significance was tested by

Table 1
Anthropometrical and biochemical variables of study subjects before and after each treatment period.

| | Omega-3 | | Placebo | | <i>p</i> |
|----------------------------|--------------|-------------------------|--------------|-------------------------|----------|
| | Baseline | After 3-month treatment | Baseline | After 3-month treatment | |
| Height (m) | 1.70 ± 0.08 | 1.71 ± 0.08 | 1.71 ± 0.07 | 1.71 ± 0.08 | 0.04 |
| Weight (kg) | 98.8 ± 17.5 | 100.5 ± 18.7 | 100.1 ± 18.5 | 101.5 ± 18.2 | 0.67 |
| BMI (kg/m ²) | 34.0 ± 4.0 | 34.2 ± 4.4 | 34.2 ± 4.4 | 34.5 ± 4.2 | 0.90 |
| Waist (cm) | 109.9 ± 12.0 | 104.6 ± 12.6 | 110.1 ± 13.6 | 103.2 ± 12.6 | 0.80 |
| Hip (cm) | 116.5 ± 9.4 | 113.4 ± 9.1 | 118.3 ± 7.4 | 115.0 ± 8.8 | 0.84 |
| W/H ratio | 0.95 ± 0.07 | 0.92 ± 0.08 | 0.93 ± 0.09 | 0.90 ± 0.07 | 0.81 |
| Glucose (mmol/L) | 4.5 ± 0.4 | 4.9 ± 0.4 | 4.7 ± 0.4 | 4.8 ± 0.4 | 0.07 |
| Insulin (μU/L) | 11.7 ± 5.6 | 13.6 ± 6.9 | 12.9 ± 6.6 | 12.2 ± 8.8 | 0.20 |
| Hemoglobin (g/L) | 143 ± 11 | 135 ± 12 | 142 ± 12 | 134 ± 9 | 0.90 |
| Total cholesterol (mmol/L) | 4.2 ± 0.7 | 4.0 ± 0.6 | 4.2 ± 0.6 | 3.9 ± 0.7 | 0.22 |
| Triacylglycerol (mmol/L) | 1.3 ± 0.6 | 1.0 ± 0.4 | 1.3 ± 0.6 | 1.1 ± 0.6 | 0.14 |
| HDL cholesterol (mmol/L) | 1.3 ± 0.2 | 1.3 ± 0.2 | 1.3 ± 0.2 | 1.3 ± 0.2 | 0.15 |
| LDL cholesterol (mmol/L) | 2.4 ± 0.6 | 2.2 ± 0.5 | 2.4 ± 0.6 | 2.1 ± 0.6 | 0.05 |
| Apolipoprotein A1 (g/L) | N/A | 1.08 ± 0.10 | N/A | 1.09 ± 0.10 | 0.58 |
| Apolipoprotein B (g/L) | N/A | 0.78 ± 0.16 | N/A | 0.77 ± 0.19 | 0.52 |
| Apolipoprotein B/A1 | N/A | 0.73 ± 0.17 | N/A | 0.70 ± 0.18 | 0.22 |
| Apolipoprotein C III (g/L) | N/A | 0.14 ± 0.03 | N/A | 0.14 ± 0.02 | 0.41 |
| Free fatty acids (mmol/L) | N/A | 0.49 ± 0.16 | N/A | 0.53 ± 0.19 | 0.04 |
| Ascorbyl radicals (μM) | N/A | 0.070 ± 0.016 | N/A | 0.072 ± 0.020 | 0.65 |

Data are presented as mean ± SD. Significance was calculated as the difference between the baseline and post-treatment value for each treatment arm tested by paired *t* test. For all variables, no significant differences were found between the baseline means of the two treatment periods. For variables measured solely after treatment, significance was tested by paired *t* test or Wilcoxon Signed ranks test. BMI: body mass index; W/H ratio: waist/hip ratio; N/A: not available.

paired *t* test of the post-treatment values. Normal distribution was determined by Shapiro–Wilk's test, and for variables which did not follow the normal distribution pattern, Wilcoxon Signed Ranks test was used. Associations between variables were analyzed using simple correlation. GraphPad Prism 4.03 (GraphPad Software, Inc., San Diego, CA, USA) was used for all curve analyses. Given that the primary outcome of this study was to evaluate whether omega-3 supplement would improve vascular function, we present power calculation for the RH-index. Based on the fact that the within-patient standard deviation of the RHI is 0.260, we estimated that 23 patients were needed for this cross-over study to achieve a minimum treatment effect of 0.2 units in RHI, one sided 5% significance level, 80% power.

