Addition of marine omega-3 fatty acids to statins in familial hypercholesterolemia does not affect in vivo or in vitro endothelial function

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Abstract

Background

Pre-statin trials reported positive effects of omega-3 fatty acids (n-3 PUFA) in cardiovascular disease (CVD), whereas recent studies and meta-analyses have not reproduced these results. The effect of n-3 PUFA in patients with familial hypercholesterolemia (FH), a group with particularly high risk of CVD, is not well established.

Objective

We investigated the effect of n-3 PUFA on the early stage of atherosclerosis in FH patients by evaluating *in vivo* (peripheral arterial tonometry (PAT)) and *in vitro* (plasma asymmetric dimethylarginine (ADMA) and E-selectin) endothelial function.

Methods

This was a double blind, placebo-controlled crossover study with 34 FH patients on statin treatment (mean age 46.6 years). In random order, all individuals were treated for three months with high dose n-3 PUFA (2g x2) and three months placebo (olive oil, 2g x2), separated by a three months washout period. Anthropometric data, blood samples and PAT were collected at four time points.

Results

There were no significant changes in reactive hyperemia index (RHI) measured by PAT after n-3 PUFA compared to placebo, median RHI after n-3 PUFA was 1.98 and after placebo 1.96, p=0.51. No significant changes were detected in the soluble endothelial marker ADMA (in two different assays) when comparing n-3 PUFA and placebo (p=0.92 and 0.14, respectively). Finally, the level of E-selectin did not change significantly during the trial, p=0.26.

Conclusion

Addition of n-3 PUFA to standard lipid-lowering treatment in genetically verified FH patients did not affect the in vivo endothelial function or soluble endothelial markers.

Keywords:

Fatty acid, familial hypercholesterolemia, triglycerides, atherosclerosis, statin.

Abbreviations and acronyms

ADMA	Asymmetric dimethylarginine
ASCEND Omega-3	A Study of Cardiovascular Events in Diabetes, Omega-3
CHD	Coronary heart disease
CVD	Cardiovascular disease
EPA	Eicosapentaenoic acid
FH	Familial hypercholesterolemia
JELIS	Japanese EPA Lipid Intervention Study
n-3 PUFA	Omega-3 polyunsaturated fatty acid
PAT	Peripheral arterial tonometry
REDUCE-IT	Reduction of cardiovascular events with icosapent ethyl-intervention trial
RHI	Reactive hyperemia index
TG	Triglycerides

Introduction

The cardiovascular effect of marine omega-3 fatty acid (n-3 PUFA) supplement is debated. Most trials have focused on secondary prevention of cardiovascular disease (CVD), hence, the knowledge regarding primary CVD prevention and n-3 PUFA is limited.[1] Early secondary CVD prevention n-3 PUFA trials showed reduction in CVD and sudden cardiac death,[2-6] whereas the majority of the later studies have failed to confirm these results.[7-12] However, the Reduction of cardiovascular events with icosapent ethyl-intervention trial (REDUCE-IT) demonstrated a positive effect of eicosapentaenoic acid (EPA) in secondary prevention.[13] Statin treatment and varied dosage of the n-3 PUFA supplement are among the explanations for the disparate trial results.[14] Thus, the uncertainty regarding n-3 PUFA supplement and CVD is reflected in the current international guidelines from American Heart Association and European Atherosclerosis Society.[1, 15] American Heart Association recommends n-3 PUFA supplement for patients with coronary heart disease (CHD) and heart failure with reduced ejection fraction, while European Atherosclerosis Society awaits further evidence on the n-3 PUFA efficacy before justifying its recommendation. A recent meta-analysis including the ten largest n-3 PUFA trials did not support supplement of n-3 PUFA as secondary CVD prevention.[16] This meta-analysis did not include the newly published results from the REDUCE-IT trial.

Individuals with familial hypercholesterolemia (FH) have a high risk of early coronary heart disease (CHD), premature mortality and increased morbidity compared to the general population.[17, 18] Low-density lipoprotein cholesterol lowering is crucial for patient outcome, and the introduction of statins has markedly improved the prognosis for FH patients. Nonetheless, FH individuals have a high residual risk of CHD despite moderate to high intensity treatment with statins,[19] and finding efficient means to reduce this residual risk, might improve patient outcome. The role of n-3 PUFA-supplements in the FH population is not well established. Among the n-3 PUFA trials in FH, lipid changes in response to n-3 PUFA supplement are the ones most studied.[20-23] Endothelial dysfunction is considered to be one of the primary events in the atherosclerotic process.[24] This dysfunction can be evaluated both by measuring soluble markers in plasma and by investigating *in vivo* endothelial response when the patient is exposed to various challenges.

