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ORIGINAL CONTRIBUTION

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Supplementation with long-chain n-3 fatty acids in non-insulin-dependent diabetes mellitus (NIDDM) patients leads to the lowering of oleic acid content in serum phospholipids

Summary *Background:* The dietary supplementation with EPA (eicosapentaenoic acid; 20:5n3) and DHA (docosahexaenoic acid; 22:6n3) has been recommended because of their favourable effects on the cardiovascular system (including complications of NIDDM). Oleic acid (18:1n9) from olive oil has some analogous and complementary effects. Potential competitive relations between long-chain n-3 fatty acids (FAs) and the oleic acid would therefore mean a problem.

Aim of the study: We focused primarily on the oleic acid changes in serum phospholipids (SPL) after a supplementation with EPA and DHA.

Methods: Thirty-five patients with type 2 diabetes mellitus (NIDDM) were supplemented for 28 days with 1.7 g of EPA plus 1.15 g of DHA/day (as Maxepa® capsules, Seven Seas®, U. K.). After that, a 3-month wash-out control period with 21 patients followed. A fatty acid composition of serum phospholipids (SPL) was determined by capillary gas-chromatography. Values were calculated as relative percentages of all FAs.

Results: After the supplementation with the Maxepa® capsules, there was a very strong increase in EPA, docosapentaenoic acid (22:5n3) and DHA content in SPL. It was followed by a strong decrease after the wash-out (all $p < 0.0001$). The oleic acid SPL content after the intervention significantly decreased from 10.105 ± 0.307 % (mean ± S. E. M.) to 9.082 ± 0.276 % ($p < 0.0003$). During the wash-out, the change was in the opposite direction ($p < 0.0001$). When the intervention and the wash-out periods were taken together, changes in the oleic acid were inversely correlated with changes in EPA, docosapentaenoic acid and DHA ($r = -0.729$; $r = -0.552$; $r = -0.629$, respectively; $p < 0.0001$; $n = 56$). On the background of the overall n-6 FA reduction, the decline in the arachidonic acid after the supplementation ($p < 0.0001$) and its rise after the wash-out ($p < 0.0003$) were similar. There were no significant changes in the saturated FA spectrum.

Conclusions: Supplementation with long-chain n-3 FAs in NIDDM patients leads to the lowering of oleic acid SPL content. Whereas the reduction of the arachidonic acid may have some desirable aspects (e. g. suppression of thromboxane TxA_2 or 4 series leukotriene production), the decline of the former is to be regarded as a potential problem. Therefore, the search for optimally balanced blends of n-3 polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs) seems to be more promising than a supplementation with only *one type* of FA.

Key words: n-3 Fatty acids – fish oil – olive oil – oleic and arachidonic acid

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Introduction

There is growing evidence that n-3 fatty acids (n–3 FAs) and monounsaturated fatty acids (MUFAs) modify an array of key risk factors for cardiovascular disease (1, 2, 3, 4, 5). Many of them emerge in clusters, especially in type 2 diabetes mellitus (6, 7, 8) and, therefore, the effects and mutual interactions of different FAs are especially interesting in this type of patients.

Long-chain n-3 FAs, especially eicosapentaenoic (EPA, 20:5n3) and docosahexaenoic (DHA, 22:6n3) acids

The results of coronary angiography showed that dietary intake of n–3 FAs mitigated the course of coronary atherosclerosis in humans (2). Improvement of survival rates after myocardial infarction and the reduction in sudden death from myocardial infarction has also been well documented (9, 10, 11). A decrease of blood pressure (12), inhibition of pathologically elevated platelet aggregation (13), improvement of blood rheology (14), vasodilatory and anti-inflammatory effects (14) have been observed. DHA is essential for the growth and functional development of the brain in infants and also in adults it improves learning ability (9). Decreases in DHA in the brain were found to be associated with cognitive decline during ageing and with onset of the sporadic Alzheimer disease (9). Diabetic nephropathy and periferal neuropathy were also benefecially affected (15).