Global fitting was used to compare the pair-wise RH response curves after n-3 and placebo treatment for each subject. This method is a form of non-linear regression in which one curve

(placebo) for each subject is used as model (or baseline), and the discrepancy between this model and the other curve is evaluated. The technique has the same function as a paired samples *t* test, but it is used for a pair of curves instead of a pair of values.

3. Results

No differences were found between n-3 and placebo treatment periods regarding anthropometrical measures, including weight, waist, hip, and the waist/hip (W/H) ratio, though a significant difference was found for height (Table 1). LDL cholesterol levels were reduced after n-3 treatment, but even more so after placebo treatment (8% vs. 13%, respectively, $p=0.047$). Free fatty acids were lower after n-3 treatment compared to placebo treatment (reduced by 7.5%, $p=0.05$). No difference was found between treatments in regards to total cholesterol, triacylglycerol, HDL cholesterol,

Table 2
Inflammatory cells, adhesion molecules, and cytokines before and after each treatment.

| | Omega-3 | | Placebo | | <i>p</i> |
|----------------------------------|-------------|-------------------------|-------------|-------------------------|----------|
| | Baseline | After 3-month treatment | Baseline | After 3-month treatment | |
| WBC (10 ⁹ /L) | 7.4 ± 1.1 | 6.9 ± 1.3 | 7.1 ± 2.0 | 7.1 ± 1.4 | 0.50 |
| Neutrophils (10 ⁹ /L) | 3.8 ± 0.9 | 3.6 ± 1.0 | 3.6 ± 1.1 | 3.9 ± 1.0 | 0.19 |
| Lymphocytes (10 ⁹ /L) | 2.7 ± 0.7 | 2.5 ± 0.6 | 2.5 ± 0.6 | 2.5 ± 0.7 | 0.037 |
| Monocytes (10 ⁹ /L) | 0.61 ± 0.14 | 0.54 ± 0.13 | 0.53 ± 0.16 | 0.57 ± 0.16 | 0.021 |
| sICAM-1 (pg/mL) | N/A | 0.68 ± 0.53 | N/A | 0.91 ± 0.76 | 0.24 |
| sVCAM-1 (pg/mL) | N/A | 2.2 ± 2.0 | N/A | 1.3 ± 1.2 | 0.016 |
| CRP (pg/mL) | N/A | 1.06 ± 0.92 | N/A | 1.11 ± 0.71 | 0.87 |
| GM-CSF (pg/mL) | N/A | 0.46 ± 0.28 | N/A | 0.46 ± 0.21 | 0.91 |
| IFN-γ (pg/mL) | N/A | 0.70 ± 0.28 | N/A | 0.75 ± 0.23 | 0.55 |
| TNF-α (pg/mL) | N/A | 5.2 ± 1.0 | N/A | 5.7 ± 1.5 | 0.008 |
| IL-1β (pg/mL) | N/A | 0.18 ± 0.02 | N/A | 0.40 ± 0.32 | 0.023 |
| IL-2 (pg/mL) | N/A | 1.3 ± 0.9 | N/A | 1.0 ± 0.8 | 0.004 |
| IL-6 (pg/mL) | N/A | 1.8 ± 0.5 | N/A | 2.1 ± 0.8 | 0.035 |
| IL-8 (pg/mL) | N/A | 2.2 ± 0.8 | N/A | 2.5 ± 0.7 | 0.17 |
| IL-10 (pg/mL) | N/A | 2.0 ± 1.1 | N/A | 2.0 ± 1.2 | 0.88 |
| IL-12 p70 (pg/mL) | N/A | 2.6 ± 1.2 | N/A | 2.6 ± 1.4 | 0.84 |
| SAA (pg/mL) | N/A | 2.2 ± 1.5 | N/A | 2.1 ± 1.8 | 0.44 |

