Moderate oral supplementation with docosahexaenoic acid improves platelet function and oxidative stress in type 2 diabetic patients

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Running title: DHA amends platelet function and redox status

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Summary

Platelets from patients with type 2 diabetes are characterized by hyperactivation and high level of oxidative stress. Docosahexaenoic acid (DHA) may have beneficial effects on platelet reactivity and redox status. We investigated whether moderate DHA supplementation, given as a triglyceride form, may correct platelet dysfunction and redox imbalance in patients with type 2 diabetes. We conducted a randomized, double-blind, placebo-controlled, two-period crossover trial (n=11 post-menopausal women with type 2 diabetes) to test the effects of 400 mg/day of DHA intake for 2 weeks on platelet aggregation, markers of arachidonic acid metabolism, lipid peroxidation status, and lipid composition. Each 2 week-period was separated from the other by a 6-week washout. Daily moderate dose DHA supplementation resulted in reduced platelet aggregation induced by collagen (-46.5%, p<0.001), and decreased platelet thromboxane B$_2$ (-35%, p<0.001), urinary 11-dehydrothromboxane B$_2$ (-13.2%, p<0.001) and F2-isoprostane levels (-19.6%, p<0.001) associated with a significant increase of plasma and platelet vitamin E concentrations (+20% and +11.8%, respectively, p<0.001). The proportions of DHA increased both in plasma lipids and in platelet phospholipids. After placebo treatment, there was no effect on any parameters tested. Our findings support a significant beneficial effect of low intake of DHA on platelet function and a favorable role in reducing oxidative stress associated with diabetes.

Keywords: platelet aggregation; arachidonic acid; vitamin E; isoprostane; plasma and platelet lipid composition
Introduction

In 2035, the number of people affected by diabetes mellitus could reach more than 550 million in the world (1). Moreover, cardiovascular disease is one of mortality causes in type 2 diabetes (2). Indeed, it is fully recognized that long-term macrovascular complications are the main cause of morbidity and mortality associated with diabetes. In type 2 diabetes, a complex interplay occurs between hyperglycemia and oxidative stress. Factors that may contribute to increased oxidative stress in diabetes mellitus include protein glycation, increased formation of reactive oxygen species, and antioxidant deficiencies (3,4). On the other hand, it is generally admitted that platelet activation contributes to the increased incidence of thrombotic and atherosclerotic diseases. During platelet activation, several steps occur including the release of arachidonic acid (ARA) from membrane phospholipids. Once released, ARA can be oxygenated into thromboxane A₂ (TxA₂), a potent pro-aggregatory and vaso-constricting compound (5) that rapidly breaks down to form the stable and inactive product thromboxane B₂ (TxB₂). Moreover, we have shown that increased platelet aggregation is already detectable in diabetic patients who do not suffer from vascular complications linked with impairment of antioxidant mechanisms (6).

For several decades, there has been accumulating evidence that n-3 polyunsaturated fatty acids (PUFA) could protect against cardiovascular diseases and interestingly, there is a low prevalence of diabetes and coronary heart disease in populations known for high intake of n-3 fatty acids (7,8). Because of the pivotal role of platelets in the regulation of hemostasis and athero-thrombosis, some studies have focused on the effects of n-3 PUFA on these cells. It is widely accepted that eicosapentaenoic (EPA or 20:5n-3) and docosahexaenoic (DHA or 22:6n-3) acids can decrease platelet function (9). Moreover, DHA could be associated with reduced progression of coronary atherosclerosis (10). Despite these described protective
effects, some studies failed to report a beneficial association between n-3 PUFA consumption and cardiovascular events (11). Among some explanations for these discordant results, some pertain to the high unsaturation of these PUFA. Indeed, DHA is highly oxidisable owing to the presence of 6 double bonds, and it is known that lipid peroxidation provides some peroxidised lipids increasing the oxidative stress. Such a lipid peroxidation has been implicated to the pathogenesis of cardiovascular diseases (12). Previously, we have shown a bimodal in vitro effect of DHA with antioxidant and pro-oxidant effects at low and high concentrations, respectively (13). More recently, we have conducted an ex-vivo study, with healthy volunteers, which has shown the validity of our in vitro findings (14). Following ingestion of increasing doses of DHA (200, 400, 800, 1600 mg/day of DHA for 2 weeks each), we have shown that platelet reactivity was decreased after 400 and 800 mg DHA/day. It was concluded that low consumption of DHA (400 to 800 mg/day) could be an effective “treatment” to protect healthy volunteers from platelet-related cardiovascular events.

