Effects of supplementation with n-3 polyunsaturated fatty acids on cognitive performance and cardiometabolic risk markers in healthy subjects of middle and older age: a randomized cross-over controlled study

Anne Nilsson*\textsuperscript{1}, Karl Radeborg\textsuperscript{2}, Ilkka Salo\textsuperscript{2}, and Inger Björck\textsuperscript{1}

\textsuperscript{1}Division of Applied Nutrition and Food Chemistry, Department of Food Technology, Engineering and Nutrition, Lund University, Sweden (where the study was performed),

\textsuperscript{2}Department of Psychology, Lund University, Sweden

*Anne Nilsson (corresponding author): anne.nilsson@appliednutrition.lth.se

Karl Radeborg: karl.radeborg@telia.com

Ilkka Salo: ilkka.salo@psychology.lu.se

Inger Björck: inge.bjorck@appliednutrition.lth.se
ABSTRACT

Background: Higher plasma n-3 polyunsaturated fatty acids (PUFA) have been associated with a lower risk of age related cognitive decline, and to beneficially affect cardiometabolic risk factors. A relation exists between metabolic disorders such as diabetes type 2 and cognitive decline. Results regarding the potential effects of n-3 PUFA on risk factors in healthy subjects are divergent, and studies regarding the possible relation between cardiometabolic parameters and cognitive performance are scarce. The objective was to evaluate the effects of 5 weeks intake of long chain n-3 PUFA on cognitive performance in healthy individuals, and to exploit the possible relation between outcomes in cognitive tests to effects on cardiometabolic risk parameters.

Methods: Fish oil n-3 PUFA (3 g daily) were consumed during 5 weeks separated by a 5 week washout period in a cross-over placebo controlled study, including 40 healthy middle aged to elderly subjects. Cognitive performance was determined by tests measuring working memory (WM) and selective attention.

Results: Supplementation with n-3 PUFA resulted in better performance in the WM-test compared with placebo ($P < 0.05$). In contrast to placebo, n-3 PUFA lowered plasma triacylglycerides and serum total cholesterol ($P < 0.05$), and systolic blood pressure ($P < 0.0001$). Systolic blood pressure ($P < 0.05$), f-glucose ($P = 0.05$), and s-TNF-$\alpha$ ($P = 0.05$), were inversely related to the performance in cognitive tests.

Conclusions: Intake of n-3 PUFA improved cognitive performance in healthy elderly subjects after five weeks compared with placebo. In addition, inverse relations were obtained between cardiometabolic risk factors and cognitive performance, indicating a potential of dietary prevention strategies to delay onset of metabolic disorders and associated cognitive
decline.

**Keywords:** omega-3 PUFA, DHA, EPA, fish oil, dietary prevention, cognitive performance, working memory, metabolic disorders, ageing
BACKGROUND

Long chain n-3 polyunsaturated fatty acids (n-3 PUFA, docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA)) are important for optimal brain function and mental health [1, 2]. In prospective cohort- [3] and cross-sectional studies [4] of middle aged and elderly populations, higher proportions of n-3 PUFA in plasma were linked to a lower risk of cognitive decline. A number of studies further reveal that higher fish consumption promoted less decline and better cognitive functions [5-9]. However, controlled intervention trials of the effect of n-3 PUFA on cognitive functions in healthy subjects are scarce, and results from the limited number of studies available are divergent. A double-blind, placebo-controlled trial in 302 cognitively healthy subjects (65 y) revealed no effects on cognitive domains of attention, sensorimotor speed, memory, and executive function after 26 weeks supplementation with 1800 mg/d or 400 mg/d EPA+DHA [10]. Contrary, a randomized, double-blind, placebo-controlled trial in 458 healthy subjects resulted in beneficial effects on a visuospatial learning and episodic memory test after 24 weeks supplementation with 900 mg daily intake of DHA [11]. In a smaller study, 33 subjects were supplemented with n-3 PUFA from fish oil (daily, 1.6g EPA + 0.8g DHA) for 35 days whereas 16 subjects were supplemented with olive oil (placebo) [12]. Supplementation with n-3 PUFA was associated with improved attentional functions and mood.

