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Direktor: Prof. Dr. med. Dietrich Reinhardt

Evaluation of two supplementation strategies to improve long-chain omega-3 fatty acid status in healthy subjects

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Julia Geppert

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Berichterstatter:	Prof. Dr. B. Koletzko
Mitberichterstatter:	Prof. Dr. C. Keller Prof. Dr. C. von Schacky auf Schönefeld
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Dekan:	Prof. Dr. med. D. Reinhardt
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Abbreviations

A	absorbance
$A^{1\%}_{1cm}$	extinction coefficient
AA	arachidonic acid (20:4n-6)
AdrA	adrenic acid (22:4n-6)
ALA	alpha-linolenic acid (18:3n-3)
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BHT	butylated hydroxytoluene
BMI	body mass index
BP	blood pressure
C	carbon atom
CE	cholesterol ester
CETP	cholesterol ester transfer protein
CHD	coronary heart disease
CHE	cholinesterase
CK	creatinekinase
CRP	C-reactive protein
CV	coefficient of variation
DHA	docosahexaenoic acid (22:6n-3)
DGLA	dihomo-gamma-linolenic acid (20:3n-6)
DPA	docosapentaenoic acid (22:5n-6 or 22:5n-3)
EDTA	ethylene diamine tetraacetic acid
EFA	essential fatty acids
ELISA	enzyme-linked immunosorbent assay
EPA	eicosapentaenoic acid (20:5n-3)
EPO	evening primrose oil
FA	fatty acid
FAME	fatty acid methyl esters
FO	fish oil
GC	gas chromatography

GGT	gamma-glutamyl transpeptidase
GLA	gamma-linolenic acid (18:3n-6)
GLM	general linear model
HCl	hydrochloric acid
HDL	high density lipoprotein
HPLC	high performance liquid chromatography
IQR	interquartile range (25^{th} – 75^{th} percentiles)
LA	linoleic acid (18:2n-6)
LCPUFA	long-chain polyunsaturated fatty acid
LDH	lactate dehydrogenase
LDL	low density lipoprotein
MA	Mead acid (20:3n-9)
MCH	mean cellular haemoglobin
MCHC	mean cellular haemoglobin concentration
MCV	mean cellular volume
MI	myocardial infarction
MUFA	monounsaturated fatty acids
NaCl	sodium chloride
n.d.	not detected
NIST	National Institute of Standards and Technology
NN	non-normally distributed
n.s.	not specified
ns	non-significant
OA	oleic acid (18:1n-9)
PA	palmitic acid (16:0)
PAI-1	plasminogen activator inhibitor-1 activity
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PFA-ADP	platelet function analysis with adenosine diphosphate
PFA-EPI	platelet function analysis with epinephrine
PL	phospholipids
PROCAM	Prospective Cardiovascular Münster study
PTT	partial thromboplastin time
PUFA	polyunsaturated fatty acids

RBC	red blood cells
SCD	sudden cardiac death
SFA	saturated fatty acids
SD	standard deviation
SEM	standard error of the mean
TG	triglycerides
TL	total lipids
TLC	thin layer chromatography
UV	ultraviolet
VLDL	very low density lipoprotein
vs.	versus
vWF	von Willebrand factor

1 Introduction

Endogenous synthesis of long-chain polyunsaturated fatty acids. The two essential fatty acids (EFA) linoleic acid (LA, Figure 1.1) and alpha-linolenic acid (ALA) and their further desaturated, longer-chain derivatives, the long-chain polyunsaturated fatty acids (LCPUFA), are indispensable for human development and optimal health.

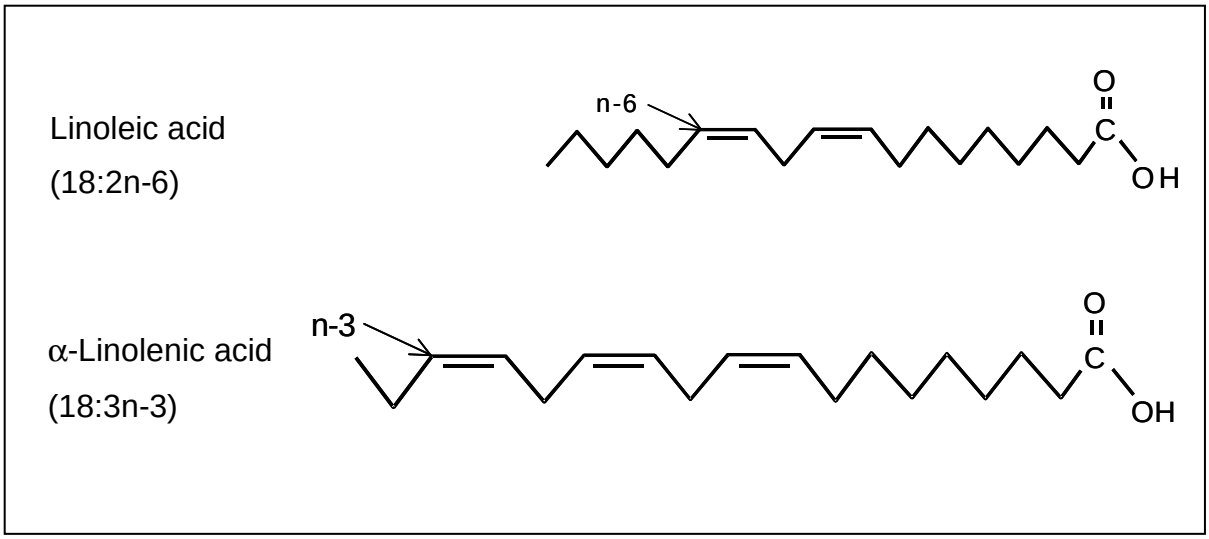

Figure 1.1. Chemical structure of linoleic acid and alpha-linolenic acid.

Arachidonic acid (AA), dihomo-gamma-linolenic acid (DGLA), and eicosapentaenoic acid (EPA) all serve as precursors for eicosanoids, while docosahexaenoic acid (DHA) is an important structural component of the gray matter of the brain and the retina of the eye. Although they can be synthesised in the body from LA or ALA through a series of elongation and desaturation (and additionally β-oxidation for DHA synthesis), this synthesis is restricted by the activity of the rate-limiting enzyme Δ6-desaturase (Figure 1.2). Conversion of ALA to EPA and DHA is not very efficient and depends on the level of ALA and its ratio to LA in the diet, because n-6 and n-3 fatty acids compete for the same enzymes responsible for their conversion [1]. Previous studies with stable isotopically labelled ALA have shown conversion of ALA to EPA

varying from 6-21% [2-4] to much lower values (0.1-0.2%; [5-7]). Reports on the conversion of ALA to DHA range from 4-9% [2,4] to 0.04% [8] or undetectable DHA synthesis [3]. Thus, endogenous formation of EPA and especially DHA is thought insufficient under certain circumstances [9], and additional dietary intake of preformed n-3 LCPUFA is needed to cover metabolic requirements.

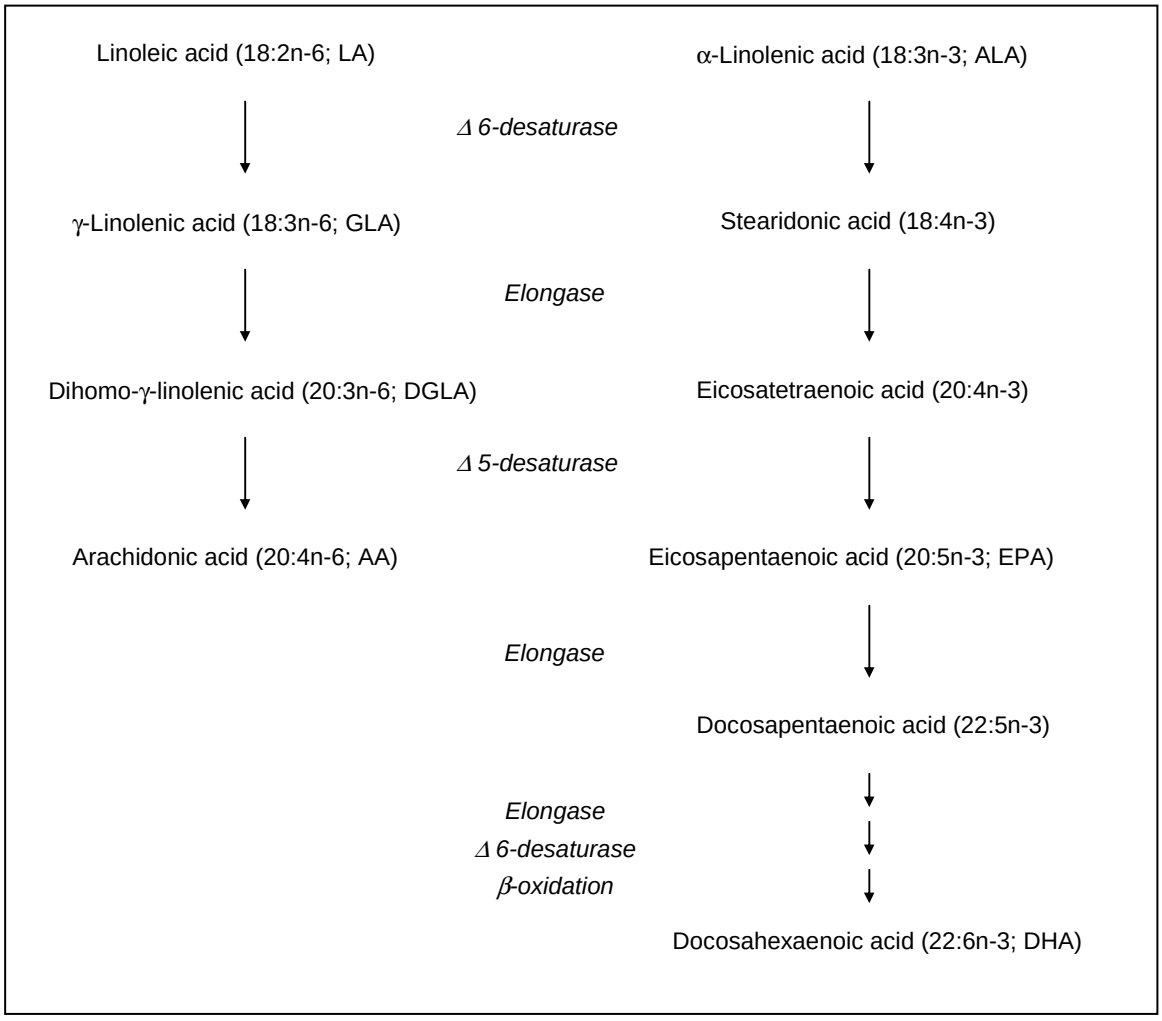

Figure 1.2. Synthesis of polyunsaturated fatty acids (modified from [10]).

Dietary intake and sources of n-3 LCPUFA in the Western diet. The vast majority of dietary EPA and DHA in a typical Western diet is consumed in the form of fish and seafood (68%) with much smaller contributions coming from eggs (12%), poultry (7%), meat and sausages (7%) [11]. Plant foods and vegetable oils are devoid of EPA and DHA, but contain varying amounts of ALA. Major sources of ALA are vegetable oils and selected nuts and seeds (e.g. soybean and canola oil, flaxseed,

English walnuts) [12,13]. The typical Western diet provides ~1-2 g ALA per day but only 0.1-0.2 g/d EPA and DHA combined [12,14,15]. For German adults, a recent study reported the median dietary intake of EPA and DHA combined to be 141 mg/d among women and 186 mg/d among men [11]. The lowest median intake was observed among women aged 18-24 years (84 mg/d, Figure 1.3).

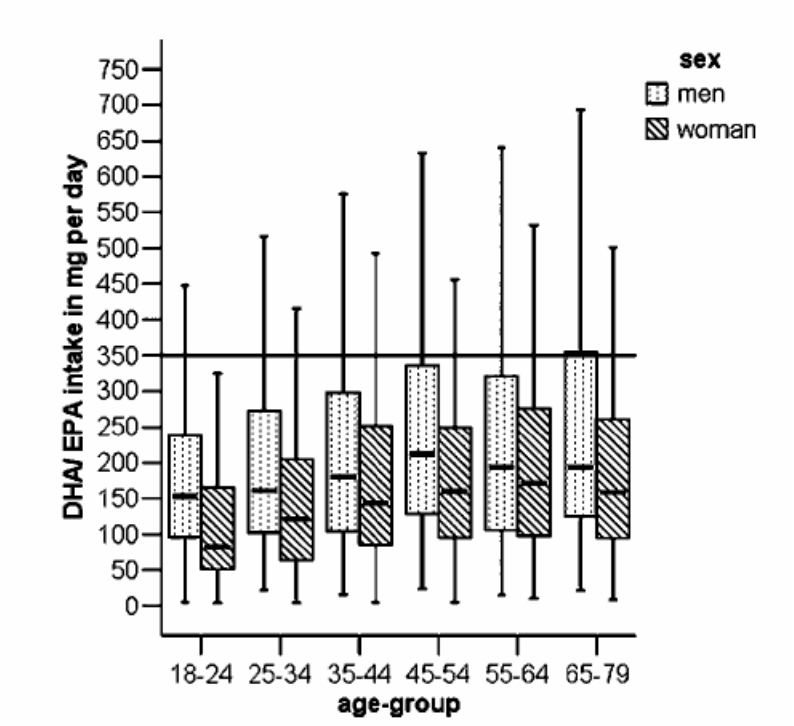

Figure 1.3. Distribution of EPA + DHA intake in German adults stratified by sex and age (bottom and top edges of the box are located at 25th and 75th percentiles, centre horizontal line is drawn at the median and whiskers mark the data points that are nearest to the 1.5 interquartile ranges, outliers are not shown). The average daily DHA and EPA intake corresponding to two servings of fatty fish per week (150-300 g fish, containing 1 g EPA/DHA per 100 g) is marked by a horizontal line.

* Figure from Bauch *et al.* [11].

The high intake of n-6 fatty acid LA (5-7% of daily energy or 11-16 g/day) yields a mean overall n-6:n-3 dietary ratio of about 9.8:1 in the United States [12] and 7.2:1 - 8.6:1 in Germany [16]. The German Society of Nutrition has recommended [17] that this ratio should be lowered < 5:1 to reduce the competitive influence of high LA intakes on ALA conversion to EPA and DHA. Attaining the proposed recommended combined EPA and DHA intake ≥ 650 mg/d for healthy adults [18] will require an approximately 4-fold increase in fish consumption in the United States and other Western countries. Such a change in dietary habits is not realistic for all persons (e.g.

vegetarians, fish allergic persons) or might not be adequate for persons with increased n-3 LCPUFA requirements (patients with coronary heart diseases, pregnant women). Additionally, individual gustatory preferences may also limit additional fish consumption. Furthermore, an increase in fish consumption is ecologically (overfishing) and regarding heavy metal contamination problematic. Alternative strategies, such as n-3 fatty acid supplements, food enrichment and use of biotechnology will become increasingly important in raising n-3 fatty acid intake in the Western diet over the long term.

N-3 LCPUFA intake and status of vegetarians. Total n-3 fatty acid intakes are similar for vegans, vegetarians, and omnivores (< 1-3 g/d, with the current average being ~1.1-1.6 g/d) [19]. However, the intakes of n-3 LCPUFA vary appreciably: ovolacto vegetarians consume minimal amounts of EPA and varying amounts of DHA from eggs, milk and dairy products (average intakes < 33 mg/d) [20]. Vegans consume negligible amounts of n-3 LCPUFA and rely entirely on in vivo biosynthesis of n-3 LCPUFA from the precursor ALA. N-6 fatty acid intakes are significantly higher in vegan and vegetarian populations than in omnivorous populations, ranging from 5-7% of energy in omnivores to about 10-12% of energy in vegans. As a result, the n-6:n-3 ratio is generally considered to be elevated in vegans (14:1 – 20:1) and ovolacto vegetarians (10:1 – 16:1) compared with omnivores (< 10:1) [19].

Lack of EPA and DHA in vegetarian and especially vegan diets is reflected in reduced amounts of these fatty acids in platelets, red blood cells (RBC) and plasma [21-26].

N-3 LCPUFA and coronary heart diseases. The risk for many chronic diseases, including coronary heart diseases (CHD), is influenced by dietary fatty acid intake [27-29]. A higher degree of incorporation of n-3 LCPUFA into myocardial membranes reduces deaths following myocardial ischemia [30]. In a nested case-control study (94 men with incident CHD and 94 men without incident CHD), levels of DHA in plasma phospholipids were inversely correlated with CHD risk in a multivariate model that controlled for the effects of HDL:LDL cholesterol ratio (standardized odds ratio = 0.57; 95% confidence interval 0.36-0.90) [31]. Harris *et al.* [32] showed that n-3 LCPUFA content in RBC membranes reflects that of cardiac membranes. The percentage content of EPA + DHA in RBC membranes (hereafter called omega-3 index) has recently been identified as a risk indicator for death from CHD [33]. The relationship between the omega-3 index and the risk for CHD death, especially

sudden cardiac death (SCD), was evaluated using the data from several published primary and secondary prevention studies. An omega-3 index ≥ 8 wt% was associated with the greatest cardioprotection, whereas an index ≤ 4 wt% was associated with the least. Harris & von Schacky [33] observed significant increases of the omega-3 index after EPA and DHA supplementation (1 g/d) for 5 months, ranging after intervention from 5 wt% to 13 wt%. These different levels in people consuming the same amount of EPA + DHA for several months might be caused by individual differences in baseline values of the omega-3 index or differences in digestion, absorption, transport, uptake in target tissues, metabolism from storage sites and in vivo conversion of ALA to LCPUFA derivatives. Consequently, a subject with low baseline omega-3 index may require a larger dose than a person with a higher baseline value. Further studies investigating the dose-response relationship between EPA + DHA intake and the omega-3 index in subjects with different background diets are needed. For clinical studies it is in addition of interest to know whether RBC total lipid fatty acids (especially omega-3 index) correspond to values found in other lipid classes (plasma phospholipids, RBC phosphatidylcholine, RBC phosphatidyl-ethanolamine) in subjects with a stable background diet.

Circulating triacylglycerol (TG) levels in the fasting and postprandial states are associated with the severity and progression of atherosclerosis [34] and are recognised as independent risk factors for CHD [35]. According to Assmann [36], the need to include factors other than LDL cholesterol in risk assessment of myocardial infarction (MI) is indicated by multivariate analysis of data from the Prospective Cardiovascular Münster (PROCAM) study in which HDL cholesterol, LDL cholesterol, TG level, age, systolic blood pressure, family history of MI, cigarette smoking, and diabetes were each identified as independent risk variables for MI. From Assmann's point of view, the impact of curvilinear relation between LDL cholesterol level and risk of MI, as shown in Figure 1.4 and demonstrated in other epidemiological studies [37-41], is frequently underestimated and it goes unrecognised that curves of different steepness are generated depending on global risk of the individuals. A change in one of the 7 identified risk factors beside LDL cholesterol (e.g. TG decrease) could result in the transfer of an individual to a lower-risk group without necessarily modifying LDL cholesterol level (Figure 1.4 and Figure 1.5).

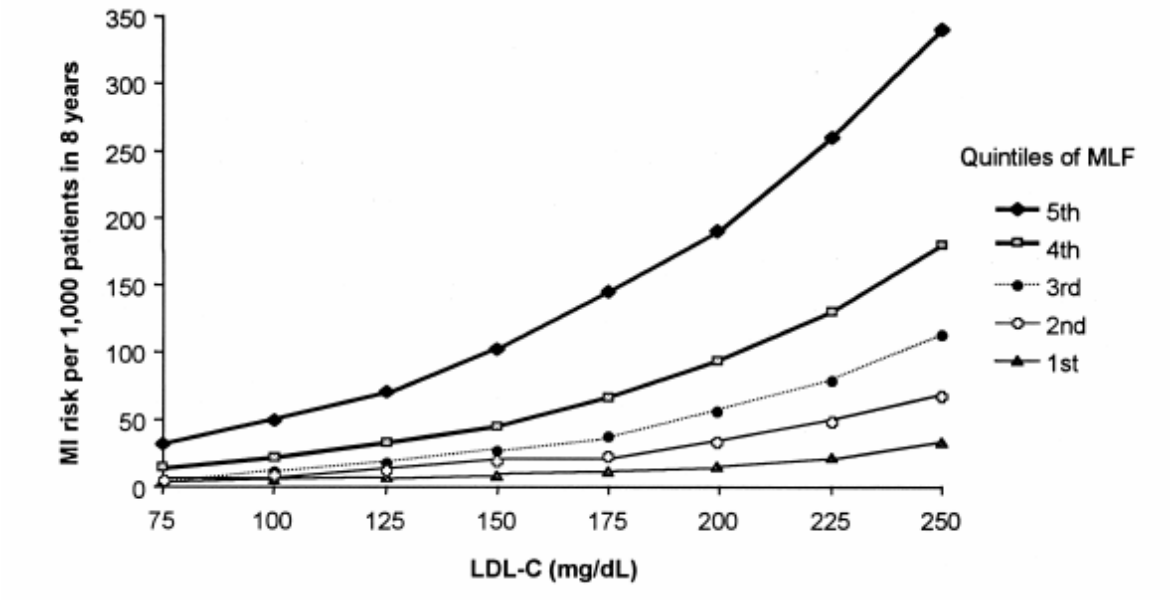

Figure 1.4. Myocardial infarction (MI) risk within multiple logistic function (MLF) quintiles according to LDL cholesterol (LDL-C) level in the Prospective Cardiovascular Münster study population.*

* Figure from Assmann [36].

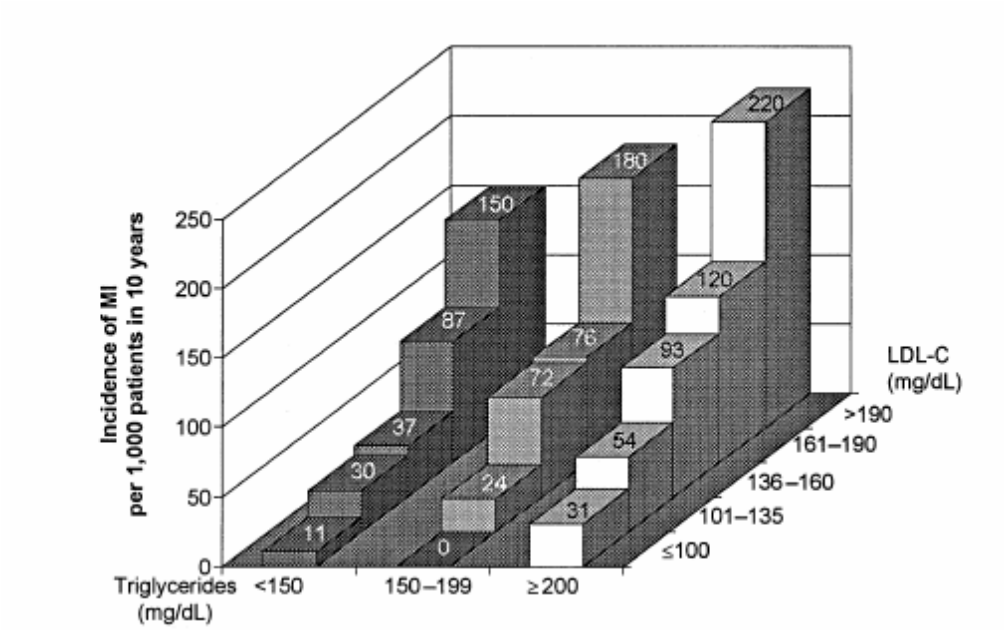

Figure 1.5. Incidence of myocardial infarction (MI) in 10 years according to baseline triglyceride level and LDL cholesterol (LDL-C) level in the Prospective Cardiovascular Münster study.*

* Figure from Assmann [36].

A meta-analysis by Austin *et al.* [42] suggested that after adjustment for HDL cholesterol and other risk factors, each 1 mM increase in TG is associated with a 14% increase in CHD in men and a 37% increase in women. According to Griffin [43] there is convincing evidence to show that even moderately raised plasma TG (> 1.5 mM), which has a predicted frequency of between 25-30% in middle-aged men and postmenopausal women, confer increased cardiovascular risk in otherwise normal, healthy individuals.

N-3 LCPUFA have TG-lowering effects in humans [44-49]. In a meta-analysis of 65 studies, Harris [50] concluded that an average dose of 4 g EPA and DHA per day results in a 25-30% decrease of fasting TG in both normolipidaemic and hypertriacylglycerolaemic subjects. It has been assumed that the hypotriglyceridaemic effect of n-3 LCPUFA is mediated by several mechanisms such as increased hepatic fatty acid oxidation, inhibition of fatty acid and TG synthesis and reduced assembly and secretion of VLDL triacylglycerol [51]. Most studies of n-3 LCPUFA have generally used oils containing mixtures of EPA and DHA in the range of 1-5 g/d, but studies investigating specifically the effects of individual n-3 LCPUFA on TG and lipoproteins in humans are rare. Few studies have compared the effects of purified EPA and DHA (as ethyl esters) showing inconsistent results: in a study by Rambjor *et al.* [52] supplementing normolipidaemic subjects with 3 g EPA or DHA/d for 3 weeks, EPA, but not DHA had a TG-lowering effect. Other studies show that both EPA and DHA lower serum TG concentrations at intakes of 3.0-4.9 g/d [47,48,53,54], whereas HDL cholesterol concentrations tend to increase only with DHA supplementation [47,52,53].

Studies applying single-cell oil as DHA source, which contain only trace amounts of EPA and other n-3 fatty acids, reported a significant reduction in TG concentrations and an increase in HDL cholesterol levels at DHA intakes in the range of 1.25-2.5 g/d in normo- or hyperlipidaemic volunteers [44,46,49,55], whereas other studies with DHA intakes of 0.7 g/d [56] and 0.75-1.5 g/d [57] did not find any significant effects on plasma TG and lipoproteins in normolipidaemic subjects. Future trials will be needed to determine minimum effective dosages of EPA and DHA over lengthy periods and to show reduction of CHD by intervention.

N-3 LCPUFA and infantile development. DHA and AA are found in high concentrations in structural lipids of the central nervous system and have been shown to be important for brain development and function [58-60]. Adequate accretion of both DHA and AA in brain and retina is particularly important during the rapid brain growth, which takes place in the perinatal period [60]. However, since pregnancy is associated with a decrease in the biochemical and functional LCPUFA status of the mother, the foetal and neonatal LCPUFA status may not be optimal [9].

Numerous studies have been conducted over recent years to evaluate the functional effects of dietary DHA supplementation on visual and cognitive development of preterm and term infants. It is now generally accepted that postnatal DHA administration promotes, at least temporarily, cognitive and visual development of infants born preterm [61-66]. Developmental benefit of postnatal DHA supplementation has been observed in term infants also, but the results are less consistent [67-69]: Some studies have shown improved development of visual acuity and cognitive functions in term infants [70-76], whereas others have shown no significant effects [77-80].

Two studies were performed in children with hyperphenylalaninaemia or phenylketonuria (mean subject age: 6 years and 11 years respectively [81,82]), whose habitual diets contain nearly no n-3 LCPUFA. These investigators observed significant improvements in visual evoked potential latencies after supplementation with LCPUFA / fish oil, suggesting that n-3 LCPUFA are essential substrates for the nervous system function even beyond infancy.

Dietary supplementation with DHA not only improves the DHA status in the recipient, but at the same time it reduces the availability of AA [83]. AA is the second-most abundant LCPUFA in the brain, and AA-derived eicosanoids are important functional mediators [84]. AA is involved in cell signalling [85] and is one of the major fatty acid moieties of anandamides, which serve important functions as ligands of the cannabinoid receptor in the brain [86]. Information on the importance of AA for development and function of the central nervous system is less clear than for DHA. Only limited evidence points to a positive effect of this fatty acid on brain development and function, when given in combination with DHA to term infants [71,87]. But cerebral AA may be required for the prevention of neuromental disorders like schizophrenia [87]. Whether or not the AA reduction associated with DHA supplementation diminishes DHA-induced functional improvements is not known.

Because of this lack of knowledge, any supplementation of mothers or infants intended to improve their DHA availability should not compromise maternal and neonatal AA status.

A potentially simple way to achieve this may be the administration of gamma-linolenic acid (GLA). This fatty acid is the Δ6-desaturated derivative of LA (see Figure 1.2) and has been suggested to be more readily converted to AA than LA [88]. Since the EFA/LCPUFA status of neonates is positively correlated with that of their mothers, a higher maternal GLA status can be expected to increase the neonatal GLA status. This may be of importance for the prevention of obesity, insulin resistance and hypertriglyceridaemia in later life, since at seven years of age these have been shown to be inversely related to GLA status at birth [89,90]. In addition, a significant, positive relationship has been observed between the DGLA status of the neonate and its birth weight [91]. According to the Barker hypothesis [92,93], a higher birth weight may be associated with a lower risk for certain chronic diseases in later life. In addition, GLA may serve essential functions in the transcription of genes involved in glucose and lipid metabolism [94,95] and is thought to play a role in atopy [96,97]. Dietary intake of GLA is very low and endogenously synthesised GLA may be expected to be converted to DGLA and possibly AA. Therefore, supplementation with GLA to pregnant women may increase the GLA and DGLA status of mothers and their neonates and, thereby, promote infantile development and support disease prevention.

Most formulas for preterm infants and some term formulas already contain a combination of DHA and AA and some formulas are enriched with GLA as well. However, infants should preferably be fed with breast milk and, consequently, the EFA and LCPUFA content of mother's milk should also be adequate for optimal postnatal development. Because the LCPUFA content of breast milk can be very low, it may be desirable to "fortify" mother's milk with LCPUFA. This can be achieved i.e. by supplementing lactating mothers with these fatty acids or their precursors. Cant *et al.* [98] were the first to show that GLA supplementation results in GLA enrichment of breast milk lipids. This was confirmed by Thijs and co-workers, who demonstrated that supplementation of lactating atopic mothers with 230 or 460 mg per day of GLA from borage oil caused a dose dependent increase in milk lipid concentrations of GLA and DGLA [99]. AA concentrations, however, were not significantly affected. ALA supplementation increases ALA, EPA and DPA, but not DHA concentration in

breast milk lipids [100]. Supplementation with DHA-rich single cell oil in doses up to 1.3 g DHA per day for 3 months increased the breast milk DHA concentration more than 5-fold (from 0.21 to 1.13 % of total fatty acids), whereas the AA content decreased slightly, but significantly [101]. Infant DHA levels in plasma and RBC phospholipids after 12 weeks of lactation were significantly and positively related to the DHA dose their mothers were supplemented with, and reached values up to 9.1 and 9.8% for plasma and erythrocyte phospholipids respectively [102]. Infant plasma and erythrocyte AA contents reduced significantly in the supplemented groups and in proportion to the maternal DHA supplementation doses. Therefore, there is need to design a fatty acid supplement for pregnant women and lactating mothers which enhances GLA, DGLA, and DHA status in the neonates and later on in the breastfed infants while not compromising their AA status.

Mothers may also functionally benefit from postnatal LCPUFA supplementation themselves, since their postnatal DHA status is lower than that of non-lactating mothers [103] and they may need to recover from possible cognitive impairments during pregnancy [104,105]. In addition, a low maternal DHA status has been suggested to increase the risk of post partum depression [106,107]. This hypothesis is supported by recent findings that a higher DHA status at delivery [107] and a better improvement of the maternal DHA status after delivery [108] are associated with less depressive symptoms in the post partum period. In a longitudinal study it appeared that post partum depression is often preceded by depression during the 32nd week of pregnancy already [109], which may also be DHA-related. Therefore, a higher DHA status during early pregnancy may be beneficial in the prevention of depressive symptoms during pregnancy and after delivery.

Objectives of this work. This work is intended to evaluate two supplementation strategies to improve n-3 LCPUFA status in healthy adults. Two randomised, double blind, placebo-controlled intervention studies with parallel design were performed. In the first study (*DHAVEG study*), healthy adult vegetarians with low basal n-3 LCPUFA status consumed a vegetarian, DHA-rich oil (delivering 1.05 g DHA and < 7 mg of each GLA, AA, and EPA as triglycerides per day) or a placebo for 8 weeks.

In the second study (*FO/EPO study*), healthy non-pregnant women were supplemented with a fish oil/evening primrose oil mixture (FO/EPO, providing not only 419 mg DHA and 72 mg EPA but also 14 mg AA and 337 mg GLA as triglycerides per day) or a placebo.