The results of animal experiments are even more promising. In isolated neonatal rat cardiac myocytes, n-3 FAs have been shown to prevent tachyarrhythmias caused by elevated calcium concentration or a β-adrenergic agent (3, 16). An increase of sarcolemmal membrane fluidity in rat cardiomyocytes (17) was also measured. In hyperlipidemic rats treatment with DHA improved the recovery of coronary flow and LV (left ventricular) dp/dt (18).

The mechanisms underlying these benefits are still not fully understood (19). It seems, however, that at least three or four of them are already generally accepted:

1. The lipid modification. The triacylglycerol (and VLDL)-lowering and HDL-increasing effect is well known (1, 19, 20, 21). The real problem is that n–3 PUFAs have no effect on the total cholesterol and the LDL-cholesterol concentrations have even been noted to rise (19, 21).
2. The eicosanoid spectrum is affected in two principal directions:

2a. EPA is a precursor for the prostaglandin PGI_3 (and other 3 series prostaglandins) (14, 22, 23). Beneficial effects of PGI_3 on the cardiovascular system are even stronger than those of the PGI_2 (a product of the arachidonic acid) (14). Effects of the further products of EPA, thromboxane TxA_3 and 5 series leukotrienes, are almost indifferent (14) (cf. TxA_2 or 4 series leukotrienes).

2b. Supplementation with n-3 FAs leads to the lowering of n-6 FAs, including arachidonic acid (14, 19, 23, 24). After these diets, TxA_2 failed to stimulate proliferation of smooth muscle cells in the vessel wall and its production was also inhibited (25).

3. Beneficial effects on some hemostatic factors. In our earlier work (26), we found a decrease of pathologically elevated vWF (von Willebrand factor) blood plasma levels and an inhibition of pathologically elevated platelet aggregation was also documented (13). On the other hand, it seems that n-3 FAs have no significant effect, e. g., on factor VII (27).
4. Effects on the electrical excitability of the cardiomyocytal membranes, on the transmembrane ionic currents and membrane fluidity which may explain the antiarrhythmic action (3, 16, 17).

Monounsaturated FAs (MUFAs, including oleic acid, typically from olive oil)

They are no match for the n-3 FAs only as far as the *direct* eicosanoid production is concerned. This does not rule out, however, some mediated action (e. g. due to the "competition" among FAs) (24, 28). On the other hand, a majority of beneficial effects, noted above in context with n–3 FAs, may also be attributed to MUFAs. The oleic acid (18:1n9) also decreases the triacylglycerols (29, 30) and it even surpasses n-3 FAs by also lowering the total- and LDL-cholesterol (5, 29, 31). Moreover, LDL enriched with oleic acid and reduced in polyunsaturated FAs may be less easily converted to a proinflammatory, minimally modified LDL (5). An inverse correlation between the oleic acid content of LDL and stimulation of monocyte chemotaxis was also found (5, 31). Incorporation of oleic acid into cultured endothelial cells stimulated by cytokines, decreased the expression of several endothelial leukocyte adhesion molecules (5). Postprandial factor VII activation and the concentration of the factor VII antigen were significantly lower after the MUFA diet (29, 32), this being another important protective effect missed by n-3 FAs (27). MUFA-enriched hypocaloric diets potentiate the beneficial effects of weight loss to ameliorate cardiovascular risk factors in obese patients with type 2 diabetes (6).

Taking into account all these effects of MUFAs, potential competitive relations between long-chain n-3 fatty acids and the oleic acid would mean a potential problem. Indeed, oleic acid content in rat erythrocyte membranes after a fish oil diet was found to be lower in comparison with the sunflower oil diet (33) and different types of interactions among FAs were also found in humans (19, 24, 28). That is why we have focused primarily on the oleic acid changes in serum phospholipids (SPL) after a supplementation with EPA and DHA.

Methods

The investigated group included 35 out-patients with type 2 diabetes mellitus (13 men and 22 postmenopausal women). The mean age was 63.63 years (95 % CI 60.77, 66.49), the mean duration of diagnosed diabetes was 13.37 years (95 % confidence interval (CI) 10.57, 16.17).