These data prompted us to perform a randomized double-blind study to determine whether a modest intake of DHA (400 mg/day for 2 weeks) may be also beneficial in type 2 diabetic patients, a population characterized by platelet hyperactivity, impaired antioxidant defense, and increased oxidative stress, in opposing that oxidative stress or synergizing with it.

**Materials and methods**

**Materials**

All chemicals were purchased from Sigma-Aldrich (Saint-Quentin, Fallavier, France). All solvents were provided by Carlo Erba-Reactifs (Val de Reuil, France). Silica gels 60 plates were supplied by Merck (VWR International, Strasbourg, France).
Ethics statement

Informed, written consents were obtained from all participants and the protocol was approved by the local ethic committee, the “Comité de Protection des Personnes Sud-Est II” (ClinicalTrials.gov Identifier is NCT01150292).

Study design

Eleven post-menopausal women with type 2 diabetes participated. Subjects with diabetes had recent glycated hemoglobin (HbA1c: 7.89 ± 1.27 %, 62.8 ± 14.1 mmol/mol, n=11), anti-diabetic drugs but no drugs known to affect platelet function (acetylsalicylic acid, clopidogrel, gliclazide, ticlopidine, non-steroidal anti-inflammatory drugs). A randomized, placebo-controlled clinical trial was conducted. The subjects were randomly assigned in double-blind fashion to DHA or placebo, administered for 2 weeks. After baseline visit, subjects were randomly assigned to take 2 capsules/day of DHA or placebo with meals for 2 weeks, then, after 6 weeks of wash out, subjects took 2 capsules/day of placebo or DHA (Figure 1). Capsules of DHA (Pro-Mind) were supplied by Decola (Maldegem, Belgium). Each capsule contained 200 mg DHA, as the only PUFA, in triglycerides from algal oil, 175 mg gelatin, 0.125 mg DL-alpha-tocopherol, and 0.125 mg ascorbic palmitate. Placebo capsules were made, specifically upon our request, by Ayanda GmbH & Co.KG (Pritwalk, Germany) and contained the same quantities of antioxidants and triglycerides of sunflower oil. Placebo and DHA capsules were indistinguishable and independently packaged by the Central Pharmacy of the hospital (Hospices Civils de Lyon). Pharmacists dispensed placebo or DHA capsules in a random process. All study personnel and participants were blinded to treatment assignment and the code was only revealed to the researchers after laboratory analyses were completed. A dietary record was obtained on the first visit and dietary changes monitored at each visit.
Blood and urine samples were collected after overnight fasting before and after each supplementation. Urine samples were stored immediately at -80°C in presence of butylated hydroxy-toluene (BHT).

**Plasma lipid measurements**

Total cholesterol, HDL-, LDL- cholesterol and triglyceride concentrations were measured using commercial enzymatic kits. Intra-assay and inter-assay coefficients of variation were <10%.

**Plasma preparation and isolation of platelets**

Blood samples were collected in tubes containing acid-citrate-dextrose (0.8% citric acid, 2.2% sodium citrate, and 2.45% dextrose, 6:1 v/v) and platelets were isolated as described previously (15). The supernatant removed from above the platelet pellet was centrifuged for 10 min at 18°C to obtain platelet-poor plasma.

**Platelet aggregation**

Collagen (Horm, from Nycomed, Linz, Austria) was used as agonist to induce platelet aggregation in a Chronolog dual-channel aggregometer (Coulter, Margency, France) according to the turbidimetric method of Born (16). Intra-assay and inter-assay coefficients of variation were 5% and 6%, respectively, with a lower detection limit of < 0.1%.

**Arachidonic acid metabolism**

Metabolism of exogenous arachidonic acid

The oxygenation of ARA through the lipoxygenase and cyclooxygenase pathways was determined by incubating 2.2 microM of [1-^{14}C] ARA (specific activity 2.07 GBq/mmol, G.E. Healthcare, Vélisy-Villacoublay) with platelets for 4 min at 37°C. Following lipid
extraction and thin-layer chromatography (TLC) separation, oxygenated products were visualized and quantified with a TLC analyser radioscanner (Raytest, Paris, France) (17). Intra-assay and inter-assay coefficients of variation were 9% and 10%, respectively, with a lower detection limit of <0.1%.