The objectives of this work are as follows:

- Evaluation of a method for the analysis of fatty acids in RBC total lipids.
- Validation of RBC total fatty acid analyses as a marker of essential fatty acid status.
- Evaluation of a method for the analysis of vitamin A and E in plasma.
- Effects of a supplementation with DHA alone or combined with GLA on fatty acid composition (RBC and/or plasma lipids).
- Effects of DHA supplementation on plasma triglycerides and lipoproteins.
- Tolerance and safety assessment of the two used fatty acid supplements (microalgae oil and fish oil/evening primrose oil respectively).

2 Subjects and Methods

2.1 DHAVEG study

2.1.1 Subjects and study design

Ethical permission of the study was obtained from the Bavarian Board of Physicians. All volunteers completed a health and lifestyle questionnaire before entering the study. Eligible for the study were persons, who fulfil the following inclusion criteria:

- Adherence to a vegetarian diet since at least one year (no meat, less than one fish meal per month)
- Age ≥ 18 years
- Body mass index (BMI) between 18 and 25 kg/m^2
- No presence of metabolic, cardiovascular, renal or neurological diseases
- No intake of medication with known influence on the lipid metabolism during the last 3 months
- No use of n-3 fatty acid supplements
- No pregnancy or lactation
- No participation in another scientific study

Written informed consent was obtained from all subjects. Participants received financial compensation for their participation in the study. The study was conducted between June and November 2003 as a randomised double blind, placebo-controlled intervention study with two parallel groups. The subjects consumed 2.3 g daily of either a vegetarian, DHA-rich oil from microalgae *Ulkenia sp.* (providing 1.05 g DHA as triglyceride per day) or the same amount of olive oil (as placebo) for 8 weeks. Subjects were randomly assigned to one of the intervention groups with stratification for gender on the basis of a computer-generated random table.
At baseline (day 0) and after 56 to 60 days of intervention, fasted blood samples were collected and body weight, height, blood pressure (BP), and heart rate were measured (Figure 2.1).

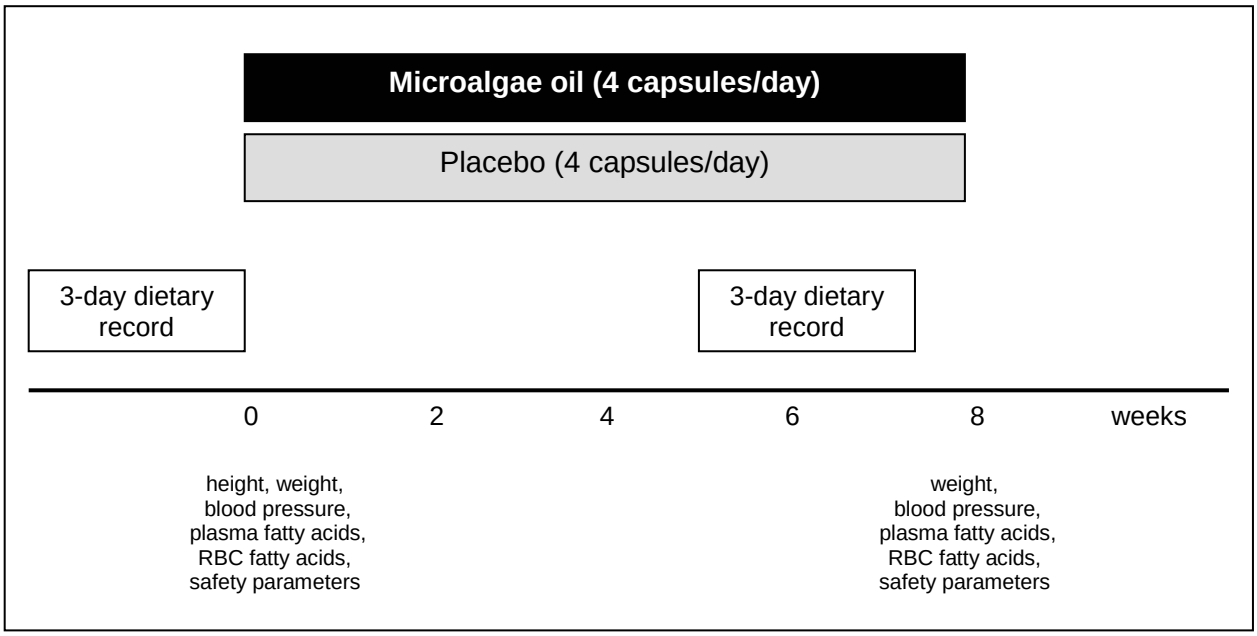

Figure 2.1. Study design, *DHAVEG study.*

RBC, red blood cells.

Before the first investigation (day 0), the subjects recorded their diet for 3 consecutive days (two weekdays and one weekend day). During the intervention, the subjects noted side effects, signs of illness, intake of medication and the number of capsules not consumed. At the end of the intervention period, they recorded their diet again for 3 consecutive days. Telephone interviews were performed every two weeks of the intervention period to monitor study compliance and side effects. Compliance was assessed by counting leftover capsules and by the plausibility of the DHA increase in RBC phosphatidylethanolamine (PE).

2.1.2 Study oils

Each DHA capsule contained on average 571 mg oil derived from microalgae *Ulkenia sp.* (Nutrinova® DHA, Nutrinova GmbH, Frankfurt/Main, Germany). Nutrinova®DHA is a highly concentrated, triglyceride-based oil, containing at least 43 g DHA/100 g total fatty acids (wt%) and almost no EPA. Matching placebo capsules contained on average 562 mg olive oil, which is free of n-3 LCPUFA. The fatty acid composition of the two study oils as determined during intervention period is given in Table 2.1.

Table 2.1. Major fatty acids of microalgae and placebo oils, *DHAVEG study* (wt%).

	Microalgae oil (*n* = 12)		Placebo oil (*n* = 12)	
	Mean	SD	Mean	SD
SFA	39.99	0.25	14.62	0.98
18:1n-9	0.52	0.03	75.86	0.76
18:1n-7	0.08	0.01	2.37	0.04
18:2n-6	1.21	0.08	5.32	0.14
18:3n-6	0.22	0.01	n.d.	
20:4n-6	0.09	0.01	n.d.	
22:5n-6	9.70	0.14	n.d.	
18:3n-3	0.11	0.01	0.68	0.02
20:5n-3	0.29	0.01	n.d.	
22:5n-3	0.09	0.01	n.d.	
22:6n-3	46.13	0.20	n.d.	

n.d., not detected; SFA, saturated fatty acids.

One thousand ppm mixed natural tocopherols (equals 2.2-2.3 mg mixed natural tocopherols per day) were added for stabilisation of the capsule content. The volunteers were required to take one capsule each during breakfast and dinner and two capsules during lunch (a total of four capsules per day). Capsules were stored refrigerated or at room temperature in a dry, dark place.

2.1.3 Measurements, blood sampling and storage

All anthropometrical measurements followed standardised procedures. Subjects were weighed without shoes and outdoor clothing on a digital scale at week 0 and week 8; height was measured only at study entry. A lump sum of 1 kg was subtracted for the weight of the clothes. The BMI was calculated as body weight (kg) divided by the square of body height (m). Seated blood pressure was determined using a stethoscope and a standard sphygmomanometer. Heart rate was counted for 15 sec at the wrist.

Venous blood samples were collected from an antecubital vein of the forearm into EDTA, citrate, and lithium heparin containing tubes (Sarstedt, Nümbrecht, Germany) as well as special tubes for platelet function analysis (containing sodium citrate, 1:10) after an overnight fast. For factor VII, von Willebrand factor, and PAI-1 analyses, citrated blood, and for vitamin A/E and fatty acid analyses, EDTA blood was centrifuged at 1300 *g* for 7 minutes at room temperature within 2 hours. The obtained plasma was divided into aliquots, and stored at -80℃ until analyses.

Blood cells from EDTA blood were washed 3 times with 0.9% sodium chloride (NaCl) solution. After lysis with distilled water (at least 200 µl/0.5 ml RBC) for 5 min, 8 ml isopropanol with BHT (50 mg/l) was added under constant shaking in 100 µl steps, and the tubes were frozen at -80℃. The analyses of fatty acids, vitamin A and E, factor VII, von Willebrand factor, and PAI-1 of a given subject were performed during 12 months of storage within the same analytical run. The other biochemical parameters were analysed at the day of blood drawing using established routine methods in the clinical chemistry laboratories of the University of Munich hospital.

2.1.4 Analytical methods

2.1.4.1 Chemicals, consumables and equipment

Table 2.2. Chemical list (fatty acid analysis).

Chemicals	Source	Quality
2.7 Dichlorfluorescein	Merck, Darmstadt	for analysis
2-Propanol	Merck, Darmstadt	for analysis
Acetic acid	Merck, Darmstadt	for analysis
Ammonia solution 25%	Merck, Darmstadt	for analysis
Butylated hydroxytoluene (BHT)	Fluka, Neu-Ulm	≥ 99% GC
Chloroform	Merck, Darmstadt	extra pure
Diisopropyl ether	Merck, Darmstadt	for analysis
Distilled water	Braun, Melsungen	ad iniectabilia
Ethanol	Baker, Deventer, Holland	absolute
Methanol	Merck, Darmstadt	for analysis
Methanolic HCl (3N)	Supelco, Bellefonte, USA	for analysis
n-Heptane	Merck, Darmstadt	for analysis
n-Hexane	Merck, Darmstadt	for analysis
Sodium carbonate	Merck, Darmstadt	anhydrous for analysis
Sodium chloride	Merck, Darmstadt	for analysis
Sodium chloride solution (0.9%)	Braun, Melsungen	ad iniectabilia
Sodium hydrogen carbonate	Merck, Darmstadt	for analysis
Sodium sulfate	Merck, Darmstadt	for analysis

Table 2.3. Chemical list (vitamin A and E analysis).

Chemicals	Source	Quality
Acetonitrile	Merck, Darmstadt	LiChrosolv
Ammonium acetate	Merck, Darmstadt	Fractopur
Butylated hydroxytoluene (BHT)	Fluka, Neu-Ulm	≥ 99% GC
Chloroform	Merck, Darmstadt	LiChrosolv
Distilled water	Braun, Melsungen	ad iniectabilia
Ethanol	Baker, Deventer, Holland	absolute
Methanol	Merck, Darmstadt	LiChrosolv
n-Hexane	Merck, Darmstadt	LiChrosolv
Precipitation reagent	Immundiagnostik, Bensheim	
Tetrahydrofuran	Merck, Darmstadt	LiChrosolv

Table 2.4. List for consumables (fatty acid analysis).

Consumables	Source
Brown glass bottle R1, G4	CS-Chromatographie Service, Langerwehe
Crimp cap R11-1.0	CS-Chromatographie Service, Langerwehe
Filter paper grade 388	Sartorius, Göttingen
Micro inlay G30/6	CS-Chromatographie Service, Langerwehe
Pasteur pipette	VWR International, Darmstadt
Pipette tip CP100	Gilson, Villiers-le-Bel, France
Pipette tip 50-1000 µl, 500-2500 µl	Eppendorf, Hamburg
Pipette tip 10-100 µl, 100-1000 µl	Greiner bio-one, Frickenhausen
Screw cap G13	CS-Chromatographie Service, Langerwehe
Sealing disc G13	CS-Chromatographie Service, Langerwehe
Test-tube 55.459 PS	Sarstedt, Nümbrecht
TLC plate, silica gel 60	Merck, Darmstadt (*DHAVEG study*)
TLC plate, silica gel 60	Macherey-Nagel, Düren (*FO/EPO study*)

Table 2.5. List for consumables (vitamin A and E analysis).

Consumables	Source
Brown glass bottle G1, G4	CS-Chromatographie Service, Langerwehe
Micro inlay G30/5	CS-Chromatographie Service, Langerwehe
Pipette tip CP100, CP250, CP1000	Gilson, Villiers-le-Bel, France
Pipette tip 50-1000 µl	Eppendorf, Hamburg
Pipette tip 10-100 µl, 100-1000 µl	Greiner bio-one, Frickenhausen
Screw cap G8-L, G13	CS-Chromatographie Service, Langerwehe
Sealing disc G13	CS-Chromatographie Service, Langerwehe
Silicone-PTFE septum	Merck, Darmstadt
Silicone-PTFE septum, slitted	Merck, Darmstadt

Table 2.6. Equipment list (fatty acid analysis).

Equipment	Source
Analytical balance type 1801	Sartorius, Göttingen
Centrifuge Beckman GPR	Beckman, Bucks, UK
Centrifuge Hettich Universal 1200	Hettich, Tuttlingen
Centrifuge Universal 30 F	Hettich, Tuttlingen
Centrifuge glass tube, conical angle 30°(12 ml)	Sc hott Duran, Mainz
Centifuge glass tube (25 ml)	Schmitz, Munich
Funnel D35m, D55mm	Duran Schott, Mainz
Membrane vacuum pump MZ 2c	Vacuubrand, Wertheim
Metal block thermostat type 2102	Bachofer, Reutlingen
Nitrogen evaporation system type 5000 6101	Bachofer, Reutlingen
Pear-shape glass flask, single-neck (50 ml)	Lenz, Wertheim
Pipette 10-100 µl, 100-1000 µl (Transferpette)	Brand, Wertheim
Pipette 50-250 µl, 500-2500 µl	Eppendorf, Hamburg
Pipette Microman M100	Gilson, Villiers-le-Bel, France
Rotary evaporator Rotavapor R-114	Büchi Labortechnik, Flawil, Switzerland
Solvent chamber for thin layer chromatography	Desaga, Heidelberg
Ultraviolet lamp	Benda, Wiesloch
Vortexer VF2	IKA-Labortechnik, Heitersheim
Waterbath B-480	Büchi-Labortechnik, Flawil, Switzerland
Gas chromatography:	
Autosampler A200S	Carlo Erba Instruments, Milan, Italy (*FO/EPO study*)
Autosampler HP 7673	Hewlett Packard, Böblingen (*DHAVEG study*)
Capillary column, BPX-70 (60 m x 0.32 mm)	SGE, Weiterstadt
Controller HP 7673	Hewlett Packard, Böblingen
Gas chromatograph HP 5890 Series II	Hewlett Packard, Böblingen
Integrator HP 3396 Series II	Hewlett Packard, Böblingen
Software EZChromEliteV2.61	Scientific Software, Pleasanton, USA

Table 2.7. Equipment list (vitamin A and E analysis).

Equipment	Source
Analytical balance type 1801	Sartorius, Göttingen
Brown glass bottle (25 ml)	Schott Duran, Mainz
Centrifuge Universal 30 F	Hettich, Tuttlingen
Nitrogen evaporation system type 5000 6101	Bachofer, Reutlingen
Pipette 10-100 µl, 100-1000µl (Transferpette)	Brand, Wertheim
Pipette 50-250µl	Eppendorf, Hamburg
Pipette Microman M100, M250, M1000	Gilson, Villiers-le-Bel, France
Quartz cuvette SUPRASIL	Hellma, Müllheim
Ultrasonic bath Sonorex Super RK 102 H	Bandelein, Berlin
UV/Vis Spectrophotometer Cary 1E	Varian, Darmstadt

Equipment (continuation)	Source
Volumetric flask 10 ml, 25 ml	Schott Duran, Mainz
Vortexer VF2	IKA-Labortechnik, Heitersheim
HPLC:	
Autosampler AS-4000A Intelligent	Merck Hitachi, Darmstadt
Column, LiChroCART 250-3, LiChrospher 100, RP18 (5 µm)	Merck, Darmstadt
Column oven STH 585	Gynkotek, Germering
Pump L-6200 Intelligent Pump	Merck Hitachi, Darmstadt
Software EZChromEliteV2.61	Scientific Software, Pleasanton, USA
UV/Vis detector L-4250	Merck Hitachi, Darmstadt

Table 2.8. List for the used standard substances (fatty acid analysis).

Standard substance	Source
Internal standard:	
Cholesteryl pentadecanoic acid	Sigma, Deisenhofen
Dipentadecanoyl phosphatidylcholine	Sigma, Deisenhofen
Pentadecanoic acid	Sigma, Deisenhofen
Tripentadecanoin	Sigma, Deisenhofen
Standards for peak identification:	
GLC-85 (reference standards)	Nu-Chek, Elysian, MN, USA
14:1t	Sigma, Deisenhofen
16:1t	Sigma, Deisenhofen
18:1n-7	Sigma, Deisenhofen
18:2tt	Sigma, Deisenhofen
18:4n-3	Sigma, Deisenhofen
20:3n-9	Sigma, Deisenhofen
20:5n-3	Sigma, Deisenhofen
22:1t	Sigma, Deisenhofen
22:4n-6	Sigma, Deisenhofen
22:5n-3	Sigma, Deisenhofen
22:5n-6	OmegaTech, CO, USA
24:0	Sigma, Deisenhofen

Table 2.9. List of the used standards and control sera (vitamin A and E analysis).

Standards/Control sera	Source	Quality
Internal standard		
Retinyl acetate	Hoffmann-La Roche, Basel, Switzerland	Purity 99.7%
Tocol	Eisai, Tokyo, Japan	for HPLC
Standards for peak identification		
alpha-Tocopherol	Supelco, Bellefonte, PA, USA	Purity 99.3%
Retinol	Merck, Darmstadt	Purity >98%
Control serum		
NIST Standard Reference Material 968c	Gaithersburg, USA	

2.1.4.2 Fatty acid analysis in study oils

Methyl esters of fatty acids (FAME) from study oil triglycerides were obtained by reaction with 3M methanolic hydrochloric acid (HCl), methanol and hexane (1+1+0.5, by vol) at 90℃ for 60 min in closed glass tubes. After adding distilled water and hexane with BHT (2 g/l), the samples were vortexed and centrifuged. The upper hexane phase containing the FAME was stored until GC analysis at -80℃.

2.1.4.3 Fatty acid analysis in plasma phospholipids

Preparation of the internal standard. For quantification of fatty acids in plasma lipid fractions, defined concentrations of dipentadecanoyl phosphatidylcholine, pentadecanoic acid, tripentadecanoin, and cholesteryl pentadecanoic acid (equalled 10 µg 15:0/100 µl for each lipid fraction) were used.

Table 2.10. Composition of the internal standard for fatty acid analysis.

	Molecular weight (g/mol)	Correction factor for non-15:0 residue	Weighted sample (g per 200 ml methanol/chloroform)
Cholesteryl pentadecanoic acid	611.0	x 2.52	50.47
Dipentadecanoyl phosphatidylcholine	706.0	x 1.46	29.22
Pentadecanoic acid	242.4	x 1.00	20.05
Tripentadecanoin	765.3	x 1.05	21.06

The in Table 2.10 presented amounts were dissolved in 200 ml methanol/chloroform (35+15, by vol, with 2 g/l BHT), aliquoted in 4 ml brown glass bottles and stored at –80°C until usage. To get a high reliability, the same internal standard mixture was used for all analyses.

Lipid extraction. Plasma lipids were extracted according to a modified method of Kolarovic & Fourier [110]. For this, 100 µl internal standard and 2 ml hexane/ isopropanol (3+2, by vol) were added to 250 µl plasma and the tube was vortexed for 30 sec. After centrifugation at 1000 *g* for 7 min the hexane phase was transferred into a 4 ml brown glass vial. Thereafter, two extractions with 2 ml hexane were performed and the combined extracts were taken to dryness under a gentle stream of nitrogen.

Separation of lipid fractions. The lipid residue was dissolved in 400 µl chloroform/ methanol (1+1, by vol) and deposited on a thin layer chromatography (TLC) plate. Phospholipids (PL), free cholesterol, non-esterified fatty acids (NEFA), TG and cholesterol esters (CE) were separated using heptane, diisopropyl ether and acetic acid (60+40+3, by vol) as mobile phase [111]. After visualisation of the lipids with 2.7-dichlorofluorescein (1% in ethanol) and UV-light, the band with the phospholipid fraction was scraped from the TLC plate and transferred into a 4 ml brown glass bottle (Figure 2.2).

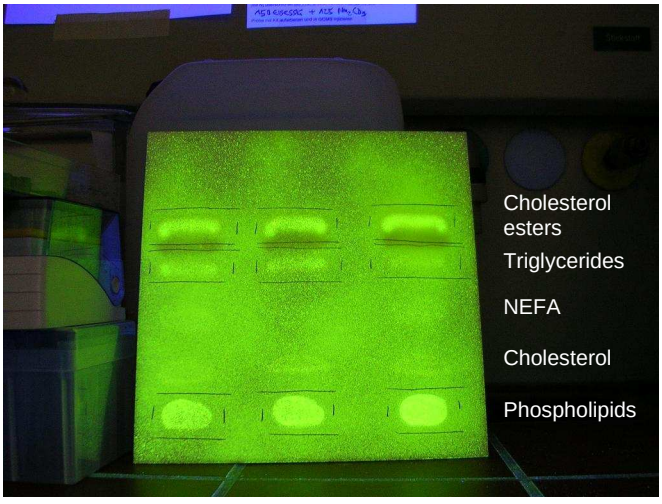

Figure 2.2. Separation of plasma lipid fractions using thin layer chromatography.

NEFA, non-esterified fatty acids.

Transesterification and extraction of FAME. FAME were obtained by reaction with 3M methanolic HCl at 85°C for 45 min. After neutralisation with sodium carbonate/sodium hydrogen carbonate/sodium sulphate buffer (1+2+2, by weight), 1 ml hexane was added. After centrifugation at 1000 *g* for 4 min the hexane layer was transferred into a 2 ml brown glass bottle. The hexane extraction was repeated and the combined extracts were taken to dryness under a gentle stream of nitrogen. For storage until GC analysis at -80°C, the samples were dissolved in 50 µl hexane + 2g/l BHT.

2.1.4.4 Fatty acid analysis in RBC total lipids and phospholipids

Lipid extraction. The RBC/isopropanol mixture (see 2.1.3) was completely transferred into a 25 ml centrifuge tube; 6 ml isopropanol and 4 ml chloroform were added in little steps under constant shaking. Thereafter, the tubes were vortexed for 10 minutes and centrifuged at 1400 *g* for 10 min. The supernatant was filtrated into a pear-shaped glass flask and a second extraction with 4 ml chloroform was performed. After shaking for 10 min, the whole sample was filtrated into the same flask and the filter was washed two times with chloroform. Four ml of the obtained lipid extract were transferred into a 4 ml brown glass bottle and frozen at -80°C until the analysis of RBC total lipids; 3 ml RBC extract was transferred into a second glass flask and taken to dryness under reduced pressure at 37°C.

Separation of lipid fractions. The lipid residue was dissolved in 400 µl of chloroform/methanol (1+1, by vol) and deposited on a TLC plate. RBC phosphatidylethanolamine (PE) and phosphatidylcholine (PC) were separated using chloroform, methanol, 25% ammonia solution, and distilled water (73+27+2.2+2.8, by vol) as mobile phase. After visualisation of the lipids with 2.7-dichlorofluorescein (1% in ethanol) and UV-light, the bands containing PE and PC were scraped separately from the TLC plate and transferred into 4 ml glass tubes.

For the analysis of RBC total lipids, 2 ml of the defrosted lipid extract was directly transferred into a brown glass tube and taken to dryness under nitrogen.

Transesterification and extraction of FAME. Transesterification of RBC PE, PC, and total lipids, extraction of FAME and storage were performed as described for plasma fatty acids (see 2.1.4.3).

2.1.4.5 Gas chromatography conditions

FAME were separated using capillary gas liquid chromatography. For this, 3 µl sample was injected into the gas chromatograph. The GC program is described in Table 2.11 and Figure 2.3.

Table 2.11. GC programme for fatty acid analysis.

Oven	
Initial temperature	130℃
Initial time	0.50 min
Rate	3.0℃/min
Final temperature	150℃
Final time	0.00 min
Rate A	1.5℃/min
Final temperature	180℃
Final time	0.00 min
Rate B	3.0℃/min
Final temperature	210℃
Final time	23.00 min (plasma PL and TG, RBC PE and PC), 33.00 min (plasma CE and total lipids, RBC total lipids)
Pressure	
Initial pressure	1.10 bar
Rate	0.025 bar/min
Final time	40.00 min
Injector / Detector	
Injector temperature	250℃
Detector temperature	300℃

CE, cholesterol esters; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, phospholipids; RBC, red blood cells.

Flame ionisation detector signals were evaluated with the software EZ-Chrom Elite, version 2.61 (Scientific Software, Pleasanton, USA) and identified by comparison with the retention times of a standard mixture run previously (Figure 3.1). The instrument was calibrated regularly by using a quantitative standards mixture (GLC-85, Nu-Chek, Elysian, MN, USA).

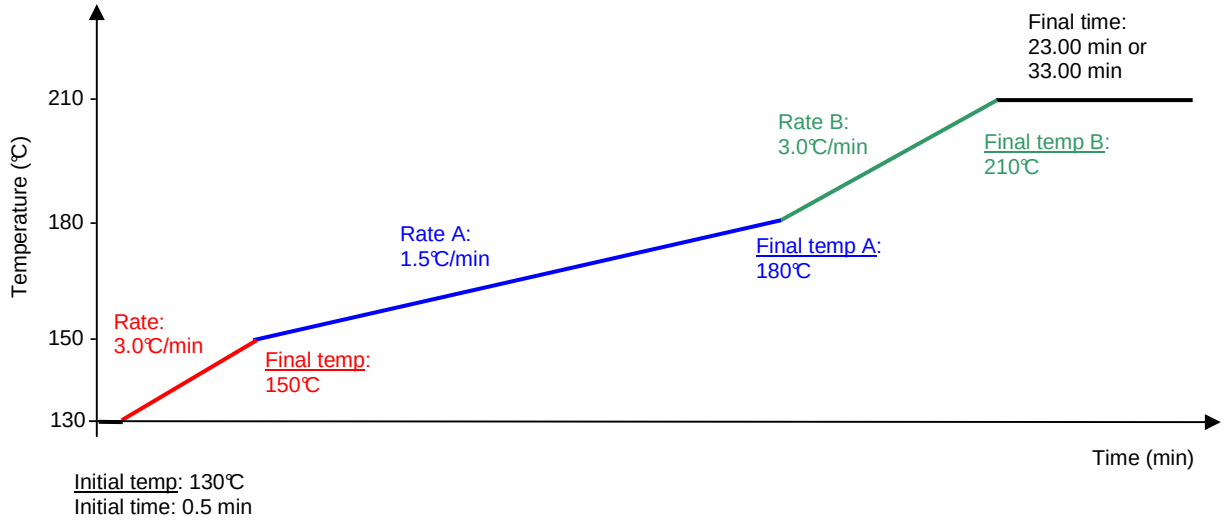

Figure 2.3. GC temperature programme for fatty acid analysis.

2.1.4.6 Calculation of fatty acid contents

Absolute fatty acid concentration (mg/l) of all identified fatty acids with 14 – 24 carbon atoms was determined via comparison with the peak area of the internal standard (15:0) and correction with the accordant response factor. In plasma lipid fractions (PL, CE, TG), the area of the internal standard equalled 10 µg 15:0/250 µl plasma = 40 mg 15:0/l plasma.

Additionally, fatty acid weight percentages (wt%) were calculated as follows:

$$\textit{Individual fatty acid (wt\%)} = \frac{\textit{Individual fatty acid (mg/l)* 100}}{\textit{Sum of all identified C14 - C24 fatty acids (mg/l)}}$$

Absolute fatty acid concentrations in RBC lipids cannot be determined, because no internal standard was added and the used RBC volume is not exactly known. For RBC, fatty acid weight percentages (wt%) were calculated by dividing the peak area of each individual peak by the peak area of all C14 – C24 fatty acids (each peak area was corrected by the accordant response factor) and multiplying the result with 100.

In addition to the fatty acid profile, the following fatty acid sums and ratios were calculated for each sample: sum of EPA and DHA (EPA + DHA), sum of all n-6 fatty acids (Σ-6 FA), sum of all n-3 fatty acids (Σn-3 FA), sum of all long-chain n-6 fatty

acids (Σ-6 LCPUFA), sum of all long-chain n-3 fatty acids (Σ-3 LCPUFA), the ratio of n-6 to n-3 fatty acids (Σn-6/Σn-3 FA), and the ratio of n-6 LCPUFA to n-3 LCPUFA (Σn-6/Σn-3 LCPUFA).

2.1.4.7 Examination of reproducibility (fatty acid analysis)

Prior to analyses of the study samples, RBC and plasma were obtained from a large blood sample as described in 2.1.3, aliquoted and stored at -80℃. Intra-assay reproducibility was assessed by analysing 8 pool samples in the same analytical run. Inter-assay reproducibilities of the used methods were assessed by analysing pool samples 11x during 6 weeks (plasma phospholipids), 14x during 9 weeks (RBC PC and PE), and 19x during 3 weeks (RBC total lipids).

2.1.4.8 Analysis of vitamin A and E in plasma

The analysis of vitamin A and E was performed according to a modified method of Schaffer [112] and Göbel *et al.* [113].

Preparation of the mobile phase. An isocratic mobile phase consisting of acetonitrile, tetrahydrofuran, methanol and 1% ammonium acetate (684+220+68+28, by vol) was used [114]. To avoid the formation of explosive peroxides from tetrahydrofuran, the mobile phase was prepared prior to use and degassed ultrasonically.

Preparation of the internal standard. To consider losses during the extraction process as well as unsteady injection amounts into the HPLC system, retinyl acetate was added as internal standard to plasma and standard samples at the beginning of the analysis. To prepare the internal standard, 250 mg retinyl acetate was dissolved in 100 ml ethanol/BHT (0.0625%). In a second step, this concentrate was diluted 1:1000 to a concentration of 2.5 mg/l to get an adequate peak height in the chromatogram. The internal standard was frozen at -80℃ until usage. To get a high reliability, the same internal standard mixture was used for analyses of all study samples.

Preparation of standard concentrates and dilutions. For preparation of the standard concentrates, the following amounts of retinol and alpha-tocopherol (Table 2.12) were dissolved in 10 ml ethanol each, aliquoted in 4 ml brown glass bottles and stored at -80℃. From these standard concentrates, dilutions were prepared as described in Table 2.12 and stored in 25 ml brown glass bottles at -80℃ until usage.

Table 2.12. Preparation of retinol and alpha-tocopherol standard concentrates and dilutions, *DHAVEG study*.

Substance	Standard concentrate	Standard dilution
Retinol	10 mg/10 ml ethanol	200 µl concentrate/10 ml ethanol ⇒ 0.02 g/l
alpha-Tocopherol	50 mg/10 ml ethanol	1000 µl concentrate/10 ml ethanol ⇒ 0.5 g/l

Preparation of the stock standard. For a new standard curve, standard dilutions were defrosted and their absorbance was measured on a spectrophotometer at 325 nm for retinol and at 292 nm for alpha-tocopherol (Table 2.13). Lambert-Beer Law was used to determine the exact concentration of the standard dilutions (c) from absorbance (A) ($A^{1\%}_{1cm}$, extinction coefficient of a compound at a certain wave length; d, thickness of cuvette).

The formula is:

$$A = A^{1\%}_{1cm} * c\ (g/dl) * d\ (cm)$$

Thus, concentration can be calculated by:

$$c\ (g/dl) = A\ /[A^{1\%}_{1cm} * d\ (cm)\ ]$$

Table 2.13. Extinction coefficients for retinol and alpha-tocopherol, *DHAVEG study*.

Substance	Molecular weight (g/mol)	Wave length (nm)	Extinction coefficient ($A^{1\%}_{1cm}$)	Source
Retinol	286.5	325	1780	[112]
alpha-Tocopherol	430.7	292	75.8	[112]

On the basis of the photometrically determined concentrations, the composition of the stock standard mixture was defined: the stock standard mixture corresponds to the highest standard of the standard curve (Std. 7) and its concentration should be higher than all expected concentrations in the plasma samples. For preparation of the stock standard mixture, certain amounts of the standard dilutions were pipetted into a 10 ml volumetric flask, dried under a gentle stream of nitrogen and redissolved in 10 ml hexane (Table 2.14).

Table 2.14. Preparation of the stock standard mixture for vitamin A/E analysis (example), *DHAVEG study*.

Substance	Concentration of standard dilution (µg/µl)*	Pipetted amount (µl)	Concentration of stock standard mixture (mg/l)
Retinol	0.0134	760	1.018
alpha-Tocopherol	0.3861	585	22.59

* Calculated by Lambert-Beer-Law.

For the calibration curve, various amounts of the stock standard mixture were evaporated after addition of 0.5% hexane/BHT and redissolved in 1 ml hexane (Table 2.15).