Data are presented as mean ± SD. Significance was calculated as the difference between the baseline and post-treatment value for each treatment arm using paired *t* test. For all variables, no significant differences were found between the baseline means of the two treatment periods. For variables measured solely after treatment, significance was tested by paired *t* test or Wilcoxon Signed Ranks test. WBC: white blood cell count, sICAM: serum inter-cellular adhesion molecule; sVCAM: serum vascular cell adhesion molecule; CRP: C-reactive protein; GM-CSF: granulocyte monocyte colony stimulating factor; IFN: interferon; TNF: tumor necrosis factor; IL: interleukin; SAA: serum amyloid A; N/A: not available.

Table 3

Fatty acid composition of serum phospholipids before and after treatment expressed as molage percent.

| | Omega-3 | | Placebo | | p |
|--------------------------|-------------|----------------|-------------|----------------|---------|
| | Baseline | After 3 months | Baseline | After 3 months | |
| Σ SFA | 47.2 ± 0.6 | 46.9 ± 1.1 | 47.7 ± 1.2 | 47.2 ± 1.0 | 0.60 |
| Σ MUFA | 12.4 ± 1.6 | 11.6 ± 1.6 | 12.3 ± 1.2 | 11.9 ± 1.2 | 0.29 |
| Σ PUFA | 40.4 ± 1.8 | 41.5 ± 2.4 | 40.1 ± 1.6 | 40.9 ± 1.7 | 0.68 |
| Σ n-6 | 34.8 ± 2.5 | 32.3 ± 2.9 | 34.0 ± 2.0 | 34.9 ± 2.6 | <0.0001 |
| 18:2n-6 | 22.2 ± 2.5 | 20.8 ± 3.1 | 21.3 ± 2.4 | 22.3 ± 3.4 | 0.009 |
| 18:3n-6 | 0.08 ± 0.04 | 0.06 ± 0.03 | 0.07 ± 0.04 | 0.07 ± 0.03 | 0.08 |
| 20:2n-6 | 0.29 ± 0.04 | 0.26 ± 0.05 | 0.28 ± 0.06 | 0.29 ± 0.05 | 0.004 |
| 20:3n-6 | 3.8 ± 0.8 | 3.2 ± 0.6 | 3.8 ± 0.7 | 3.8 ± 0.6 | 0.001 |
| 20:4n-6 (AA) | 8.4 ± 1.1 | 7.9 ± 1.1 | 8.6 ± 1.6 | 8.4 ± 1.9 | 0.49 |
| Σ n-3 | 5.5 ± 1.4 | 9.1 ± 2.3 | 5.9 ± 1.4 | 5.8 ± 2.2 | <0.0001 |
| 18:3n-3 (ALA) | 0.27 ± 0.09 | 0.23 ± 0.07 | 0.24 ± 0.11 | 0.28 ± 0.11 | 0.04 |
| 20:5n-3 (EPA) | 1.2 ± 0.5 | 3.6 ± 1.5 | 1.3 ± 0.4 | 1.4 ± 1.0 | <0.0001 |
| 22:6n-3 (DHA) | 4.0 ± 1.0 | 5.3 ± 1.1 | 4.4 ± 1.1 | 4.1 ± 1.3 | <0.0001 |
| 20:4n-6/22:6n-3 (AA/DHA) | 2.2 ± 0.6 | 1.5 ± 0.4 | 2.1 ± 0.5 | 2.2 ± 0.7 | <0.0001 |

Data are presented as mean ± SD. Significance was calculated as the difference between the baseline and post-treatment value for each treatment arm using paired t test. For all variables, no significant differences between the baseline means of the two treatment periods were found.

apolipoprotein, ascorbyl radical, glucose, insulin and hemoglobin levels (Table 1).