Basal formation of platelet thromboxane B₂ and its urinary 11-dehydro metabolite

In the absence of specific stimulation, platelets in presence of BHT as an antioxidant (5x10⁻⁵ mol/l) were immediately frozen. TxB₂ was quantified by immunoassay according to the manufacturer’s recommendations (Enzo Life Sciences, Lyon, France) (Intra-assay and inter-assay coefficients of variation were 4%). Spot urine samples were collected and stored immediately at -80°C in presence of BHT, and then 11-dehydro-TxB₂ was also quantified by immunoassay. Intra-assay and inter-assay coefficients of variation were 12% and 13%, respectively, and the limit of detection was 10 pg/ml for both kits.

**Antioxidant status**

Plasma and platelet vitamin E

Alpha- and gamma-tocopherol concentrations were determined according to a described method (18). Briefly, after extraction with hexane, tocopherol isomers were separated by reverse-phase HPLC and detected by fluorimetry (excitation and emission at 295 nm and 340 nm, respectively). Intra-assays and inter-assay coefficients of variation were <5 % and the limit of detection was 4 pmol.

**Lipid peroxidation markers**

The malondialdehyde (MDA) content of unstimulated platelets was measured by the HPLC technique of Therasse and Lemonier (19) with fluorimetric detection (excitation 515 nm,
emission 553 nm). Intra-assay and inter-assay coefficients of variation were 12% and 15%, respectively, and the limit of detection was 5 pmol.

Urinary isoprostane (8-iso-PGF$_{2\alpha}$ or 15-F2t-IsoP) and creatinine were determined by immunoassay (Enzo Life Sciences, Lyon, France) and urinary isoprostane expressed per milligram of creatinine. Intra-assays and inter-assays coefficient of variations were 5% and 9%, respectively and the limit of detection was 15 pg/ml for isoprostane kit. Intra-assay and inter-assay coefficients of variation were 4% and 3%, respectively, and the limit of detection was 0.3 µg/ml for creatinine kit.

**Lipid analysis**

Plasma lipids were extracted twice with chloroform/ethanol (2:1, v/v) containing BHT and 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine as an internal standard and transmethylated with methanol (20). Fatty acid methyl esters (FAMEs) were analyzed by gas chromatography (GC) and values were calculated as mole percentages (mol%) of all FAMEs determined (C$_{16}$ to C$_{24}$).

Platelet lipids were extracted twice with chloroform/ethanol (2:1, v/v) containing BHT and 1,2-diheptadecanoyl-sn-glycero-3-phosphocholine and 1,2-diheptadecanoyl-sn-3-phosphoethanolamine as internal standards. Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were separated by TLC, transmethylated with methanol and analyzed by GC. Intra-assay and inter-assay coefficients of variation were between 3% and 10% according to the fatty acid, and the limit of detection was 10 ng.

**Sample size calculation**

The sample size calculation was based on our previous data. Considering that, first the percent of aggregation was increased in diabetic patients compared to controls (6), and
second 400 mg-DHA ingestion in healthy volunteers decreased platelet aggregation induced by collagen (14), we estimated that 13 subjects would give 80% power to detect a 40% difference in platelet aggregation induced by collagen at a significance level of P=0.05. 14 diabetic patients were recruited but 3 voluntarily withdrew.

**Statistical analysis**

The study was designed as a two-period crossover study with double blinding to the order of randomization to DHA or placebo. Statistical analyses were performed by using StatView 5 for Windows (Abacus Corp, Baltimore, MD, USA). The non-parametric Friedman test was used to analyze the differences for all the variables. Wilcoxon’s post-test was used to identify group differences. Results are presented as mean ± SEM (n=11).

**Results**

**Subjects’ characteristics**

Eleven post-menopausal women with type 2 diabetes were included in the protocol analysis. Their mean age was 59.8 ± 4.7 years at baseline. Some anthropometric variables were measured such as BMI (34.1 ± 5.1 kg/m²), waist and hip circumferences (113.7 ± 10.1 cm and 115 ± 10.5 cm, respectively, n=9 to 11). Patients followed the DHA and placebo supplementations without any reported difficulty. According to patient self-reports, lifestyle and medication were unchanged throughout the study. As reported in Table 1, there were no significant differences in metabolic parameters measured during placebo or DHA treatment.

**Platelet aggregation**
As shown in Figure 2, platelet aggregation induced by 0.05 µg/ml collagen was significantly decreased (-46.5%) after the 400 mg/d DHA supplementation compared to baseline. No effect was observed after placebo treatment.