Table 2.15. Preparation of the 7 standard dilutions for vitamin A/E analysis, *DHAVEG study*.

	Std. 7	Std. 6	Std. 5	Std. 4	Std. 3	Std. 2	Std.1
1) Stock standard	1000 µl	844 µl	688 µl	532 µl	376 µl	220 µl	100 µl
2) Add 0.5% hexane/BHT	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl	100 µl
3) Dry under nitrogen	✓	✓	✓	✓	✓	✓	✓
4) Add hexane	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl	1000 µl

The concentrations of standards 1 – 7 were calculated according to their dilution factors (Table 2.16):

Table 2.16. Calculated concentrations of the 7 standard dilutions for retinol and tocopherol calibration curves (mg/l), *DHAVEG study*.

	Std. 7	Std. 6	Std. 5	Std. 4	Std. 3	Std. 2	Std.1
Dilution factor	x1	x0.84	x0.69	x0.53	x0.36	x0.22	x0.1
Retinol	1.018	0.856	0.700	0.541	0.383	0.224	0.102
alpha-Tocopherol	22.59	19.07	15.54	12.02	8.49	4.97	2.26

Extraction of the standards. The extraction method of the 7 standards was similar to the extraction of the plasma samples (see below): 250 µl distilled water (instead of plasma), 50 µl internal standard and 500 µl precipitation reagent were pipetted into a 4 ml brown glass tube to minimize light-induced degradation of vitamins and vortexed for 15 sec. Thereafter, 250 µl standard (standard 1 – 7 respectively) and 750 µl hexane (instead of 1 ml hexane in case of plasma samples) were added and the samples were mixed for another 30 sec. After centrifugation at 1100 *g* for 5 min, the upper phase was transferred into a 1.5 ml brown glass vial. Thereafter, a second extraction with 1 ml hexane was performed and the combined extracts were taken to dryness under a gentle stream of nitrogen. The dried extract was redissolved in 100 µl mobile phase, shaken mechanically for 10 min and transferred into microvials before injecting into a HPLC system with UV-Vis detection. The HPLC conditions are shown in 2.1.4.9. Detector signals were evaluated with the software EZ-Chrom Elite, version 2.61. Figure 2.4 exemplifies the separation of fat-soluble vitamins and carotenes with HPLC.

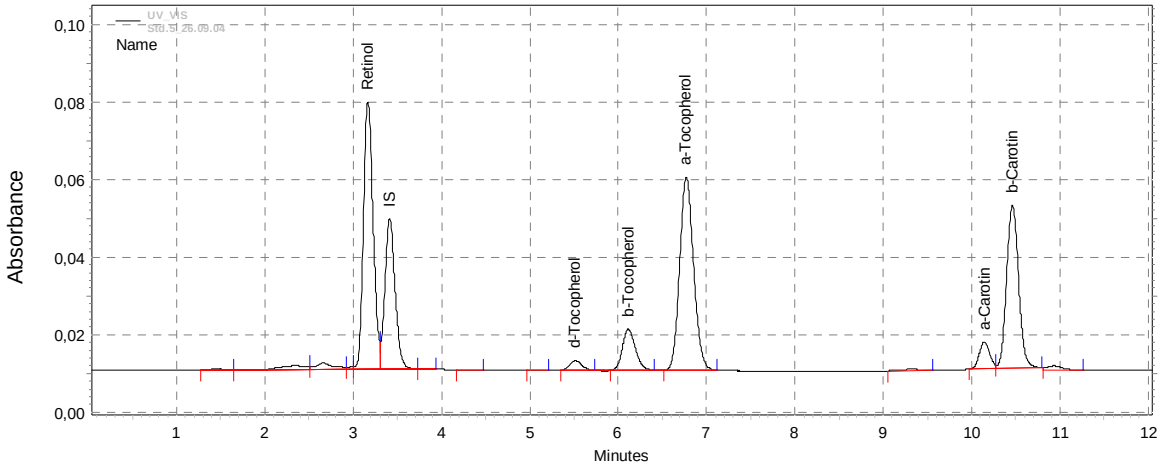

Figure 2.4. Chromatogram of a standard mixture using UV-Vis detection, *DHAVEG study*.

IS, internal standard (retinyl acetate); d-Tocopherol, delta-tocopherol; b-Tocopherol, beta/gamma-tocopherol, a-Tocopherol, alpha-tocopherol; a-Carotin, alpha-carotene; b-Carotin, beta-carotene.

Standard curve construction. A 7-point standard curve was constructed for retinol and alpha-tocopherol with the software Microsoft Excel 97 SR-2 (Microsoft GmbH, Unterschleißheim) by plotting the vitamin concentrations (Table 2.16, y-axis) against the peak-area ratios (vitamin/internal standard, x-axis) (see Figure 8.1). Subsequently, the equation of the regression line and the coefficient of determination (r^2) were calculated.

Extraction of plasma samples. 250 µl plasma, 50 µl internal standard and 500 µl precipitation reagent were pipetted into a 4 ml brown glass bottle and vortexed for 15 sec to precipitate the proteins. Subsequently, two extractions with 1 ml hexane were performed. The upper phases were transferred into a 1.5 ml brown glass bottle and taken to dryness under a gentle stream of nitrogen. The dried extract was redissolved in 100 µl mobile phase, shaken mechanically for 10 min and transferred into microvials. 50 µl were injected into a HPLC system with UV-Vis detection. The HPLC conditions are shown in 2.1.4.9. Detector signals were evaluated with the software EZ-Chrom Elite, version 2.61 and identified by comparison with the retention times of a standard mixture run previously (Figure 2.4).

Calculation of vitamin A and E concentrations. The peak-area ratios (vitamin / internal standard) were computed for retinol and alpha-tocopherol and the appropriate concentrations were calculated using the equations of the standard curves (Figure 8.1).

2.1.4.9 HPLC conditions

The column temperature was held constant at 30°C. The programs of pump and UV/Vis detector were as follows:

Table 2.17. Programme of the HPLC pump, *DHAVEG study*.

Time (min)	Flow (ml/min)
0	0.65
8	0.65
9	1.30
13	1.30
15	0.65

Table 2.18. Programme of the UV-Vis detector, *DHAVEG study*.

Time (min)	Wave length (nm)	Detection of
0	325	Retinol
4	292	Tocopherols
9	450	Carotenes
15	325	

2.1.4.10 Examination of reproducibility, recovery, and accuracy (vitamin A and E analysis)

Before analysing the study samples, plasma was obtained from a large blood sample as described in 2.1.3, aliquoted and stored at -80°C. Intra-assay reproducibility was assessed by analysing 8 pool samples in the same analytical run. Inter-assay reproducibility of the used method was assessed by analysing pool samples 11x during 2 weeks.

Recovery of the vitamin A and E measurements was assessed by performing the described method for plasma samples (2.1.4.8), but using for the first extraction instead of 1 ml hexane 50 µl stock standard and 950 µl hexane (n = 4). Recovery was calculated by dividing the mean measured concentration by the expected concentration and multiplying the result with 100%.

The accuracy of the vitamin A and E measurements was verified with lyophilised standard reference material 968c obtained from the US National Institute of

Standards and Technology (NIST), which contains the vitamins in low and high concentrations. Three samples of each concentration level were analysed and compared with the approved values. A bias between 0 and 5% is regarded as excellent, while a bias between 5 and 10% is considered acceptable by the NIST.

2.1.4.11 Plasma triglycerides and lipoproteins

TG, total and HDL cholesterol concentrations were determined in lithium heparin plasma by standard enzymatic methods on an automated sample processor with the appropriate reagent systems (Table 2.19). LDL cholesterol concentrations were calculated using the Friedewald formula [115]:

LDL cholesterol = Total cholesterol – HDL cholesterol – VLDL cholesterol
(in which VLDL cholesterol = Triglycerides / 5).

The reproducibility of the used test kits as declared from the manufacturer is shown in Table 8.1.

Table 2.19. Analyses of plasma triglycerides, total and HDL cholesterol, *DHAVEG study.*

Parameter	Test kit	Analytical equipment
Triglycerides	Cobas Integra TRIGL (Roche Diagnostics GmbH, Mannheim)	Cobas Integra 800 (Roche Diagnostics GmbH, Mannheim)
Total cholesterol	Cobas Integra CHOLL (Roche Diagnostics GmbH, Mannheim)	
HDL cholesterol	Cobas Integra HDL_C (Roche Diagnostics GmbH, Mannheim)	

2.1.4.12 Blood cell count, biochemical and haemostatic parameters

Full blood cell count and biochemical parameters. Full blood cell counts were performed in EDTA blood on an automatic analyser (Table 2.20). Bilirubin, creatinine, gamma-glutamyl transpeptidase (Gamma-GT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholinesterase (CHE), creatinekinase (CK), lactate dehydrogenase (LDH), uric acid, total protein, C-reactive protein (CRP) and glucose were measured in lithium heparin plasma on an automated sample

processor with the appropriate reagent systems. The reproducibility of the used test kits as declared from the manufacturer is shown in Table 8.2.

Table 2.20. Analyses of full blood cell count and biochemical parameters, *DHAVEG study.*

Parameter	Test kit	Analytical equipment
Full blood cell count		Coulter Micro Diff II (Beckmann Coulter, Krefeld)
	All test kits from Roche Diagnostics GmbH, Mannheim	
Bilirubin	BIL-T DPD	Roche/Hitachi 912 (Roche Diagnostics GmbH, Mannheim)
Creatinine	CREA Jaffe	
Gamma-GT	GGT flüssig	
ALT	ALT/GPT IFCC	
AST	AST/GOT IFCC	
CHE	CHE	
CK	CK NAC	
LDH	LDH IFCC	
Uric acid	UA plus	
Total protein	TP	
CRP	CRP TINA-QUANT	
Glucose	GLU HK	

Gamma-GT, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHE, cholinesterase; CK, creatinekinase; LDH, lactate dehydrogenase; CRP, C-reactive protein.

Haemostatic parameters. Platelet function analysis was performed within 2 hours of blood collection in citrated whole blood using a platelet function analyser (Table 2.21). Membranes pre-coated with collagen/epinephrine or collagen/adenosine diphosphate were used to stimulate platelet aggregation. Quick's time, partial thromboplastin time (PTT), fibrinogen, and d-dimers were measured in citrated plasma using automated analysers with commercially available kits. PAI-1 activities were measured using an ELISA assay. Factor VII and von Willebrand factor analyses were performed on automated sample processors with commercial test kits. The reproducibility of the used test kits as declared from the manufacturer is shown in Table 8.3.

Table 2.21. Analyses of haemostatic parameters, *DHAVEG study.*

Parameter	Test kit	Analytical equipment
Quick's time	Thromborel S (Dade Behring, Marburg)	Amelung Amax CS (Trinity Biotech, Darmstadt)
PTT	Pathromtin**SL (Dade Behring, Marburg)	
Fibrinogen	Multifibren U (Dade Behring, Marburg)	
D-dimers	TINA-QUANT a D-Dimer (Roche Diagnostics GmbH, Mannheim)	Roche/Hitachi 912 (Roche Diagnostics GmbH, Mannheim)
Factor VII	HemosIL, Factor VII deficient plasma, (Instrumentation Laboratory, Milan, Italy)	ACL 9000 (Instrumentation Laboratory, Milan, Italy)
Von Willebrand factor	IL Test^{TM} Von Willebrand Faktor (Instrumentation Laboratory, Milan, Italy)	BCS (Dade Behring, Marburg)
PAI-1	Coatest PAI (Chromogenix-Instrumentation Laboratory, Milan, Italy)	SPECTRA thermo (Tecan, Crailsheim)
PFA-ADP	PFA-100 reagents (Dade Behring, Marburg)	Platelet Function Analyzer PFA-100 (Dade Behring, Marburg)
PFA-EPI	PFA-100 reagents (Dade Behring, Marburg)	

PTT, partial thromboplastin time; PAI-1, plasminogen activator inhibitor-1 activity; PFA-ADP, platelet function analysis with adenosine diphosphate; PFA-EPI, platelet function analysis with epinephrine.

2.1.4.13 Dietary evaluation

Food consumption of the subjects was determined before and during the intervention period using a self-reported three-day estimated dietary record (see Attachment, pages A54 – A57). We applied a modified form of the "Freiburger Ernährungs-protokoll", which is contained as protocol template in the used nutrition software Prodi (Nutri-Science GmbH, Freiburg, Germany). Vegetarian foods were added to the original protocol and foods of animal origin were excluded with the exception of eggs, butter, milk, and dairy products.

In the used tally sheet protocol, the subjects estimated the quantities of the foods and beverages consumed using household measures (teaspoon, tablespoon, cup, slice, piece, etc.) according to the provided instructions. The diet was recorded over three consecutive days covering two weekdays and one weekend day (Thursday to Saturday or Sunday to Tuesday).

Recorded dietary intakes were entered into the software Prodi version 4.5 LE 2003, which calculated the nutrient intake based on the German Nutrient Data Base BLS, version II.3 (BgVV, Berlin, Germany).

2.1.5 Data management and statistical analyses

Statistical evaluation was carried out with SPSS 12.0 for Windows (SPSS Inc., Chicago, USA). Data are presented as mean ± SD for normally distributed data or as median with interquartile range (IQR, 25^{th} – 75^{th} percentiles) in case of non-normality.

Test for normality. Data were checked for normality by visual inspection and by Kolmogorov-Smirnov-test (with Lilliefors correction). Parametric tests were used for normally distributed variables and nonparametric tests for variables that are not normally distributed. Exact significances were calculated for all nonparametric tests.

Between-group differences. Any differences between the groups (active treatment vs. placebo) at each time point or the impact of treatment on absolute changes (week 8 minus week 0) were tested for significance using Student's unpaired t-test for normally distributed variables or the Mann-Whitney U test for variables not normally distributed.

Within-group differences. Differences between week 0 and week 8 were determined within each treatment group using Student's t-test for dependent samples and Wilcoxon nonparametric test respectively.

Correlations and bivariate tabular analysis. Correlations between parameters were estimated by computing Pearson's correlation coefficient in the case of normally distributed values and the Spearman-Rho correlation coefficient in the case of other distributions respectively. For bivariate tabular analysis the chi-square test (exact calculation) was used. In cases of expected values smaller than 5, a Fisher exact test was used instead.

Level of significance. *P* values below 5% were considered significant.

2.2 FO/EPO study

2.2.1 Subjects and study design

Ethical permission of the study was obtained from the Bavarian Board of Physicians. All volunteers completed a health and lifestyle questionnaire before entering the study. Eligible for the study were persons, who fulfil the following inclusion criteria:

- Female gender
- Age between 18 and 40 years
- BMI between 18 and 25 kg/m^2
- No hypertension (defined as a systolic blood pressure > 140 mm Hg or a diastolic blood pressure > 90 mm Hg [116])
- No presence of metabolic, cardiovascular, renal or neurological diseases
- No long-term use of medication (contraceptives excluded)
- No use of EFA or LCPUFA supplements
- No drug abuse
- No vegetarian lifestyle during the last 3 months
- Smoking of ≤ 5 cigarettes per day
- Consumption of ≤ 7 glasses alcohol per week
- Consumption of fish ≤ 2 times a week
- No pregnancy or lactation
- No participation in another scientific study

All subjects gave their written informed consent and received financial compensation for their participation. The study was conducted between June and August 2005 as a randomised double blind, placebo-controlled intervention study with two parallel groups. The subjects consumed 3.4 g daily of either a fish oil/evening primrose oil mixture (FO/EPO, delivering 419 mg DHA, 72 mg EPA, 14 mg AA, 337 mg GLA as triglycerides per day) or the same amount of a placebo (mixture of palm oil, rapeseed oil, and sunflower seed oil) for 8 weeks. Subjects were randomly assigned to one of the intervention groups on the basis of a computer-generated random table.
Fasted blood samples were collected at weeks 0 (baseline), 4, 6 and 8 (Figure 2.5). Body weight, blood pressure, and heart rate were measured at study entry and after

8 weeks of intervention; height was measured at baseline only. During the intervention, the subjects noted side effects, signs of illness, intake of medication and the number of capsules not consumed. Compliance was assessed by counting leftover capsules and calculated as the percentage of the prescribed capsules taken. To check the success of blinding, the subjects were asked at the end of the study about their assumed group assignment.

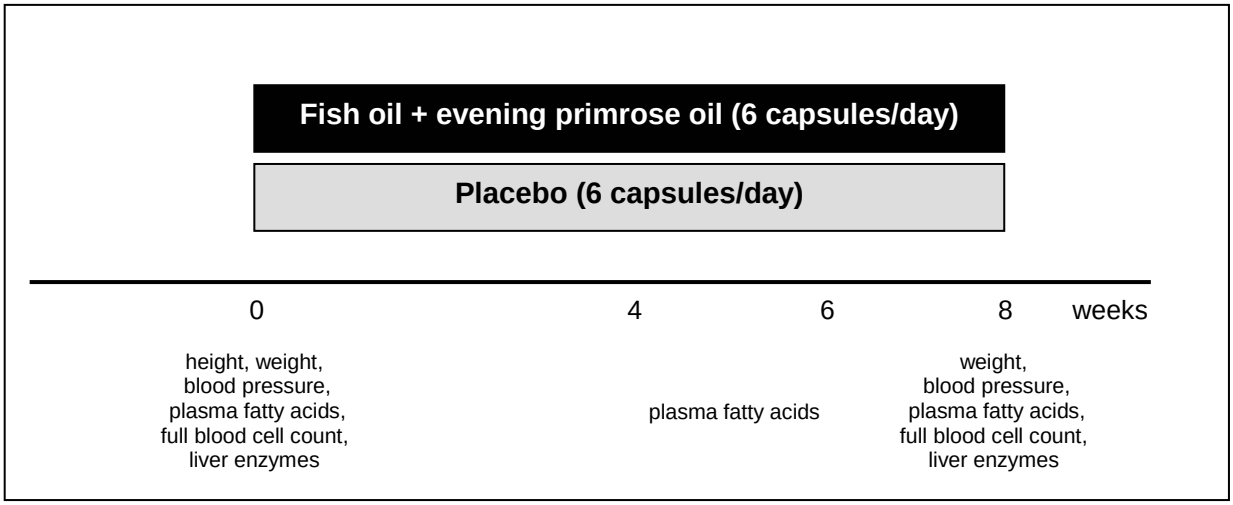

Figure 2.5. Study design, *FO/EPO study.*

2.2.2 Study oils

Each FO/EPO capsule contained 438 mg evening primrose oil (EFAMOL Ltd, Brackenholme, Selby, North Yorkshire, UK) and 129 mg fish oil (Incromega DHA 500 TG SR, Croda Chemicals Europe Ltd, Cowick Hall, East Yorkshire, UK) (Table 2.22). Matching placebo capsules consisted of palm oil, rapeseed oil, and sunflower seed oil. The study capsules contained 1% synthetic vitamin E acetate for stabilisation of the capsule content. The fatty acid composition of the two study oils as declared from the manufacturer and as determined after the intervention period in our laboratory (January 2006) is given in Table 2.23. The volunteers were required to take daily 2 x 3 capsules or 3 x 2 capsules with the meals (a total of six capsules per day). Capsules were stored refrigerated or at room temperature in a dry, dark place.

Table 2.22. Ingredients of placebo and FO/EPO capsules, *FO/EPO study.*

	Component	Percent (%)
Placebo capsule (567.2 mg)	Palm oil	59.4
	Rapeseed oil	19.8
	Sunflower seed oil	19.8
	Synthetic vitamin E acetate	1.0
FO/EPO capsule (572.7 mg)	Efamol Evening primrose oil	76.5
	Croda Incromega DHA 500 TG SR	22.5
	Synthetic vitamin E acetate	1.0

FO/EPO, fish oil and evening primrose oil mixture.

Table 2.23. Major fatty acids of FO/EPO and placebo oils (wt%), *FO/EPO study.*

	FO/EPO*	FO/EPO† (*n* = 4)	Placebo*	Placebo† (*n* = 4)
16:0	5.1	4.93 ± 0.03	27.8	28.46 ± 0.03
18:0	1.9	1.84 ± 0.01	3.8	3.63 ± 0.01
18:1n-9 + 18:1n-7	6.3	5.98 ± 0.06	42.4	42.01 ± 0.07
18:2n-6	56.4	56.64 ± 0.04	21.1	21.37 ± 0.02
18:3n-6	9.8	10.28 ± 0.03	-	-
20:4n-6	0.4	0.41 ± 0.00	-	-
18:3n-3	0.2	0.17 ± 0.01	2.0	2.06 ± 0.03
20:5n-3	2.1	2.13 ± 0.02	-	-
22:6n-3	12.2	13.28 ± 0.06	-	-

FO/EPO, fish oil and evening primrose oil mixture.

* Values are reported as means (according to manufacturer's declaration).

† As determined in our laboratory in January 2006 (mean ± SD).

2.2.3 Measurements, blood sampling and storage

All anthropometrical measurements at baseline followed standardised procedures. Height without shoes was measured to the nearest 5 millimetres and body weight without shoes and outdoor clothing to the nearest 100 g using a digital scale. A lump sum of 1 kg was subtracted for the weight of the clothes. Blood pressure and heart rate were determined in sitting position using a digital sphygmomanometer.

Venous blood samples (5 ml) were collected from the antecubital vein of the forearm into an EDTA containing tube (Sarstedt, Nümbrecht, Germany) in sitting or lying position of the subject. Blood was centrifuged at 4℃ (1500 *g* for 5 minutes) within 2 hours and the obtained plasma was aliquoted into two plastic storage vials, which

were closed tightly under a stream of nitrogen, and stored at -80℃ until analysis of total lipid (TL), phospholipid (PL), triglyceride (TG) and cholesterol ester (CE) fatty acids. At baseline and after 8 weeks of supplementation, two additional blood samples (1 ml each) were collected into a lithium heparin tube and into an EDTA tube (both Sarstedt, Nümbrecht, Germany) for liver enzyme measurements and a full blood count respectively. Fatty acid analyses of a given subject (weeks 0, 4, 6, and 8) were performed during 4 months of storage within the same analytical run. Full blood cell count and liver enzymes were analysed at the day of blood drawing using established routine methods in the clinical chemistry laboratories of the University of Munich hospital.

2.2.4 Analytical methods

2.2.4.1 Chemicals, consumables and equipment

Chemicals, consumables and equipment for fatty acid analyses as well as the preparation of the internal standard and the GC conditions are described in 2.1.4.1 and 2.1.4.5.

2.2.4.2 Fatty acid analysis in study oils

Fatty acid composition of the study oils was analysed as described in 2.1.4.2.

2.2.4.3 Fatty acid analysis in plasma lipids

Lipid extraction. Plasma lipids were extracted according to a modified Folch method [117]. One hundred µl of the internal standard solution (see 2.1.4.3), 10 ml chloroform/methanol (2+1, by vol, containing 50 mg BHT/l) and 2.5 ml distilled water were added to 250 µl plasma for total lipids, PL, TG and CE analysis. The tubes were vortexed for 15 minutes and centrifuged at 1000 *g* for 6 min. The aqueous phase was discarded and the remaining organic phase replenished with methanol up to a total volume of 10 ml. Subsequently, two extractions with 2.5 ml 2% NaCl solution were performed. The aqueous phases were discarded and the remaining organic phase containing the lipids was taken to dryness under reduced pressure.

Separation of lipid fractions. The lipid residue was dissolved in 600 µl of chloroform/methanol (1+1, by vol), of which 150 µl were directly transferred into the

reaction vial and taken to dryness under nitrogen for analyses of fatty acids in plasma total lipids. The remaining 450 µl were deposited on a TLC plate for separation of PL, free cholesterol, NEFA, TG and CE using heptane, diisopropyl ether and acetic acid (60+40+3, by vol) as mobile phase [111]. After visualisation of the lipids with 2.7-dichlorofluorescein (1% in ethanol) and UV-light, the bands with the lipid fractions of interest (PL, TG, and CE) were scraped separately from the TLC plate and transferred into 4 ml glass tubes (Figure 2.2).

Transesterification and extraction of FAME. Transesterification of plasma total lipids and lipid fractions, extraction of FAME and storage were performed as described for plasma fatty acids in the *DHAVEG study* (see 2.1.4.3).

2.2.4.4 GC conditions and calculation of fatty acid concentrations

For gas chromatography conditions see 2.1.4.5. The calculation of fatty acid concentrations (mg/l) in plasma lipid fractions (PL, CE, TG) is described in 2.1.4.6. In plasma total lipids, the area of the internal standard equalled 40 µg 15:0/250 µl plasma = 160 mg 15:0/l plasma. Fatty acid percentages (wt%) were calculated dividing the absolute concentration of an individual fatty acid by the concentration of all C14 – C24 fatty acids and multiplying the result with 100.

In addition to the fatty acid profile, the following fatty acid sums and ratios were calculated for each sample: sum of GLA and DGLA (GLA + DGLA), sum of all n-6 fatty acids (Σ-6 FA), sum of all n-3 fatty acids (Σn-3 FA), sum of all long-chain n-6 fatty acids (Σ-6 LCPUFA), sum of all long-chain n-3 fatty acids (Σ-3 LCPUFA), the ratio of n-6 to n-3 fatty acids (Σn-6/Σn-3 FA), and the ratio of n-6 LCPUFA to n-3 LCPUFA (Σn-6/Σn-3 LCPUFA).

2.2.4.5 Examination of reproducibility

Pool plasma samples were provided by the sponsor of the study and stored at -80°C. Intra-assay reproducibility was assessed by analysing 8 pool samples in the same analytical run. When analysing the study samples, inter-assay reproducibility of the used method was assessed by analysing pool plasma samples 10/11x during 7 weeks.

2.2.4.6 Blood cell count and liver enzymes

Full blood cell counts were performed in EDTA blood on an automatic analyser. Gamma-glutamyl transpeptidase, alanine aminotransferase, aspartate amino-transferase, and cholinesterase were measured in lithium heparin plasma on an automated sample processor with the appropriate reagent systems (see 2.1.4.12).

2.2.5 Data management and statistical analyses

Statistical evaluation was carried out with SPSS 12.0 for Windows (SPSS Inc., Chicago, USA). Data are presented as mean ± SD for normally distributed data (raw or transformed data) or as median with IQR in case of non-normality. For transformed data, the back-transformed mean is shown additionally.

Test for normality. Data were checked for normality by visual inspection and by Kolmogorov-Smirnov-test (with Lilliefors correction). In case of non-normality, transformation of the fatty acid data was performed to obtain a normal distribution. Parametric tests were used for normally distributed variables and nonparametric tests for variables that are not normally distributed. Exact significances were calculated for all nonparametric tests.

Between-group differences. Any differences between the groups (active treatment vs. placebo) at each time point or the impact of treatment on absolute changes (i.e. week 8 minus week 0) or relative changes from baseline (i.e. [week 8-week 0]/week 0) were tested for significance using Student's unpaired t-test for normally distributed variables or the Mann-Whitney U test for variables not normally distributed.

Within-group differences. Student's t-test for dependent samples with Bonferroni-Holm correction [118] for multiple comparisons was used to compare data from weeks 4, 6, and 8 with baseline data within each treatment group. In cases of non-normally distributed data, which could not successfully be transformed, the Wilcoxon nonparametric test with Bonferroni-Holm correction for multiple comparisons was used to compare data from week 4, 6, and 8 with baseline data within each treatment group.

General Linear Model. The overall effect of treatment on fatty acid composition was determined using the General Linear Model (GLM) for repeated measures corrected for fatty acid concentrations at baseline (week 0). The between-subjects factor had two levels ("placebo" versus "FO/EPO"), whereas the within-subjects factor "time" had three levels (weeks 4, 6, and 8).

Correlations and bivariate tabular analysis. Correlations between parameters were estimated by computing Pearson's correlation coefficient in the case of normally distributed values and the Spearman-Rho correlation coefficient in the case of other distributions respectively. For bivariate tabular analysis the chi-square test (exact calculation) was used. In cases of expected values smaller than 5, a Fisher exact test was used instead.

Level of significance. *P* values below 5% were considered significant with exception of multiple comparisons (week 4, 6 and 8 vs. week 0), in which the *p* values were corrected to < 1.67%, < 2.5%, and < 5% [118].

3 Results

3.1 DHAVEG study

3.1.1 Methods for fatty acid analysis

The GC method (2.1.4.5) enabled the separation of the following C14 – C24 fatty acids, which were identified by comparison with the peaks of a standard mixture (Figure 3.1):

Saturated fatty acids: 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 22:0, 24:0;

Trans fatty acids: 14:1t, 16:1t, 18:1t, 18:2tt, 22:1t;

Monounsaturated fatty acids: 14:1n-5, 15:1n-5, 16:1n-7, 17:1n-7, 18:1n-9, 18:1n-7, 20:1n-9, 22:1n-9, 24:1n-9;

Polyunsaturated fatty acids: 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:2n-6, 20:3n-9, 20:3n-6, 20:4n-6, 20:3n-3, 20:5n-3, 22:2n-6, 22:4n-6 (small amounts of 22:3n-3 cannot be excluded), 22:5n-6, 22:5n-3, 22:6n-3.

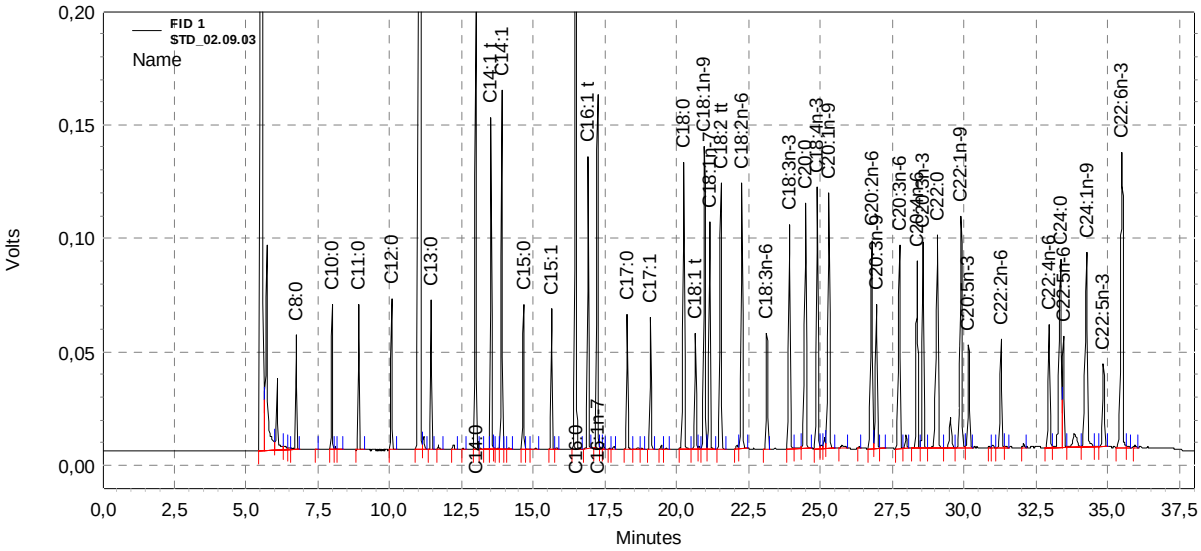

Figure 3.1. Chromatogram of the fatty acid standard mixture.

Reproducibility. In biological assays, intra-assay coefficients of variation below 5% are considered desirable. Table 3.1 and Table 3.2 show the determined coefficients of variation for selected n-6 and n-3 fatty acids in plasma PL, RBC total lipids, PE, and PC (for complete intra-assay and inter-assay CV see Table 8.4 - Table 8.6).

Table 3.1. Intra-assay CV (%) of fatty acid analyses, *DHAVEG study.*

	Plasma PL (*n* = 8)		RBC TL (*n* = 8)	RBC PE (*n* = 7)	RBC PC (*n* = 7)
	mg/l*	wt%†	wt%†	wt%†	wt%†
n-6 fatty acids					
18:2n-6	1.52	0.64	0.65	2.68	0.92
18:3n-6	8.16	8.12	2.74	3.49	n.d.
20:3n-6	1.58	0.83	1.32	0.71	1.49
20:4n-6	1.76	0.39	0.22	2.00	2.27
22:4n-6	1.85	1.33	0.44	2.77	9.49
22:5n-6	3.82	4.19	1.74	3.28	7.31
n-3 fatty acids					
18:3n-3	2.15	1.76	2.51	5.38	6.18
20:5n-3	3.02	2.29	0.93	1.85	2.07
22:5n-3	2.93	2.74	0.48	3.15	4.14
22:6n-3	4.31	4.11	0.54	3.32	3.71

n.d., not detected; PL, phospholipids; RBC, red blood cell; TL, total lipids; PE, phosphatidylethanolamine; PC, phosphatidylcholine.
* CV absolute fatty acid concentration (mg/l).
† CV relative fatty acid composition (wt%).