A previous trial has investigated the effect of n-3 PUFA on the arterial elasticity in 20 FH patients, and demonstrated reduced systolic blood pressure and improved large arterial elasticity.[25] In order to reveal a potential effect of n-3 PUFA on endothelial function in FH patients, we examined the endothelial function both *in vivo* and *in vitro*. Our primary endpoint, the *in vivo* endothelial function, was assessed noninvasively by peripheral arterial tonometry (PAT), a well-established method.[26-30] The soluble endothelial markers asymmetric dimethylarginine (ADMA) and E-selectin served as indicators of the *in vitro* endothelial function.

Patients and Methods:

The data that support the findings of this study are available from the corresponding author upon request. The individuals in this study were recruited from Nordland Hospital's lipid clinic (Bodø, Norway), the inclusion and exclusion criteria are listed in Table 1. Compliance level was set at 50% intake of study medication.

Study design

The study was a randomized double-blinded crossover trial (Figure 1). At the first hospital visit, patients were randomized to either three months treatment with n-3 PUFA or three months placebo. After treatment and a subsequent washout period of at least three months duration, the individuals received alternate medication in the second treatment period. In total there were four hospital visits (at inclusion and after three, six and nine months). All four hospital visits included measurement of anthropometric data, a physical examination, blood samples and evaluation of the *in vivo* endothelial function. The participants were instructed not to take extra trial medicine in case of a forgotten dose. A research nurse was in telephonic contact with the participants between the hospital visits. The unused medication was handed in at hospital visit number two and four. According to the returned medication, the mean compliance was 90% (range 68-100%) in both treatment periods (PUFA or placebo). All trial patients were above the pre-determined compliance level of 50%. A registration of dietary intake of n-3 PUFA was also completed by the participants. The participants were instructed to avoid dietary n-3 PUFA supplements for three months before inclusion and throughout the trial, but no specific recommendations regarding diet were given. The dietary registration form showed a mean intake of 0.8 servings of fatty fish per week, with standard deviation 0.5. The mean intake of weekly fish servings (lean and fatty fish combined) was 1.7 servings, with standard deviation 1.0.

Randomization

Apotekproduksjon A/S (Oslo, Norway) was responsible for labelling the study medication and for the randomization. The randomization was blinded to both study individuals and project members. Block randomization was used in order to ensure

apportionment of the participants starting with n-3 PUFA and placebo. Apotekproduksjon A/S kept the randomization code until the last participant had completed the study period.

Study Medication:

BASF (Lysaker, Norway) provided the n-3 PUFA ethyl esters supplement and the placebo capsules. The marine n-3 PUFA supplement was a 1000 mg capsule consisting of 460 mg of eicosapentaenoic acid (EPA) and 380 mg docosahexanoic acid and was administered in a dose of 4000 mg daily (two capsules twice a day). The placebo capsules contained olive oil and was administered in the same manner as the n-3 PUFA supplement.

Ethics

The study was approved by the Regional Ethics Committee of Northern Norway, P REK 2011/899, and by the Norwegian Medicines Agency. EUDRACTNR 2012-000505-68. ClinicalTrials.gov NCT01813006. Informed written consent was obtained from all individuals in the study.

Blood samples

Fasting blood samples were collected at each visit. Routine analyzes for the purpose of safety were analyzed consecutively, whereas samples for ADMA and E-selectin were frozen at -70°C and analyzed in batch at the end of the study. Total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol were analyzed on an ADVIA®1800 system (Siemens Medical Solutions Diagnostics, Japan) using reagents from Siemens Healthcare Diagnostics Ltd.

Assessment of *in vivo* endothelial function:

The *in vivo* endothelial function was assessed by PAT (EndoPAT2000® Itamar Medical Ltd, Caesarea, Israel). A pneumatic plethysmograph placed on the subject's index finger detects the digital pulse amplitude. During the test, the blood flow in the brachial artery is temporarily stopped by a blood pressure cuff. After five minutes occlusion of the artery, the blood flow is restored, and the PAT-device measures the changes in digital pulse amplitude during reactive hyperemia, as an expression of the subject's vasodilator function. The reactive hyperemia index (RHI) is the post-

occlusion to pre-occlusion ratio. PAT was measured at all four hospital visits, all tests were performed by the same personnel. The study participants were fasting and rested in supine position for five minutes before the examination. The test was performed according to the specifications of the manufacturer. RHI values are skewed data, hence, nonparametric test was applied.