3.1. Inflammation and adhesion molecules

The numbers of lymphocytes and monocytes were decreased after n-3 treatment as compared to placebo (Table 2). No difference was found between treatments for the change in white blood cell count or neutrophils. Serum vascular cell adhesion molecule-1 (sVCAM-1) was increased after n-3 treatment compared to placebo, but no difference was found in serum inter-cellular adhesion molecule-1 (sICAM-1). Tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β and IL-6 were all decreased after n-3 treatment compared to placebo, whereas IL-2 increased. High sensitivity C-reactive protein (hsCRP), granulocyte monocyte colony stimulating factor (GM-CSF), interferon gamma (IFN-γ), IL-8, IL-10, IL-12 p70 and serum amyloid A (SAA) levels were not different between n-3 and placebo treatments.

3.2. Serum phospholipid fatty acids

The sum of n-3 levels were increased 65% after n-3 treatment and decreased 2% after placebo treatment ($p < 0.0001$ for difference; Table 3). The sum of n-6 levels were decreased 6% after n-3 treatment and increased 3% after placebo treatment

($p < 0.0001$). N-3 treatment increased the concentration of 20:5n-3 (EPA) by 191% and 22:6n-3 (DHA) by 34%, and decreased the concentration of 18:3n-3 (ALA) by 10%. The changes measured after placebo treatment were smaller. Accordingly, the AA/DHA ratio was decreased 32% after n-3 treatment but slightly increased with placebo ($p < 0.0001$). The levels of 18:2n-6, 18:3n-6, 20:2n-6 and 20:3n-6 were all decreased after n-3 treatment and slightly increased or unchanged after placebo treatment. No difference was found regarding the sum of saturated fatty acids, MUFA or PUFA.

3.3. Vascular function

The RH response increased with n-3 treatment compared to placebo ($p = 0.01$, Fig. 1). In line with this finding, the maximum RH response (RH_{max}), RH response 60 s post-occlusion (RH_{60s}), and AUC of the RH response were also increased after n-3 treatment compared to placebo (Table 4).

3.4. High-resolution ultrasound and arterial stiffness measurements

The RA diameter was decreased after n-3 treatment compared to placebo. No changes in the RA IT, IMT or MT were found between n-3 treatment and placebo (Table 4). PWV was not affected by n-3

Table 4

Blood pressure, heart rate, pulse wave velocity, and high-resolution ultrasound measurements of the radial artery and endothelial function after treatment.

| | Omega-3 | Placebo | p |
|--|---------------|---------------|-------|
| SBP (mmHg) | 111 ± 11 | 110 ± 11 | 0.67 |
| DBP (mmHg) | 64 ± 7 | 64 ± 6 | 0.61 |
| HR (bpm) | 67 ± 11 | 68 ± 8 | 0.62 |
| PWV (m/s) | 7.0 ± 0.9 | 7.0 ± 0.9 | 0.76 |
| AI (%) | -15.0 ± 7.6 | -11.4 ± 11.2 | 0.05 |
| Intima thickness (mm) | 0.055 ± 0.007 | 0.056 ± 0.009 | 0.73 |
| Media thickness (mm) | 0.20 ± 0.05 | 0.18 ± 0.04 | 0.29 |
| Intima-media thickness (mm) | 0.25 ± 0.05 | 0.24 ± 0.04 | 0.39 |
| Diameter (mm) | 1.9 ± 0.2 | 2.0 ± 0.4 | 0.05 |
| RHI | 1.8 ± 0.4 | 2.0 ± 0.6 | 0.07 |
| F-RHI | 0.21 ± 0.16 | 0.23 ± 0.16 | 0.61 |
| RH _{max} (% of baseline at max dil) | 1.9 ± 0.9 | 1.6 ± 0.7 | 0.095 |
| RH _{60s} (% of baseline at 60 s post-occlusion) | 1.7 ± 1.0 | 1.3 ± 0.6 | 0.056 |
| AUC _{0-1 min} | 0.6 ± 0.3 | 0.5 ± 0.2 | 0.23 |
| AUC _{0-3 min} | 3.8 ± 1.9 | 3.2 ± 1.4 | 0.07 |
| AUC _{0-5 min} | 6.5 ± 2.9 | 5.5 ± 2.3 | 0.11 |

Data are presented as mean ± SD. SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; PWV: pulse wave velocity; AI: augmentation index; RHI: reactive hyperemia index; F-RHI: natural logarithm of RHI; RH_{max}: maximum hyperemic response; RH_{60s}: reactive hyperemic response at 60 s post-occlusion; AUC: area under curve, corrected for baseline.