Cardiometabolic disorders such the metabolic syndrome, impaired glucose tolerance, and diabetes are associated with higher risk of cognitive decline, e.g. decreased memory and executive functioning [13-15], information processing speed, attention [16], and overall intellectual functioning [17, 18]. Long chain n-3 PUFA intervention studies have shown benefits on several key metabolic risk factors, e.g. lowers the blood pressure and triglycerides [19], reduce inflammatory markers [20], and improve glucose metabolism [21] and insulin
sensitivity [22].

The present study undertakes to evaluate the effects of dietary supplementation with n-3 PUFA on cognitive performance in healthy individuals; and to relate cognitive outcome to cardiometabolic risk parameters. For this purpose, healthy middle aged to elderly subjects (51-72 years) with BMI 20-30 kg/m² were provided n-3 PUFA supplement from fish oil (3g/day) or placebo for 5 weeks, respectively, in a randomized cross-over design with a 5 week wash-out period. Cognitive tests of working memory and selective attention were performed after the n-3 PUFA- and placebo periods, respectively, and metabolic risk markers measured in blood prior to and after the PUFA- and placebo periods.
SUBJECTS AND METHODS

**Ethics statement**

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Regional Ethical Review Board in Lund, Sweden (protocol 2008/5). Written informed consent was obtained from all subjects.

**Study population**

Healthy subjects, 28 women and 12 men aged 51-72 y (mean ± SD: 63 ± 6 y) with BMI between 20-30 kg/m² (mean ± SD: 25 ± 3) were enrolled in the study. Exclusion criteria were blood glucose > 6.1 mmol/L, BMI > 30 kg/m² and known metabolic diseases, gastro-intestinal disorders or known cognitive decline. Due to detection of abnormal fasting blood glucose concentrations during the study period (7.0 and 6.7 mmol/L, respectively), two men were excluded, and in total 38 subjects completed the study.

**Test product and placebo**

*Test product:* The dietary supplement consisted of capsules containing 1000 mg fish oil whereof 600 mg was n-3 PUFA (EPA 300 mg, DHA 210 mg and 90 mg unspecified) (Pikasol fish oil capsules, Axellus VS, ORCLA, Oslo, Norway). Five capsules per day were consumed; resulting in a total daily intake of 3 g n-3 PUFA (EPA 1500 mg, DHA 1050 mg and 450 unspecified).

*Placebo:* The placebo supplement was provided as two tablets per day containing in total 366 mg dicalcium phosphate (E 341), 150 mg microcrystalline cellulose (E 460) and 4 mg magnesium salts of fatty acids (E 470b). The placebo was provided for the study by Axellus.
Study design

The study had a cross-over randomised but balanced design. Twenty subjects (14 women and 6 men) started with five weeks daily consumption of omega-3 PUFA and consumed placebo in a second five weeks intervention period, and 20 subjects (14 women and 6 men) were enrolled to start with five weeks consumption of the placebo. Only 18 subjects (14 women and 4 men) who started with placebo completed the study (see section “study population” above). The intervention periods were separated by a five week washout period. The subjects visited the experimental department at four occasions; in the mornings prior to start of each intervention periods and in the mornings after finished the PUFA- and placebo period, respectively.

Protocol

The evening prior to attendance, at 9.00 pm. the test subjects ate a standardized meal consisting of white wheat bread with optional spread, and had coffee, tea or water to drink. Thereafter they were fasting until the arrival at the research department. At 07.45 am, the test subjects were weighed and seated to rest for at minimum 10 minutes before the blood pressure was registered and fasting blood samples collected. A standardized breakfast was served consisting of white wheat bread (Dollar Storfranska, Lockarps bakery, Malmö, Sweden) and apricot marmalade (Ica, Sweden) corresponding to 55 g available carbohydrates. Water, 250 ml, or a plain cup of decaffeinated coffee or tea was served with the bread. The breakfast was consumed within 15 min. Thereafter, cognitive tests were performed and capillary blood tests were collected repeatedly up to 180 min post commencing the breakfast. The occupation during periods in-between the cognitive tests were standardized such that the subjects performed Sudoku.
Cognitive tests

Prior to the intervention periods, i.e. at visit no. 1 and 3, the subjects performed pilot versions of the cognitive tests to reduce learning effects and stress at the cognitive test days. Measurements of cognitive performance were performed after completion of each intervention period, i.e. at visit no. 2 and 4.