These 35 patients were treated for 28 days with 1.7 g EPA (eicosapentaenoic acid) plus 1.15 g DHA (docosahexaenoic acid)/day (10 capsules/day of MAXEPA®,

Table 1 Fatty acid (FA) content in serum phospholipids (SPL, % of all FA) after Maxepa® (ME) and wash-out (WO)

FA [%]	Before ME Mean ± SEM	After ME Mean ± SEM	p =	n =	Before WO Mean ± SEM	After WO Mean ± SEM	p =	n =
Oleic 18:1n9	10.105 ± 0.307	9.082 ± 0.276	0.0003	35	8.908 ± 0.370	11.190 ± 0.496	< 0.0001	21
ΣMUFA	12.535 ± 0.356	11.752 ± 0.329	0.0058	35	11.609 ± 0.424	13.681 ± 0.579	0.0004	21
Arach. 20:4n6	11.452 ± 0.414	9.857 ± 0.278	< 0.0001	35	9.862 ± 0.257	11.193 ± 0.346	0.0003	21
Σ n-6	37.303 ± 0.709	32.978 ± 0.781	< 0.0001	35	31.898 ± 0.787	40.531 ± 1.521	< 0.0001	21
Palmitic 16:0	27.627 ± 0.680	26.723 ± 0.451	NS (0.27)	35	27.084 ± 0.592	26.965 ± 0.461	NS (0.83)	21
Σ SFA	44.944 ± 0.968	43.267 ± 0.426	NS (0.11)	35	43.626 ± 0.649	43.981 ± 0.539	NS (0.57)	21
α-linol. 18:3n3	0.188 ± 0.011	0.159 ± 0.009	0.024	35	0.151 ± 0.011	0.194 ± 0.013	0.0059	21
EPA 20:5n3	0.907 ± 0.082	4.648 ± 0.223	< 0.0001	35	4.920 ± 0.288	0.922 ± 0.085	< 0.0001	21
Docosap 22:5n3	0.866 ± 0.043	1.345 ± 0.055	< 0.0001	35	1.411 ± 0.067	0.858 ± 0.037	< 0.0001	21
DHA 22:6n3	3.311 ± 0.174	6.367 ± 0.225	< 0.0001	35	6.702 ± 0.273	3.344 ± 0.225	< 0.0001	21

Arach. arachidonic acid (AA) MUFA monounsaturated fatty acids
α-linol α-linolenic acid (ALA) SFA saturated fatty acids
docosap docosapentaenoic acid

Seven Seas® U. K.). After that, a 3-month wash-out control period with 21 patients followed. Interruption of the follow-up in the remaining 14 patients was practically random (e. g. travelling abroad or other personal problems). During this entire follow-up, there were no other changes in patients' standard diabetic diet, treatment or habits which might interfere with final results.

Plasma fatty acid composition is known as a reliable indicator of habitual dietary fat intake (34) and, therefore, Table 1 also gives information about the intake of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) by the baseline diet.

Informed consent was obtained from all participants and the study was conducted in agreement with local ethical standards and the Helsinki Declaration of 1975, as revised in 1989.

The first step of serum phospholipid fatty acid (FA) determination was the extraction of serum lipids with chloroform-methanol (2:1, vol/vol) containing butylated hydroxytoluene (BHT) and lipid classes were separated by thin-layer chromatography (34). The phospholipid band was scraped and converted to methyl esters of fatty acids (FA) (34). The FA composition was determined by capillary gas-chromatography using a model CP 9001 gas chromatograph (Chrompack International, Middelburg, The Netherlands) equipped with a Omegawax 250 fused silica capillary column, 30 m × 0.25 mm ID, 0.25 µm film (Supelco) and a flame-ionisation detector. The identity of individual FA peaks was ascertained by comparing each peak's retention time relative to the retention times of FAs in synthetic standards of known FA compositions (Matreya, Inc.). Values were calculated as *relative percentages of all FA* determined using a MOSAIC analytical system.

Statistical analysis included mean values and the standard errors of means (SEM) and it was carried out using paired Student's test and linear regression analysis (simple linear correlation). Changes in parameters are also given as their means with 95 % confidence intervals (CI). No further distinction was made for the significance levels better than $p < 0.0001$.