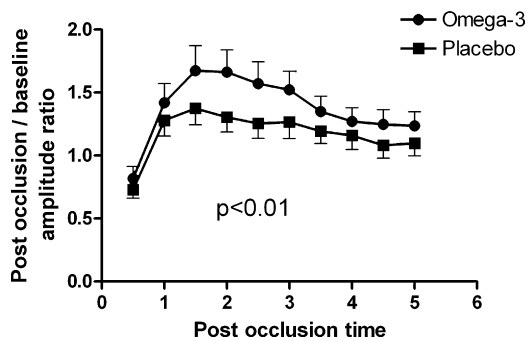


Fig. 1. Vasodilatory response to reactive hyperemia after omega-3 or placebo treatment. Pair-wise compared curves using global fitting are shown ($p=0.01$). Data points represent mean \pm SD.

treatment. The augmentation index (AI) assessed by PAT was lower with n-3 treatment compared to placebo ($p=0.05$, Table 4).

3.5. Correlations

The change in the AI was inversely correlated to changes in EPA ($r=-0.47$, $p=0.025$) and the sum of n-3 ($r=-0.47$, $p=0.02$). Inverse correlations were found between the changes in vascular function and cholesterol ($r=-0.50$, $p=0.01$ for $AUC_{0-3\text{min}}$). The measurements of vascular function also correlated with the change in 20:2n-6 ($r=0.52$, $p=0.01$ for $AUC_{0-5\text{min}}$).

Changes in the logarithmical measure F-RHI inversely correlated with the change in LDL cholesterol ($r=-0.63$, $p=0.001$) and HDL cholesterol ($r=-0.44$, $p=0.04$).

4. Discussion

The present study establishes that vascular function improves after 3 months of n-3 supplementation in obese adolescents. Obese children demonstrate elevated inflammation, which can affect the endothelium and vascular wall [14]. One may surmise that this elevated inflammation, which may be caused by relatively lower (vs. lean controls) serum n-3 concentrations, can be reduced by n-3 supplementation. Indeed, our present results showed that n-3 treatment decreases the number of lymphocytes, monocytes, and the level of TNF- α , IL-1 β , and IL-6 similar to what has been observed in healthy adults eating a Mediterranean inspired diet [15]. All of these components are involved in the atherosclerotic process in different ways [16–18], and TNF- α , IL-1 β and IL-6 are secreted from cells in the adipose tissue [19]. Decreased levels of these inflammatory factors could contribute to improved endothelial function. Also, the increased n-3 PUFA levels in serum phospholipids suggest increased incorporation of n-3 PUFAs into the membranes of circulating cells.

Vascular function is related to the dietary FA composition [20]. N-3 treatment, but not placebo treatment, has been shown to improve both forearm vasoconstrictive responses and endothelial function in adults [21]. These results can be explained by an increased level of endogenous nitric oxide (NO) [22], such as has been shown after n-3 supplementation for 3 weeks. In agreement with these studies, we demonstrated that n-3 supplementation improved vascular function in obese adolescents. Because both NO-dependent and -independent vasodilatation might be affected by n-3 treatment, we analyzed the entire vasodilatory response. In order to best illustrate the various components of the improvement in vascular function, we performed a pair-wise comparison for the entire post-occlusion hyperemic response curve. We also used RH_{max} and $RH_{60\text{s}}$ to demonstrate the maximum hyperemic response in these children. Taken together, our data suggest that

n-3 supplementation improves vasodilatory responses, beneficially affecting cardiovascular risk in obese adolescents.