The performed intra- and inter-assay variation tests proved a good reproducibility of the used methods for most fatty acids. Intra-assay CV were < 5% for the described n-3 and n-6 fatty acids (mg/l and wt%) with the exception of GLA in plasma PL (CV 8%), 22:4n-6 in RBC PC (9%) and ALA in RBC PE and PC (5.4% and 6.2% respectively), but these fatty acids account for minor proportions of total fatty acids (< 0.4 wt% each). Inter-assay CV for the described n-3 and n-6 fatty acids were < 8% in plasma PL and RBC total lipids, < 7% in RBC PE and < 10% in RBC PC.

Table 3.2. Inter-assay CV (%) of fatty acid analyses, *DHAVEG study*

	Plasma PL (*n* = 11)		RBC TL (*n* = 19)	RBC PE (*n* = 14)	RBC PC (*n* = 14)
	mg/l*	wt%†	wt%†	wt%†	wt%†
n-6 fatty acids					
18:2n-6	4.70	1.79	1.17	1.30	0.94
18:3n-6	7.97	5.17	7.96	5.64	5.30
20:3n-6	5.12	2.10	0.86	1.26	2.14
20:4n-6	7.19	5.47	0.82	1.25	3.28
22:4n-6	6.22	3.82	1.04	1.80	6.11
22:5n-6	6.15	3.72	2.31	2.44	8.82
n-3 fatty acids					
18:3n-3	4.27	2.01	3.23	6.76	2.84
20:5n-3	6.74	3.92	1.99	1.48	3.99
22:5n-3	7.46	6.72	1.78	2.19	9.74
22:6n-3	5.13	2.95	2.06	1.84	6.02

PL, phospholipids; RBC, red blood cell; TL, total lipids; PE, phosphatidylethanolamine; PC, phosphatidylcholine.
* CV absolute fatty acid concentration (mg/l).
† CV relative fatty acid composition (wt%).

3.1.2 Method for vitamin A and E analysis

Linearity of calibration curves. The calculated coefficients of determination (r^2) obtained with the described method using seven vitamin concentration levels in the stated concentration range were as follows: retinol 0.102 – 1.018 mg/l (r^2 = 0.9995), alpha-tocopherol 2.26 – 22.59 mg/l (r^2 = 0.9998) (Figure 8.1).

Reproducibility. The performed intra- and inter-assay variation tests proved good reproducibilities of the used method. Table 3.3 shows the determined coefficients of variation for retinol and alpha-tocopherol: intra-assay CV were < 2% and inter-assay CV < 4% for both substances.

Table 3.3. Reproducibility of vitamin A and E analysis in plasma, *DHAVEG study.*

	Intra-assay (*n* = 8)		Inter-assay (*n* = 11)	
	Mean (mg/l)	CV (%)	Mean (mg/l)	CV (%)
Retinol	0.50	1.89	0.49	3.79
alpha-Tocopherol	7.31	1.80	9.06	3.67

Recovery from plasma. The mean recoveries of added retinol and alpha- tocopherol to plasma (n = 4) were 100% and 97% respectively.

Table 3.4. Recoveries of retinol and alpha-tocopherol from plasma, *DHAVEG study.*

	Basal plasma (n = 4)	Plasma + standard mixture (n = 4)	Expected concentration	Recovery (%)
Retinol	0.586 ± 0.007	0.756 ± 0.008	0.755	100 ± 1
alpha-Tocopherol	9.54 ± 0.21	15.03 ± 0.21	15.46	97 ± 1

Accuracy of the measurements. The results for retinol were in excellent agreement with the approved values of the NIST standard reference material (bias < 5%), whereas the results for the lower alpha-tocopherol level corresponded acceptably to the certified values. The results for the higher alpha-tocopherol level were almost 13% lower than the certified values.

Table 3.5. Accuracy of vitamin A and E measurements, *DHAVEG study.*

	Certified value (mg/l)[†]	Measured (n = 3, mg/l)[§]	Bias (mean, %)
Retinol			
Level 1	0.841 ± 0.027	0.850 ± 0.019	0.5
Level 2	0.484 ± 0.012	0.475 ± 0.007	-1.8
alpha-Tocopherol			
Level 1	7.47 ± 0.47	6.79 ± 0.13	-9.1
Level 2	16.79 ± 0.76	14.67 ± 0.07	-12.8

The bias from the assigned value was calculated as follows:
*(mean of the 3 measured samples – certified value) * 100% / certified value*.

[†] The true concentration is expected, with 95% confidence, to be in the interval defined by the certified value ± the expanded uncertainty.

[§] Mean ± SD.

3.1.3 Subjects

One hundred-fourteen free-living, apparently healthy vegetarians aged 18 to 43 years (87 female, 27 male) were included in the study and randomly assigned to one of the intervention groups. There was no difference between the groups with respect to age, BMI, blood pressure, heart rate, duration of vegetarian diet, proportion of non-smokers and gender ratio at study entry (Table 3.6).

Table 3.6. Baseline characteristics of the subjects, *DHAVEG study* (mean ± SD or median with IQR).

	DHA (*n* = 58 - 59)*	Placebo (*n* = 55)	*P*†
Female (*n*) / male subjects (*n*)	44 / 15	43 / 12	0.667
Age (years)§	24 (22 – 27)	24 (22 – 29)	0.904
BMI (kg/m^2)	21.4 ± 1.8	21.2 ± 2.0	0.532
Blood pressure (mm Hg)			
Systolic§	98 (90 – 100)	95 (90 – 100)	0.574
Diastolic§	65 (60 – 70)	70 (60 – 70)	0.737
Heart rate (beats/min)§	68 (60 – 72)	68 (64 – 72)	0.872
Years on a vegetarian diet§	8.0 (6.0 – 13.0)	8.5 (5.0 – 10.0)	0.445
Non-smokers (%)	75	80	0.512

* For one subject, blood pressure and heart rate values are missing.
§ Median with IQR in parentheses.
† Analyses of group differences.

Two of the 114 subjects recruited into the study dropped out during the intervention period. One subject in the placebo group came down with a renal colic, which was considered unrelated to the dietary supplement, and the contact to one subject of the DHA group was lost. Three individuals in the DHA group and one in the placebo group were excluded from all analyses. The reasons for exclusions were poor compliance with study protocol (*n* = 2) and diarrhoea/vomitus for more than 6 days of the intervention period (*n* = 2, one subject from each group). Thus, 108 subjects (94.7%) are included in the statistical analysis. Two subjects were additionally excluded from the statistical analyses of triglycerides and lipoproteins, because of suspected hypertriacylglycerolaemia (basal TG > 2.5 mmol/l), resulting in 106 subjects (93.0%) for these parameters.

3.1.4 Compliance and side effects

The median number of days in the study was 56 days (range: 56 – 60 days) in both groups. Compliance as judged by capsule count was 98% (mean) in the DHA group and 99% in the placebo group, with no significant between-group difference (Table 3.7).

Side effects were reported in the DHA group by 11% and in the placebo group by 8% of the subjects (Table 3.8) including gastrointestinal upsets (flatulence, pain, diarrhoea, belching) and minor skin reactions (acne). Side effects were evenly distributed between DHA and placebo groups.

Table 3.7. Compliance, *DHAVEG study* (% of prescribed capsules taken).*

	DHA (*n* = 55)	Placebo (*n* = 53)
Mean ± SD (%)	98 ± 2	99 ± 2
Median with IQR (%)	100 (97 – 100)	100 (98 – 100)
100% compliance (number and %)	28 (51%)	32 (60%)

* No difference in compliance was observed between DHA and placebo groups ($p = 0.441$).

Table 3.8. Reported side effects, *DHAVEG study* (number and %).*

	DHA (*n* = 55)	Placebo (*n* = 53)
Total side effects	6 (11%)	4 (8%)
Skin reactions	-	1 (2%)
Flatulence	3 (5%)	2 (4%)
Stomach ache	2 (4%)	1 (2%)
Diarrhoea	-	2 (4%)
Belching	2 (4%)	1 (2%)

* Side effects were evenly distributed between DHA and placebo groups ($p > 0.05$).

3.1.5 BMI, blood pressure and heart rate

Body weight, BMI, blood pressure and heart rate did not differ between DHA and placebo groups at week 0 and week 8 (Table 8.11). An examination of within-group changes demonstrated slight but significant increases in body weight and BMI after 8 weeks of intervention in the placebo group, but no changes in the DHA group. Changes from baseline in body weight and BMI tended to be different between DHA and placebo groups ($p = 0.072$ and $p = 0.056$ respectively). In both groups, slight increases of systolic blood pressure were observed (not significant in the DHA group: $p = 0.066$); the change from baseline in systolic blood pressure was not different between the groups. Diastolic blood pressure and heart rate did not change during intervention.

3.1.6 Dietary intake

The proportion of macronutrients and the intakes of energy, alcohol, cholesterol, and n-3 LCPUFA with the background diet (not including supplements for placebo and DHA group) were not different between the two groups before the intervention and did not change (with the exception of energy intake in the DHA group) in both groups during intervention (Table 3.9).

Energy intake in the DHA group was significantly lower during intervention compared to baseline and tended to be reduced at this time point compared to placebo (p = 0.079). Dietary fibre intake was comparably high between both groups before intervention; during intervention it was significantly lower in the DHA than in the placebo group. Baseline DHA intakes were 23 mg/d (median) in the DHA group and 20 mg/d in the placebo group. Before intervention, DHA and placebo groups differed in dietary LA intake (% of energy) (medians: 3.3% vs. 5.0%) and LA:ALA ratios (median: 6.6:1 vs. 7.9:1). Dietary LA intake and LA:ALA ratio decreased in the placebo group during the study, resulting in values comparable to the DHA group at study end.

Table 3.9. Dietary intake with the background diet before and during intervention, *DHAVEG study* (mean ± SD or median with IQR).

	DHA group (n = 55)		Placebo group (n = 53)	
	Before intervention	During intervention	Before intervention	During intervention
Energy (MJ)[§]	8.8 (7.2 – 10.3)	7.7 (6.5 – 9.2) [1]	8.8 (7.0 – 10.1)	8.1 (7.0 – 10.9)
Protein (% of energy)[§]	13.0 (10.9 – 15.2)	12.4 (11.1 – 15.2)	13.2 (10.8 – 14.7)	12.5 (11.0 – 14.2)
Total fat (% of energy)[§]	28.9 (23.1 – 32.9)	28.5 (24.1 – 31.9)	31.0 (24.1 – 38.4)	29.8 (24.6 – 35.0)
Carbohydrates (% of energy)	54.2 ± 1.1	54.3 ± 1.0	51.6 ± 1.2	52.9 ± 1.1
Fibre (g)[§]	28.1 (21.1 – 35.9)	24.8 (19.5 – 35.9)[a]	30.5 (22.6 – 44.8)	30.1 (24.3 – 41.3)
Alcohol (g)[§]	2.0 (0.2 – 9.2)	1.3 (0.1 – 10.9)	2.0 (0.1 – 10.9)	5.1 (0.1 – 11.1)
Cholesterol (mg)[§]	150 (84 – 231)	137 (88 – 200)	146 (76 – 208)	143 (73 – 256)
LA (% of energy)[§]	3.3 (2.5 – 4.6)[a]	3.3 (2.6 – 5.2)	5.0 (3.3 – 7.3)	3.5 (2.5 – 6.2) [1]
ALA (% of energy)[§]	0.51 (0.42 – 0.79)	0.52 (0.42 – 0.72)	0.57 (0.42 – 0.87)	0.52 (0.42 – 0.81)
LA:ALA ratio[§]	6.6 (4.5 – 8.6)[a]	6.7 (4.7 – 9.1)	7.9 (6.1 – 10.4)	6.1 (5.2 – 9.0) [1]
EPA (mg)[§]	0.0 (0.0 – 3.3)	0.0 (0.0 – 3.3)	0.0 (0.0 – 3.3)	0.0 (0.0 – 3.3)
DHA (mg)[§]	23.3 (6.7 – 53.3)	16.7 (6.7 – 36.7)	20.0 (3.3 – 40.0)	20.0 (5.0 – 45.0)

ALA, alpha-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid.

[§] Median with IQR in parentheses.

[1] $p < 0.05$ vs. week 0;

[a] $p < 0.05$ vs. placebo at the same time point.

Spearman-Rho correlation coefficients between dietary LA intake (g/d) and LA (wt%) in RBC total lipids, RBC PE, RBC PC, and plasma PL at baseline were 0.454, 0.470, 0.391 and 0.318 respectively (*p* always < 0.01).

3.1.7 Plasma triglycerides and lipoproteins

Measured lipid metabolism parameters were not different between the two groups at study entry and did not change in the placebo group (Figure 3.4, Table 8.12). A significant 20% decrease in plasma TG concentrations from 0.96 to 0.77 mmol/l (medians) was observed in the DHA group. Absolute TG changes from baseline were significantly different between DHA (-0.15 mmol/l, median) and placebo groups (-0.02 mmol/l). After DHA supplementation, observed TG concentrations did not exceed 1.5 mmol/l (Figure 3.2, •), whereas after placebo intervention, the values were distributed beyond 2.5 mmol/l (o). The number of subjects with plasma TG levels > 1.5 mmol/l did not differ between DHA and placebo group at week 0 (17% vs. 15%), but after the intervention, the frequency was significantly lower in the DHA supplemented group compared to placebo (0% vs. 21%).

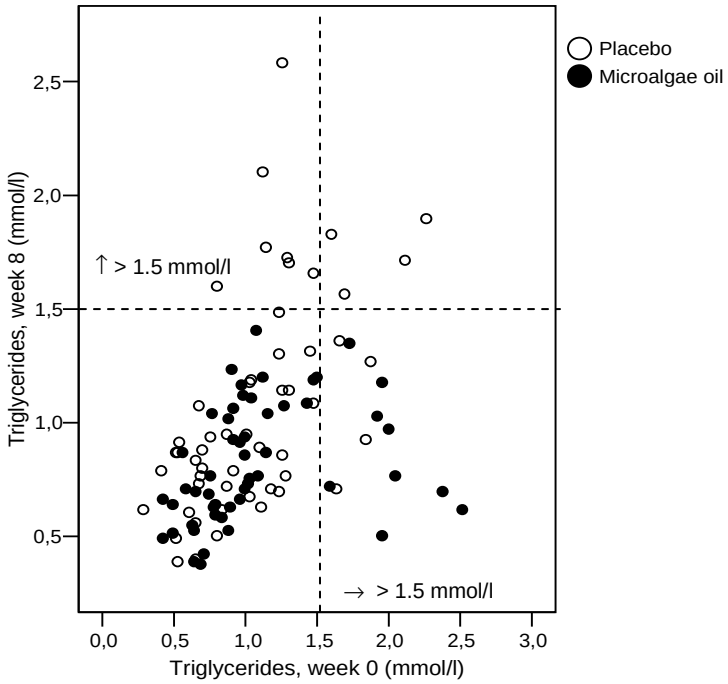

Figure 3.2. Plasma triglycerides at week 0 and week 8, *DHAVEG study* (*n* = 53 in both groups).

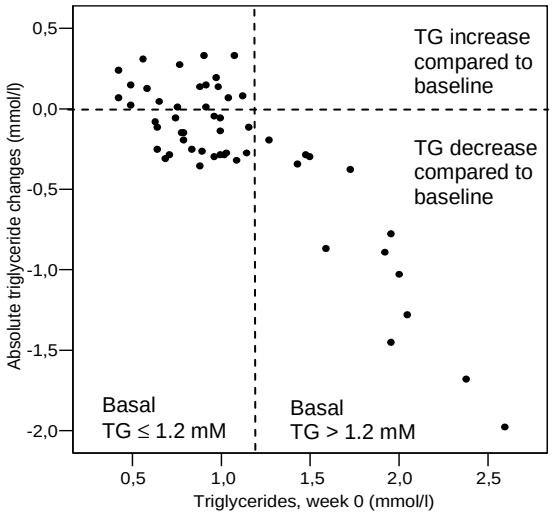

Figure 3.3. Plasma triglyceride changes after DHA supplementation according to baseline triglyceride levels, *DHAVEG study* (*n* = 53).

Figure 3.3 shows that in subjects with basal TG levels ≤ 1.2 mmol/l, DHA supplementation had no influence on TG levels (*n* = 40; mean TG levels 0.79 mmol/l at week 8 vs. 0.83 mmol/l at baseline; *p* = 0.161), but that in subjects with basal TG levels > 1.2 mmol/l, DHA decreased TG consistently (*n* = 13; mean TG levels 0.95 mmol/l at week 8 vs. 1.83 mmol/l at baseline; *p* < 0.001).

Plasma total, LDL and HDL cholesterol increased significantly in the DHA group; the changes from baseline in these parameters were significantly different between DHA and placebo groups (Figure 3.4, Table 8.12). We found a slight, negative correlation between absolute changes in TG and in HDL concentrations after DHA supplementation (*r* = -0.307, *p* = 0.025). In the DHA group, there were no correlations between individual DHA changes in plasma/RBC and changes in plasma TG, total, LDL or HDL cholesterol concentrations.

The ratio of TG to HDL cholesterol was significantly reduced from 0.57 to 0.43 (medians) in the DHA group and remained unchanged in the placebo group; these changes from baseline were significantly different between the two groups. Total cholesterol:HDL cholesterol and LDL cholesterol:HDL cholesterol ratios were not affected by intervention (Table 8.12).

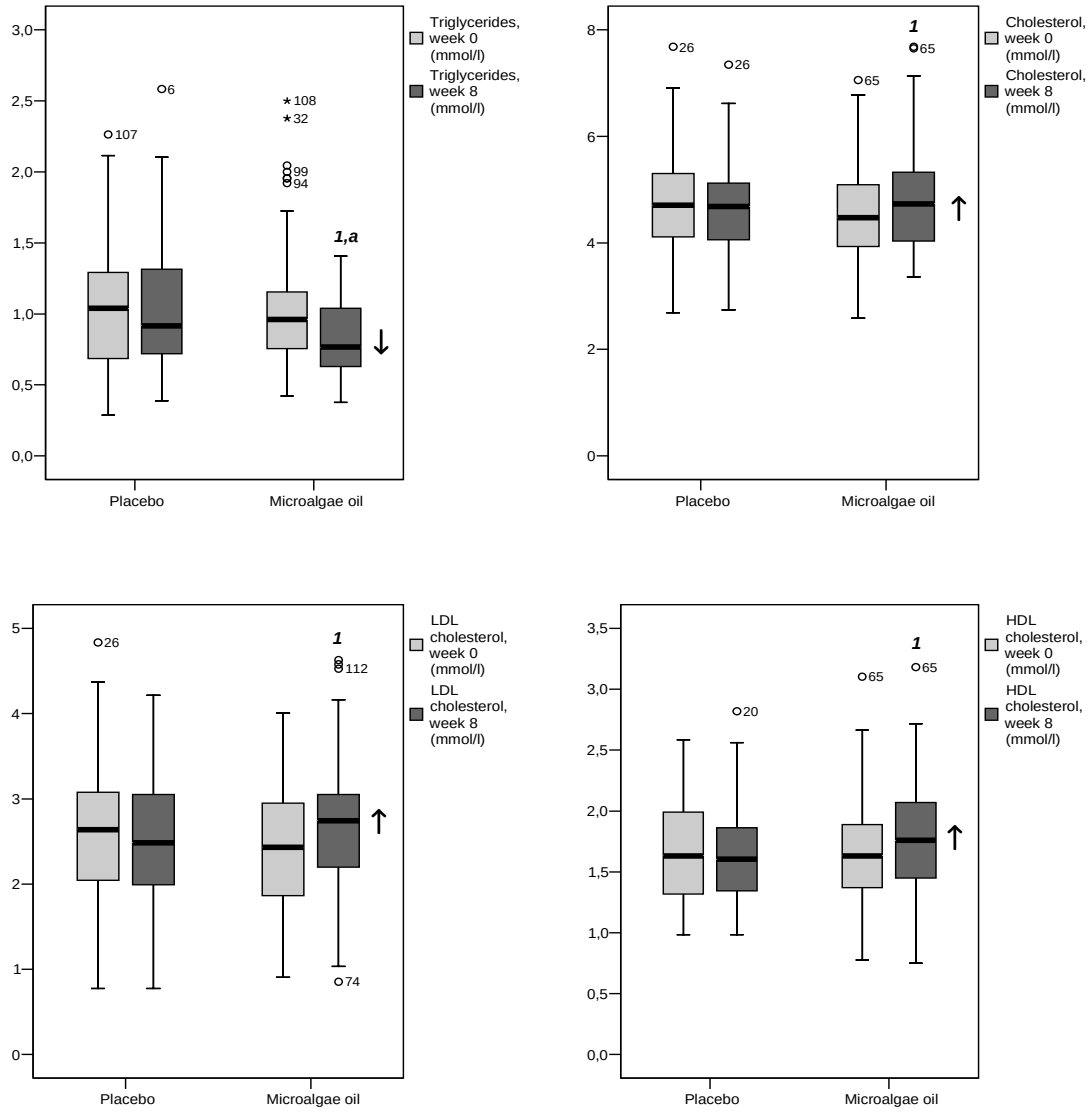

Figure 3.4. Plasma triglycerides and lipoproteins at week 0 and week 8, *DHAVEG study* (*n* = 53 in both groups).

Bottom and top edges are located at 25th and 75th percentiles, centre horizontal line is drawn at the median, whiskers mark the data points nearest to the 1.5 interquartile ranges, outliers are shown as points together with subject code.

[1] $p < 0.05$ vs. week 0; [a] $p < 0.05$ vs. placebo at the same time point.

3.1.8 Vitamin A and E

Absolute retinol and alpha-tocopherol levels as well as lipid-adjusted alpha-tocopherol levels were not different between the two groups at study entry and did not change in both groups (Table 8.13). Retinol changes showed greater increases from baseline values with microalgae oil than with placebo; changes in absolute and lipid-adjusted alpha-tocopherol levels were not different between DHA and placebo groups.

3.1.9 Plasma haemostatic factors

All measured haemostatic parameters were not different between the two groups at week 0 and week 8 (Table 8.14). Minor changes from baseline with no significant group differences were observed for fibrinogen, PTT, and PAI-1 as well as ADP-induced platelet aggregation time. In the DHA group, a reduction of von Willebrand factor was observed and changes from baseline in this haemostatic factor were significantly different between the two intervention groups.

With the exception of von Willebrand factor, incidence of haemostatic factors out of the laboratory's reference range did not differ neither at baseline nor at week 8 between DHA and placebo groups and within-group incidences did not differ between baseline and week 8 (Table 3.10). Concerning von Willebrand factor, we observed values out of reference range less often after DHA supplementation than at baseline (1 vs. 12 subjects), and incidence of such values was lower in the DHA group than in the placebo group at week 8 (1 vs. 11 subjects).

Table 3.10. Haemostatic factors out of laboratory's reference range, *DHAVEG study* (number).

		DHA			Placebo		
	Reference range[†]	*n**	Week 0	Week 8	*n**	Week 0	Week 8
Quick's test (%)	> 70	53	0	0	52	1	1
PTT (sec)	25 - 42	53	0	0	52	2	0
Fibrinogen (mg/dl)	160 - 400	54	3	4	52	8	7
D-dimers (µg/ml)	< 0.5	55	5	3	53	4	2
Factor VII (%)	50 - 130	55	10	9	53	13	9
Von Willebrand factor (%)	50 - 130	55	12	1[1,a]	53	11	11
PAI-1 (AU/ml)	< 15	55	6	3	53	2	2
PFA-ADP	< 110	43	8	9	40	8	9
PFA-EPI	< 160	42	4	4	34	3	6

PTT, partial thromboplastin time; PAI-1, plasminogen activator inhibitor-1 activity; PFA-ADP, platelet function analysis with adenosine diphosphate; PFA-EPI, platelet function analysis with epinephrine.

[†] Reference range as specified from the clinical chemistry laboratories of the University of Munich hospital.

* Some samples are missing because of technical problems during the measurements or coagulation of the blood samples.

[1] $p < 0.05$ vs. week 0; [a] $p < 0.05$ vs. placebo at the same time point.

3.1.10 Full blood cell count and biochemical parameters

Parameters of haematology and blood chemistry were not different between the two groups at week 0 and week 8 with the exception of bilirubin levels at baseline, which were slightly, but significantly higher in the DHA than in the placebo group (0.7 vs. 0.6 mg/dl, medians) (Table 8.15).

Small changes from baseline within the normal ranges were observed in uric acid, total protein, leucocytes, haemoglobin and MCH with no significant group differences. ALT levels increased from 13.0 to 16.0 U/l (medians) and CHE levels decreased from 7.5 to 7.2 kU/l (medians) with DHA supplementation; changes from baseline were significantly different between the two intervention groups. RBC count decreased in both groups compared to baseline, but reduction was significantly higher in the placebo group. The haematocrit value decreased with placebo treatment and changes from baseline were significantly different between the two groups.

All observed changes in haematology and biochemical parameters were minor and within the normal ranges. Incidence of liver and cardiac enzymes out of the laboratory's reference range did not differ neither at baseline nor at week 8 between DHA and placebo groups; within-group incidences did not differ between baseline and week 8 (Table 3.11).

Table 3.11. Liver and cardiac enzymes out of laboratory's reference range, *DHAVEG study* (number).

		DHA			Placebo		
	Reference range[†] (male / female)	*n**	Week 0	Week 8	*n**	Week 0	Week 8
GGT (U/l)	< 55 / < 38	55	2	2	53	1	0
ALT (U/l)	< 45 / < 35	55	1	2	53	0	1
AST (U/l)	< 40 / < 33	55	0	1	53	2	2
CHE (kU/l)	5.00 – 13.30	54	2	3	51	4	5
CK (U/l)	< 180 / < 155	55	4	5	53	5	8
LDH (U/l)	< 250	54	0	0	53	0	0

GGT, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHE, cholinesterase; CK, creatinekinase; LDH, lactate dehydrogenase.

[†] Reference range as specified from the clinical chemistry laboratories of the University of Munich hospital.

* Some samples are missing because of technical problems during the measurements.

3.1.11 Fatty acids in plasma and RBC

Fatty acid composition of RBC and plasma lipids was not different between groups at baseline (with the exception of Σn-3 LCPUFA in plasma PL) and changed negligibly in the placebo group (Table 8.16 - Table 8.19). After DHA supplementation, no changes or little increases were observed in saturated fatty acids 16:0 and 18:0. Microalgae oil supplementation resulted in significant increases of n-6 DPA, EPA, and DHA levels and significant decreases of 18:1n-9, 20:3n-9, 18:2n-6, 18:3n-6, 20:3n-6, AA, 22:4n-6 and n-3 DPA levels in all measured fractions relative to baseline values (Figure 3.5).

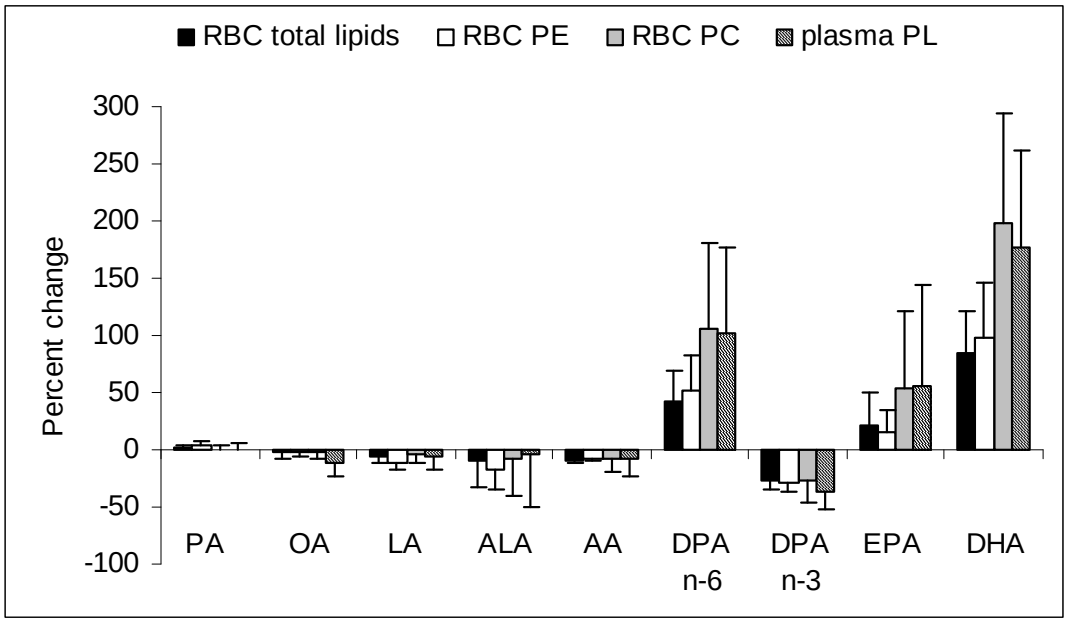

Figure 3.5. Relative changes of selected fatty acids in RBC total lipids, RBC PE, RBC PC and plasma PL in the DHA group compared to baseline values, *DHAVEG study* (*n* = 52 in RBC total lipids, otherwise *n* = 55).

Values were calculated as individual's percent change in each lipid fraction and are reported as mean +/- SD.
PA, 16:0; OA, 18:1n-9; LA, 18:2n-6; ALA, 18:3n-3; AA, 20:4n-6; DPA n-6, 22:5n-6; DPA n-3, 22:5n-3; EPA, 20:5n-3; DHA, 22:6n-3.

Relative to placebo, DHA supplementation resulted in significantly higher contents of 16:0 (not significant in RBC PC), n-6 DPA, EPA (not significant in RBC PE) and DHA, and lower contents of 18:1n-9 (not significant in RBC PE), 20:3n-9, 18:2n-6, 18:3n-6, 20:3n-6, AA, 22:4n-6, 18:3n-3 (not significant in plasma PL and RBC PC) and n-3 DPA. After DHA supplementation, the highest relative fatty acid changes were observed in DHA, n-6 DPA, and EPA in all measured lipid classes (Figure 3.5). The

degree of these changes was different in the various lipid classes with higher percentage changes in RBC PC and plasma PL than in RBC total lipids and RBC PE for DHA, n-6 DPA and EPA.

The sums of n-6 FA and n-6 LCPUFA decreased and Σn-3 FA and Σn-3 LCPUFA increased significantly compared to baseline values in all measured plasma and RBC lipids after 8 weeks of DHA supplementation resulting in remarkable decreases of Σn-6/Σn-3 FA and Σn-6/Σn-3 LCPUFA ratios. At week 8, Σn-6 FA, Σn-6 LCPUFA as well as the ratios of Σn-6/Σn-3 FA and Σn-6/Σn-3 LCPUFA were significantly lower and Σn-3 FA and Σn-3 LCPUFA were significantly higher in the DHA than in the placebo group.

Statistically significant increases in EPA + DHA levels versus baseline values were observed after 8 weeks of microalgae oil supplementation in all investigated lipid fractions. The omega-3 index rose significantly from 4.8 ± 1.2 wt% to 8.4 ± 1.3 wt% (mean ± SD) in the DHA supplemented group, ranging after 8 weeks from 4.7 wt% to 11.0 wt%. Relative to placebo application, supplementation with DHA-rich microalgae oil resulted in significantly higher contents of EPA + DHA in all tested lipid fractions. At baseline, 29% of the DHA group and 33% of the placebo group volunteers had an omega-3 index ≤ 4% and no one reached a desirable omega-3 index ≥ 8% (Figure 3.6). After 8 weeks of intervention, an omega-3 index ≤ 4% did not occur anymore in the DHA group but remained in 39% of the placebo group subjects ($p < 0.001$). 69% of the DHA supplemented subjects had an omega-3 index ≥ 8%, while no subject of the placebo group showed an omega-3 index above 8% ($p < 0.001$).