Assessment of *in vitro* endothelial function:

ADMA

ADMA was analyzed in two different assays, ADMA from Cusabio (Houston, TX) and ADMA Xpress from Immundiagnostik (Bensheim, Germany). The detection range for ADMA was 7.8-500 ng/m and for ADMA Xpress 0-2 μmol/L. Both ADMA assays used ethylenediaminetetraacetic acid plasma and were enzyme-linked immunosorbent assays. The ADMA assay from Immundiagnostik utilizes the competitive enzyme-linked immunosorbent assay technique, whereas the assay from Cusabio uses the sandwich enzyme-linked immunosorbent assay technique.

E-selectin

E-selectin (CD62) was analyzed as a singleplex from eBioscience (Thermo Fisher Scientific, San Diego, CA) using ethylenediaminetetraacetic acid plasma. E-selectin level in plasma was analyzed using the microsphere-based human E-selectin.

All analyzes were performed according to instructions provided by the manufacturers.

Statistics:

The RHI-results, blood samples and anthropometric measurements after the n-3 PUFA period and the placebo period respectively, were grouped and statistically analyzed independently of intervention order. For RHI, ADMA, ADMA Xpress and E-selectin, values after the n-3 PUFA period and values after the placebo period were compared statistically. Anthropometric data, blood pressure, heart rate and lipids were analyzed statistically by comparing baseline values and values after n-3 PUFA, and baseline values and values after the placebo period, respectively. The crossover study design required paired statistical analysis. Shapiro-Wilk normality test was applied on the differences between the periods. For normally distributed differences, paired *t*-test was applied, whereas Wilcoxon Matched-Pairs Signed Rank test was

used for not normally distributed differences. A two-tailed P level < 0.05 was considered statistically significant. Correction for multiple comparisons was not performed. A possible carry-over effect was evaluated by a treatment-period interaction analysis, calculated by a two-sample *t* test or a Mann-Whitney test, comparing the patient's average response in the group starting with n-3 PUFA and the group starting with placebo. The statistic work and graphs were done with Prism version 7.0a (GraphPad Software Inc, La Jolla, CA).

Results

Study population and baseline characteristics

Thirty-eight patients were enrolled in the study. One person left due to abdominal adverse effects (reflux, abdominal pain), one person started fertility treatment, and one patient chose to leave the study for unknown reason. Additionally, one of the individuals became pregnant at the end of the trial period and was excluded. Thus, 34 patients completed the nine-month follow-up, 17 females and 17 males. The baseline characteristics of the study population are listed in Table 2.

Anthropometry, blood pressure, heart rate and lipids:

The results of the effect of n-3 PUFA compared to placebo on anthropometric data, blood pressure, heart rate and lipids are presented in Table 3. The levels of total cholesterol, low-density lipoprotein cholesterol and triglycerides (TG) significantly changed after n-3 PUFA compared to baseline. Apart from a slight, but statistically significant, increase in hip circumference after treatment with placebo there were no changes in the remaining parameters.

Peripheral arterial tonometry:

The median RHI (first quartile, third quartile) after n-3 PUFA was 1.98 (1.58, 2.46) and after placebo 1.96 (1.60, 2.52). There was no significant difference in RHI between the n-3 PUFA treatment and the placebo period, p=0.51, median of differences 0.07. (Figure 2).

Eight of the 34 individuals in the present study had one or two RHI measurements below 1.35. Among these eight, two individuals had established CHD. None of the study participants had RHI results below 1.35 at all four hospital visits. According to the manufacturer, an RHI level <1.67 indicates endothelial dysfunction. Twenty-two of the 34 participants had RHI levels below 1,67 (11 had one measurement <1.67, four had two measurements <1.67, four individuals had three RHI measurements <1.67 and three persons had all four measurements of RHI <1.67). Three of the study participants with one RHI measurement <1.67 were smokers, the rest were non-smokers.

ADMA

Three study participants had ADMA-levels below lower limit of detection. In these cases, the individual's value was set at lower limit of detection (7.8 ng/mL) for use in the statistics. The differences between the ADMA-results after n-3 PUFA and placebo were not normally distributed and non-parametric method was applied. Median (first quartile, third quartile) ADMA after n-3 PUFA was 22.5 ng/mL (13 ng/mL, 49.3 ng/mL), and the median (first quartile, third quartile) ADMA after placebo was 25 ng/mL (14 ng/mL, 49 ng/mL). No significant difference between n-3 PUFA and placebo was detected, p=0.92, the median of differences was 0.85 (Figure 3).