Oxidative stress and free radicals are increased in obese individuals and have been suggested to play an important role in the development of both atherosclerosis and insulin resistance [23]. The fact that the n-3 concentration increases not only in the serum phospholipids, as in the present study, but also in muscle and adipose tissue phospholipids (Dangardt et al., unpublished data), makes it conceivable that n-3 PUFAs are incorporated into the membranes of vascular wall cells, which could beneficially influence NO release, leukocyte adhesion, free radical formation, and inflammatory status. This reasoning is further supported by Thies et al., who found that n-3 supplementation increases plaque stability and decreases macrophage infiltration, suggesting a lower degree of plaque inflammatory activity [24].

We recently showed that obese children have an increased RA IT compared to lean children. Moreover, the obese children also show a decreased PWV, possibly reflecting a compensatory mechanism in response to increased blood volume in the obese state [4]. Woo et al. found that both IMT and endothelial function may be improved in obese children with long-term dietary modification, but even more efficiently by a combination of diet and exercise [25]. In our study, we did not find any changes in the IT, MT or IMT. Three months of n-3 supplementation might be enough to unravel functional changes in the vasculature, but structural vascular changes may require more long-term intervention. The collective improvement of the AI and vascular function in these obese adolescents may constitute a moderate anti-hypertensive effect of n-3 treatment, effects that may not be detectable by sphygmomanometry.

The subjects received 1.2 g of pure n-3 PUFAs per day, which is a relatively low dose for healthy adults [26]. Considering the influence of the large amount of adipose tissue in obese children, the dose might be too low for optimal results. In obese adults, 4 g/day seems to be an adequate dosage [26], which is almost four times higher than the dose we used. Because there are no previous studies of n-3 supplementation in obese children, we can only speculate that a higher dose might have influenced the results further.

It would certainly be possible to provide 1.2 g of n-3 in only 2 capsules using a more concentrated formula, which would facilitate a more long-term study design. One would surmise that at least 1 year of omega-3 supplementation would be required in such a study to detect structural vascular changes. Most importantly, however, obese adolescents should be encouraged to increase their daily dietary n-3 intake. Hence, we demonstrated that omega-3 fatty acid supplementation improves vascular function in obese adolescents and, if continued long-term, it may beneficially influence cardiovascular risk.

Conflict of interest

The authors declare that they do not have any conflicts of interest to declare.