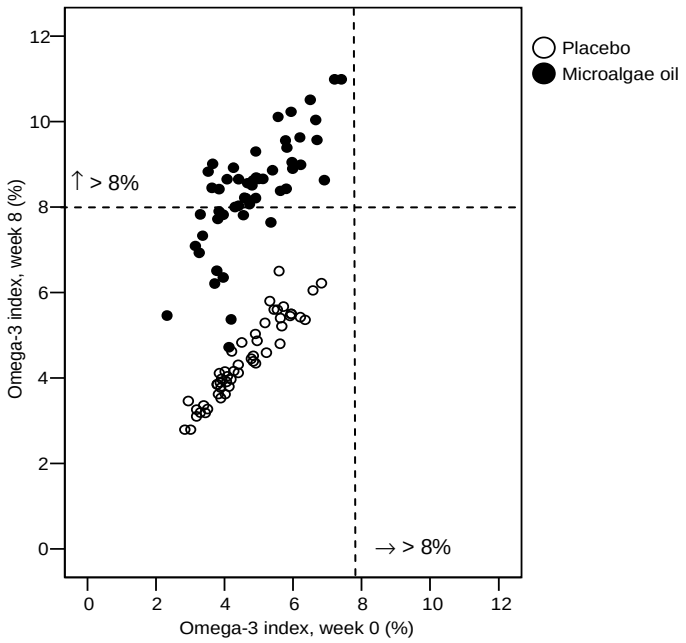

Figure 3.6. Omega-3 index (%) at week 0 and week 8, *DHAVEG study* (Placebo: *n* = 51; Microalgae oil: *n* = 52).

3.1.12 Correlations between relative fatty acid concentrations

Spearman-Rho correlation coefficients were computed between the relative fatty acid levels in RBC total lipids and fatty acid proportions in RBC PE, RBC PC, and plasma PL for all subjects (Table 8.20). Relative fatty acid content of selected fatty acids in RBC total lipids correlated significantly with the corresponding fatty acid content in the other lipid classes (plasma PL, RBC PC and RBC PE) (*r* always > 0.49). RBC total lipid DHA and RBC total lipid EPA + DHA consistently showed the strongest correlations with the other lipid fractions at both time points (*r* always ≥ 0.90).

At baseline, correlation coefficients between omega-3 index and EPA + DHA from RBC PE, RBC PC and plasma PL were almost identical in DHA and placebo groups (Figure 8.2). After the intervention, correlation coefficients remained similar to baseline in the placebo group, but became lower in the DHA group.

The correlations between EPA + DHA levels at week 0 and those at week 8 were lower in the DHA than in the placebo group in all tested lipid fractions (in the placebo group *r* = 0.841 - 0.970; in the DHA group *r* = 0.464 - 0.856, *p* always < 0.001).

3.1.13 Correlations between percentage fatty acid changes

In the DHA group, Spearman-Rho correlations were computed between relative fatty acid changes from baseline values in RBC total lipid / plasma PL and percentage changes in the other lipid fractions for LA, AA, n-6 DPA, ALA, EPA, n-3 DPA, DHA, and EPA + DHA (Table 8.21). Relative changes in RBC total lipid n-6 and n-3 fatty acids correlated significantly and positively with changes in the other lipid classes (*r* always > 0.48); here, the correlations were highest for n-6 DPA, EPA, DHA, and EPA + DHA (*r* always > 0.75). Relative changes in RBC PE correlated better with RBC total lipid than with plasma PL changes for all tested fatty acids. Relative changes in plasma PL correlated best with changes in RBC PC for all tested n-6 and n-3 fatty acids with exception of ALA.

3.2 FO/EPO study

3.2.1 Method for fatty acid analysis

Reproducibility. The GC method (2.1.4.5) enabled the separation and identification of the in 3.1.1 described fatty acids. The used method for determination of fatty acids in plasma total lipids and lipid fractions showed good intra-assay and inter-assay reproducibilities. Table 3.12 and Table 3.13 describe the determined coefficients of variation for selected n-6 and n-3 fatty acids (for complete intra-assay and inter-assay CV see Table 8.7 - Table 8.10).

Table 3.12. Intra-assay CV (%) of fatty acid analyses, *FO/EPO study.*

	Plasma PL (*n* = 8)		Plasma CE (*n* = 8)		Plasma TG (*n* = 8)		Plasma TL (*n* = 8)	
	mg/l*	wt%†	mg/l*	wt%†	mg/l*	wt%†	mg/l*	wt%†
n-6 fatty acids								
18:2n-6	0.70	0.35	1.22	0.08	1.38	0.28	1.31	0.98
18:3n-6	2.46	2.78	1.17	0.33	1.88	1.24	1.85	1.55
20:3n-6	0.74	0.69	1.49	0.41	1.37	0.74	2.06	1.76
20:4n-6	1.21	1.12	1.37	0.38	1.35	0.22	1.43	0.90
22:4n-6	0.88	1.15	n.d.	n.d.	3.29	2.36	1.97	1.50
22:5n-6	1.49	1.44	6.37	5.57	2.20	1.24	2.04	1.86
n-3 fatty acids								
18:3n-3	0.96	1.15	1.54	0.58	1.76	1.16	1.17	0.98
20:5n-3	1.27	1.71	1.56	0.62	3.16	2.90	1.71	1.09
22:5n-3	1.39	1.76	n.d.	n.d.	2.06	2.02	2.16	1.84
22:6n-3	1.62	1.85	2.21	0.46	1.82	1.44	2.21	1.90

n.d., not detected, PL, phospholipids; CE, cholesterol esters; TG, triglycerides; TL, total lipids.

* CV absolute fatty acid concentration (mg/l).

† CV relative fatty acid composition (wt%).

Intra-assay CV were < 4% for the described n-3 and n-6 fatty acids (mg/l and wt%) with the exception of 22:5n-6 in plasma CE (CV < 7%), but this fatty acid accounted only for a minor proportion of total fatty acids in plasma CE (0.05 wt%, mean). Inter-assay CV for the described n-3 and n-6 fatty acids were < 5% in plasma PL and <6% in plasma CE, TG and TL (exception: 22:5n-6 in plasma CE with an inter-assay CV <10%).

Table 3.13. Inter-assay CV (%) of fatty acid analyses, *FO/EPO study.*

	Plasma PL (*n* = 11)		Plasma CE (*n* = 10)		Plasma TG (*n* = 10)		Plasma TL (*n* = 10)	
	mg/l*	wt%†	mg/l*	wt%†	mg/l*	wt%†	mg/l*	wt%†
n-6 fatty acids								
18:2n-6	3.22	0.71	3.37	0.43	2.87	0.45	3.35	1.22
18:3n-6	4.55	2.85	3.53	2.23	3.47	2.38	3.55	1.96
20:3n-6	3.52	1.29	4.90	2.88	4.88	3.38	4.06	3.12
20:4n-6	3.80	1.53	3.97	1.37	4.07	2.56	3.32	1.68
22:4n-6	4.36	2.52	n.d.	n.d.	5.30	4.44	4.48	3.06
22:5n-6	3.39	2.26	9.26	7.58	5.37	4.01	5.23	4.67
n-3 fatty acids								
18:3n-3	4.07	2.50	3.88	1.45	2.84	2.92	3.16	0.88
20:5n-3	3.92	1.73	5.18	4.18	4.14	3.70	3.85	2.81
22:5n-3	4.86	2.81	n.d.	n.d.	5.23	4.06	4.24	3.29
22:6n-3	4.91	2.97	5.66	4.42	5.13	3.91	3.81	2.57

n.d., not detected, PL, phospholipids; CE, cholesterol esters; TG, triglycerides; TL, total lipids.

* CV absolute fatty acid concentration (mg/l).
† CV relative fatty acid composition (wt%).

3.2.2 Subjects

Forty free-living, apparently healthy, non-pregnant women aged 19 to 36 years were included in the study and randomly assigned to one of the intervention groups. There were no significant differences in subject characteristics between the two intervention groups at study entry (Table 3.14).

Table 3.14. Baseline characteristics of the subjects, *FO/EPO study* (mean ± SD).

	FO/EPO (*n* = 20)	Placebo (*n* = 20)	*P*†
Age (years)	24.6 ± 4.2	24.8 ± 2.9	0.863
BMI (kg/m^2)	21.8 ± 1.9	21.9 ± 2.0	0.823
Blood pressure (mm Hg)			
Systolic	118 ± 13	119 ± 11	0.743
Diastolic	74 ± 7	73 ± 8	0.741
Heart rate (beats/min)	76 ± 13	74 ± 14	0.767
Non-smokers (%)	90	70	0.235
Use of oral contraceptives (%)	60	60	1.000

† Analyses of group differences.

One subject from the FO/EPO group dropped out during the first 4 weeks of the intervention period, because she came down with an abdominal influenza and could not take the capsules for more than 7 days. Thus, 39 subjects (97.5%) were included in the statistical analysis.

3.2.3 Compliance, side effects and success of blinding

Compliance as judged by capsule count was 98% (mean) for both groups with no significant group difference (Table 3.15).

Table 3.15. Compliance, *FO/EPO study* (% of prescribed capsules taken).*

	FO/EPO (*n* = 19)	Placebo (*n* = 20)
Mean ± SD (%)	98 ± 2	98 ± 3
Median with IQR (%)	99 (98 – 100)	99 (97 – 99)
100% compliance (number and %)	5 (26%)	3 (15%)

IQR, interquartile range.

* No difference in compliance was observed between FO/EPO and placebo groups ($p = 0.533$).

Three subjects from each group reported mild adverse effects including gastrointestinal upsets (indigestion, belching), minor skin reactions (acne) and prolonged bleeding time (Table 3.16). Side effects were evenly distributed between FO/EPO and placebo groups. Group assignment was guessed correctly in the FO/EPO group by 63% and in the placebo group by 50% of the subjects with no significant group difference ($p = 0.523$).

Table 3.16. Reported side effects, *FO/EPO study* (number).*

	FO/EPO (*n* = 19)	Placebo (*n* = 20)
Total side effects	3	3
Skin reactions	1	1
Prolonged bleeding time	-	1
Indigestion	1	-
Diarrhoea	-	1
Belching	1	-

* Side effects were evenly distributed between FO/EPO and placebo groups ($p > 0.05$).

3.2.4 BMI, blood pressure and heart rate

Body weight, BMI, blood pressure and heart rate did not differ between FO/EPO and placebo groups at week 0 and week 8 and absolute changes from baseline after 8 weeks of intervention were not significantly different between the two groups (Table 8.22). The diastolic blood pressure decreased slightly after 8 weeks of FO/EPO supplementation compared to baseline ($p = 0.046$), but changes from baseline were not significantly different between the groups.

3.2.5 Full blood cell count and liver enzymes

Liver enzymes and haematological parameters did not differ between FO/EPO and placebo groups at week 0 and week 8 with the exception of platelet counts at both time points ($p < 0.05$) and cholinesterase (CHE) levels at baseline (trend: $p = 0.053$, Table 8.23). Small changes from baseline within the normal ranges were observed in MCV and MCHC with no significant group differences. CHE levels were significantly increased after 8 weeks of placebo intervention and changes from baseline were significantly different between the two groups. The haematocrit value decreased with FO/EPO supplementation compared to baseline and changes from baseline were significantly different between FO/EPO and placebo groups. Changes from baseline in haemoglobin levels showed a greater reduction with FO/EPO treatment compared to placebo.

All observed changes in haematology and liver enzymes were minor and within the normal ranges. Incidence of liver enzymes and haematological parameters out of the laboratory's reference range did not differ neither at baseline nor at week 8 between FO/EPO and placebo groups; within-group incidences did not differ between baseline and week 8 (Table 3.17).

Table 3.17. Liver enzymes and haematological parameters out of reference range, *FO/EPO study* (number).

		FO/EPO (*n* = 19)		Placebo (*n* = 20)	
	Reference range*	Week 0	Week 8	Week 0	Week 8
GGT (U/l)	< 38	0	0	0	0
ALT (U/l)	< 35	2	1	1	0
AST (U/l)	< 33	0	1	2	0
CHE (kU/l)	5.0 - 13.3	3	3	7	5
Blood cell count					
Leucocytes (G/l)	4.0 - 11.0	0	0	0	0
Erythrocytes (T/l)	4.1 - 5.1	1	2	1	1
Haemoglobin (g/dl)	12.0 - 15.5	1	0	0	1
Haematocrit (%)	36.0 - 46.0	1	0	0	1
MCV (fl)	80.0 - 96.0	1	3	2	2
MCH (pg)	28.0 - 33.0	2	3	0	1
MCHC (%)	32.0 - 36.0	3	0	1	2
Platelets (G/l)	150 - 400	0	1	0	1

GGT, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHE, cholinesterase; MCV, mean cellular volume; MCH, mean cellular haemoglobin; MCHC, mean cellular haemoglobin concentration; G = Giga, 10^9; T = Tera, 10^{12}.

* Reference range as specified from the clinical chemistry laboratories of the University of Munich hospital.

3.2.6 Relative fatty acid composition

The GLM could not be performed for the following fatty acids, sums and ratios, because the data could not successfully be transformed into a normal distribution: EPA in TL and PL, DHA in PL and TG, Σn-6 FA in PL, Σn-3 FA in CE, Σn-6 LCPUFA in PL, and Σn-6/Σn-3 LCPUFA in TL (Table 3.18).

The GLM for repeated measures (weeks 4, 6, and 8) corrected for fatty acid percentages at baseline (week 0) revealed significant increasing effects of FO/EPO treatment compared to placebo on the following fatty acids, sums and ratios: GLA, DGLA, AA (in plasma TG), adrenic acid (in plasma TG), EPA, DHA, GLA + DGLA, Σn-3 FA, Σn-6 LCPUFA, and Σn-3 LCPUFA (Table 3.18, ↑).

The GLM for repeated measures corrected for fatty acid percentages at baseline indicated significant decreasing effects of FO/EPO treatment compared to placebo on the following fatty acids, sums and ratios: Mead acid (MA), LA (in plasma PL), adrenic acid (in plasma PL), n-6 DPA (in plasma TL, PL, and CE), ALA, n-3 DPA (in plasma PL), Σn-6/Σn-3 FA, and Σn-6/Σn-3 LCPUFA (↓).

The GLM for repeated measures indicated no different effects of FO/EPO and placebo treatment on LA (in plasma TL, CE, and TG), AA (in plasma TL, PL, and CE), adrenic acid (plasma TL), n-6 DPA (in plasma TG), n-3 DPA (in plasma TL and TG), and Σn-6 FA.

Table 3.18. Effects of treatment on fatty acid proportions, sums, and ratios, *FO/EPO study.**

	Total lipids	Phospholipids	Cholesterol esters	Triglycerides
20:3n-9	↓	↓	↓	↓
18:2n-6	=	↓	=	=
18:3n-6	↑	↑	↑	↑
20:3n-6	↑	↑	↑	↑
20:4n-6	=	=	=	↑
22:4n-6	=	↓	n.d.	↑
22:5n-6	↓	↓	↓	=
18:3n-3	↓	↓	↓	↓
20:5n-3	NN	NN	↑	↑
22:5n-3	=	↓	n.d.	=
22:6n-3	↑	NN	↑	NN
GLA + DGLA	↑	↑	↑	↑
∑n-6 FA	=	NN	=	=
∑n-3 FA	↑	↑	NN	↑
∑n-6 LCPUFA	↑	NN	↑	↑
∑n-3 LCPUFA	↑	↑	↑	↑
∑n-6/∑n-3 FA	↓	↓	↓	↓
∑n-6/∑n-3 LCPUFA	NN	↓	↓	↓

GLA, gamma-linolenic acid; DGLA, dihomo-gamma-linolenic acid; ∑n-6 FA, sum of n-6 fatty acids; ∑n-3 FA, sum of n-3 fatty acids; ∑n-6 LCPUFA, sum of all long-chain n-6 fatty acids; ∑n-3 LCPUFA, sum of all long-chain n-3 LCPUFA; ∑n-6/∑n-3 FA, ratio of n-6 to n-3 fatty acids; ∑n-6/∑n-3 LCPUFA, ratio of n-6 to n-3 long-chain polyunsaturated fatty acids; n.d., not detected; NN, non-normally distributed (no GLM could be performed); ↓, decrease compared to placebo; ↑, increase compared to placebo; =, same effect as placebo.

* Indicated by GLM.

The effects of treatment were further described by comparing data from weeks 4, 6, and 8 with baseline data within each group and by determining differences among treatment groups at each time point (Table 8.24 - Table 8.31 and Figure 8.3 - Figure 8.7). The obtained significances are summarised for fatty acids of main interest in Table 3.19.

Table 3.19. Plasma fatty acids (wt%): differences from baseline at weeks 4, 6, and 8 within each group or differences between treatments at the same time point, *FO/EPO study*.

		FO/EPO versus baseline			Placebo versus baseline			FO/EPO versus placebo			
		Week 4	Week 6	Week 8	Week 4	Week 6	Week 8	Week 0	Week 4	Week 6	Week 8
GLA	TL	↑	↑	↑	ns	ns	ns	ns	**aaa**	**aa**	**aaa**
	PL	↑	ns	↑	ns	ns	ns	ns	**aaa**	ns	**aaa**
	CE	↑	↑	↑	ns	ns	ns	ns	**aaa**	**aa**	**aaa**
	TG	↑	↑	↑	ns	ns	ns	ns	**aaa**	**a**	**a**
DGLA	TL	↑	↑	↑	ns	ns	ns	**a**	**aa**	**aa**	**aaa**
	PL	↑	↑	↑	ns	ns	ns	**a**	**aa**	**aa**	**aaa**
	CE	↑	↑	↑	ns	ns	ns	**a**	**aa**	**aa**	**aaa**
	TG	↑	↑	↑	ns	ns	ns	ns	**aaa**	**aaa**	**aaa**
AA	TL	ns	ns	ns	ns	↓	↓	ns	ns	ns	ns
	PL	ns	ns	ns	ns	↓	↓	ns	ns	ns	ns
	CE	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	TG	↑	ns	ns	ns	ns	ns	**a**	ns	ns	ns
EPA	TL	↑	↑	↑	ns	ns	↑	ns	**aa**	**aa**	**a**
	PL	↑	↑	↑	ns	ns	ns	ns	**aa**	**aa**	**a**
	CE	ns	ns	ns	ns	ns	↑	**a**	**aa**	**aaa**	**a**
	TG	↑	↑	↑	ns	ns	ns	ns	**aa**	**aa**	**aa**
DHA	TL	↑	↑	↑	ns	ns	ns	ns	**aaa**	**aaa**	**aaa**
	PL	↑	↑	↑	ns	ns	ns	ns	**aaa**	**aaa**	**aaa**
	CE	↑	↑	↑	ns	ns	ns	ns	**aaa**	**aaa**	**aaa**
	TG	↑	↑	↑	ns	ns	ns	ns	**aaa**	**aaa**	**aaa**

AA, arachidonic acid; CE, cholesterol ester; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; ns, non-significant; PL, phospholipids, TG, triglycerides; TL, total lipids.

↑ Significantly higher than baseline value (Bonferroni-Holm adjusted significances).
↓ Significantly lower than baseline value (Bonferroni-Holm adjusted significances).
a, **aa** and **aaa** Higher than placebo value at the same time point with significances $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

3.2.6.1 Plasma total lipids

Baseline values of DGLA, ALA, Σn-3 FA, and Σn-6/Σn-3 FA were significantly different between FO/EPO and placebo groups; the other fatty acids, sums and ratios of interest differed not significantly between the two groups at study entry (Table 3.19, Table 8.24, Table 8.25, and Figure 8.3 - Figure 8.7).

Most investigated fatty acids, their sums and ratios in plasma TL were not significantly affected by placebo treatment. Exceptions were ALA, EPA, AA, adrenic acid, n-6 DPA, Σn-6 LCPUFA, and Σn-6/Σn-3 LCPUFA, which were significantly higher (ALA, EPA) or lower (AA, adrenic acid, n-6 DPA, Σn-6 LCPUFA, Σn-6/Σn-3 LCPUFA) at week 4, 6, and/or 8 than at baseline.

In the FO/EPO group, the following fatty acids, sums and ratios decreased significantly during intervention (week 4, 6 and/or 8) compared to baseline: Mead acid, adrenic acid, n-6 DPA, ALA as well as the ratios Σn-6/Σn-3 FA and Σn-6/Σn-3 LCPUFA. Significant increases compared to baseline were observed for GLA, DGLA, EPA, DHA, GLA + DGLA, Σn-3 FA, and Σn-3 LCPUFA throughout FO/EPO supplementation. The sum of all n-6 LCPUFA was not affected by FO/EPO intake.
Proportions of GLA, DGLA, EPA, DHA, GLA + DGLA, Σn-3 FA, and Σn-3 LCPUFA were significantly higher at weeks 4, 6, and 8 in the FO/EPO than in the placebo group. Mead acid, adrenic acid (only at week 6) as well as the ratios of Σn-6/Σn-3 FA and Σn-6/Σn-3 LCPUFA were significantly lower throughout intervention period with FO/EPO than with placebo supplementation.

3.2.6.2 Plasma phospholipids

Baseline values of DGLA, ALA, and GLA + DGLA were significantly higher in the FO/EPO than in the placebo group; the other fatty acids, sums and ratios of interest differed not significantly between the two groups at study entry (Table 3.19, Table 8.26, Table 8.27, and Figure 8.3 - Figure 8.7).
Most investigated fatty acids, their sums and ratios in plasma PL were not significantly affected by placebo treatment. Exceptions were AA, adrenic acid, n-6 DPA, and Σn-6 LCPUFA, which were significantly lower at weeks 4, 6, and/or 8 compared to baseline values.
In the FO/EPO group, the following fatty acids, sums and ratios were significantly lower at ≥ 1 time point during intervention compared to baseline: Mead acid, adrenic acid, n-6 DPA, ALA, n-3 DPA, Σn-6 FA as well as the ratios Σn-6/Σn-3 FA and Σn-6/Σn-3 LCPUFA. Increases compared to baseline were observed for GLA (not significant at week 6), DGLA, EPA, DHA, GLA + DGLA, Σn-3 FA, and Σn-3 LCPUFA throughout FO/EPO supplementation. The sum of all n-6 LCPUFA was not affected by FO/EPO intake.
The proportions of GLA (not significant at week 6), DGLA, EPA, DHA, GLA + DGLA, Σn-3 FA, and Σn-3 LCPUFA were significantly higher at weeks 4, 6, and 8 in the FO/EPO than in the placebo group. With FO/EPO treatment, Mead acid, LA, adrenic acid, n-6 DPA, ALA, Σn-6 FA as well as the ratios Σn-6/Σn-3 FA and Σn-6/Σn-3

LCPUFA were significantly lower at ≥ 1 time point of the intervention period compared to placebo.

3.2.6.3 Plasma cholesterol esters

Baseline values of DGLA, ALA, EPA, Σn-3 FA, Σn-3 LCPUFA, and Σn-6/Σn-3 FA were significantly different between FO/EPO and placebo groups; the other fatty acids, sums and ratios of interest differed not significantly between the two groups at study entry (Table 3.19, Table 8.28, Table 8.29, and Figure 8.3 - Figure 8.7).

Most investigated fatty acids, their sums and ratios in plasma CE were not significantly affected by placebo treatment; exceptions were n-6 DPA, EPA, and Σn-6/Σn-3 LCPUFA, which were significantly higher (EPA) or lower (n-6 DPA, Σn-6/Σn-3 LCPUFA) at week 8 than at baseline.

In the FO/EPO group, the following fatty acids, sums and ratios were significantly lower at ≥ 1 time point during intervention compared to baseline: Mead acid, n-6 DPA, ALA as well as the ratios Σn-6/Σn-3 FA and Σn-6/Σn-3 LCPUFA. Significant increases compared to baseline were observed for GLA, DGLA, DHA, GLA + DGLA, Σn-3 FA, and Σn-3 LCPUFA throughout FO/EPO supplementation. The sum of all n-6 LCPUFA was not affected by FO/EPO intake.

The proportions of GLA, DGLA, EPA, DHA, GLA + DGLA, Σn-3 FA, and Σn-3 LCPUFA were significantly higher at weeks 4, 6, and 8 in the FO/EPO than in the placebo group. With FO/EPO treatment, the ratios Σn-6/Σn-3 FA and Σn-6/Σn-3 LCPUFA were significantly lower throughout intervention compared to placebo.

3.2.6.4 Plasma triglycerides

Baseline values of ALA, Σn-3 FA, Σn-3 LCPUFA and Σn-6/Σn-3 FA were significantly different between FO/EPO and placebo groups; the other fatty acids, sums and ratios of interest differed not significantly between the two groups at study entry (Table 8.30, Table 8.31, and Figure 8.3 - Figure 8.7).

Most investigated fatty acids, their sums and ratios in plasma TG were not significantly affected by placebo treatment; exceptions were ALA levels, Σn-3 FA, Σn-6/Σn-3 FA, Σn-6/Σn-3 LCPUFA, which were significantly higher (ALA, Σn-3 FA) or

lower (Σn-6/Σn-3 FA, Σn-6/Σn-3 LCPUFA) at ≥ 1 time points during intervention compared to baseline.

In the FO/EPO group, the following fatty acids and ratios were significantly lower throughout intervention compared to baseline: Mead acid as well as the ratios Σn-6/Σn-3 FA and Σn-6/Σn-3 LCPUFA. Significant increases compared to baseline were observed for LA, GLA, DGLA, AA, EPA, DHA, GLA + DGLA, Σn-6 FA, Σn-3 FA, and Σn-3 LCPUFA at ≥ 1 time points of FO/EPO supplementation (Table 3.19). The sum of all n-6 LCPUFA was not affected by FO/EPO intake.

The proportions of GLA, DGLA, AA, EPA, n-3 DPA, DHA, GLA + DGLA, Σn-3 FA, Σn-6 LCPUFA, and Σn-3 LCPUFA were significantly higher at ≥ 1 time point during intervention in the FO/EPO than in the placebo group. With FO/EPO treatment, Mead acid (only significant at week 6) as well as the ratios Σn-6/Σn-3 FA and Σn-6/Σn-3 LCPUFA were significantly lower during intervention compared to placebo.

3.2.7 Relative changes in fatty acid composition

Relative changes from baseline in GLA, DGLA, AA, EPA, and DHA levels (wt%/wt%) are shown in Figure 8.8 - Figure 8.12. For GLA, percentage changes from baseline were significantly different between FO/EPO and placebo groups at weeks 4 and 8 in all investigated plasma lipid domains and additionally at week 6 in plasma TG (Figure 8.8). In the placebo group, GLA changes from baseline at weeks 4, 6, and 8 were between -25.7% and 6.6% in all lipid domains (medians). With FO/EPO supplementation, GLA increased in plasma TL by 34.6 – 63.0%, in PL by 16.5 – 42.5%, in CE by 25.8 – 56.8%, and in TG by 27.4 – 49.7%.

For DGLA, percentage changes from baseline were significantly different between the two intervention groups at week 8 in plasma PL and at weeks 4, 6 (in TL: p = 0.050), and 8 in plasma TL, CE, and TG (Figure 8.9). The proportion of DGLA changed negligible with placebo treatment: median changes were between –7.1% and 3.8% in all investigated lipid domains. FO/EPO supplementation increased DGLA in plasma TL by 9.5 – 13.3%, in plasma PL by 12.2 – 14.8%, in plasma CE by 14.0 – 19.9%, and in plasma TG by 29.3 – 33.3%.

AA changes from baseline at weeks 4, 6, and 8 did not differ between FO/EPO and placebo groups in plasma TL, PL, and CE (median changes were between -8.7% and 5.9%), but were higher in plasma TG at all time points with FO/EPO supplementation than with placebo (Figure 8.10). The active treatment increased AA

in plasma TG by 14.8 – 17.3%, whereas median changes were between –7.6% and –5.0% with placebo intake.

Percentage changes from baseline in EPA levels were not significantly different between the two groups at weeks 4, 6 or 8 (Figure 8.11). EPA levels increased in plasma TL by 5.9 – 21.6% with placebo and by 32.7 – 44.7% with FO/EPO supplementation. In plasma PL, median increases were between 5.6 – 23.7% with placebo and between 30.3 – 38.3% with FO/EPO treatment. Placebo supplementation increased EPA in plasma CE by 7.5 – 21.8% as it did FO/EPO treatment by 28.6 – 42.9%. In plasma TG, median EPA increases were 15.2 - 29.4% with placebo and 47.0 – 60.7% with the active treatment.

DHA changes from baseline were significantly higher throughout intervention in the FO/EPO than in the placebo group (Figure 8.12). Placebo treatment changed DHA levels in plasma TL, PL, and CE by –2.2 to 4.0%, and in plasma TG by 16.8 – 25.2%. FO/EPO increased DHA levels in plasma TL by 57.3 – 66.2%, in plasma PL by 45.5 – 60.3%, in plasma CE by 52.4 – 67.0%, and in plasma TG by 130.8 – 155.8%.

3.2.8 Correlations between relative fatty acid concentrations

Spearman-Rho correlation coefficients were computed for all subjects (n = 39/40) between relative fatty acid levels (wt%) of the various lipid domains. Proportions of LA, GLA, ALA, DGLA, AA, EPA, and DHA were significantly correlated between TL, CE, PL, and TG, with the exception of LA levels in plasma TG at week 4, which were not correlated with LA in TL, PL, or CE (Table 8.32).

The proportions of LA correlated strongly between TL, CE, and PL at all time points ($r \geq 0.785$). GLA levels showed the strongest correlations between TL and CE ($r \geq 0.936$ at all time points). Correlation coefficients for ALA were ≥ 0.760 between TL – CE, TL – PL, TL - TG, and CE - PL. Proportions of DGLA were in general highly correlated between TL – CE ($r \geq 0.887$), TL – PL ($r \geq 0.954$), and CE – PL ($r \geq 0.860$). AA levels showed strong correlations between TL – CE ($r \geq 0.857$), TL – PL ($r \geq 0.875$), and CE – PL ($r \geq 0.839$). Correlation coefficients between EPA levels in TL, CE or PL were ≥ 0.887 at all time points. The proportions of DHA were highly correlated between TL – CE ($r \geq 0.921$), TL – PL ($r \geq 0.951$), and CE – PL ($r \geq 0.927$) at all time points.

3.2.9 Correlations between percentage fatty acid changes

Spearman-Rho correlations for the percentage fatty acid changes from baseline (wt%/wt%) during FO/EPO supplementation in plasma TL and lipid fractions are given in Table 8.33. Percentage fatty acid changes from baseline (calculated for GLA, DGLA, AA, EPA, and DHA) at weeks 4, 6, and 8 were significantly correlated between TL – CE, TL – PL, and CE - PL. The correlations with fatty acid changes in plasma TG were in general not as strong as between the other lipid fractions or not significant. Correlations for DGLA, AA, and DHA percentage changes were highest between plasma TL and PL (r > 0.81), whereas GLA changes correlated best between TL and CE (r > 0.87). Correlation coefficients for EPA changes were comparable high between TL – CE and TL – PL (r > 0.87 and r > 0.88 respectively).

4 Discussion

4.1 Method development

4.1.1 Fatty acids in RBC total lipids

The described method enabled the analysis of relative concentrations of fatty acids in RBC total lipids with satisfactory reproducibility. Even though each lipid fraction revealed a distinct and characteristic fatty acid pattern, RBC total lipid fatty acids were significantly correlated with fatty acids from RBC PC, RBC PE, and plasma PL. In agreement, also Vlaardingerbroek *et al*. [119] and Harris & von Schacky [33] reported strong correlations between RBC and plasma PL LCPUFA. In contrast, a study by Leichsenring *et al*. [120] did not find any relation of RBC DHA (PE, PC, and total lipids) values to DHA levels in plasma PL, which might be due to a greater heterogeneity of their subjects with respect to age and diet.

After 8-week supplementation, correlation coefficients between omega-3 index and RBC PE, RBC PC, and plasma PL EPA + DHA were higher in the placebo than in the DHA group. Furthermore, correlations between omega-3 index from weeks 0 and 8 were stronger for the placebo group. These findings may indicate that EPA + DHA contents in plasma and RBC lipid fractions are not in a steady state after 8 weeks of DHA supplementation. Another explanation may be that there are individual differences in digestion of the supplemented fatty acids, their absorption, beta-oxidation or preferential incorporation into the various lipid fractions.

Our study shows that RBC total lipids are a good biomarker for dietary n-3 LCPUFA intake as they reflect increased DHA intake. Furthermore, the fatty acid composition and the relative fatty acid changes of RBC total lipids correlate well with that in other biomarkers of n-3 fatty acid intake (plasma phospholipids, RBC PC and PE), and the analytical procedure required for RBC total fatty acid analyses is less demanding than the analyses of fatty acids in isolated RBC lipid fractions. Thus, RBC total lipid fatty acid analyses can be used instead of RBC lipid fraction analyses for assessing PUFA status.