Working memory (WM) test: The WM was estimated by test of the type originally used by Daneman and Carpenter [23]. The tests employed in the present study represent an extension of the methodology developed by Radeborg et al. [24]. There are several reasons for choosing WM as a measure of cognitive performance in this study. WM can be defined as a system responsible for simultaneous temporary short term storing and processing of information, and is involved in many everyday activities; such as mathematical problem solving where one often has to remember part of the result in a calculation while performing further mathematical operations. WM represent a fundamental ability for higher-level cognitive processes. Thus, measures of WM capacity have been shown to correlate significantly with activities as diverse as e.g. reading comprehension [23], note taking [25], the following of directions [26], reasoning [27], and complex learning [28]. Some authors [29, 30], even claim that WM and general problem solving ability or intelligence, as measured by e.g. Raven’s Matrices, reflect nearly identical constructs. However, whereas intelligence tests generally only can be administered once due to risk of considerable learning effects, WM can be measured repeatedly. In total, three oral WM-tests were included at each experimental day (performed at 60, 110, and 160 min). Two of the tests (at 60 and 160 min) were executed principally as described previously [31], modified by including 12 sets of 3-5 short declarative sentences (four of each number) instead of 4 sentences in all sets. As previously, the sentences could be either semantically meaningful of the type ‘the boy brushed his teeth’,
or nonsensical, such as ‘the rabbit struck the idea’. The test leader was blind to the product provided to the test subjects. The sentences were read one by one to the subjects. Immediately after a sentence, he/she had to indicate if it was a semantically meaningful sentence or not. After each set of sentences the subjects had to repeat, in any order, the first noun in each of the sentences. One test could at maximum generate 48 credits. The tests consisted of equal number of sentences that were semantically meaningful (24 credits) and nonsensical (24 credits). It has been described that remembering of a noun in a semantically nonsensical sentence is more demanding [24]. The WM-test could therefore be divided into two parts differing in degree of difficulty. The third WM-test was performed at 110 min and was similar to the tests just described with the exception of that instead of short sentences, the test was composed of simple additions of two single digit numbers. The test leader presented orally the two figures to be added and the test subjects were supposed to immediately give an oral answer to the addition. After a set of 3-5 additions the subjects had to repeat the first figure in each addition. The test could at maximum generate 48 credits. One WM-test took approximately 8-10 min to perform. Four different but comparable WM-tests composed of sentences and two different but comparable WM-tests composed of figures were included in the study.

Selective attention (SA) test: The test was computerized and primarily measured the ability to sustain attention and to control and split the attention to the entire picture on the computer screen. Alike the WM-test, the SA-test also include aspects of the WM. The storing time allotted was however shorter compared with the WM-test, whereas the time pressure was higher. The test was performed as described previously [31] modified by instead of including 72 pictures, each shown for two seconds on the screen, the test included 96 pictures. The SA-test was performed at fasting and at 45, 95, 145 min after start of the breakfast. The test began
with a short training session, and took approximately 10 min to perform. It was scored with the number of correct responses (total 95 credits) and for the reaction time needed to give the answer (i.e. press one of the keys).

**Metabolic risk markers**

Physiological test variables were determined prior to and after each intervention period. Blood pressure was determined with an automatic blood pressure cuff (Digital Automatic Blood Pressure Monitor, Model M3 Intelligence, OMRON HEALTHCARE CO., LTD, Kyoto, Japan. Finger-prick capillary blood was withdrawn at fasting and at 15, 30, 45, 60, 90, 125, 160 and 180 min after the start of the standardized breakfast for determination of glucose concentrations and glucose tolerance (HemoCue®B-glucose, HemoCue AB, Ängelholm, Sweden). Venous blood was withdrawn for determination of fasting levels of serum (s) insulin, s-TNF-α, s-adiponectin, s-free fatty acids (s-FFA), s-triacylglycerol, s-LDL-C, s-HDL-C, s-total cholesterol and plasma (p) malondialdehyde (MDA). The venous blood samples were centrifuged and plasma and serum separated and stored in a freezer (-40 °C) until analyzed.