**Platelet metabolism**

Incubations of platelets with exogenous ARA allowed us to determine the specific oxygenation of this fatty acid by cyclooxygenase and 12-lipoxygenase. Interestingly, DHA intake affected significantly the formation of TxB\(_2\) with a decrease of 35% (Table 2). Moreover, 12-hydroxy-5,8,10-heptadecatrienoic acid (HHT), the other main cyclooxygenase product in platelets was decreased by 16.4% (p=0.06). Altogether, a significant decrease of the cyclooxygenase pathway was noted whereas the formation of 12-HETE, the lipoxygenase end-product, was not affected by DHA supplementation (Table 2). In the absence of specific stimulation, lower amount of basal TxB\(_2\) was found after DHA intake compared to before DHA (Table 2). One major urinary metabolite of TxB\(_2\) (11-dehydro-TxB\(_2\)) was also significantly decreased after DHA supplementation (Table 2). No effect was observed after placebo treatment.

**Antioxidant status and lipid peroxidation**

To evaluate the antioxidant status, alpha-tocopherol level, an effective lipophilic antioxidant and free radical scavenger, was determined in both plasma and platelets. As shown in Table 2, significant increases of plasma and platelet alpha-tocopherol levels were observed after DHA intake compared to tocopherol levels before supplementation. A tendency to the increase was observed for platelet gamma-tocopherol levels but the difference did not reach statistical significance (pre DHA: 34.1 ± 1.7 vs post DHA: 39.5 ± 2.8 pmol/mg prot., p=0.09, n=10). To assess the overall lipid peroxide levels in platelets, platelet MDA contents were determined and a tendency to decrease was observed (p=0.06) (Table 2) after DHA
supplementation compared to pre DHA. Finally, we measured a urinary marker of oxidative stress, 8-iso-PGF$_{2\alpha}$ (or 15-F2t-IsoP). The level of this marker was significantly decreased after DHA intake compared to the control before supplementation of DHA (Table 2). No effect on the antioxidant status and lipid peroxidation was observed after placebo treatment as compared to the situation before placebo.

**Fatty acid composition of plasma and platelet lipids**

In plasma, supplementation of the diet with 400 mg DHA/d for 2 weeks, induced a significant increase of DHA (+40%) in total lipids (Fig. 3A). This result reflects acceptable compliance for capsule intake. Interestingly, this change was accompanied by a concomitant slight but significant decrease of 22:5n-3 (-9.9%) while 20:5n-3 proportion did not change significantly. No effect on the main n-6 fatty acids (18:2, 20:3 and 20:4) was observed (Fig. 3B). Regarding platelets, a significant increase of DHA in PC (+36%) was detected (Fig. 3C) as well as in PE (+29%) (Fig. 3E). As already found in plasma, the 22:5n-3 proportion decreased significantly in PE (-18.7%) and a tendency to decrease could be observed in PC (-10.7%). No effect on the main n-6 fatty acids (18:2, 20:3 and 20:4) was observed in PC (Fig. 3D) and PE (Fig. 3F) after DHA supplementation. Placebo supplementation had no significant effect either on plasma lipid composition or platelet phospholipids (Fig. 3 A to F). Proportions of saturated fatty acids (and 16:0 and 18:0 dimethyl-acetals for PE, representing plasmalogens), and monounsaturated fatty acids remained constant for all plasma and platelet lipids (results not shown).

**Discussion**
Platelet aggregation is a complex process involving multiple receptors and signaling pathways. Collagen-induced platelet shape change and aggregation involves the participation of its receptors, the release of endogenous arachidonic acid and formation of thromboxane A₂, and the response to the latter lipid mediator. Indeed, whole reactivity to collagen could not take into account the reactivity to other specific agonists such as ADP and epinephrine. Because we have previously shown that low supplementation of DHA can reduce platelet aggregation in response to collagen, in healthy volunteers (14), the primary objective of this study was to investigate the efficacy of 400 mg DHA daily supplementation for 2 weeks on platelet function in type 2 diabetic patients, who are characterized by platelet hyperactivity (6). One of the main findings of the study is the demonstration that this modest oral dose of DHA was able to reduce platelet aggregation in response to collagen in post-menopausal women with type 2 diabetes mellitus. In such a population, Woodman et al. (21) and Venkatakrishnan et al. (22) already showed that DHA supplementation reduced collagen-induced platelet aggregation, but our result is the first one obtained after such a low DHA supplementation. In addition and in agreement with our data on platelet aggregation, we show for the first time that the conversion of exogenous ARA into cyclooxygenase products was decreased after DHA intake while conversion of ARA by the lipoxygenase pathway was not affected as already observed in vitro (23). Moreover, the basal formation of platelet TxB₂ was significantly reduced after DHA as well as the concentration of one major urinary metabolite (11-dehydro-TxB₂), which represents a biomarker of platelet activity (24), and reflects the whole biosynthesis of TxA₂ by platelets and extra-platelet sources. Altogether, our results indicate that moderate DHA intake efficiently decreased platelet reactivity in patients with type 2 diabetes mellitus.