4.1.2 Vitamin A and E in plasma

The used method enabled the analysis of absolute concentrations of retinol and alpha-tocopherol in plasma with good reproducibility. The performed recovery tests from added standard substances to plasma were satisfactory, but accuracy of alpha-tocopherol determination was not acceptable for high levels (bias –13%). One reason for the bad accuracy of the method could be that the chosen internal standard substance retinyl acetate was not optimal: first, its retention time was so close to that of retinol that the peaks could not be completely separated with the mobile phase used (Figure 2.4), and furthermore, the extraction behaviour of retinyl acetate (a vitamin A analogue) might be comparable with that of retinol, but might rather differ from that of alpha-tocopherol. Other explanations might be that the used precipitation reagent was not optimal, so that vitamins were partly enclosed in the precipitate, or that there were imprecisions when preparing the standard dilutions for the calibration curve.

To improve the method, we tried the synthetic vitamin E analogue tocol as an internal standard: its peak can be well separated from the tocopherols (Figure 4.1) and its extraction behaviour might be more comparable with that of alpha-tocopherol. To optimise the extraction procedure, we used ethanol/BHT (0.0625%) for protein precipitation: Göbel *et al.* [113] tested four precipitation reagents (ethanol, acid ethanol, isopropanol and acid isopropanol) for their suitability for increasing vitamin yields in pool plasma. While obtained retinol levels showed no differences, highest yields of tocopherol were achieved with ethanol used as precipitation reagent. Because Göbel *et al.* reported greater precision when three extraction steps were employed, we enhanced the number of extraction steps from two to three and placed additionally the tubes after centrifugation on ice to improve phase separation.

We also changed the preparation of the standard curves: instead of doing a standard mixture (containing retinol and alpha-tocopherol) as stock standard and preparing from it 7 standard dilutions, we did separate stock standards for each vitamin (equals the highest standard of the standard curve). The advantages were as follows: two pipette steps and one drying step less per vitamin means less imprecision; furthermore, it was now possible to determine the exact concentrations of the two stock standards photometrically, and to use these stock standards (with known concentrations) directly for preparation of the other 6 standard dilutions.

The improved method for quantification of vitamin A and E in plasma showed good reproducibilities: intra-assay CV (*n* = 8) were 1.0% for retinol and 0.9% for alpha-tocopherol; inter-assay CV (*n* = 7 during 3 weeks) were 1.5% for retinol and 1.4% for alpha-tocopherol. Recoveries from added standard substances to plasma (*n* = 4) were 96% for vitamin A and 102% for vitamin E. The accuracy of the vitamin A and E measurements was verified with lyophilised standard reference material 968c obtained from the US National Institute of Standards and Technology (level 1, *n* = 3). The results for retinol and alpha-tocopherol were in good agreement with the approved values (bias: +1% for retinol and +4% for alpha-tocopherol).

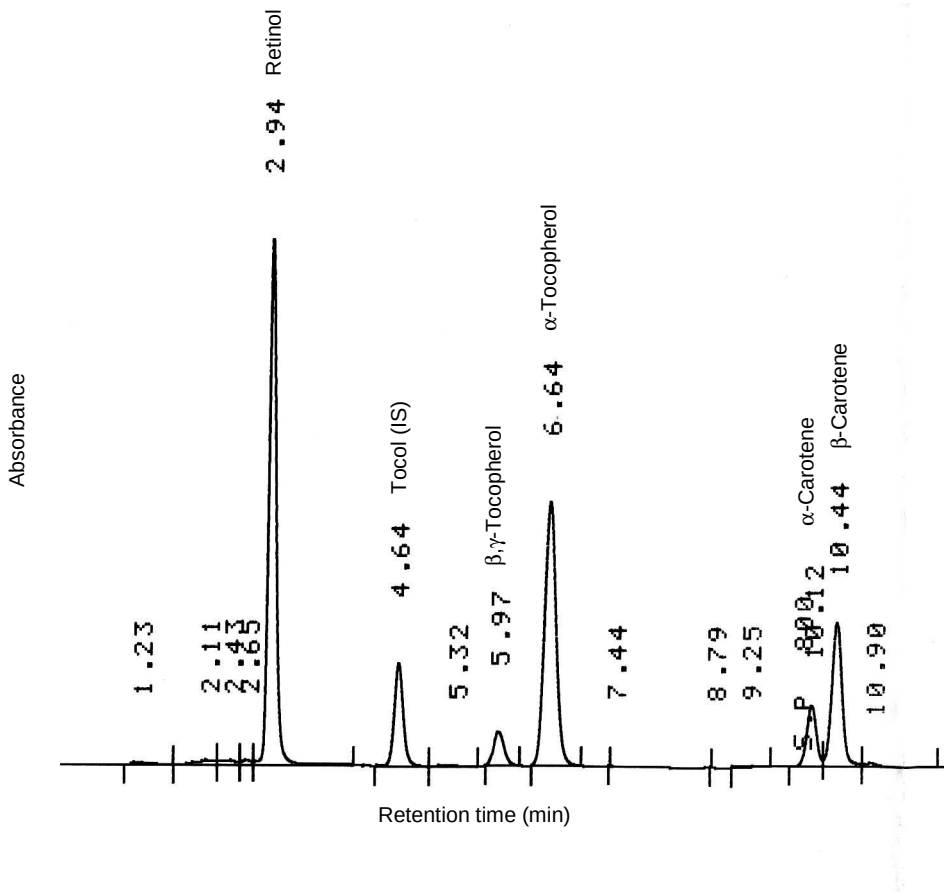

Figure 4.1. Chromatogram of a standard mixture (containing retinol, tocopherols, and carotenes) with tocol as internal standard (IS) using UV-Vis detection, *DHAVEG study*.

4.2 DHAVEG study

A daily intake of 1.05 g DHA (as triglyceride) from Ulkenia oil markedly increased the DHA content of plasma and RBC phospholipids. Previous studies reported a two to threefold increase of the DHA content of serum and platelet phospholipids after supplementation with 0.75 g, 1.50 g or 1.62 g DHA/d for 6 wk [44,57,121] and a rise of DHA levels in plasma PL by 76% and in RBC phosphoglycerides by 58% respectively, after 3 months of supplementation with 0.7 g DHA/d [56]. The observed increase in EPA in RBC and plasma lipids after DHA supplementation in our study appears to reflect retroconversion of DHA, as suggested also by other investigators [44,45,54,121]. We found a decrease in the n-6 LCPUFA 20:3n-6, 20:4n-6, and 22:4n-6, accompanied by a decrease in n-3 DPA, consistent with other findings [44,45,57,121]. In contrast, n-3 DPA shows considerable enrichment in serum and platelet phospholipids when fish or seal oils (providing EPA, DHA and some n-3 DPA) are fed [122,123]. Thus, dietary DHA and possibly also n-6 DPA appear to partially replace n-3 DPA in circulating and cellular phospholipids in human subjects. In contrast to other studies using DHA-rich oils from an algal source, which reported significant decreases in n-6 DPA [44,45,121], we observed an increase of n-6 DPA in plasma and RBC phospholipids, which is explained by the significant n-6 DPA content in the tested oil derived from microalgae *Ulkenia sp.* (9.7 wt% or 0.22 g/d). Sanders *et al.* [124] also reported an increase of n-6 DPA and DHA levels in plasma and RBC lipids after 4-week supplementation with an algal triglyceride providing 1.5 g DHA and 0.6 g n-6 DPA per day.

The omega-3 index rose significantly from 4.8 ± 1.2 wt% to 8.4 ± 1.3 wt% (mean ± SD) in the DHA supplemented group. A similar increase (from 4.7 to 9.9 wt%, means) of the omega-3 index was reported by Harris & von Schacky [33] after EPA and DHA supplementation (1 g/d) for 5 months. At baseline, no one of our subjects reached a desirable omega-3 index ≥ 8 wt%, although more than 50% of the subjects reached or exceeded recommended intakes for ALA (0.5% of energy) and 26% had a dietary LA:ALA ratio below 5:1 as considered desirable [17]. These results suggest that the in vivo conversion of ALA to n-3 LCPUFA is not adequate to reach a desirable EPA + DHA status even with adequate ALA intake and low dietary LA:ALA ratio. Thus, a dietary intake of pre-formed n-3 LCPUFA seems to be necessary to enhance the omega-3 index. After 8-week supplementation with microalgae oil, 69% of the

subjects had an omega-3 index ≥ 8%, while no subject of the placebo group showed an omega-3 index above 8%.

Relative changes of n-6 DPA, EPA and DHA were greater in plasma PL and RBC PC than in RBC total lipids or RBC PE, with strong correlations between RBC PC and plasma PL fatty acid changes. RBC PE and plasma PL fatty acid changes were less closely related. Plasma PL fatty acids change relatively fast and reflect dietary intakes over the past few days [125]. RBC PL are asymmetrically distributed between the outer and the inner layer of the membrane [126]: the major part of PE is located at the inner layer, whereas PC occurs predominantly on the outer layer and can exchange more easily with plasma lipids. Thus, PC reflects more directly the fatty acid composition of PL of the plasma [127], whereas the fatty acid composition of PE appears to be more independent from the plasma fatty acids and depends on selective incorporation of PUFA and LCPUFA into PE [128].

Relative changes in RBC total lipid n-6 DPA, EPA and DHA were lower than their average changes in RBC PE and RBC PC, indicating that these fatty acids were preferentially incorporated into PE and PC, which comprise about 23% and 22% of total membrane lipids (modified from [129], calculated without cholesterol), but less in the other lipids of the RBC membrane like sphingomyeline, phosphatidylserine, glycolipids and other lipids, comprising about 23%, 9%, 4%, and 18% of total membrane lipids respectively.

Dietary intake of EPA and DHA combined with the background diet was as expected very low in these vegetarian subjects (23 mg/d in both groups, median). The observed values are in agreement with a study by Conquer & Holub, who reported that vegetarians consume minimal EPA (< 5 mg/d) and varying amounts of DHA depending on egg consumption (< 33 mg/d) [20]. For comparison, the dietary intake of EPA and DHA combined in the German population has recently been estimated to be 141 mg/d among women and 186 mg/d among men (medians) [11]. Reported energy intake in the DHA group was significantly lower during intervention compared to baseline (7.7 MJ vs. 8.8 MJ, medians), but remained unchanged in the placebo group. We observed no changes in body weight over the 8-week study period in the DHA group, but a slight weight gain in the placebo group (+ 0.5 kg). An explanation for the reduced energy intake without weight loss in the DHA group and the weight gain without (recorded) increased energy intake in the placebo group could be that energy requirements decreased in both groups during the intervention period, e.g.

because of less physical activity related to seasonal influences. The participation in a nutritional study and the intake of oil-containing capsules might have caused larger reductions in dietary intakes and/or a greater “underreporting” of consumed foods than before the intervention in both groups at the days of the dietary record. Since we observed a trend towards lower energy intakes in the DHA group relative to placebo, the tested microalgae oil might have had a different effect on appetite or regulation of food intake than olive oil in the vegetarian subjects studied.

In the present study, heart rate and diastolic blood pressure were not affected by DHA supplementation. Similar results were shown previously with DHA intakes of 0.7 g and 3.0 g/d [48,57]. Mori *et al.* [130] reported a decrease of systolic and diastolic BP after DHA supplementation in men with otherwise normal BP, whereas Grimsgaard *et al.* [131] did not detect a reduction of BP in comparable subjects. Both groups observed a significant influence of DHA on heart rate in healthy men. Very clear effects in respect to n-3 LCPUFA on BP control have been observed in hypertensive patients [132-135], but these investigators used extremely large amounts of omega-3 fatty acids ranging from 3.4 to 15 g per day. In the present study, systolic and diastolic BP at baseline were very low (DHA group: 100/65 mm Hg; placebo group: 95/70 mm Hg, medians); therefore, a further decrease of BP could not be expected and would also be considered undesirable. The observed increase of systolic BP in the placebo group and the same trend in the DHA group may be caused by a systematic change in BP measurement or by environmental influences.

Sanders & Hinds [136] reported that plasma alpha-tocopherol concentrations fell below the normal range during a period of fish oil supplementation (2.1 g DHA and 0.8 g EPA per day), suggesting that fish oil increases the requirement for antioxidants. No significant changes in plasma alpha-tocopherol were evident following fish oil treatment (3 g EPA + DHA per day) providing 21 mg/d total tocopherol of which 16 mg was alpha-tocopherol [137]. In the present study, the olive oil and microalgae oil capsules provided a total of 2.2-2.3 mg mixed natural tocopherols per day. No changes of alpha-tocopherol levels and lipid-adjusted alpha-tocopherol concentrations were observed after intervention in both groups, suggesting that 2.3 mg mixed natural tocopherols are sufficient to maintain alpha-tocopherol levels at a DHA intake of ~1 g/d.

For safety and tolerance evaluation, side effects were reported and several haematology and biochemistry parameters were analysed. Self-reported side effects were equally distributed between DHA and placebo group. We observed significant changes in some blood parameters after DHA supplementation within the normal ranges (e.g. alanine aminotransferase and cholinesterase levels), which are presumed to be of no clinical relevance. Incidence of liver and cardiac enzymes out of the laboratory's reference range did not differ neither at baseline nor at week 8 between DHA and placebo groups; within-group incidences did not differ between baseline and week 8. Slight increases in the serum activity of liver enzymes during n-3 LCPUFA supplementation has been noted repeatedly [138,139]; the mechanisms remain unclear.

With the exception of von Willebrand factor (vWF), incidence of haemostatic factors out of the laboratory's reference range did not differ neither at baseline nor at week 8 between DHA and placebo groups; within-group incidences did not differ between baseline and week 8. Concerning vWF, we observed values out of reference range less often after DHA supplementation than at baseline (1 vs. 12 subjects), and incidence of such values was lower in the DHA than in the placebo group at week 8 (1 vs. 11 subjects). Other studies supplementing n-3 LCPUFA observed decreased [140,141] or unchanged [122,124,142,143] vWF levels. Plasma vWF levels have been proposed as risk factor for CHD and stroke, especially in high-risk populations with previous cardiovascular events, diabetes or old age [144]. Our results might indicate a normalisation of prior abnormal vWF levels with DHA supplementation. Further research is needed to clarify the possible beneficial effects of DHA on haemostasis and the impact on CHD or stroke risk in normolipidaemic subjects.

Our results show that a supplementation with ~1 g DHA/d for 8 weeks significantly lowered TG in normolipidaemic (basal TG < 2.5 mM) subjects. Previous studies with single-cell oil sources of DHA reported a significant reduction in TG concentrations and an increase in HDL cholesterol levels with DHA intakes in the range of 1.6 - 2.4 g/d in normolipidaemic volunteers [44,49,55], whereas other studies with DHA intakes of 0.7 g/d [56] and 0.75 - 1.5 g/d [57] did not find any significant effects on plasma TG and lipoproteins. In persons with combined hyperlipidaemia, significant reductions in TG and increases in LDL and HDL cholesterol concentrations were observed after supplementation with 1.25 g or 2.5 g DHA per day [46]. N-3 LCPUFA lower plasma TG concentrations by several mechanisms such as increased hepatic

fatty acid oxidation, inhibition of fatty acid and TG synthesis and depressed assembly and secretion of VLDL TG [51]. The TG-lowering effect of DHA is greater in subjects with higher initial TG concentrations [50]. Considering the normolipidaemic subjects in our study, it also seems that DHA lowers plasma TG only in subjects with higher basal TG (> ~1.2 mmol/l).

HDL cholesterol concentrations increased after 8 weeks of DHA supplementation. Plasma TG and HDL cholesterol of all subjects at baseline were slightly inversely correlated (r = -0.207, p = 0.033), and absolute changes in TG correlated inversely with HDL concentrations after DHA supplementation (r = -0.308, p = 0.025). The mechanisms by which DHA supplementation increases HDL cholesterol are not known, but may be related to alterations in lipid transfer protein activity [145]. A decrease of cholesteryl ester transfer protein (CETP) activity would reduce the exchange from HDL cholesterol esters and VLDL triglycerides, resulting in larger, more cholesterol-rich HDL cholesterol particles. Indeed, other studies applying purified DHA (3.6 g/d and 4.9 g/d respectively) observed a decrease in the apoA1:HDL-cholesterol ratio [47,54]. These findings suggest an effect of DHA supplementation on HDL particle size with a shift towards the larger, more cholesterol-rich HDL-2 particle. Other investigators observed an increased HDL-2:HDL-3 cholesterol ratio [52,55] or increased HDL-2 cholesterol [53] after DHA supplementation.

In the present study, total and LDL cholesterol levels increased after DHA intake. Other studies did not detect any significant changes in total cholesterol after supplementation with 0.75 - 4.9 g DHA/d [47,48,52,54,57] or increased total cholesterol levels after supplementation with an algal triglyceride providing 1.5 g DHA and 0.6 g n-6 DPA per day [124]. Inconsistent effects of DHA on LDL cholesterol levels were reported in previous studies: some investigators observed LDL cholesterol-raising effects of DHA [46,53,56,124], whereas others found no effects [44,47-49,52,54,57]. The repeatedly observed increase in plasma LDL cholesterol after DHA / fish oil supplementation must be due to either decreased clearance or increased production of LDL. The limited amount of TG available for packaging into VLDL after supplementation with n-3 LCPUFA could result in smaller VLDL particles that contain less TG. Small VLDL are more readily converted to LDL, which may cause increased LDL cholesterol levels [43]. However, previous studies did not find increases in smaller VLDL subspecies with n-3 fatty acid supplementation [146,147].

Lu *et al.* [147] suggested that n-3 fatty acids could enhance the conversion of VLDL to LDL via increased inherent susceptibility of VLDL particles to lipolysis and/or increased in vivo lipolytic activity.

Decreased clearance of LDL might be due to decreases in LDL receptor binding activity or reduced LDL receptor expression. Animal experiments feeding fish oil with DHA to hamsters and primates showed decreased receptor-mediated clearance of LDL cholesterol [148,149]. Binding of LDL to the LDL receptor decreased because of an altered LDL structure and a reduced affinity of LDL for its receptor. Lindsey *et al.* [150] observed an *in vitro* depression of LDL receptor activity and expression in human hepatoma HepG2 cells after supplementation with 3.6 g EPA/d and 2.9 g DHA/d for only 2 weeks. Whether n-3 FA alter LDL receptor activity or expression in humans is not known, but LDL kinetic studies showed no decrease in fractional catabolic rate relative to a high saturated fat diet [151] or a vegetable diet [152], providing no evidence for a change in LDL receptor activity in humans. Further research is needed to clarify the reasons for the increase in LDL cholesterol with moderate intakes of DHA and the possible consequences for CHD risk in normolipidaemic subjects.

Kinosian *et al.* [153,154] proposed that changes in total cholesterol:HDL cholesterol and LDL:HDL cholesterol ratios are better predictors of risk for CHD than changes in total or LDL cholesterol alone. The ratio of TG to HDL cholesterol is additionally considered a predictor of risk for myocardial infarction [155]. In our study, the total cholesterol:HDL cholesterol ratio as well as the LDL:HDL cholesterol remained unchanged in both intervention groups, but the TG:HDL cholesterol ratio was significantly lower after DHA supplementation. Stark & Holub [45] also reported a significant decrease of TG:HDL cholesterol after supplementation with 2.8 g DHA/d over 4 weeks in postmenopausal women. Decreased ratios of total:HDL cholesterol [44,47] and LDL:HDL cholesterol [44] or no changes in LDL:HDL or total:HDL cholesterol ratios [45,57] were observed with DHA supplementation in other studies.

In conclusion, DHA-rich oil from microalgae *Ulkenia sp.* is well tolerated and can be considered a suitable vegetarian source of n-3 LCPUFA. Eight-week supplementation with this microalgae oil can achieve a desirable omega-3 index ≥ 8% in subjects with low basal n-3 LCPUFA status. The DHA intake was associated with improvements in some CHD risk factors (plasma TG, TG:HDL cholesterol ratio,

vWF), but others, notably LDL cholesterol, worsened. Therefore, the overall effects of this treatment on CHD risk are unclear and should be further investigated.

4.3 FO/EPO study

The objective of this pilot study was to investigate the effects of a fatty acid mixture (providing 419 mg DHA, 337 mg GLA, 72 mg EPA, and 14 mg AA as triglycerides per day) on plasma fatty acid composition in healthy, non-pregnant women. Previous studies showed that supplementation with DHA alone increased plasma lipid concentrations of DHA and (in most studies) EPA, but decreased GLA, DGLA, and AA concentrations [44-48,53,54,56,57,121,156]. Supplementation with GLA on the other hand increased plasma concentrations of DGLA as well as (in most studies) AA and GLA levels, but did not enhance EPA and DHA levels [10,157-161]. We hypothesised that the tested n-3 LCPUFA/GLA mixture (FO/EPO) would result in an increase of plasma DHA, GLA, and DGLA levels without impairing AA status.

This pilot study showed that 8-week supplementation with FO/EPO increased the proportions of GLA and its elongation product DGLA in plasma total lipids and in all measured plasma lipid fractions (CE, PL, and TG) compared to baseline, suggesting that some of the GLA is elongated before incorporation into PL, CE or TG. These observations are consistent with those of Laidlaw & Holub [123], who found significant increases from baseline in the proportions of GLA and DGLA (measured in plasma PL) in healthy women given EPA + DHA and GLA in a ratio of 1:1 or 1:0.5 for 4 weeks; no GLA and DGLA changes could be observed in this study when the ratio of n-3 LCPUFA to GLA was 1:0.25. Miles *et al.* [10] reported increases in the proportion of GLA in plasma TG and CE, but not in PL, after supplementation with a n-3 LCPUFA/GLA mixture (1:0.625). Other studies supplementing 8 – 28 subjects with n-3 LCPUFA and GLA (in a ratio from 1:0.33 to 1:1.14) could not detect any changes in absolute GLA and DGLA levels of plasma total lipids or in relative concentrations of plasma PL [159,160,162,163]. The effects on GLA and DGLA might be influenced by the total GLA amount or by the ratio of n-3 LCPUFA to GLA in the used supplement: Laidlaw & Holub [123] observed increasing DGLA values with increasing amounts of GLA and constant EPA + DHA dosages. Concerning the changes in GLA levels, a reason for the different findings between the studies may be the lipid fraction studied: as demonstrated by several investigators [10,164], GLA is mainly found in CE and TG, but hardly in PL (< 0.1 wt%), so that changes would be more readily detected in these fractions than in plasma PL (depending also on the used method for fatty acid analyses and on the number of subjects).

In the present study, the proportion of AA increased in plasma TG, whereas AA levels did not change in plasma TL, CE, and PL. Other investigators reported decreased [123,162] or unchanged [10,159,163] plasma AA levels in TL or PL with n-3 LCPUFA/GLA mixtures compared to baseline. The inconsistent effects on AA levels may relate on the one hand to the lipid fraction studied, on the other hand to differences in the GLA, AA, and EPA contents or in the EPA:GLA ratio of the used supplements: EPA and DGLA compete with AA for esterification into phospholipids and furthermore, the (n-3) fatty acid product of the Δ5-desaturase reaction, EPA, attenuates the conversion of (GLA-derived) DGLA to AA [159,165]. In the current study, the decrease of AA levels in plasma, which is often observed with n-3 LCPUFA supplementation, could be prevented in all measured plasma lipid fractions as anticipated by adding GLA (and AA) to the supplement (n-3 LCPUFA:GLA = 1: ~0.7; EPA:GLA = 1: ~4.7); thus, the GLA amount in the supplement seems to be adequate.

FO/EPO supplementation increased the proportions of EPA and DHA in all investigated plasma lipid fractions (exception: EPA levels in plasma CE were not significantly changed from baseline values). Consistent with our results, other studies supplementing n-3 LCPUFA combined with GLA also observed increased levels of EPA and DHA in plasma TL and PL [10,123,159,160,162,163]. The observed increase in plasma EPA levels after FO/EPO supplementation in our study may relate to the EPA content of the supplement (~72 mg/day) and/or could reflect retroconversion of DHA, as suggested also by other investigators [44,45,54,121].

As we observed increases in ALA and EPA levels and decreases in long-chain n-6 fatty acids (AA, adrenic acid, n-6 DPA) in some plasma lipid fractions with placebo treatment, the fatty acid composition of the placebo oil might not have been sufficiently comparable to the habitual fatty acid composition of the subjects' diet. In a previous study conducted in November 2003 at our hospital, we determined the dietary intake in 23 female omnivores (age: 23.8 ± 3.2 years, height: 1.68 ± 0.06 m, BMI: 20.9 ± 1.9 kg/m^2, mean ± SD for all). These subjects were well comparable with the subjects in the present study (predominantly medicine students with comparable age, height and BMI). In this previous study, we evaluated the median EPA intake with the normal diet to be 20 mg per day (IQR 10 – 30 mg/d). If we assumed that ALA conversion to EPA could be up to 20% in young women [2], the additional ALA supply with placebo capsules (about 69 mg/d) would deliver about 14 mg EPA, what

accounts for an additional EPA supply of 47% or more in ¾ of the subjects (30 mg EPA/d = 100% ⇒ 44 mg EPA/d = 146.7%). This might have caused the observed increases in plasma EPA and decreases in plasma long-chain n-6 fatty acids. Another possibility might be that the subjects changed their dietary habits during the intervention period, because with this study they became aware of the benefits of n-3 fatty acids. As the subjects did not record their diets before and during the intervention period of this study, we cannot prove or disprove one of these speculations. For future intervention studies, dietary records might be helpful to detect possible changes in the background diet. A possible change in fatty acid composition of the placebo oil should be taken into account for the planned main study supplementing pregnant and lactating women with the FO/EPO mixture.

The present pilot study also serves to compare the fatty acid responses in the various lipid domains. This information will then be used to decide which lipid domain will be investigated in future studies supplementing n-3 and n-6 fatty acids. The proportions of GLA, DGLA, EPA, and DHA in all investigated plasma lipids (total lipids and lipid fractions) reached their maximum already after 4 - 6 weeks of FO/EPO supplementation; therefore, the intervention period of 8 weeks is definitely adequate and could possibly be reduced to 6 weeks in future studies. Regarding all subjects at study entry (*n* = 40), GLA was mainly found in cholesterol esters, triglycerides, and total lipids (medians: 0.85 wt%, 0.31 wt%, and 0.37 wt% respectively), but hardly in phospholipids (< 0.1 wt%) as demonstrated by several investigators [10,164]. DGLA, AA, and DHA concentrations, on the other hand, were highest in phospholipids (medians: 3.43 wt%, 9.34 wt% and 3.09 wt% respectively) and considerably lower in cholesterol esters and triglycerides. Relative GLA changes from baseline (wt%/wt%) with FO/EPO supplementation were comparably high in plasma TL, CE, and TG (median changes at weeks 4, 6, and 8 ranged between 25 – 63%) and little lower in PL (16 – 43%). DGLA, EPA, and DHA changes from baseline were highest in plasma TG (29 – 33%, 47 – 61%, 131 – 156% respectively), and for each fatty acid comparably high in plasma TL, CE, and PL (about 10 – 20% for DGLA, 29 – 45% for EPA, and 45 – 67% for DHA). Percentage changes from baseline in these fatty acids of main interest at weeks 4 – 8 were significantly correlated between TL – CE, TL – PL, and CE - PL.

As proportions and changes of the tested n-6 and n-3 fatty acids were correlated between TL – CE and TL - PL, fatty acid analyses might be limited to plasma total

lipids, provided that the subjects are healthy (no dyslipidaemias), non-pregnant and fasting. In our subjects, the proportion of PL, CE, and TG total fatty acids (in % of plasma total lipid fatty acids) changed only negligible between weeks 0, 4, 6, and 8 (PL: 44.7 – 45.0% of TL, CE: 26.0 – 26.7% of TL, TG: 19.5 – 20.0% of TL, means, $p > 0.05$ between the four time points); this finding is probably an important condition for the strong correlations between fatty acids of TL and lipid fractions. Plasma total lipids as biomarkers for n-6 and n-3 fatty acid status have the following positive attributes: all n-6 and n-3 fatty acids are present in adequate amounts in plasma TL, fatty acid composition in plasma TL responds to increasing EFA/LCPUFA intakes and is correlated with fatty acids in plasma PL, CE, the analyses are less time-consuming (no separation of the lipid fractions), and the required sample volume is smaller. A disadvantage is that the fatty acid composition in plasma total lipids is influenced by fasting or fed state; therefore, it might be problematical in pregnant women, because of the observed raise in plasma triglycerides during pregnancy [166], as well as in neonates, in whom it is not possible to take fasted blood samples. For these subjects, fatty acid analyses in plasma PL and CE might be the best choice as markers for fatty acid status.

For safety and tolerance evaluation, side effects were reported and several haematological and biochemical parameters were analysed. The supplementation with the FO/EPO mixture did not result in any physiologically relevant changes of safety parameters: incidence of liver enzymes and haematological parameters out of the laboratory's reference range did not differ neither at baseline nor at week 8 between FO/EPO and placebo groups; within-group incidences did not differ between baseline and week 8. The observed different changes in cholinesterase levels with DHA/EPO or placebo intake might be due to the lower basal cholinesterase levels in the placebo group and their normalisation over the intervention period. Self-reported side effects were equally distributed between FO/EPO and placebo groups.

In conclusion, in women of childbearing age the tested fatty acid supplement is well tolerated and appears safe. FO/EPO intake resulted in the anticipated increase of plasma GLA, DGLA, and DHA levels without impairing AA status. These data provide a basis for testing this FO/EPO mixture in pregnant women for its efficacy to optimise maternal and neonatal LCPUFA status, and for its effects on infantile development, early markers of allergy risk and prevention of obesity, insulin resistance, hypertriglyceridaemia or other chronic diseases in later life.

4.4 Comparison of the two used supplements

The fatty acid profiles of plasma and/or RBC lipids followed closely the fatty acid composition of the supplements and illustrate the competitive interactions between n-3 and n-6 metabolic pathways: in the *DHAVEG study*, main fatty acids in the supplement were DHA (~1050 mg/d as triglyceride) and n-6 docosapentaenoic acid (~222 mg/d as triglyceride). Median percentage changes from baseline in plasma phospholipids after 8 weeks of intervention were highest (and significantly different from changes with placebo intervention) for these two fatty acids with a +159% increase for DHA and +87% increase for n-6 DPA (Figure 4.2, blue bars). Reductions in GLA, DGLA, AA levels as well as in n-6 LCPUFA were greater with microalgae oil than with placebo intake.

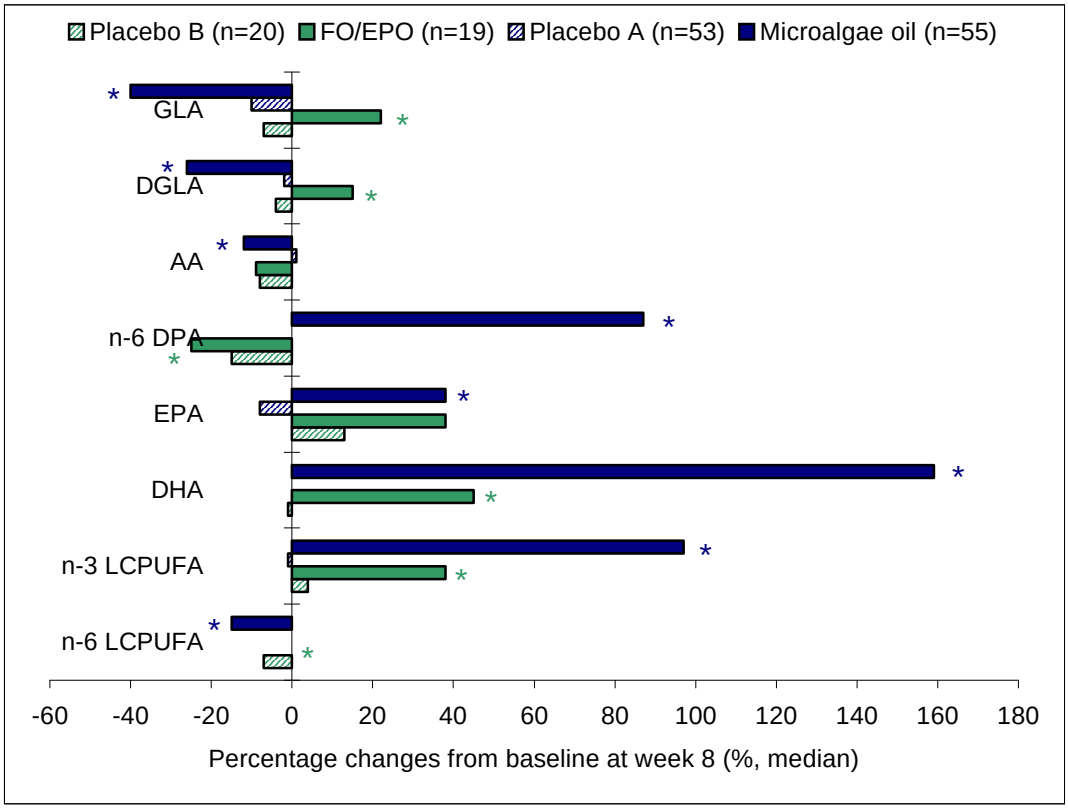

Figure 4.2. Percentage changes from baseline in plasma phospholipids after 8 weeks of supplementation with microalgae oil, FO/EPO or placebo (%, medians).