Methods for analyses of insulin, FFA, adiponectin and triacylglycerols are described elsewhere [32]. S-TNF-α was determined with a sandwich enzyme immunoassay kit (TNF-α ELISA Kit, Immunodiagnostik AG, Germany). S-HDL-C and s-LDL-C were assayed with an enzymatic selective protection method Kit (WAKO Chemicals GMBH, Germany). Total cholesterol was calculated from the results of HDL-C, LDL-C and triacylglycerol using Friedewald’s equation [33]. Plasma MDA was determined by measure of lipid peroxidation as TBARS as is described in [34], modified by excluding the n-butanol.
**Calculations and statistical methods**

The results are expressed as means ± SEM. The influence of the test- and placebo products on the cognitive tests was investigated by repeated measures ANOVA at the test points, with order of test meals and test meals as independent variables and performance on cognitive tests as dependent variables. Statistical calculations were performed in Stat View 5.0 and SuperAnova 1.11. Treatment effects on physiological test parameters (based on changes from baseline in the intervention- and placebo period, respectively) and time effects on cognitive tests were assessed with analysis of variance (ANOVA general linear model) followed by Tukey’s pairwise multiple comparison method for means, in MINITAB Statistical Software (release 13.32; Minitab inc., State College. PA. USA). Participants acted as their own control. GraphPad Prism (version 4.03; GraphPad Software, San Diego, CA, USA) was applied for calculation of blood glucose incremental areas under the curves (IAUC). Blood glucose IAUC (0-90 min) was applied to determine glucose tolerance. Pearson correlations were used to study relations between physiological test parameters and results in the cognitive tests. Generally, n = 38 in the calculations. Values of $P \leq 0.05$ were considered statistically significant.
RESULTS

**Cognitive tests**

*WM-tests:* The outcomes from the WM-tests are presented in Table 1. Five weeks dietary supplement with n-3 PUFA from fish oil improved performance in the WM-test at 60 min compared with the placebo ($P = 0.04$). There was a tendency towards improvement in the total WM-test based on sentences (WM-tests at 60 + 160 min, $P = 0.07$). When including only the most demanding part in the statistical calculations, i.e. the semantically nonsensical sentences, the differences in performance after n-3 PUFA compared with placebo became statistically significant (WM-tests at 60 + 160 min, $P = 0.015$, Table 2).

There were no differences in the performance of the WM-tests depending on the consumption sequence of the test product (word retrieval: $P = 0.85$, figure retrieval $P = 0.45$). However, there was a [treatment*consumption sequence] interaction in the WM-tests (total word retrieval $P = 0.021$ and figure retrieval $P = 0.015$), that revealed better performance after n-3 PUFA compared with the placebo in the subject group (20 subjects) that had PUFA in the first intervention period (word retrieval: $P = 0.007$, figure retrieval: $P = 0.048$), whereas there were no significant differences depending on treatment in the 18 subjects that started with the placebo (word retrieval: $P = 0.69$, figure retrieval: $P = 0.29$). There were no significant time effects in performance between the WM-tests performed at 60 min and 160 min ($P = 0.15$). The absence of improvement with time indicates that there were no learning effects in the WM-tests.

*SA-tests:* The results from the SA-tests are displayed in Table 3. Even if not significant, there was a tendency towards better performance after n-3 PUFA supplementation compared with the placebo in the total SA-test (SA-test 1-4, $P = 0.087$). No differences in the performance
were seen in the SA-tests depending on the consumption sequence ($P = 0.15$) but there were [treatment*consumption sequence] interactions (total SA-test (tests 1-4), $P = 0.0001$), with better performance after the placebo ($P = 0.007$) or PUFA ($P = 0.019$), depending on being consumed in the second intervention period. The improvements in performance from the first to the second test occasion indicate learning effects in the SA-test. In addition, there was also a time effect during the test day meaning that the subjects performed inferior ($P = 0.001$) in the first SA-test (fasting) compared with the other three SA-tests.