Because of the high susceptibility of DHA to oxidative damage, care was taken to ensure that DHA supplementation did not increase lipid peroxidation. Indeed, numerous studies support
the conclusion that there is an association between diabetes and oxidative stress. It is known
that oxidative stress may be an important factor in the pathogenesis of cardio-vascular
diseases (3), and also precedes the development of atherosclerosis as already shown in
patients affected by type 1 (25) or type 2 diabetes without vascular complications (6).
Previously, it was shown that plasma MDA significantly decreased after n-3 PUFA
(EPA+DHA) supplement in patients with type 2 diabetes mellitus (26) or did not increase
after DHA or EPA intervention (27). Platelet MDA is considered as a global oxidative stress
marker (produced by both enzymatic and non-enzymatic oxidation). Interestingly, we
observed a tendency, close to significance, to a decrease of platelet MDA after the 400 mg/d
DHA supplement. Accordingly, F2-isoprostanes, products of free radical peroxidation of
ARA, released by PLA2 activity to the blood stream and finally excreted into urine, provide a
reliable measure of in vivo oxidative stress (28). As already shown by Mori et al. (29), we
also found a significant decrease of 15-F2t-isoprostane excretion following DHA intake.
These results are also in agreement with those of McDonald et al. (30) who found an n-3
PUFA-mediated decrease in platelet isoprostane levels in a group of patients with type 2
diabetes. However, both studies (29,30) dealt with much higher intake of EPA plus DHA.
The augmented oxidative stress reported in diabetic patients (3,6) may be the result of higher
free radical production but also caused by decreased antioxidant defenses. For a long time, it
has been reported that alpha-tocopherol level was decreased in plasma (31) as well as in
platelets from patients with diabetes (6). Interestingly, our evaluation of plasma and platelet
alpha-tocopherol levels before and after 400 mg/day DHA supplementation confirmed the
efficacy of the intervention. Indeed, consistent with decreased lipid peroxidation, plasma and
platelet vitamin E levels were significantly increased as already observed in platelets after
low intake of EPA (18) or DHA (14).
Supplementation of patients with 400 mg/day DHA had no effect on the plasma lipid profile with no changes in the concentrations of total cholesterol, cholesterol fractions and triglycerides but it should be noted that the baseline values were already quite in the normality or borderline high. In agreement, several reports have indicated that DHA supplementation reduced plasma triglycerides with doses superior to 2g/day (32) and had no significant effects on total cholesterol and lipid subfractions (33).

Baseline plasma DHA proportion is generally low because this fatty acid is consumed in small quantities in dietary lipids and the synthesis from alpha-linolenic and eicosapentaenoic acids is not efficient in humans. Consequently, a low supplementation like 400 mg/day DHA induced a substantial increase in plasma lipids vs baseline. We have indeed previously reported that a 200 mg/day DHA dose was sufficient to increase DHA proportion in both plasma phospholipids and cholesteryl esters in healthy men (14). As already reported (14,34,35), we found a decreased 22:5n-3 proportion in response to DHA intake. To explain this decrease, Conquer and Holub (36) had proposed a possible competition between DHA and 22:5n-3 to esterification into phospholipids while other modifications of metabolism could not be ruled out.

The biological actions of DHA may probably be due to different mechanisms induced by the fatty acid itself, esterified or not. Indeed, we cannot exclude some effects of DHA on the molecular organization of plasma membrane according to its incorporation into phospholipids and the remodeling of cholesterol-enriched lipid microdomains (37). The effects of such small doses of DHA on the regulation of numerous gene products (38) have not been shown, but they cannot be excluded either. Moreover, since more than ten years, there is a growing interest for the effects of DHA oxygenated metabolites either on oxidative stress (39) or on platelet aggregation (40). Thus, additional mechanisms may mediate the effects observed in
the current study. Nevertheless, our results reinforce recommendations for a low intake of
DHA but the duration of such a supplement remains to be established.