GLA, gamma-linolenic acid; DGLA, dihomo-gamma-linolenic acid; AA, arachidonic acid; n-6 DPA, n-6 docosapentaenoic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; n-3 LCPUFA, sum of all long-chain n-3 fatty acids; n-6 LCPUFA, sum of all long-chain n-6 fatty acids; FO/EPO, fish oil/evening primrose oil.

* $p < 0.05$ vs. changes with the appropriate placebo (A in *DHAVEG study*; B in *FO/EPO study*).

In the *FO/EPO study*, the FO/EPO mixture provided 419 mg DHA and 337 mg GLA as triglycerides per day. Percentage changes from baseline values of plasma phospholipid fatty acids showed a significantly greater increase with FO/EPO than with placebo for GLA (medians: +22% vs. –7%), its elongation product DGLA (+15% vs. –4%), and DHA (+45% vs. –1%) (Figure 4.2, green bars).

In contrast to the supplementation with DHA alone (*DHAVEG study*), the expected dietary DHA-induced decrease of n-6 fatty acids (e.g. GLA, DGLA, AA, Σn-6 LCPUFA) in plasma phospholipids was attenuated in the *FO/EPO study* by the presence of GLA in the supplement. The combination of DHA and GLA may be of added benefit especially in pregnant or lactating women, in whom it is desirable to enhance n-3 LCPUFA status without compromising GLA, DGLA, or AA status. Further studies should compare the effects of DHA alone and in combination with GLA on neonatal and infantile brain development and function.

Concerning the CHD risk, it is noteworthy that increasing concentrations of DHA in serum phospholipids have been inversely correlated with risk of CHD [31]. In this regard, Simon *et al.* [31] reported that a standard deviation increase (+1.22 wt%) in plasma PL DHA resulted in an odds ratio of 0.66 for CHD risk (95% confidence interval 0.46 - 0.94). In our two studies, 8-week supplementation resulted in absolute DHA increases from baseline values ≥ 1.22 wt% in 96% (53/55) of the microalgae oil supplemented subjects and in 74% (14/19) of the FO/EPO supplemented subjects.

The omega-3 index has been also identified as a risk indicator for death from CHD and especially SCD [33]. To convert plasma fatty acid data of the *FO/EPO study* to the correspondent omega-3 index, we used the following equation which was determined from fatty acid data of the *DHAVEG study* (Figure 4.3):

Omega-3 index (wt%) = Plasma phospholipid EPA+DHA (wt%) x 0.7612 + 2.1649

In both studies, no subject had a desirable omega-3 index ≥ 8 wt% at baseline. After 8-week supplementation with microalgae oil, 69% of the subjects reached an omega-3 index ≥ 8% (median omega-3 index increased from 4.70 wt% at baseline to 8.54 wt% at week 8), whereas FO/EPO treatment did not result in omega-3 index values ≥ 8 wt% (median (estimated) omega-3 index increased from 5.02 wt% at baseline to 6.57 wt% at week 8).

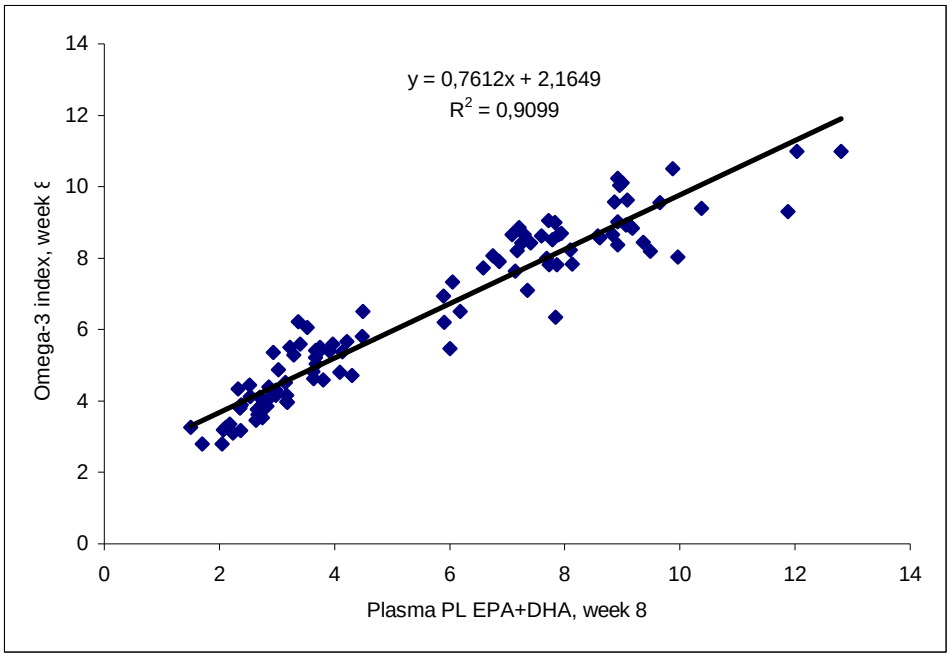

Figure 4.3. RBC vs. plasma phospholipid EPA + DHA, *DHAVEG study*.

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PL, phospholipids.
These two parameters were measured in 103 pairs of blood samples (*DHAVEG study*, week 8, Spearman-Rho $r = 0.94$, $p < 0.001$).

Regarding only the changes in plasma phospholipid DHA or omega-3 index, there might be a better reduction of CHD risk with microalgae oil than with the tested combination of fish oil and evening primrose oil. But a study by Laidlaw & Holub [123] indicated added benefit from combined supplementation of n-3 LCPUFA and GLA on reduction of CHD risk. These investigators observed reduced serum TG concentrations by 40%, 39%, and 35% with EPA+DHA supplementation alone (4 g) or in conjunction with GLA (1 or 2 g), whereas TG concentrations did not change significantly when GLA supplementation increased to 4 g/d. Interestingly, the combination of EPA+DHA and GLA in the 4:2 and 4:4 groups resulted in a tendency of LDL cholesterol to decrease, whereas EPA+DHA alone typically has no effect [44,47-49,52,54,57] or a modest elevating effect [46,53,56,124,156] on LDL concentrations.

The international task force for the prevention of CHD uses the variables age, systolic blood pressure, cigarette smoking, diabetes, family history of MI, and LDL cholesterol, HDL cholesterol, and TG concentrations with the PROCAM risk calculator to predict the risk of MI within the next 10 years [167]. Laidlaw & Holub [123] reported that on the basis of this risk calculator, the lipid changes observed

(before and after supplementation) in the four groups studied yielded estimated reductions in the risk of MI over a 10-y period of 37%, 33%, 43%, and 24% (group means) in the 4:0, 4:1, 4:2, and 4:4 groups respectively. Thus, the 4:2 group (4 g EPA + DHA and 2 g GLA) had the greatest overall reduction in MI risk on the basis of the PROCAM risk calculator. Further studies are needed investigating and comparing the effects of DHA alone and in combination with GLA on CHD risk.

5 Summary

5.1 DHAVEG study

Background: Low red blood cell (RBC) membrane content of eicosapentaenoic and docosahexaenoic acids (EPA + DHA, hereafter called omega-3 index) has recently been described as an indicator for increased risk of death from coronary heart disease (CHD). Previous studies demonstrated repeatedly beneficial effects of n-3 long-chain polyunsaturated fatty acids (n-3 LCPUFA) on plasma triglyceride (TG) levels, but studies investigating the individual effects of EPA or DHA on plasma TG and lipoproteins in humans are rare.

Objectives: This study aimed to investigate the influence of a vegetarian DHA-rich oil from microalgae *Ulkenia sp.* (almost free of EPA) on plasma and RBC DHA status, omega-3 index, and plasma lipids as well as its tolerance and safety in healthy adults with low basal DHA status.

Design: A randomised, double blind, placebo-controlled intervention study with two parallel groups was performed. One hundred fourteen vegetarians (87 f, 27 m) aged 18 to 43 years consumed 2.28 g daily of either microalgae oil providing ~1 g DHA (as triglyceride) or about the same amount of olive oil (as placebo) for 8 weeks. RBC and plasma fatty acids, plasma lipids, and safety parameters (blood cell count, liver and cardiac enzymes, haemostatic parameters, plasma vitamin E) were measured at week 0 and week 8.

Results: DHA supplementation significantly increased DHA in RBC total lipids (from 4.4 to 7.9 wt%, means), in RBC phosphatidylethanolamine (from 6.5 to 12.1 wt%), in RBC phosphatidylcholine (from 1.4 to 3.8 wt%), and in plasma phospholipids (from 2.8 to 7.4 wt%), whereas EPA levels rose to a much lesser extent. The mean omega-3 index rose significantly from 4.8 to 8.4 wt% in the DHA group. After intervention, 69% of DHA supplemented subjects but no subject of the placebo group reached an omega-3 index above the desirable value of 8 wt%. Microalgae oil supplementation resulted in significant decreases of 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:4n-6 and 22:5n-3 levels in all measured fractions relative to baseline values.

Plasma TG decreased by 20% from 0.96 to 0.77 mmol/l (medians) with DHA treatment. Plasma total, LDL and HDL cholesterol increased significantly in the DHA group, resulting in lower TG:HDL cholesterol and unchanged LDL:HDL and total cholesterol:HDL cholesterol ratios.

Self-reported side effects were minor und evenly distributed between DHA and placebo groups (11% vs. 8%). The intake of DHA-rich microalgae oil did not result in any physiologically relevant changes of safety and haemostatic factors with exception of von Willebrand factor (vWF) levels. Concerning vWF, we observed values out of reference range less often after DHA supplementation than at baseline (1 vs. 12 subjects), and incidence of such values was lower in the DHA than in the placebo group at week 8 (1 vs. 11 subjects). Absolute and lipid-adjusted alpha-tocopherol levels did not change with DHA supplementation.

Conclusions: In healthy adults DHA-rich oil from microalgae *Ulkenia sp.* is well tolerated, appears safe and can be considered a suitable vegetarian source of n-3 LCPUFA. Eight-week supplementation with the tested oil can achieve a desirable omega-3 index ≥ 8% in subjects with low basal n-3 LCPUFA status. The DHA intake was associated with improvements in some CHD risk factors (plasma TG, TG:HDL cholesterol ratio, vWF), but others, notably LDL cholesterol, worsened. Therefore, the overall effects of this treatment on CHD risk are unclear and should be further investigated.

5.2 FO/EPO study

Background: Fish oil supplementation can enhance docosahexaenoic acid (DHA) blood levels in pregnancy, but this might compromise maternal and neonatal arachidonic acid (AA) status, which is also important for infantile development. **Objectives:** This pilot study investigated whether co-administration of fish oil (FO) and evening primrose oil (EPO) will enhance n-3 long-chain polyunsaturated fatty acid (LCPUFA) status without reduction of AA levels. We also evaluated the effects on plasma gamma-linolenic acid (GLA) and dihomo-GLA (DGLA) concentrations, since these fatty acids have been shown to be associated with increased birth weight (DGLA) and reduced risk of certain aspects of metabolic syndrome (GLA). Tolerance and safety of this fatty acid mixture were assessed as well.

Design: A randomised, double blind, placebo-controlled intervention study with two parallel groups was performed. Forty non-pregnant women aged 19 – 36 years consumed 3.4 g daily of either a FO/EPO mixture (providing 419 mg DHA, 337 mg GLA, and 72 mg eicosapentaenoic acid as triglycerides per day) or a placebo (mixture of palm oil, rapeseed oil, and sunflower seed oil) for 8 wk. Fatty acids were quantified in plasma total lipids (TL), phospholipids (PL), cholesterol esters (CE), and triglycerides (TG) at baseline and after 4, 6 and 8 weeks. Safety parameters (e.g. blood cell count, liver enzymes) were measured at weeks 0 and 8.

Results: Eight-week supplementation with FO/EPO significantly increased plasma total lipid DHA (from 2.0 to 3.1 wt%, means), DGLA (from 2.0 to 2.3 wt%), and GLA (from 0.36 to 0.52 wt%), whereas AA levels did not change. After 8 weeks, mean percentage changes from baseline values of plasma total lipid fatty acids showed a significantly greater increase with FO/EPO than with placebo for GLA (+49.9 vs. +2.1%), DGLA (+13.8 vs. +0.7%) and DHA (+59.6 vs. +5.5%), while there was no difference for AA (-2.2 vs. -5.9%). Results were largely comparable for the other lipid fractions (PL, CE, and TG).

Three subjects each both in the active and in the placebo group reported mild adverse effects (skin reactions, gastrointestinal upsets). FO/EPO supplementation did not result in any physiologically relevant changes of liver enzymes and full blood cell count.

Conclusions: In women of childbearing age the tested FO/EPO mixture is well tolerated and appears safe. FO/EPO intake resulted in the anticipated increase of plasma GLA, DGLA, and DHA levels without impairing AA status. These data provide a basis for testing this FO/EPO mixture in pregnant women for its efficacy to optimise maternal and neonatal LCPUFA status, and for its effects on infantile development, early markers of allergy risk and prevention of obesity, insulin resistance, hypertriglyceridaemia or other chronic diseases in later life.

5.3 Conclusions from both studies

In contrast to supplementation with DHA alone (*DHAVEG study*, microalgae oil), the expected dietary DHA-induced decrease of n-6 fatty acids (e.g. GLA, DGLA, AA, Σn-6 LCPUFA) in plasma lipids is attenuated in the *FO/EPO study* by the presence of GLA in the supplement (fish oil/evening primrose oil mixture). The combination of DHA and GLA may be of added benefit especially in pregnant or lactating women, in whom it is desirable to enhance n-3 LCPUFA status without compromising GLA, DGLA, and AA status. Further studies should compare the effects of DHA alone and in combination with GLA on neonatal and infantile brain development and function.

Increases from baseline in plasma phospholipid DHA, n-3 LCPUFA and (probably) omega-3 index are smaller with FO/EPO than with microalgae oil treatment. Further studies are needed investigating and comparing the effects of DHA alone and in combination with GLA on CHD risk.

6 Zusammenfassung

6.1 DHAVEG-Studie

Hintergrund: Der Gehalt an Eicosapentaensäure (EPA) und Docosahexaensäure (DHA) in der Erythrozytenmembran (von nun an „Omega-3 Index“ genannt) wurde kürzlich als Risikoindikator für den Tod durch kardiovaskuläre Erkrankungen (CHD) beschrieben. Für den günstigen Effekt von langkettigen n-3 Fettsäuren (n-3 LCPUFA) wird u.a. ihre Triglyzerid-senkende Wirkung diskutiert. Nur wenige Studien untersuchten die individuellen Effekte von EPA oder DHA auf Triglyzerid- (TG) und Lipoproteinspiegel im Plasma.

Ziele: In dieser Studie sollte der Einfluss eines vegetarischen, DHA-reichen (praktisch EPA-freien) Öles der Mikroalge *Ulkenia sp.* auf den DHA-Gehalt von Plasma und Erythrozyten (RBC), den Omega-3 Index, Plasmalipide sowie seine Verträglichkeit und Unbedenklichkeit bei gesunden Erwachsenen mit niedrigem basalen DHA-Status untersucht werden.

Studiendesign: Es wurde eine randomisierte, doppelt-blinde, Plazebo-kontrollierte Interventionsstudie mit zwei parallelen Gruppen durchgeführt. Einhundertvierzehn Vegetarier (87 Frauen, 27 Männer) zwischen 18 und 43 Jahren nahmen über 8 Wochen täglich 2,28 g Mikroalgenöl (entspricht einer täglichen Aufnahme von ~1 g DHA als TG) oder dieselbe Menge Olivenöl (als Plazebo) zu sich. An Woche 0 (Baseline) und Woche 8 wurden RBC- und Plasmafettsäuren, Plasmalipide und verschiedene Sicherheitsparameter (u.a. Blutbild, Leberwerte, Gerinnungsparameter, Vitamin E im Plasma) bestimmt.

Ergebnisse: Die Supplementierung mit DHA erhöhte signifikant den DHA-Gehalt in RBC-Gesamtlipiden (von 4,4 auf 7,9 wt%, Mittelwerte), in RBC-Phosphatidyl-ethanolamin (von 6,5 auf 12,1 wt%), in RBC-Phosphatidylcholin (von 1,4 auf 3,8 wt%) und in Plasma-Phospholipiden (von 2,8 auf 7,4 wt%), während der EPA-Anstieg geringer ausfiel. Der mittlere Omega-3 Index erhöhte sich in der DHA-Gruppe signifikant von 4,8 auf 8,4 wt%. Nach 8-wöchiger Intervention erreichten 69% der DHA-supplementierten Probanden, aber kein Proband der Plazebo-Gruppe, einen wünschenswerten Omega-3 Index größer 8 wt%. Die Supplementierung mit

Mikroalgenöl führte zu signifikanten Abnahmen von 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:4n-6 und 22:5n-3 in Plasma und RBC.

Die DHA-Gabe senkte die Plasma-TG um 20% von 0,96 auf 0,77 mmol/l (Mediane). Plasma-Gesamtcholesterol sowie LDL- und HDL-Cholesterol stiegen in der DHA-Gruppe signifikant an, was zu einem niedrigeren Verhältnis von TG:HDL-Cholesterol und unveränderten LDL:HDL- sowie Gesamtcholesterol:HDL-Cholesterol-Ratios führte.

Leichte Nebenwirkungen (Hautreaktionen und gastrointestinale Beschwerden) traten gleichhäufig in DHA- und Plazebo-Gruppe auf (11% vs. 8%). Bei der Einnahme von DHA-reichem Mikroalgenöl wurden keine physiologisch relevanten Veränderungen der Sicherheitsparameter beobachtet. Ausnahme waren die Plasmaspiegel des von Willebrand-Faktors (vWF), die nach DHA-Gabe seltener außerhalb des Referenzbereiches lagen als zu Studienbeginn (1 vs. 12 Probanden) sowie seltener als in der Plazebo-Gruppe zum selben Zeitpunkt (1 vs. 11 Probanden). Die absoluten und auf Gesamtlipide bezogenen Alpha-Tocopherolspiegel im Plasma veränderten sich nicht durch die DHA-Gabe.

Schlussfolgerungen: DHA-reiches Öl der Mikroalge *Ulkenia sp.* ist bei gesunden Erwachsenen gut verträglich und anscheinend unbedenklich und kann als geeignete vegetarische Quelle für n-3 LCPUFA angesehen werden. Durch 8-wöchige Supplementierung des getesteten Öles lässt sich bei Personen mit niedrigem basalen n-3 LCPUFA-Status ein wünschenswerter Omega-3 Index ≥ 8% erzielen. Die DHA-Aufnahme war mit Verbesserungen einiger CHD-Risikofaktoren verbunden (Plasma-TG, TG:HDL-Cholesterol, vWF), aber andere Risikofaktoren, insbesondere das LDL-Cholesterol, verschlechterten sich leicht. Deshalb ist der Gesamteffekt dieser Intervention auf das CHD-Risiko unklar und sollte weiter untersucht werden.

6.2 FO/EPO-Studie

Hintergrund: Eine Supplementierung mit Fischöl kann den Docosahexaensäure (DHA)-Status während der Schwangerschaft erhöhen, aber gleichzeitig zu einer Verminderung der maternalen und neonatalen Arachidonsäure (AA)-Konzentrationen führen. Da auch AA für die Entwicklung des fötalen Gehirns und anderer Gewebe benötigt wird, ist eine Verschlechterung ihrer Verfügbarkeit nicht wünschenswert.

Ziele: In dieser Pilotstudie sollte untersucht werden, ob eine Supplementierung von Fischöl (FO) und Nachtkerzenöl (EPO) die Plasmaspiegel an langkettigen n-3 Fettsäuren (n-3 LCPUFA) erhöht, ohne den AA-Status zu beeinträchtigen. Desweiteren waren die Auswirkungen auf Gamma-Linolensäure (GLA)- und Dihomo-GLA (DGLA)-Konzentrationen von Interesse, da gezeigt werden konnte, dass diese Fettsäuren mit erhöhtem Geburtsgewicht (DGLA) und geringerem Risiko für bestimmte Aspekte des metabolischen Syndroms (GLA) assoziiert sind. Auch die Verträglichkeit und Unbedenklichkeit dieser Fettsäuremischung sollten beurteilt werden.

Studiendesign: Es wurde eine randomisierte, doppelt-blinde, Plazebo-kontrollierte Interventionsstudie mit zwei parallelen Gruppen durchgeführt. Vierzig nicht-schwangere Frauen zwischen 19 und 36 Jahren nahmen über 8 Wochen täglich 3,4 g einer FO/EPO-Mischung (entspricht einer täglichen Aufnahme von 419 mg DHA, 337 mg GLA und 72 mg Eicosapentaensäure) oder ein Plazebo (Mischung aus Palmöl, Rapsöl und Sonnenblumenöl) zu sich. Zu Studienbeginn und nach 4, 6 und 8 Wochen wurden die Fettsäurekonzentrationen in Plasma-Gesamtlipiden (TL), Phospholipiden (PL), Cholesterolestern und Triglyzeriden (TG) gemessen. Zusätzlich wurden an Woche 0 und Woche 8 verschiedene Sicherheitsparameter (Blutbild, Leberwerte) bestimmt.

Ergebnisse: Die 8-wöchige Supplementierung mit FO/EPO erhöhte in den Plasma-Gesamtlipiden die Spiegel an DHA (von 2,0 auf 3,1 wt%, Mittelwerte), DGLA (von 2,0 auf 2,3 wt%) und GLA (von 0,36 auf 0,52 wt%), während sich die AA-Level nicht veränderten. Die relativen Veränderungen in den Gesamtlipiden nach 8 Wochen Intervention (% der Baselinewerte) von GLA (+49,9 vs. +2,1% mit Plazebo, Mittelwerte), DGLA (+13,8 vs. +0,7%) und DHA (+59,6 vs. +5,5%) waren nach FO/EPO-Gabe signifikant größer als mit Plazebo, wohingegen sich die Veränderungen der AA-Spiegel nicht zwischen den beiden Interventionen

unterschieden (-2,2 vs. –5,9%). Die Fettsäureveränderungen in den untersuchten Plasma-Lipidfraktionen (PL, CE, TG) waren in Richtung und Ausmaß ähnlich wie in den Gesamtlipiden.

Je drei Probandinnen aus der FO/EPO- sowie der Plazebogruppe berichteten von leichten Nebenwirkungen (z.B. Hautreaktionen, leichte gastrointestinale Beschwerden). Die FO/EPO-Supplementierung führte zu keinen physiologisch relevanten Veränderungen der Leberwerte oder im Blutbild.

Schlussfolgerungen: Die getestete FO/EPO-Mischung ist bei Frauen im gebärfähigen Alter gut verträglich und anscheinend unbedenklich. Das Supplement erhöht die Plasmaspiegel von DHA, GLA und DGLA, ohne den AA-Status zu beeinträchtigen. In einer weiteren Studie sollten nun die Effekte dieser FO/EPO-Mischung auf den LCPUFA-Status schwangerer Frauen und ihres Föten sowie die Auswirkungen auf Gemütsverfassung und Gehirnleistung der Mutter, auf die geistige Entwicklung des Kindes, das Allergierisiko sowie die Prävention von Übergewicht, Insulinresistenz, Hypertriglyzeridämie oder anderen chronischen Erkrankungen im weiteren Leben untersucht werden.

6.3 Schlussfolgerungen aus beiden Studien

Im Gegensatz zur alleinigen DHA-Gabe in der *DHAVEG-Studie* (Mikroalgenöl) wird die durch Nahrungs-DHA induzierte Abnahme der n-6 Fettsäuren im Plasma (insbesondere von GLA, DGLA, AA sowie Σn-6 LCPUFA) durch den GLA-Gehalt im Supplement (Mischung aus Fischöl und Nachtkerzenöl, *FO/EPO-Studie*) verhindert. Die Kombination von DHA und GLA könnte von zusätzlichem Nutzen insbesondere für schwangere und stillende Frauen sein, bei denen eine Erhöhung des n-3 LCPUFA-Status ohne Beeinträchtigung der GLA-, DGLA- und AA-Level erwünscht ist. Zukünftige Studien sollten die Auswirkungen von DHA alleine sowie in Kombination mit GLA auf die neonatale und kindliche Gehirnentwicklung und -funktion untersuchen und vergleichen.

Die Erhöhung der DHA- und n-3 LCPUFA-Gehalte in Plasmaphospholipiden sowie (vermutlich) auch des Omega-3 Indexes ist mit FO/EPO-Gabe geringer als mit Mikroalgenöl-Gabe. Weitere Studien sind notwendig, um die Effekte von DHA alleine und in Kombination mit GLA auf das CHD-Risiko zu untersuchen.

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8 Attachment

8.1 Tables

Table 8.1. Reproducibility of triglyceride and lipoprotein analyses, *DHAVEG study.**

	Intra-assay			Inter-assay		
	n	Mean	CV (%)	*n*	Mean	CV (%)
Triglycerides (mg/dl)						
Level 1	20	84.9	1.6	20	84.9	1.9
Level 2	20	143.0	1.6	20	143.0	1.9
Total cholesterol (mg/dl)						
Level 1	20	205	1.3	20	205	2.2
Level 2	20	259	1.1	20	259	2.5
HDL cholesterol (mg/dl)						
Level 1	21	21.7	0.75	21	21.7	2.1
Level 2	21	46.0	0.85	21	46.0	1.7

n, n-fold determination of one sample.

* According to manufacturer's declarations.

Table 8.2. Reproducibility of test kits for biochemical parameters, *DHAVEG study* and *FO/EPO study.**

	Intra-assay			Inter-assay		
	n	Mean	CV (%)	*n*	Mean	CV (%)
Bilirubin (mg/dl)				n.s.		
Human serum	21	2.1	1.3		2.1	1.9
Precinorm U	21	2.1	1.8		2.1	2.1
Precipath U	21	5.0	1.2		5.0	1.6
Creatinine (mg/dl)				n.s.		
Human serum	21	1.67	0.7		1.09	2.3
Precinorm U	21	1.95	0.6		1.92	1.5
Precipath U	21	3.69	0.6		3.70	1.7
Gamma-GT (U/l)				n.s.		
Human serum	21	51.6	1.5		53.7	1.4
Precinorm U	21	40.6	0.5		39.1	1.9
Precipath U	21	185	0.3		173	1.8
ALT (U/l)						
Human serum	21	48	1.8	10	40	3.2
Precinorm U	21	61	2.9	10	55	2.1
Precipath U	21	131	1.1	10	124	0.5
AST (U/l)						
Human serum	21	143	0.7	10	101	2.9
Precinorm U	21	81	4.0	10	74	3.4
Precipath U	21	174	0.8	10	161	1.9
Cholinesterase (U/l)						
Human serum	21	15998	0.7	10	16117	1.4
Precinorm U	21	3358	1.4	10	3402	1.4
Precipath U	21	4756	1.1	10	4759	1.7
Creatinekinase (U/l)						
Human serum	21	31	2.6	10	150	2.1
Precinorm U	21	224	0.4	10	220	1.7
Precipath U	21	484	0.5	10	475	1.7
LDH (U/l)				n.s.		
Human serum	21	132.3	1.4		195.9	2.3
Precinorm U	21	144.7	1.3		160.6	1.8
Precipath U	21	243.7	1.0		247.3	1.3
Uric acid (mg/dl)				n.s.		
Human serum	21	5.57	0.5		7.21	1.7
Precinorm U	21	4.67	0.5		4.86	1.3
Precipath U	21	10.18	0.4		9.39	1.6
Total protein (g/dl)				n.s.		
Human serum	21	4.4	0.60		6.4	0.95
Precinorm U	21	5.0	0.47		5.1	1.21
Precipath U	21	4.8	0.70		4.9	1.22
CRP (mg/dl)						
Human serum	21	2.3	1.3	8	2.2	6.0
Precinorm U	21	5.4	1.0	8	4.2	2.9
Precipath U	21	9.4	0.6	8	11.5	1.3
Glucose (mg/dl)				n.s.		
Human serum	21	127	1.0		126	1.7
Control serum I	21	66	1.1		118	1.9
Control serum II	21	274	0.8		253	1.9

n, n-fold determination of one sample; gamma-GT, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; CRP, C-reactive protein; n.s., not specified. * According to manufacturer's declarations.

Table 8.3. Reproducibility of test kits for haemostatic parameters, *DHAVEG study.**

	Intra-assay			Inter-assay		
	n	Mean	CV (%)	*n*	Mean	CV (%)
Quick's test (%)						
Behring Coagulation Timer	8		0.7 – 1.2	5		1.5 – 2.2
Bering Fibrintimer A	8		1.2 – 6.0	5		0.7 – 3.3
PTT (sec)						
Normal plasma	4			10		
Pathological plasma	4		0.6 – 2.0	10		0.3 – 2.8
Heparine plasma pool	4			10		
Fibrinogen (mg/dl)	n.s.			n.s.		
Normal plasma			1.5 - 5			2.0 - 5
Pathological plasma			3 - 6			3 - 6
D-dimeres (µg/ml)				n.s.		
Plasma	21	0.19	7.3		0.30	6.5
Low control	21	0.86	1.7		0.87	8.3
High control	21	5.11	0.8		4.58	3.2
Factor VII (%)	n.s.			n.s.		
Normal		99.4	0.8		99.7	2.6
Low abnormal		47.6	1.4		47.6	3.5
Von Willebrand factor (%)	n.s.			n.s.		
Level 1		33.7	2.2			?
Level 2		80.8	1.3			?
Level 3		101.5	1.4			?
PAI-1 (AU/ml)						
Level 1	9	12	2.4	10	12	7.6
Level 2	9	23	0.4	10	23	1,6
PFA-ADP			?			?
PFA-EPI			?			?

n, n-fold determination of one sample; PTT, partial thromboplastin time; PAI-1, plasminogen activator inhibitor-1 activity; PFA-ADP, platelet function analysis with adenosine diphosphate; PFA-EPI, platelet function analysis with epinephrine; n.s., not specified; ?, no informations available.

* According to manufacturer's declarations.

Table 8.4. Reproducibility of fatty acid analysis in plasma PL, *DHAVEG study* (mean, CV).