ADMA Xpress

The mean (standard deviation) ADMA Xpress after n-3 PUFA was 177.0 ng/mL (36.0 ng/mL). After placebo mean (standard deviation) ADMA Xpress was 168.5 ng/mL (41.0 ng/mL). The differences between the groups were normally distributed and there was no significant difference in ADMA Xpress levels after n-3 PUFA compared to placebo, p=0.14, mean of differences -8.7 with 95% confidence interval (-20.6;2.9) (Figure 4).

E-selectin:

The mean (standard deviation) E-selectin after n-3 PUFA was 44.6 ng/mL (19 ng/mL) and after placebo mean (standard deviation) E-selectin was 42.9 ng/mL (18 ng/mL). The differences between the n-3 PUFA and placebo levels of E-selectin were normally distributed, and no statistically significant difference between the periods was found, p=0.26, mean of differences -1.7 with 95% confidence interval (-4.7;1.3). (Figure 5).

No carry-over effects were detected for any of the parameters examined, as judged by treatment-period interaction calculations.

Discussion

Identification of drugs reducing the development of atherosclerosis in statin treated FH patients is important, considering the residual risk of CVD in this patient group.[19] Furthermore, limiting the use of dietary supplements and medication without documented effects on the process of atherosclerosis is necessary to avoid polypharmacy in the FH population.

In the present study the *in vivo* endothelial function (change in RHI) as measured by PAT was unaffected by a three month administration of a high dose n-3 PUFA in FH patients, consistent with previous n-3 PUFA and PAT studies.[26, 29] The lack of change in RHI suggests that n-3 PUFA in addition to statins in FH patients does not influence the peripheral hyperemic response. The peripheral hyperemic response was linked to coronary microvascular endothelial dysfunction by Bonetti et al, and they proposed a reference level of RHI <1.35 to recognize individuals with coronary endothelial dysfunction.[31] FH patients have impaired endothelial function,[24] however only three study participants had RHI <1,67 at all four hospital visits. This might imply that statin treatment has improved the endothelial function in 31 of the study participants in line with a previous report,[28] but there was no additional effect after supplement with n-3 PUFA.

Endothelial function *in vitro* assessed by the soluble endothelial markers ADMA (in two different assays) and E-selectin were not influenced by addition of n-3 PUFA to statins in this trial. The lack of change in ADMA levels is consistent with previous studies in children with hyperlipidemia (docosahexanoic acid supplement) and in patients with myocardial infarction,[32, 33] yet knowledge concerning n-3 PUFA and ADMA is limited. ADMA is considered to be an indirect way to investigate the endothelial function as ADMA is an inhibitor of nitric oxide synthase, and thus decreases the level of the potent vasodilator nitric oxide and subsequently impairs the endothelial function. Increased levels of ADMA have previously been linked to atherosclerosis risk factors such as hypercholesterolemia and hypertriglyceridemia.[34, 35] However, the participants in these studies were not on statin treatment, and a later meta-analysis concluded that statin treatment significantly reduced ADMA concentrations.[36]

The level of soluble E-selectin (CD62) also remained unchanged during the trial. E-selectin is a cell adhesion molecule expressed by cytokine-activated endothelial cells and is present both as membrane-bound E-selectin and soluble E-selectin. Membrane-bound E-selectin binds to ligands on circulating cells, especially monocytes, thus playing an important role in recruiting cells to the endothelium in inflammatory processes such as atherosclerosis.[37] Soluble E-selectin is considered a marker for activated endothelium, and the effect of n-3 PUFA on E-selectin has previously been studied in several populations. No effect of n-3 PUFA on E-selectin levels was found in a meta-analysis by Yang et al.[38] Interestingly, one trial showed a significant reduction in E-selectin after seven months of n-3 PUFA supplements in a population with hypertriglyceridemia.[39] Accordingly, the trial period in our study may have been too short to demonstrate E-selectin changes. However, atorvastatin treatment in patients with hypercholesterolemia has previously demonstrated significant reduction in levels of E-selectin,[40] thus statin treatment might have reduced the E-selectin levels in our study population prior to the n-3 PUFA supplement.