**Relations between cognitive performance and metabolic risk markers**

The systolic blood pressure and s-triglycerides were significantly more suppressed (based on differences from the basal values measured prior to start of the intervention periods) after 5 weeks supplementation with omega-3 PUFA compared with after the placebo ($P = 0.04$ and $P = 0.05$, respectively). The results of the effects of n-3 PUFA on metabolic test markers are compiled in Table 4. As a general feature, the systolic blood pressure was inversely related to the performance in the cognitive tests. This relation was most pronounced in the oral WM-tests after the intervention with n-3 PUFA (WM 60 min: $r = -0.35$, $P = 0.034$, WM 110 min: $r = -0.38$, $P = 0.022$, WM 160 min (in the most difficult part): $r = -0.36$, $P = 0.029$). The fasting glucose concentrations were inversely related to the performance in the WM test at 110 min after n-3 PUFA ($r = -0.32$, $P = 0.05$). There was also a trend towards an inverse relation between f-glucose concentrations after the placebo period and WM test at 60 min (the most difficult part, $r = -0.23$, $P = 0.069$). Concentrations of triacylglycerides also tended to be inversely related to cognitive performance; the strongest relation seen following n-3 PUFA at WM 60 min after start of the standardized breakfast ($r = -0.30$, $P = 0.066$). Serum TNF-α concentrations were inversely related to the performance in the SA-test (time = 0) after the placebo period ($r = -0.33$, $P = 0.05$).
DISCUSSION

The results show that daily intake of n-3 PUFA from fish oil significantly improved cognitive functions (working memory capacity) in healthy subjects after five weeks. In addition there was a tendency towards better performance in the selective attention test after the n-3 PUFA period (SA-test no. 1-4, \( P = 0.087 \)). DHA + EPA are involved in a number of brain functions that may modulate cognitive functions, e.g. neurotransmission and regulation of signal transduction pathways [1], and are also important structural components in neuronal cell membranes. In addition n-3 PUFA possess several anti-inflammatory properties [35]. A growing body of data link chronic inflammation to poorer cognitive functions [36]. For example, in a middle-aged group of healthy subjects, circulating levels of IL-6 were inversely related to performance on a cluster of cognitive tests evaluating auditory recognition memory, attention, working memory, and executive function [36]. Interestingly, there was an inverse relation between TNF-\( \alpha \) concentrations and performance in the SA-test in the present study. The relationship between inflammation and cognitive performance indicate that n-3 PUFA may be beneficial to cognitive functions due to a general anti-inflammatory effect; involving also effects on neuro-inflammation. Low grade chronic inflammation is increasingly also recognised as an important factor in the development of metabolic disorders such as diabetes type 2 [37] and cardiovascular disease [38] (i.e. conditions that predispose for cognitive decline [13, 39-41]).

In addition to improved cognitive performance, n-3 PUFA improved acknowledged cardiometabolic risk markers, i.e. systolic blood pressure and triglycerides. The systolic blood pressure was inversely related to performance on cognitive tests and there was also a tendency toward an inverse relation between cognitive performance and triglycerides (\( P = 0.066 \)). The reductions in triglycerides and systolic blood pressure in the present cohort of healthy mature
subjects were similar to those previously described in hyper-triglyceridaemic subjects after daily intake of 1g of fish- or seal oil for six weeks [19], highlighting the cardioprotective properties of n-3 PUF in healthy subjects.

The novelty of the present investigation is the simultaneously evaluated effects of n-3 PUFA on cognition, and the relation to cardiometabolic risk markers in healthy subjects. The relation between higher levels of cardiometabolic risk markers and inferior cognitive performance in healthy subjects, as observed in the present study, highlights the potential of a preventive dietary approach in the combat of both metabolic disorders and associated cognitive decline.

Available studies of effects of n-3 PUFA have mainly used different fatty acids as placebo. In the current study we included a non oil based placebo product. The rationale for not choosing oil for placebo is that several fatty acids possess known or suggested metabolic and/or cognitive effects, and are therefore not inert to the test variables investigated in studies of metabolism and cognition [8, 42-44]. A potential limitation of our study relates to the fact that the n-3 PUFA was administered in the form of a capsule, whereas the placebo treatment was in tablet form, since it was impossible to seal a capsule containing water. However, the test subjects were uninformed as to the activity of the PUFA and placebo supplement. It should also be noted that it is difficult to blind an intake of fish oil due to side effects such as ‘fishy burps’ [45].