Altogether, in this randomized study, our findings indicate that a modest DHA intake, not
only decreases platelet reactivity, but offers an additional therapeutic benefit in reducing
redox status in patients with type 2 diabetes mellitus. Our results reinforce the antioxidant
potential of low doses of DHA already suggested in healthy subjects (14).

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Humaine Rhône-Alpes for their expert blood drawing and some lipid analyses.

Duality of Interest. No potential conflicts of interest relevant to this article were reported.

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Figure legends

Figure 1 -Study design.

Figure 2 Influence of DHA or placebo intake on platelet aggregation.

Percentages of aggregation were assessed 4 min after the addition of 0.05 microg/ml collagen.

All values are means ± SEM, n=11. ** p<0.001 post DHA vs. pre DHA.
Figure 3-Effect of the DHA or placebo intake on plasma and platelet lipid composition.

A: Effect of the DHA or placebo supplementation on main n-3 fatty acids in plasma. B: Effect of the DHA or placebo supplementation of DHA on main n-6 fatty acids in plasma. C: Effect of the DHA or placebo supplementation on main n-3 fatty acids in platelet phosphatidylcholine (PC). D: Effect of the DHA or placebo supplementation of DHA on main n-6 fatty acids in platelet PC. E: Effect of the DHA or placebo supplementation on main n-3 fatty acids in platelet phosphatidylethanolamine (PE). F: Effect of the DHA or placebo supplementation on main n-6 fatty acids in platelet PE.

** p<0.001 post DHA vs pre DHA.

| Table 1-Characteristics of participants before and after DHA or placebo supplementation |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Weight (kg)                     | Pre DHA                        | Post DHA                       | Pre placebo                    | Post placebo                    |
|                                 | 84.2 ± 4.8                     | 83.3 ± 5                       | 83.7 ± 4.6                     | 83.8 ± 4.8                     |
| Diastolic blood pressure (mmHg) | 75.8 ± 1.5                     | 77.8 ± 3                       | 79 ± 3                         | 74.2 ± 2.8                     |
| Systolic blood pressure (mmHg)  | 137 ± 5.9                      | 141.2 ± 5.2                    | 137.8 ± 3.7                    | 137.3 ± 6.3                    |
| Triglycerides (mmol/l)          | 1.67 ± 0.8                     | 1.51 ± 0.16                    | 1.75 ± 0.17                    | 1.91 ± 0.24                    |
| Total cholesterol (mmol/l)      | 4.37 ± 0.23                    | 4.47 ± 0.33                    | 4.59 ± 0.30                    | 4.30 ± 0.25                    |
| HDL cholesterol (mmol/l)        | 1.18 ± 0.06                    | 1.18 ± 0.08                    | 1.13 ± 0.07                    | 1.12 ± 0.07                    |
| LDL cholesterol (mmol/l)        | 2.41 ± 0.26                    | 2.53 ± 0.30                    | 2.54 ± 0.33                    | 2.35 ± 0.21                    |

Data are means ± SEM (n=11). There were no significant differences between pre DHA and post DHA values and between pre placebo and post placebo values.
Table 2- Effect of DHA and placebo on platelet arachidonic acid metabolism, antioxidant status and lipid peroxidation