Apart from a minor increase in hip circumference after the placebo period, anthropometric data such as waist circumference and body mass index, as well as blood pressure and heart rate did not change significantly during the present trial (table 3). These finding are in line with previous publications, both in prior randomized controlled trials[26, 41] and a recent Cochrane review including 79 n-3 PUFA -trials of at least 12 months duration.[42] In contrast, multiple physiologic effects have been linked to n-3 PUFA, such as blood pressure-lowering and reduction of heart rate.[43, 44] However, these meta-analyses are partly based on pre-statin trials which might explain this inconsistency. A consistent finding in n-3 PUFA trials is a reduction in TG,[45] and this effect was also present in our study. Although the absolute reduction was small, this change in TG was statistically significant ($p<0.001$) and represented a 27% reduction in TG after n-3 PUFA supplementation compared to placebo, in line with previous publications.[29, 46] Furthermore, the reduction in TG levels in the treatment group provides evidence that the participants had a high level of compliance.

The treatment period was 12 weeks both in the n-3 PUFA and the placebo periods, because previous studies have shown effect on endothelial function in shorter or

equal trial periods.[41, 47, 48] In order to minimize the carryover effect, the blood samples after intervention and placebo period were separated by at least six months (washout three months + three months treatment). A high dosage of n-3 PUFA was chosen in this trial, given the known effect on TG and limited results in selected prior studies applying low dosage. Our study population consisted of nine individuals with established CHD and 25 individuals without known CHD. The number of individuals with CHD was too small to do statistical comparisons between the two groups.

Studies on n-3 PUFA and endothelial function report inconsistent results. Possible explanations have previously been pointed out, such as the diversity in study design and end points, different study populations (e.g. diabetes mellitus type 2, hypertriglyceridemia, healthy individuals, children, obese populations, statin-treated patients), various dosage of n-3 PUFA and duration of intervention.
Several modalities to assess endothelial function have been utilized in n-3 PUFA - trials. Among the noninvasive modalities, estimation of flow-mediated dilation by ultrasound is the one most commonly applied.[29, 48-50] PAT has the advantage of being operator independent and has been used extensively in CVD research, but not so much in trials evaluating the effect of n-3 PUFA on endothelial function. Two previous trials assessing individuals not treated with statins, found no significant difference in RHI after n-3 PUFA measured by PAT,[26, 29] whereas flow-mediated dilation-studies have shown positive effects on endothelial function after n-3 PUFA administration to cigarette smokers and adults with metabolic syndrome not treated with statins.[47, 48] In contrast, n-3 PUFA supplement had no effect on endothelial function evaluated by flow-mediated dilation in statin treated patients with previous myocardial infarction and type 2 diabetes.[46, 51]
The same inconsistency is reflected in randomized controlled trials investigating n-3 PUFA and CVD endpoints.[2-11] The recently published results from the A Study of Cardiovascular Events in Diabetes, Omega-3-trial (ASCEND Omega-3) concluded that n-3 PUFA supplements did not reduce the incidence of serious vascular events in diabetic patients without CVD.[12] On the other hand, the results from the REDUCE-IT trial demonstrated reduced risk in the primary end point by supplement of a high dose of EPA (4 g icosapent ethyl) in statin treated patients with hypertriglyceridemia and established CVD or risk factors.[13] The Japanese EPA Lipid Intervention study also showed an effect of EPA on major coronary events in a study population using

statins[4]. The EPA dose in our trial medication was 1.84 g, comparable with the EPA dose in the JELIS-trial. However, our study medication also contained 1.52 g of docosahexanoic acid, and we did not find any significant impact on the endothelial function in our FH population. Future studies should investigate the effect of high dosage of EPA in the FH population. The potential antiatherogenic effects of n-3 PUFA are not fully elucidated.[52] High levels of TG were linked to decreased endothelial function in a report by Kajikawa et al,[49] nonetheless, the risk reduction of CVD in the REDUCE-IT-trial was not associated with normalization of TG level.[13]

Strengths and Limitations

This was a single center study with well-characterized participants. A limited number of study personnel performed the measurements and examinations of the patients. The study length was three months in both the n-3 PUFA -period and the placebo-period, which is comparable to previous studies.

Yet, there are several limitations to consider in the present study. First, our trial sample was quite small, but the crossover design increased the power. Second, our study population was a mix of primary and secondary prevention of CVD. Third, our results may not apply to FH patients with a lower habitual dietary intake of marine n-3 PUFAs. Last, this study did not measure the n-3 PUFA levels in the blood, however all the participants were above the level of compliance set at 50% according to the unused medication and the level of TG changed significantly after n-3 PUFA period compared to baseline levels.