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References

- [1] Harris WS, Miller M, Tighe AP, Davidson MH, Schaefer EJ. Omega-3 fatty acids and coronary heart disease risk: clinical and mechanistic perspectives. *Atherosclerosis* 2008;197:12–24.
- [2] Karlsson M, Marild S, Brandberg J, Lonn L, Friberg P, Strandvik B. Serum phospholipid fatty acids, adipose tissue, and metabolic markers in obese adolescents. *Obesity (Silver Spring)* 2006;14:1931–9.
- [3] Osika W, Dangardt F, Gronros J, et al. Increasing peripheral artery intima thickness from childhood to seniority. *Arterioscler Thromb Vasc Biol* 2007;27:671–6.
- [4] Dangardt F, Osika W, Volkmann R, Gan LM, Friberg P. Obese children show increased intimal wall thickness and decreased pulse wave velocity. *Clin Physiol Funct Imaging* 2008;28:287–93.
- [5] Tounian P, Aggoun Y, Dubern B, et al. Presence of increased stiffness of the common carotid artery and endothelial dysfunction in severely obese children: a prospective study. *Lancet* 2001;358:1400–4.
- [6] Leeson CP, Mann A, Kattenhorn M, Deanfield JE, Lucas A, Muller DP. Relationship between circulating n-3 fatty acid concentrations and endothelial function in early adulthood. *Eur Heart J* 2002;23:216–22.
- [7] Korotkova M, Gabrielson BG, Holmang A, Larsson BM, Hanson LA, Strandvik B. Gender-related long-term effects in adult rats by perinatal dietary ratio of n-6/n-3 fatty acids. *Am J Physiol Regul Integr Comp Physiol* 2005;288:R575–579.
- [8] Richardson AJ, Montgomery P. The Oxford-Durham study: a randomized, controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. *Pediatrics* 2005;115:1360–6.
- [9] Stefansson BV, Haraldsson B, Nilsson U. Ascorbyl free radical reflects catalytically active iron after intravenous iron saccharate injection. *Free Radic Biol Med* 2008;45:1302–7.
- [10] Bonetti PO, Lerman LO, Lerman A. Endothelial dysfunction: a marker of atherosclerotic risk. *Arterioscler Thromb Vasc Biol* 2003;23:168–75.
- [11] Donald AE, Halcox JP, Charakida M, et al. Methodological approaches to optimize reproducibility and power in clinical studies of flow-mediated dilation. *J Am Coll Cardiol* 2008;51:1959–64.
- [12] Hamburg NM, Keyes MJ, Larson MG, et al. Cross-sectional relations of digital vascular function to cardiovascular risk factors in the Framingham Heart Study. *Circulation* 2008;117:2467–74.
- [13] De Angelis L, Millasseau SC, Smith A, et al. Sex differences in age-related stiffening of the aorta in subjects with type 2 diabetes. *Hypertension* 2004;44:67–71.
- [14] Meyer AA, Kundt G, Steiner M, Schuff-Werner P, Kienast W. Impaired flow-mediated vasodilation, carotid artery intima-media thickening, and elevated endothelial plasma markers in obese children: the impact of cardiovascular risk factors. *Pediatrics* 2006;117:1560–7.
- [15] Ambring A, Johansson M, Axelsen M, Gan L, Strandvik B, Friberg P. Mediterranean-inspired diet lowers the ratio of serum phospholipid n-6 to n-3 fatty acids, the number of leukocytes and platelets, and vascular endothelial growth factor in healthy subjects. *Am J Clin Nutr* 2006;83:575–81.
- [16] Burstein SA, Peng J, Friesse P, et al. Cytokine-induced alteration of platelet and hemostatic function. *Stem Cells* 1996;14(Suppl. 1):154–62.
- [17] Lyon CJ, Law RE, Hsueh WA. Minireview: adiposity, inflammation, and atherogenesis. *Endocrinology* 2003;144:2195–200.
- [18] Apostolakis S, Vogiatzi K, Krambovitis E, Spandidos DA. IL-1 cytokines in cardiovascular disease: diagnostic, prognostic and therapeutic implications. *Cardiovasc Hematol Agents Med Chem* 2008;6:150–8.
- [19] Chudek J, Wiecek A. Adipose tissue, inflammation and endothelial dysfunction. *Pharmacol Rep* 2006;58(Suppl.):81–8.
- [20] Lopez-Garcia E, Schulze MB, Manson JE, et al. Consumption of (n-3) fatty acids is related to plasma biomarkers of inflammation and endothelial activation in women. *J Nutr* 2004;134:1806–11.
- [21] Shah AP, Ichiuji AM, Han JK, et al. Cardiovascular and endothelial effects of fish oil supplementation in healthy volunteers. *J Cardiovasc Pharmacol Ther* 2007;12:213–9.
- [22] Harris WS, Rambjor GS, Windsor SL, Diederich D. n-3 Fatty acids and urinary excretion of nitric oxide metabolites in humans. *Am J Clin Nutr* 1997;65:459–64.
- [23] Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress-activated signaling pathways mediators of insulin resistance and beta-cell dysfunction? *Diabetes* 2003;52:1–8.
- [24] Thies F, Garry JM, Yaqoob P, et al. Association of n-3 polyunsaturated fatty acids with stability of atherosclerotic plaques: a randomised controlled trial. *Lancet* 2003;361:477–85.
- [25] Woo KS, Chook P, Yu CW, et al. Effects of diet and exercise on obesity-related vascular dysfunction in children. *Circulation* 2004;109:1981–6.
- [26] Wang C, Harris WS, Chung M, et al. n-3 Fatty acids from fish or fish-oil supplements, but not alpha-linolenic acid, benefit cardiovascular disease outcomes in primary- and secondary-prevention studies: a systematic review. *Am J Clin Nutr* 2006;84:5–17.