<table>
<thead>
<tr>
<th></th>
<th>Pre DHA</th>
<th>Post DHA</th>
<th>Pre placebo</th>
<th>Post placebo</th>
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</thead>
<tbody>
<tr>
<td><strong>TxB₂</strong> (nmol/mg prot.)</td>
<td>0.92 ± 0.2</td>
<td>0.60 ± 0.16**</td>
<td>0.68 ± 0.11</td>
<td>0.55 ± 0.07</td>
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<td><strong>HHT</strong> (nmol/mg prot.)</td>
<td>0.55 ± 0.09</td>
<td>0.46 ± 0.10</td>
<td>0.47 ± 0.06</td>
<td>0.46 ± 0.05</td>
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<tr>
<td><strong>TxB₂ + HHT</strong> (nmol/mg prot.)</td>
<td>1.48 ± 0.28</td>
<td>1.07 ± 0.26*</td>
<td>1.15 ± 0.17</td>
<td>1.02 ± 0.12</td>
</tr>
<tr>
<td><strong>12-HETE</strong> (nmol/mg prot.)</td>
<td>0.27 ± 0.08</td>
<td>0.24 ± 0.07</td>
<td>0.21 ± 0.02</td>
<td>0.23 ± 0.02</td>
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<tr>
<td><strong>Basal TxB₂</strong> (pmol/mg prot.)</td>
<td>102.9 ± 14</td>
<td>70.9 ± 12.1**</td>
<td>81.2 ± 13.6</td>
<td>85.1 ± 13.3</td>
</tr>
<tr>
<td><strong>11-dehydro-TxB₂</strong> (ng/mg creat.)</td>
<td>6.1 ± 0.5</td>
<td>5.3 ± 0.4**</td>
<td>5.8 ± 0.6</td>
<td>6.2 ± 0.8</td>
</tr>
<tr>
<td><strong>Plasma vitamin E</strong> (nmol/ml) n=10</td>
<td>8.5 ± 0.9</td>
<td>10.2 ± 0.8**</td>
<td>11.2 ± 1.1</td>
<td>10.6 ± 1.2</td>
</tr>
<tr>
<td><strong>Platelet vitamin E</strong> (pmol/mg prot.)</td>
<td>220.1 ± 13.5</td>
<td>246.1 ± 17.7**</td>
<td>251.1 ± 11.9</td>
<td>234.6 ± 12.1</td>
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<td><strong>MDA</strong> (pmol/mg prot.)</td>
<td>211.9 ± 36.4</td>
<td>147.2 ± 29.3</td>
<td>228.2 ± 48.1</td>
<td>219.1 ± 37.6</td>
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<tr>
<td><strong>15-F₂t-IsoP</strong> (ng/mg creat.)</td>
<td>5.1 ± 0.9</td>
<td>4.1 ± 0.8**</td>
<td>4.1 ± 0.7</td>
<td>5.2 ± 1</td>
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</table>

Formation of the main ARA metabolites through cyclooxygenase (TxB₂ and HHT) and lipoxygenase (12-HETE) produced after stimulation with 2.2 microM [¹⁴C] ARA during 4 min. at 37°C (the first four lines). Platelet lipids were extracted and separated by TLC, and metabolites of [¹⁴C] ARA were quantified by radio-chromatography. Basal formations of TxB₂ in unstimulated platelets (in grey) and of one major urinary metabolites of TxB₂, 11-dehydro-TxB₂, were quantified by immunoassays. Plasma, platelet vitamin E (a-tocopherol) and platelet MDA were extracted and quantified after HPLC separation. Urinary isoprostanes were quantified by immunoassays. There were no significant differences between pre placebo and post placebo values. All values are means ± SEM, n=11. *p< 0.05, **p<0.001 compared to values before DHA (pre DHA).
What is known about this topic?

- Increased platelet function and oxidative stress are observed in patients with type 2 diabetes.
- DHA may exert athero-protective properties under certain conditions.
- DHA may have a bimodal effect with antioxidant and pro-oxidant at low and high concentrations, respectively.

What this paper add?

- Moderate dose of DHA reduced platelet hyper-aggregation of patients with type 2 diabetes.
- In these conditions, DHA reduced oxidative stress associated with type 2 diabetes.
- Our study supports a beneficial effect of low intake of DHA in patients with type 2 diabetes.
Fig. 1

Baseline visit → Randomized

400 mg DHA
w0 → w2
placebo
w0 → w2

400 mg DHA
w8 → w10
placebo
w8 → w10

wash-out period
Fig. 2

% aggregation

Pre DHA  Post DHA  Pre placebo  Post placebo

**
Fig. 3

Panel A: PLASMA
- **Pre DHA**
- **Post DHA**
- **Pre placebo**
- **Post placebo**

Panel B: PLASMA
- **Pre DHA**
- **Post DHA**
- **Pre placebo**
- **Post placebo**

Panel C: Platelet PC
- **Pre DHA**
- **Post DHA**
- **Pre placebo**
- **Post placebo**

Panel D: Platelet PC
- **Pre DHA**
- **Post DHA**
- **Pre placebo**
- **Post placebo**

Panel E: Platelet PE
- **Pre DHA**
- **Post DHA**
- **Pre placebo**
- **Post placebo**

Panel F: Platelet PE
- **Pre DHA**
- **Post DHA**
- **Pre placebo**
- **Post placebo**