Results

The fatty acid composition of serum phospholipids (SPL) before and after the supplementation with EPA plus DHA (Maxepa®) is presented in Table 1. Elevation of EPA and DHA was extremely significant ($p < 0.0001$). The SPL content of the docosapentaenoic acid (22:5n3, a direct product of EPA) also increased sharply ($p < 0.0001$). An extremely significant decline in all of these ($p < 0.0001$) was observed during the wash-out (Table 1).

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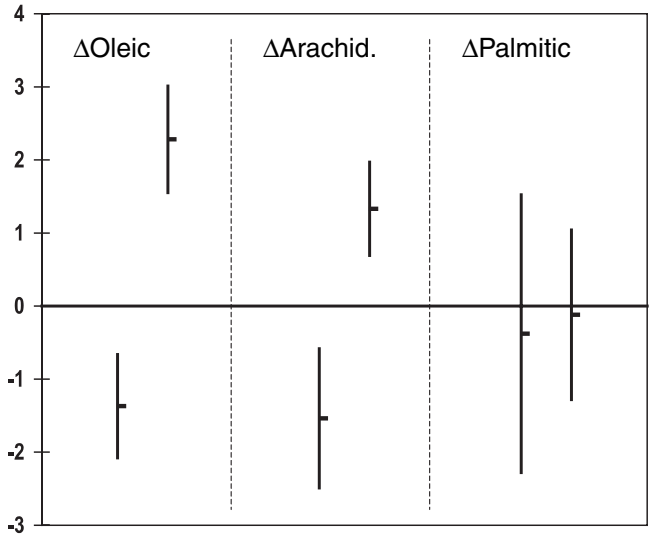

Fig. 1 Changes (Δ, % of serum phospholipid content) of selected FAs. 21 patients who were followed-up also during the wash-out. Mean changes (central dash) with their 95 % CI (vertical segments). In each pair: first segment … mean change after the treatment with Maxepa® (ME); second segment … mean change during the wash-out (WO). Significance level of difference between mean changes during ME vs. WO was $p < 0.0001$ for Δoleic and Δarachidonic but not significant ($p = 0.81$) for Δpalmitic acid

The α-linolenic acid (18:3n3), the first FA in the n-3 PUFA cascade, behaved inversely, although the significance levels ($p < 0.024$ and $p < 0.0059$, respectively) were relatively lower (Table 1).

The *oleic* acid SPL content after the intervention with Maxepa® significantly *decreased* from 10.105 ± 0.307 % (relative percentage of all FA; mean ± S. E. M.) to 9.082 ± 0.276 % ($p < 0.0003$; Table 1). During the wash-out, the change was in the opposite direction ($p < 0.0001$; Table 1; Fig. 1). The significance level of the difference between mean changes in 21 patients during these two periods was also extremely high ($p < 0.0001$; Fig.1). This 'mirror-like' behaviour of the oleic acid after the supplementation with EPA plus DHA vs. wash-out is also illustrated in Fig. 1. When the changes during the entire follow-up (i. e. the intervention plus wash-out; 35 + 21 samples) were taken together, a significant inverse correlation between changes in EPA (ΔEPA) and the oleic acid (ΔOL) emerged ($r = -0.729$; $p < 0.0001$; Table 2). The changes in the docosapentaenoic acid (Δ22:5n3) and docosahexaenoic acid (ΔDHA, 22:6n3) resulted in analogous correlations with ΔOL ($r = -0.552$, $r = -0.629$; $p < 0.0001$, $p < 0.0001$, respectively; Table 2).

On the background of the overall n-6 FA reduction, the decline in the arachidonic acid (AA) after the supplementation ($p < 0.0001$; Table 1) and its rise after the wash-out ($p < 0.0003$; Table 1, Fig. 1) were similar. There was even a significant positive correlation between its changes (ΔAA) and ΔOL ($r = 0.608$; $p < 0.0001$; Table 2).

The palmitic acid (16:0) and the sum of all saturated fatty acids (Σ SFA) were not affected either by Maxepa®, or by the wash-out (Table 1; Fig. 1). Figure 1 shows the mean 'changes' of the palmitic acid with their 95 % CI as being almost evenly distributed in respect of the zero line.