In conclusion, the present study reveals that 5 weeks daily intake of omega-3 PUFA from fish oil has the potential to improve cognitive functions and cardiometabolic risk factors in a healthy middle aged to elderly cohort. The relationship between outcome in cognitive tests and several cardiometabolic risk factors highlights the importance of early dietary prevention to prevent cognitive decline secondary to cardiometabolic disorders. The dietary prevention strategy should preferably include fish in quantities to supply sufficient amounts of PUFA, in
addition to other food groups with potential metabolic benefits e.g. whole grain, low-glycaemic index foods, fruits, berries, vegetables, and prebiotics [32, 46-49]. Further studies are needed to clarify the underlying mechanism of the enhanced cognitive effect of omega-3 PUFA, and the relationship to cardiometabolic risk markers.
LIST OF ABBREVIATIONS

n-3 PUFA: long chain n-3 polyunsaturated fatty acids; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; BMI: body mass index; WM: Working memory; SA: selective attention.
COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS’ CONTRIBUTIONS

Contributors: AN coordinate the study, was responsible for the study design, carried out the experimental work, the collection-, analysis and statistical calculations regarding the blood tests, and the evaluation and writing of the paper. KR and IS had the primary responsibility for the cognitive tests and the statistical analysis of cognitive test variables, and was involved in the evaluation and writing of the paper. IB was involved in the study design, and the evaluation, and writing of the paper. All authors read and approved the final manuscript.

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Table 1. Results in the WM-tests following 5 weeks daily dietary supplementation with 3 g omega-3 PUFA from fish oil or a placebo product, respectively.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>WM-tests ² (max 48 credits)</th>
<th>Omega-3</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>60 min (word)</td>
<td>31.4±0.8²</td>
<td>30.0±1.0²</td>
<td></td>
</tr>
<tr>
<td>110 min (figures)</td>
<td>33.8±1.4²</td>
<td>33.3±1.4²</td>
<td></td>
</tr>
<tr>
<td>160 min (words)</td>
<td>30.4±0.9²</td>
<td>29.6±1.0²</td>
<td></td>
</tr>
</tbody>
</table>

¹Data are given as means per treatment ± SEM, n = 38, but only 37 subjects performed the WM-test at 110 min (19 subjects started with placebo and 18 subjects started with PUFA) due to one subject did not perform the test in time. Labeled means in a row without a common letter differ, P < 0.05.

² At 60 min and 160 min the subjects were supposed to recall nouns and at 110 min the subjects were supposed to recall figures.
Table 2. Results in the most demanding part of the WM-tests following 5 weeks daily dietary supplementation with 3 g omega-3 PUFA from fish oil or a placebo product, respectively.\(^1\)

<table>
<thead>
<tr>
<th>WM-test (max 24 credits)</th>
<th>Treatments</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Omega-3</td>
<td>Placebo</td>
<td></td>
</tr>
<tr>
<td>60 min(^2)</td>
<td>14.8±0.6(^a)</td>
<td>13.7±0.5(^b)</td>
<td></td>
</tr>
<tr>
<td>160 min</td>
<td>14.5±0.5(^a)</td>
<td>13.8±0.5(^a)</td>
<td></td>
</tr>
<tr>
<td>Total (60+160 min)(^3)</td>
<td>29.3±0.9(^a)</td>
<td>27.5±1.0(^b)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)The data shows results for the most demanding part of the tests, i.e. recall of a noun in semantically nonsensical sentences. Data are given as means per treatment ± SEM, \(n = 38\), Labeled means in a row without a common letter differ, \(^2P=0.01\), \(^3P=0.015\).
Table 3. Results in the SA-tests following 5 weeks daily dietary supplementation with 3 g omega-3 from fish oil or a placebo product, respectively\(^1\).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Omega-3</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA-test (max 95 credits)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>77.39±2.57</td>
<td>75.28±2.79</td>
</tr>
<tr>
<td>45 min</td>
<td>80.84±1.92</td>
<td>79.97±2.29</td>
</tr>
<tr>
<td>95 min</td>
<td>81.16±2.02</td>
<td>81.43±1.90</td>
</tr>
<tr>
<td>145 min</td>
<td>81.73±1.77</td>
<td>81.62±2.08</td>
</tr>
<tr>
<td>Total (0-145min)(^2)</td>
<td>322.5±7.80</td>
<td>318.3±8.78</td>
</tr>
</tbody>
</table>