Conclusion

In the present study we found no effect on *in vivo* or *in vitro* endothelial function by adding marine n-3 PUFA to statins in a FH population. Our results do not support supplementing FH patients with marine n-3 PUFA.

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Figure Legends

Figure 1. Illustration of the crossover trial design.
1: First hospital evaluation (HE), 2: Second HE, 3: Third HE, 4: Final HE

Figure 2. Reactive hyperemia index (RHI)-values after Omega-3 fatty acid supplement versus placebo, no significant difference. SEM – standard error of mean.

Figure 3. Asymmetric dimethylarginine (ADMA) levels after Omega-3 fatty acid supplement versus placebo, no significant difference. SEM – standard error of mean.

Figure 4. Asymmetric dimethylarginine Xpress (ADMA Xpress) levels after Omega-3 fatty acid supplement versus placebo, no significant difference. SEM – standard error of mean.

Figure 5. E-selectin levels after Omega-3 fatty acid supplement versus placebo, no significant difference. SEM – standard error of mean.

Tables

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Age 18-75 yrs	Non-compliance
Genetically verified FH	Intake of PUFAs (requires three months washout period before enrollment)
Clinically stable, with and without CVD	Pregnancy or planned pregnancy / fertility treatment
Statin treatment >12 months	Breastfeeding
	Cancer and/or severe illness
	Blood samples at inclusion outside ref. range*

*If lab values outside normal range in control blood samples, the study patient was excluded from the study

Table 2. Population characteristics at inclusion

n	34
Female	17
Age (range)	46.6 (18-71)
Current smoker (%)	5 (15)
Established coronary disease (%)	9 (27)
Statin treatment*	
Atorvastatin (%)	22 (65)
Rosuvastatin (%)	8 (24)
Simvastatin (%)	4 (12)
Statin+Ezetimibe (%)	*12 (35)*
Mutation	
D200N	8
C210G	6

L380V	6
Del Exon 11-14	4
136C-T	3
R3500Q	2
Gujerat	2
Del Exon 2-3	1
R395W	1
T705I	1

All mutations are low density lipoprotein receptor mutations, except R3500Q which is a mutation of the apolipoprotein B gene. A recent Norwegian study reported that 97% of familial hypercholesterolemia was due to mutations in the low density lipoprotein receptor LDL receptor,[53] and the Gujerat mutation has previously been described in Norway.[54]

Table 3. Effect of Omega-3 fatty acids versus placebo on anthropometric characteristics, blood pressure, heart rate and lipids

	Baseline	**After Omega-3 fatty acids**	**After placebo**
BMI (kg/m$_2$)	27.6 ± 5.0	27.6 ± 5.2	27.8 ± 5.2
Waist (cm)	90 ± 14	91 ± 14	91 ± 15
Hip (cm)	102 ± 9	103 ± 9	104 ± 10*
sBP (mmHg)	133 ± 20	131 ± 16	134 ± 21
dBP (mmHg)	76.6 ± 10	76 ± 10	76 ± 12
HR (bpm)	68 ± 12	66 ± 8	67 ± 10
Total cholesterol			
mmol/L	5.0 ± 1.1	4.6 ± 0.8*	5.0 ± 1.1
mg/dL	193.4 ± 42.5	177.9 ± 30.9*	193.4 ± 42.5
LDL-C			
mmol/L	3.2 ± 1.0	2.8 ± 0.9**	3.2 ± 0.9
mg/dL	123.7 ± 38.7	108.3 ± 34.8**	123.7 ± 34.8
HDL-C			
mmol/L	1.4 ± 0.5	1.4 ± 0.4	1.4 ± 0.4
mg/dL	54.1 ± 19.3	54.1 ± 15.5	54.1 ± 15.5

TG			
mmol/L	1.09 ± 0.59	0.84 ± 0.39***	1.15 ± 0.86
mg/dL	96.5 ± 52.3	74.4 ± 34.5***	101.9 ± 76.2

Data are presented as mean ±SD.

BMI – body mass index, Waist – waist circumference, Hip – Hip circumference, sBP – systolic blood pressure, dBP – diastolic blood pressure, HR – heart rate, LDL-C – low-density lipoprotein cholesterol, HDL-C – high density lipoprotein cholesterol, TG – triglycerides.

The p-value was calculated as the difference between baseline values and after Omega-3 fatty acids period or the difference between baseline values and after placebo period using paired *t*-test (normally distributed difference) or Wilcoxon Matched Pairs Signed Rank test (not normally distributed difference).

* $p \leq 0.05$

** $p \leq 0.01$

*** $p \leq 0.0001$