Discussion

This study showed that the supplementation with EPA and DHA resulted in a significant reduction in the oleic acid SPL content (Table 1; Fig. 1). The causality of this relation is supported by an almost 'mirror-like' behaviour of the oleic acid between the two observation periods (i. e. intervention with Maxepa® vs. wash-out; Fig. 1) and by the inverse correlations between all three long-chain n-3 FA changes and ΔOL (Table 2). The extremely high significance levels of these relations ($p < 0.0003$ or $p < 0.0001$) also reflect this fact. Our findings are in agreement with results from animal experiments (33) and also with other human studies dealing with FA interactions in general (19, 24, 28).

On the background of the *overall* FA spectrum this reduction was 'only' approximately 1 %, but in respect of the oleic acid *itself* this decline meant about 10 % and, in view of its specific effects mentioned in the 'Introduction', it would be better not to underestimate the possible impact.

The changes in the arachidonic acid (ΔAA), a n-6 PUFA, were similar to those of the oleic acid (ΔOL; Table 1, Fig. 1). There was even a positive correlation between ΔAA and ΔOL (Table 2), which might be explained by a common cause, i. e. changes in n-3 FAs.

The clinical impacts of the decline in arachidonic acid and oleic acid are, however, completely different. The former is a precursor not only for the 2 series prostaglandins (which are predominantly cardioprotective) but also for the thromboxane TxA_2 and for the strongly proinflammatory 4

Table 2 Correlations between changes in selected pairs of fatty acids. ME and WO periods were taken together (n = 56)

Δ FA(x)	Δ FA(y)	r =	p =	Regr.eq. y =
Δ EPA	Δ Oleic	– 0.729	< 0.0001	0.558–0.406 x
Δ Docosapent.	Δ Oleic	– 0.552	< 0.0001	0.403–2.028 x
Δ DHA	Δ Oleic	– 0.629	< 0.0001	0.481–0.406 x
Δ Arachidonic	Δ Oleic	0.608	< 0.0001	0.516 + 0.601 x
Δ EPA	Δ Arachidonic	– 0.563	< 0.0001	– 0.231–0.318 x

Docosapent docosapentaenoic acid (22:5n3)

series leukotrienes (14, 19, 23, 25). Under circumstances, the reduction in AA may, thus, be regarded as even clinically beneficial. The concise comparison of n-3 FA vs. oleic acid benefits and drawbacks as presented in the introduction to this article shows, however, that the reduction of oleic acid is not desirable.

There was a certain decline also in the α-linolenic acid (18:3n3) after the intervention with EPA and DHA (Table 1) although its statistical significance was distinctly lower in comparison with OL or AA. On the background of the dramatic increase in other n-3 FAs (Table 1), we would not expect this effect to be of great importance clinically.

We found no significant changes either in the palmitic acid (16:0) or in the sum of saturated fatty acids (Σ SFA; Table 1, Fig. 1) during the two periods. It rules out the possibility that the mechanism of n-3 FA effect on other fatty acids might be only of 'arithmetic' nature (i. e. that an increase in one type of FAs must result in reduction of all others). Earlier results from animal experiments (35) showed that Δ^9 desaturase activity was inhibited in rats fed with n-3 or n-6 PUFAs. This interference with the oleic acid related metabolism was accepted as a possible explanation for oleic acid decrease in rat erythrocyte membranes after a fish-oil diet (33).

In conclusion, this study showed a significant reduction of SPL oleic acid content after a supplementation with EPA and DHA in humans. It should be seen in context with the fact that, regardless of an array of outstanding benificial effects attributable to the long-chain n-3 PUFAs, they still lack several effects manageable by MUFAs. That is why the search for optimally balanced blends of n-3 PUFAs and MUFAs seems to be even more promising than a supplementation with only *one type* of FA. First recommendations concerning best relations between dietary long-chain n-3 FAs and n-6 FAs have been available since the early 1990s (10, 14); the recommended ratio was 1:5 or 1:10. The presented study has been intended also as a 'challenge', calling attention to the necessity of searching for analogous optimal ratio between n-3 FAs and MUFAs.

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