\(^1\)Data are given as means per treatment ± SEM, \(n=36\) at fasting (18 subject started with PUFA and 18 with placebo), \(n=37\) at the rest of the time points (19 subject started with PUFA and 18 with placebo) due to 2 and 1 subjects, respectively, performed the test incorrectly.

\(^2\) The total SA-test (test 1-4), \(P=0.087\). The statistical calculations for the total SA-test is based on \(n=36\), the number of subjects that performed all four tests.
Table 4. Results of the physiological test parameters before and after 5 weeks interventions with 3g/d omega-3 PUFA from fish oil and placebo, respectively.

| Physiological parameters | Omega-3 | | | Placebo | | | | Δ | Δ |
|--------------------------|---------|---|------|---------|---|------|---|
|                          | Before  | After | Δ-Omega-3 | Before  | After | Δ-Placebo | Δ |
| Weigh (kg)               | 72.1±2.0 | 72.3±2.0 | 0.2±0.1 | 72.3±2.0 | 72.2±2.0 | -0.1±0.1 | ns |
| Systolic BP (mmhg)       | 134±3   | 127±3  | -7±2*** | 132±3   | 131±3  | -1±2     | P≤0.05 |
| Diastolic BP (mmhg)      | 79.2±1,4 | 76.9±1,4 | -2.24±1.0* | 78.8±1,2 | 77.3±1,4 | -1.46±0.8^ | ns |
| f-Glucose (mmol/L)       | 5.4±0,1 | 5.5±0,1 | 0.1±0.1 | 5.5±0,1 | 5.4±0,1 | -0.1±0.1 | ns |
| ΔGlucose peak (mmol/L)   | 4.2±0,2 | 4.0±0,2 | -0.2±0,2 | 4.3±0,2 | 4.2±0,2 | -0.1±0,2 | ns |
| Glucose 90 min IAUC (mmol*min/L) | 217±13 | 213±11 | -4±1 | 225±10 | 226±11 | 1±10 | ns |
| Insulin (pmol/L)         | 35±3    | 40±3   | 5±2* | 37±3    | 43±04  | 6±3^ | ns |
| FFA (mmol/L)             | 0.28±0,02 | 0.26±0,02 | -0.03±0,02 | 0.28±0,02 | 0.31±0,02 | 0.03±0,02 | P=0.055 |
| Triglycerides (mmol/L)   | 1.63±0,10 | 1.45±0,09 | -0.19±0,07* | 1.58±0,10 | 1.66±0,11 | 0.08±0,07 | P≤0.05 |
| Total Cholesterol (mmol/L) | 7.0±0,2 | 6.7±0,2 | -0.32±0,14* | 7.0±0,2 | 6.9±0,2 | -0.04±0,17 | ns |
Values are mean±SEM, n=38.

1 Changes (△) in test variables from baseline (prior to start of PUFA) after 5 weeks PUFA supplementation. 2 Changes (△) in test variables from baseline (prior to start of placebo) after 5 weeks placebo supplementation.

*: P<0.05, ***: P<0.001 (ANOVA) with respect to differences from baseline after 5 weeks intervention with PUFA. †: P<0.05 (ANOVA) with respect to differences from baseline after 5 weeks intake of the placebo product.

3 P-values for differences between effects of PUFA (△-PUFA) and effects of placebo (△-placebo). 4 P=0.077 for the difference in diastolic BP.
from baseline after 5 weeks intake of placebo. $^5$P=0.097 for the difference in LDL from baseline after 5 weeks intake of PUFA.

ns: no significant differences between Δ-PUFA and Δ-placebo (ANOVA).