	Intra-assay (*n* = 8)				Inter-assay (*n* = 11)			
	mg/l	CV (%)	wt%	CV (%)	mg/l	CV (%)	wt%	CV (%)
Saturated fatty acids								
14:0	4.84	1.98	0.45	1.66	6.71	4.74	0.44	3.47
15:0	IS		IS		IS		IS	
16:0	327.15	1.63	30.23	0.43	437.57	2.19	28.82	2.12
17:0	4.23	2.02	0.39	0.64	4.69	6.36	0.31	3.81
18:0	147.82	1.94	13.66	0.83	190.92	2.59	12.57	2.53
20:0	7.44	1.81	0.69	0.83	6.77	11.04	0.45	10.44
22:0	20.18	1.47	1.86	0.69	19.00	15.07	1.25	14.74
24:0	16.37	1.58	1.51	1.36	15.47	12.00	1.02	12.00
Trans fatty acids								
14:1t	n.d.		n.d.		n.d.		n.d.	
16:1t	1.15	5.50	0.11	5.08	0.78	16.71	0.05	15.45
18:1t	2.91	3.66	0.27	3.32	2.59	18.59	0.17	16.55
18:2tt	0.94	3.94	0.09	3.52	0.65	12.61	0.04	13.27
22:1t	1.02	9.64	0.09	9.79	n.d.		n.d.	
MUFA								
14:1n-5	n.d.		n.d.		n.d.		n.d.	
15:1n-5	n.d.		n.d.		n.d.		n.d.	
16:1n-7	7.13	1.57	0.66	0.73	20.49	5.58	1.35	2.85
17:1n-7	n.d.		n.d.		n.d.		n.d.	
18:1n-7	17.04	1.61	1.57	0.42	22.17	5.56	1.46	2.75
18:1n-9	112.05	1.44	10.35	0.57	177.37	5.50	11.67	2.92
20:1n-9	1.81	3.44	0.17	2.75	2.39	8.37	0.16	5.49
22:1n-9	2.70	9.92	0.25	9.33	2.61	34.48	0.17	33.04
24:1n-9	33.65	1.96	3.11	1.61	30.06	15.79	1.98	14.83
PUFA								
20:3n-9	1.29	23.62	0.12	23.73	3.85	11.01	0.25	8.25
18:2n-6	211.35	1.52	19.53	0.64	270.19	4.70	17.78	1.79
18:3n-6	0.91	8.16	0.08	8.12	2.15	7.97	0.14	5.17
20:2n-6	5.16	7.11	0.48	6.80	7.47	9.43	0.49	6.87
20:3n-6	29.11	1.58	2.69	0.83	69.84	5.12	4.60	2.10
20:4n-6	72.26	1.76	6.68	0.39	127.79	7.19	8.41	5.47
22:2n-6	n.d.		n.d.		n.d.		n.d.	
22:4n-6	2.58	1.85	0.24	1.33	9.23	6.22	0.61	3.82
22:5n-6	2.61	3.82	0.24	4.19	7.29	6.15	0.48	3.72
18:3n-3	1.39	2.15	0.13	1.76	4.02	4.27	0.26	2.01
18:4n-3	0.47	7.88	0.04	7.64	0.21	22.49	0.01	22.19
20:3n-3	0.65	22.83	0.06	22.83	2.39	15.59	0.16	13.94
20:5n-3	5.96	3.02	0.55	2.29	7.57	6.74	0.50	3.92
22:5n-3	5.36	2.93	0.50	2.74	11.60	7.46	0.76	6.72
22:6n-3	23.42	4.31	2.16	4.11	53.18	5.13	3.50	2.95

CV, coefficient of variation; IS, internal standard; n.d., not detected, PL, phospholipids.

Table 8.5. Reproducibility of fatty acid analysis in RBC PC and PE, *DHAVEG study* (mean, CV).

	Intra-assay (*n* = 7*)				Inter-assay (*n* = 14)			
	RBC PC		RBC PE		RBC PC		RBC PE	
	wt%	CV (%)	wt%	CV (%)	wt%	CV (%)	wt%	CV (%)
Saturated fatty acids								
14:0	0.60	2.96	0.26	5.98	0.74	3.95	0.23	11.29
15:0	n.d.		n.d.		n.d.		n.d.	
16:0	37.42	0.86	17.36	2.71	37.62	1.30	17.36	1.56
17:0	0.38	1.34	0.28	3.74	0.33	3.49	0.23	4.64
18:0	8.76	1.17	8.02	1.10	8.78	2.17	7.47	1.75
20:0	n.d.		n.d.		n.d.		n.d.	
22:0	n.d.		n.d.		n.d.		n.d.	
24:0	0.06	22.1	n.d.		0.22	35.11	n.d.	
Trans fatty acids								
14:1t	n.d.		n.d.		n.d.		n.d.	
16:1t	0.07	33.63	0.44	3.92	0.11	8.48	0.10	14.87
18:1t	0.13	12.08	0.18	4.82	0.08	21.70	0.13	13.85
18:2tt	0.06	10.41	n.d.		0.05	9.22	0.05	9.18
22:1t	n.d.		n.d.		n.d.		n.d.	
MUFA								
14:1n-5	n.d.		n.d.		n.d.		n.d.	
15:1n-5	n.d.		n.d.		n.d.		n.d.	
16:1n-7	0.68	2.28	0.37	5.32	1.33	2.26	0.52	3.42
17:1n-7	n.d.		n.d.		n.d.		n.d.	
18:1n-7	1.64	0.86	0.78	2.22	2.08	1.64	1.27	7.93
18:1n-9	15.63	1.32	16.48	3.18	15.29	1.61	16.58	1.20
20:1n-9	0.19	3.25	0.34	1.80	0.18	4.40	0.37	2.66
22:1n-9	n.d.		n.d.		0.24	3.83	n.d.	
24:1n-9	0.232	22.14	n.d.		0.23	41.38	n.d.	
PUFA								
20:3n-9	0.09	3.30	0.09	4.84	0.15	3.78	0.14	4.35
18:2n-6	19.53	0.92	5.64	2.68	18.54	0.94	5.02	1.30
18:3n-6	0.07	3.49	n.d.		0.08	5.64	0.09	5.30
20:2n-6	0.32	2.04	0.22	2.93	0.48	4.36	0.20	3.21
20:3n-6	2.87	1.49	1.24	0.71	3.67	2.14	1.79	1.26
20:4n-6	7.54	2.27	25.93	2.00	5.62	3.28	23.55	1.25
22:2n-6	n.d.		n.d.		n.d.		n.d.	
22:4n-6	0.32	9.49	7.99	2.77	0.34	6.11	7.43	1.80
22:5n-6	0.14	7.31	0.99	3.28	0.23	8.82	1.16	2.44
18:3n-3	0.14	6.18	0.16	5.38	0.26	2.84	0.17	6.76
18:4n-3	n.d.		n.d.		n.d.		n.d.	
20:5n-3	0.45	2.07	1.26	1.85	0.30	3.99	0.83	1.48
22:5n-3	0.31	4.14	4.25	3.15	0.42	9.74	4.66	2.19
22:6n-3	1.57	3.71	7.50	3.32	1.85	6.02	9.71	1.84

* One sample was lost; n.d., not detected; PC, phosphatidylcholine, PE, phosphatidylethanolamine.

Table 8.6. Reproducibility of fatty acid analysis in RBC total lipids, *DHAVEG study* (mean, CV).

	Intra-assay (*n* = 8)		Inter-assay (*n* = 19)	
	wt%	CV (%)	wt%	CV (%)
Saturated fatty acids				
14:0	0.36	1.40	0.37	2.14
15:0	0.21	0.58	0.21	3.14
16:0	22.24	0.29	22.26	0.88
17:0	0.32	1.10	0.32	2.52
18:0	13.80	0.19	13.64	0.80
20:0	0.36	2.31	0.37	4.08
22:0	1.33	1.31	1.35	2.64
24:0	4.45	2.08	4.61	5.93
Trans fatty acids				
14:1t	n.d.		n.d.	
16:1t	n.d.		n.d.	
18:1t	0.19	7.98	0.17	5.41
18:2tt	n.d.		n.d.	
22:1t	n.d.		n.d.	
MUFA				
14:1n-5	n.d.		n.d.	
15:1n-5	n.d.		n.d.	
16:1n-7	0.45	1.00	0.46	1.42
17:1n-7	n.d.		n.d.	
18:1n-7	1.49	1.13	1.48	5.46
18:1n-9	11.55	0.20	11.44	0.94
20:1n-9	0.21	1.50	0.20	2.11
22:1n-9	0.17	34.69	0.19	34.03
24:1n-9	3.96	1.55	4.17	4.31
PUFA				
20:3n-9	0.10	2.33	0.10	3.79
18:2n-6	9.20	0.65	9.23	1.17
18:3n-6	0.07	2.74	0.06	7.96
20:2n-6	0.23	1.16	0.25	4.32
20:3n-6	2.05	1.32	2.05	0.86
20:4n-6	15.29	0.22	15.07	0.82
22:2n-6	n.d.		n.d.	
22:4n-6	3.77	0.44	3.82	1.04
22:5n-6	0.71	1.74	0.76	2.31
18:3n-3	0.09	2.51	0.09	3.23
18:4n-3	n.d.		n.d.	
20:3n-3	0.17	6.41	0.15	10.43
20:5n-3	0.49	0.93	0.49	1.99
22:5n-3	2.82	0.48	2.80	1.78
22:6n-3	3.79	0.54	3.76	2.06

n.d., not detected.

Table 8.7. Reproducibility of fatty acid analysis in plasma phospholipids, *FO/EPO study* (mean, CV).

	Intra-assay (*n* = 8)				Inter-assay (*n* = 11)			
	mg/l	CV (%)	wt%	CV (%)	mg/l	CV (%)	wt%	CV (%)
Saturated fatty acids								
14:0	9.52	0.94	0.54	0.74	9.43	3.37	0.52	3.65
15:0	IS		IS		IS		IS	
16:0	611.67	1.00	34.75	0.40	620.63	2.17	34.41	1.43
17:0	6.14	1.78	0.35	1.61	6.21	2.11	0.34	2.42
18:0	154.98	1.34	8.80	0.72	159.99	2.42	8.87	1.16
20:0	8.25	4.81	0.47	4.83	9.10	4.50	0.50	4.37
22:0	24.47	1.39	1.39	1.35	25.60	10.78	1.42	10.46
24:0	13.59	1.55	0.77	1.61	15.27	4.12	0.85	3.40
Trans fatty acids								
14:1t	n.d.		n.d.		n.d.		n.d.	
16:1t	1.98	5.07	0.11	5.15	1.66	17.54	0.09	18.74
18:1t	1.75	8.53	0.10	8.51	2.40	11.06	0.13	10.27
18:2tt	0.81	11.71	0.05	11.42	0.84	8.06	0.05	7.53
22:1t	4.37	2.43	0.25	2.17	4.63	5.76	0.26	4.61
MUFA								
14:1n-5	n.d.		n.d.		n.d.		n.d.	
15:1n-5	n.d.		n.d.		n.d.		n.d.	
16:1n-7	32.26	0.96	1.83	0.77	32.53	3.08	1.80	1.44
17:1n-7	n.d.		n.d.		n.d.		n.d.	
18:1n-7	32.62	1.15	1.85	0.57	33.32	3.32	1.85	1.12
18:1n-9	195.64	0.90	11.11	0.40	200.06	3.05	11.09	0.52
20:1n-9	2.11	2.68	0.12	2.13	2.24	4.68	0.12	4.62
22:1n-9	3.72	28.11	0.21	27.35	4.96	41.79	0.27	41.60
24:1n-9	36.98	0.80	2.10	0.63	39.22	3.45	2.17	2.69
PUFA								
20:3n-9	5.73	1.09	0.33	1.27	6.02	2.79	0.33	2.02
18:2n-6	246.23	0.70	13.99	0.35	250.87	3.22	13.90	0.71
18:3n-6	1.94	2.46	0.11	2.78	1.98	4.55	0.11	2.85
20:2n-6	7.61	0.52	0.43	0.69	7.87	3.17	0.44	1.72
20:3n-6	77.27	0.74	4.39	0.69	79.37	3.52	4.40	1.29
20:4n-6	178.25	1.21	10.13	1.12	184.12	3.80	10.20	1.53
22:2n-6	n.d.		n.d.		n.d.		n.d.	
22:4n-6	9.57	0.88	0.54	1.15	9.96	4.36	0.55	2.52
22:5n-6	10.23	1.49	0.58	1.44	10.16	3.39	0.56	2.26
18:3n-3	3.11	0.96	0.18	1.15	3.15	4.07	0.17	2.50
18:4n-3	0.68	11.17	0.04	11.68	0.70	10.92	0.04	10.92
20:3n-3	2.31	3.67	0.13	3.34	2.30	9.30	0.13	9.19
20:5n-3	11.70	1.27	0.66	1.71	11.88	3.92	0.66	1.73
22:5n-3	15.38	1.39	0.87	1.76	16.09	4.66	0.89	2.81
22:6n-3	49.59	1.62	2.82	1.85	51.51	4.91	2.85	2.97

IS, internal standard; n.d., not detected.

Table 8.8. Reproducibility of fatty acid analysis in plasma CE, *FO/EPO study* (mean, CV).

	Intra-assay (*n* = 8)				Inter-assay (*n* = 10)			
	mg/l	CV (%)	wt%	CV (%)	mg/l	CV (%)	wt%	CV (%)
Saturated fatty acids								
14:0	12.01	1.09	1.11	0.86	12.18	3.02	1.15	4.02
15:0	IS		IS		IS		IS	
16:0	136.00	1.04	12.62	0.23	135.16	2.23	12.72	1.89
17:0	0.92	6.15	0.09	5.49	1.14	6.63	0.11	7.35
18:0	6.68	1.75	0.62	1.24	6.92	4.27	0.65	4.96
20:0	1.06	2.85	0.10	3.43	0.93	14.34	0.09	14.86
22:0	n.d.		n.d.		n.d.		n.d.	
24:0	n.d.		n.d.		n.d.		n.d.	
Trans fatty acids								
14:1t	n.d.		n.d.		n.d.		n.d.	
16:1t	n.d.		n.d.		n.d.		n.d.	
18:1t	n.d.		n.d.		n.d.		n.d.	
18:2tt	n.d.		n.d.		n.d.		n.d.	
22:1t	1.21	7.82	0.11	7.08	1.16	9.78	0.11	8.12
MUFA								
14:1n-5	0.82	5.83	0.08	6.26	0.88	7.28	0.08	7.29
15:1n-5	n.d.		n.d.		n.d.		n.d.	
16:1n-7	111.46	1.17	10.34	0.41	110.40	2.86	10.39	1.83
17:1n-7	n.d.		n.d.		n.d.		n.d.	
18:1n-7	15.77	1.16	1.46	0.35	16.38	4.27	1.54	2.51
18:1n-9	241.77	1.25	22.43	0.16	238.52	3.17	22.43	0.49
20:1n-9	n.d.		n.d.		n.d.		n.d.	
22:1n-9	1.06	15.56	0.10	16.26	1.26	38.92	0.12	38.84
24:1n-9	n.d.		n.d.		n.d.		n.d.	
PUFA								
20:3n-9	1.29	3.61	0.12	3.79	1.34	22.84	0.13	23.82
18:2n-6	417.23	1.22	38.70	0.08	409.68	3.37	38.53	0.43
18:3n-6	14.50	1.17	1.35	0.33	14.42	3.53	1.36	2.23
20:2n-6	n.d.		n.d.		n.d.		n.d.	
20:3n-6	10.46	1.49	0.97	0.41	10.13	4.90	0.95	2.88
20:4n-6	82.07	1.37	7.61	0.38	79.75	3.97	7.50	1.37
22:2n-6	n.d.		n.d.		n.d.		n.d.	
22:4n-6	n.d.		n.d.		n.d.		n.d.	
22:5n-6	0.58	6.37	0.05	5.57	0.56	9.26	0.05	7.58
18:3n-3	7.59	1.54	0.70	0.58	7.40	3.88	0.70	1.45
18:4n-3	0.65	5.12	0.06	4.90	0.63	20.19	0.06	19.50
20:3n-3	0.52	10.53	0.05	9.81	0.47	12.77	0.04	11.92
20:5n-3	7.87	1.56	0.73	0.62	7.46	5.18	0.70	4.18
22:5n-3	n.d.		n.d.		n.d.		n.d.	
22:6n-3	4.97	2.21	0.46	1.59	4.80	5.66	0.45	4.41

CE, cholesterol esters; CV, coefficient of variation; IS, internal standard; n.d., not detected.

Table 8.9. Reproducibility of fatty acid analysis in plasma triglycerides, *FO/EPO study* (mean, CV).

	Intra-assay (*n* = 8)				Inter-assay (*n* = 10)			
	mg/l	CV (%)	wt%	CV (%)	mg/l	CV (%)	wt%	CV (%)
Saturated fatty acids								
14:0	25.90	1.21	2.79	1.02	26.32	3.02	2.95	4.40
15:0	IS		IS		IS		IS	
16:0	305.63	1.04	32.92	0.37	303.36	2.65	33.94	0.85
17:0	2.50	2.83	0.27	2.20	2.62	3.54	0.29	3.44
18:0	21.64	1.59	2.33	1.29	20.72	5.31	2.32	3.70
20:0	1.46	7.77	0.16	7.59	1.16	13.60	0.13	12.72
22:0	0.82	5.22	0.09	4.36	0.32	11.13	0.04	10.41
24:0	n.d.		n.d.		n.d.		n.d.	
Trans fatty acids								
14:1t	n.d.		n.d.		n.d.		n.d.	
16:1t	0.72	6.64	0.08	6.89	0.74	8.95	0.08	8.40
18:1t	3.25	3.07	0.35	2.30	3.27	17.37	0.37	15.24
18:2tt	0.81	10.44	0.09	10.38	0.69	8.20	0.08	8.20
22:1t	0.80	4.99	0.09	4.62	0.76	9.05	0.09	7.31
MUFA								
14:1n-5	2.65	1.07	0.29	1.09	2.78	7.02	0.31	8.46
15:1n-5	n.d.		n.d.		n.d.		n.d.	
16:1n-7	82.78	1.21	8.91	0.31	81.89	3.00	9.16	2.98
17:1n-7	n.d.		n.d.		n.d.		n.d.	
18:1n-7	41.39	1.42	4.46	0.37	38.50	3.59	4.31	1.28
18:1n-9	278.87	1.42	30.03	0.30	263.09	3.42	29.43	1.02
20:1n-9	4.40	1.57	0.47	0.72	3.94	6.57	0.44	5.11
22:1n-9	1.29	8.23	0.14	8.02	1.43	25.61	0.16	25.84
24:1n-9	n.d.		n.d.		n.d.		n.d.	
PUFA								
20:3n-9	3.34	0.91	0.36	0.95	3.10	7.36	0.35	6.16
18:2n-6	95.60	1.38	10.30	0.28	89.97	2.87	10.07	0.45
18:3n-6	2.40	1.88	0.26	1.24	2.31	3.47	0.26	2.38
20:2n-6	2.45	1.05	0.26	0.58	2.28	6.63	0.26	5.52
20:3n-6	4.81	1.37	0.52	0.74	4.24	4.88	0.48	3.38
20:4n-6	17.60	1.35	1.90	0.22	15.91	4.07	1.78	2.56
22:2n-6	n.d.		n.d.		n.d.		n.d.	
22:4n-6	2.68	3.29	0.29	2.36	2.48	5.30	0.28	4.44
22:5n-6	3.57	2.20	0.38	1.24	3.16	5.37	0.35	4.01
18:3n-3	6.81	1.76	0.73	1.16	6.40	2.84	0.72	2.92
18:4n-3	n.d.		n.d.		n.d.		n.d.	
20:3n-3	0.80	4.99	0.09	5.00	0.71	8.44	0.08	6.54
20:5n-3	1.97	3.16	0.21	2.90	1.66	4.14	0.19	3.70
22:5n-3	2.54	2.06	0.27	2.02	2.14	5.23	0.24	4.06
22:6n-3	8.69	1.82	0.94	1.44	7.45	5.13	0.83	3.91

CV, coefficient of variation; IS, internal standard; n.d., not detected.

Table 8.10. Reproducibility of fatty acid analysis in plasma total lipids, *FO/EPO study* (mean, CV).

	Intra-assay (*n* = 8)				Inter-assay (*n* = 10)			
	mg/l	CV (%)	wt%	CV (%)	mg/l	CV (%)	wt%	CV (%)
Saturated fatty acids								
14:0	50.70	1.20	1.26	0.56	51.65	2.69	1.27	2.86
15:0	IS		IS		IS		IS	
16:0	1134.12	1.17	28.11	0.61	1140.11	2.06	27.95	1.13
17:0	10.56	2.56	0.26	2.13	10.48	3.08	0.26	2.03
18:0	206.16	2.20	5.11	1.78	205.02	3.41	5.03	2.58
20:0	9.32	6.07	0.23	6.13	11.82	5.16	0.29	4.59
22:0	27.84	2.93	0.69	2.60	26.72	15.69	0.66	15.51
24:0	15.06	3.08	0.37	2.45	15.95	4.94	0.39	3.97
Trans fatty acids								
14:1t	n.d.		n.d.		n.d.		n.d.	
16:1t	2.96	4.09	0.07	3.08	2.75	9.81	0.07	9.85
18:1t	5.48	2.59	0.14	2.69	6.24	4.98	0.15	4.76
18:2tt	1.92	7.72	0.05	7.73	1.87	4.13	0.05	4.26
22:1t	7.36	3.07	0.18	2.76	7.18	5.50	0.18	3.63
MUFA								
14:1n-5	4.16	2.06	0.10	2.47	4.33	3.67	0.11	4.52
15:1n-5	n.d.		n.d.		n.d.		n.d.	
16:1n-7	235.78	1.33	5.84	1.07	241.01	3.09	5.91	1.98
17:1n-7	n.d.		n.d.		n.d.		n.d.	
18:1n-7	97.16	1.22	2.41	0.68	97.87	2.81	2.40	0.86
18:1n-9	753.10	1.48	18.66	1.20	772.53	3.57	18.94	1.75
20:1n-9	7.02	3.09	0.17	3.42	7.42	4.68	0.18	3.41
22:1n-9	5.20	68.11	0.13	66.65	5.33	32.00	0.13	31.93
24:1n-9	42.40	2.87	1.05	2.50	42.27	5.06	1.04	4.48
PUFA								
20:3n-9	11.40	1.64	0.28	1.13	11.57	5.38	0.28	3.43
18:2n-6	787.38	1.31	19.51	0.98	800.91	3.35	19.63	1.22
18:3n-6	19.34	1.85	0.48	1.55	19.94	3.55	0.49	1.96
20:2n-6	11.92	1.60	0.30	1.43	12.00	4.49	0.29	2.36
20:3n-6	104.38	2.06	2.59	1.76	103.07	4.06	2.53	3.12
20:4n-6	308.30	1.43	7.64	0.90	307.09	3.32	7.53	1.68
22:2n-6	n.d.		n.d.		n.d.		n.d.	
22:4n-6	14.40	1.97	0.36	1.50	14.30	4.48	0.35	3.06
22:5n-6	17.16	2.04	0.43	1.86	16.21	5.23	0.40	4.67
18:3n-3	19.28	1.17	0.48	0.98	19.41	3.16	0.48	0.88
18:4n-3	2.02	8.40	0.05	8.96	2.16	18.89	0.05	18.62
20:3n-3	3.98	5.45	0.10	4.93	3.82	9.75	0.09	9.03
20:5n-3	24.36	1.71	0.60	1.09	23.74	3.85	0.58	2.81
22:5n-3	21.36	2.16	0.53	1.84	20.90	4.24	0.51	3.29
22:6n-3	73.60	2.21	1.82	1.90	73.60	3.81	1.80	2.57

CV, coefficient of variation; IS, internal standard; n.d., not detected.

Table 8.11. BMI, blood pressure and heart rate at weeks 0 and 8, DHAVEG study (mean ± SD or median with IQR).

	DHA group (*n* = 54 - 55)*		Placebo group (*n* = 53)		
	Week 0	Week 8	Week 0	Week 8	*P†*
Weight (kg)[§]	60.8 (56.2 – 67.3)	60.9 (54.8 – 67.7)	60.2 (53.7 – 69.0)	60.7 (54.0 – 70.4)[1]	0.072
BMI (kg/m^2)	21.23 ± 1.69	21.23 ± 1.75	21.18 ± 1.94	21.36 ± 1.90[1]	0.056
Systolic BP (mm Hg)[§]	100 (90 – 100)	100 (94 – 110)	95 (90 – 100)	100 (95 – 105)[1]	0.675
Diastolic BP (mm Hg)[§]	65 (60 – 70)	70 (60 – 70)	70 (60 – 70)	70 (65 – 70)	0.700
Heart rate (beats/min)[§]	68 (63 – 72)	68 (64 – 72)	68 (64 – 72)	68 (64 – 72)	0.664

BMI, body mass index; BP, blood pressure; IQR, interquartile range.

* For one subject, blood pressure and heart rate values are missing; [§] Median with IQR in parentheses.

[1] $p < 0.05$ vs. week 0.

† Analyses of group differences in absolute changes from baseline (week 8 – week 0).

Table 8.12. Plasma triglycerides and lipoproteins at weeks 0 and 8, *DHAVEG study* (mean ± SD or median with IQR).

	DHA group (*n* = 53)		Placebo group *(n* = 53)		
	Week 0	Week 8	Week 0	Week 8	*P†*
Triglycerides (mmol/l)[§]	0.96 (0.75 – 1.21)	0.77 (0.63 – 1.05)[1,a]	1.04 (0.68 – 1.30)	0.91 (0.71 – 1.34)	0.033
Total cholesterol (mmol/l)[§]	4.47 (3.89 – 5.18)	4.73 (4.02 – 5.39)[1]	4.71 (4.05 – 5.30)	4.68 (4.03 – 5.13)	0.004
LDL cholesterol (mmol/l)[§]	2.43 (1.86 – 2.96)	2.74 (2.19 – 3.07)[1]	2.64 (2.03 – 3.10)	2.48 (1.95 – 3.07)	0.003
HDL cholesterol (mmol/l)	1.65 ± 0.47	1.77 ± 0.47[1]	1.67 ± 0.44	1.66 ± 0.43	0.002
LDL:HDL cholesterol	1.58 ± 0.54	1.64 ± 0.60	1.64 ± 0.65	1.65 ± 0.69	0.441
Total:HDL cholesterol	2.92 ± 0.70	2.87 ± 0.68	2.95 ± 0.76	2.97 ± 0.82	0.486
TG:HDL chosterol[§]	0.57 (0.43 – 0.91)	0.43 (0.35 – 0.64)[1,a]	0.63 (0.36 – 0.87)	0.57 (0.43 – 0.86)	0.021

IQR, interquartile range; TG, triglycerides.

[§] Median with IQR in parentheses.

[1] $p < 0.05$ vs. week 0.

[a] $p < 0.05$ vs. placebo at the same time point.

† Analyses of group differences in absolute changes from baseline (week 8 – week 0).

Table 8.13. Vitamin A and E levels at weeks 0 and 8, *DHAVEG study* (mean ± SD).

	DHA group (*n* = 55)		Placebo group *(n* = 53)		
	Week 0	Week 8	Week 0	Week 8	*P†*
Retinol (µmol/l)	1.78 ± 0.44	1.81 ± 0.42	1.81 ± 0.43	1.74 ± 0.43	0.020
alpha-Tocopherol (µmol/l)	20.80 ± 3.99	20.32 ± 3.79	21.22 ± 4.17	21.11 ± 4.02	0.450
alpha-Tocopherol/ (Cholesterol+TG) (µmol/mmol)	3.67 ± 0.58	3.60 ± 0.59	3.72 ± 0.54	3.72 ± 0.55	0.736

TG, triglycerides.
† Analyses of group differences in absolute changes from baseline (week 8 – week 0).

Table 8.14. Plasma haemostatic factors at weeks 0 and 8, *DHAVEG study* (median with IQR).

	DHA group			Placebo group			
	*n**	Week 0	Week 8	*n**	Week 0	Week 8	*P†*
PTT (sec)	53	32.0 (31.0 – 34.0)	32.0 (29.5 – 35.0)	52	31.5 (30.0 – 34.7)	31.5 (29.0 – 34.0)[1]	0.212
Fibrinogen (mg/dl)	53	233 (209 – 264)	258 (230 – 301)[1]	51	230 (203 – 292)	268 (226 – 327)[1]	0.608
D-dimers (µg/ml)	55	0.2 (0.1 – 0.3)	0.2 (0.1 – 0.3)	53	0.2 (0.1 – 0.3)	0.2 (0.1 – 0.3)	0.838
Factor VII (%)	55	103.0 (94.0 – 120.0)	104.0 (90.0 – 119.0)	53	105.0 (88.0 – 130.5)	107.0 (88.5 – 122.0)	0.330
Von Willebrand factor (%)	55	91.0 (75.0 – 119.0)	89.0 (71.0 – 105.0)[1]	53	92.0 (65.5 – 116.0)	96.0 (67.0 – 109.5)	0.033
PAI-1 (AU/ml)	55	2.75 (0.00 – 6.08)	3.57 (1.31 – 6.15)	53	2.25 (0.00 – 6.39)	5.42 (1.90 – 8.32)[1]	0.083
PFA-ADP	43	82.0 (70.0 – 101.0)	92.0 (81.0 – 105.0)[1]	40	85.0 (74.0 – 96.8)	88.0 (74.0 – 109.5)	0.728
PFA-EPI	42	117.5 (104.0 – 134.0)	128.0 (108.8 – 147.0)	34	116.0 (101.0 – 139.0)	129.5 (105.8 – 149.5)	0.761

IQR, interquartile range; PTT, partial thromboplastin time; PAI-1, plasminogen activator inhibitor-1 activity; PFA-ADP, platelet function analysis with adenosine diphosphate; PFA-EPI, platelet function analysis with epinephrine.

* Some samples are missing because of technical problems during measurement or coagulation of the blood samples.

[1] $p < 0.05$ vs. week 0.

† Analyses of group differences in absolute changes from baseline (week 8 – week 0).

Table 8.15. Biochemical parameters and full blood cell count at weeks 0 and 8, *DHAVEG study* (mean ± SD or median with IQR).

	DHA group (*n* = 54 - 55)*		Placebo group (*n* = 51 - 53)*		
	Week 0	Week 8	Week 0	Week 8	*P†*
Creatinine (mg/dl)[§]	0.8 (0.8 – 0.9)	0.9 (0.8 – 0.9)	0.8 (0.8 – 0.9)	0.8 (0.8 – 0.9)	0.184
Bilirubin (mg/dl)[§]	0.7 (0.5 – 1.0)[a]	0.8 (0.5 – 1.0)	0.6 (0.4 – 0.8)	0.6 (0.4 – 0.8)	0.328
GGT (U/l)[§]	15.0 (12.0 – 20.0)	15.0 (12.0 – 20.0)	14.0 (13.0 – 20.0)	15.0 (12.0 – 18.5)	0.069
ALT (U/l)[§]	13.0 (10.0 – 18.0)	16.0 (12.0 – 20.0)[1]	14.0 (10.5 – 19.0)	13.0 (11.0 – 19.0)	0.031
AST (U/l)[§]	20.0 (18.0 – 25.0)	21.0 (18.0 – 26.0)	21.0 (18.5 – 26.5)	21.0 (19.0 – 24.0)	0.267
CHE (kU/l)[§]	7.5 (6.6 – 8.6)	7.2 (6.5 – 8.0)[1]	6.9 (5.9 – 8.1)	6.9 (6.2 – 7.7)	<0.001
CK (U/l)[§]	79.0 (57.0 – 104.0)	80.0 (64.0 – 102.0)	84.0 (66.0 – 102.5)	77.0 (64.0 – 117.0)	0.567
LDH (U/l[§]	144.0 (135.5 – 154.3)	146.5 (131.8 – 158.0)	147.0 (128.5 – 165.5)	140.0 (128.0 – 165.5)	0.566
Uric acid (mg/dl)	4.56 ± 1.15	4.55 ± 0.95	4.95 ± 1.20	4.73 ± 1.23[1]	0.140
Total protein (g/dl)	7.60 ± 0.45	7.44 ± 0.53[1]	7.69 ± 0.45	7.43 ± 0.42[1]	0.308
CRP (mg/dl)[§]	0.20 (0.10 – 0.30)	0.20 (0.05 – 0.30)	0.20 (0.10 – 0.30)	0.20 (0.10 – 0.40)	0.326
Glucose (mg/dl)[§]	93.3 ± 7.5	92.2 ± 7.6	95.9 ± 6.1	94.6 ± 7.8	0.926
Blood cell count					
Leucocytes (G/l)[§]	5.80 (4.90 – 6.60)	5.60 (4.40 – 7.00)	5.60 (4.95 – 6.60)	5.50 (4.85 – 6.15)[1]	0.397
Erythrocytes (T/l)[§]	4.40 (4.30 – 4.70)	4.30 (4.10 – 4.70)	4.40 (4.30 – 4.80)	4.30 (4.05 – 4.45)[1]	0.028
Haemoglobin (g/dl)[§]	13.7 (12.9 – 14.5)	13.4 (12.8 – 14.5)[1]	13.7 (12.7 – 14.5)	13.2 (12.6 – 13.9)[1]	0.255
Haematocrit (%)	40.0 ± 3.1	39.5 ± 3.6	40.1 ± 3.4	38.5 ± 3.3[1]	0.044
MCV (fl)	88.9 ± 4.5	89.3 ± 4.5	89.4 ± 4.7	89.8 ± 4.7	0.821
MCH (pg)[§]	30.6 (29.4 – 32.0)	30.9 (29.8 – 32.0)	30.6 (29.6 – 31.5)	30.9 (30.2 – 32.3)[1]	0.194
MCHC (%)[§]	34.7 (33.0 – 35.3)	34.5 (33.6 – 35.4)	34.1 (33.0 – 35.1)	34.7 (33.5 – 35.6)	0.439
Platelets (G/l)	233.3 ± 57.0	236.7 ± 60.6	238.9 ± 53.3	239.3 ± 59.2	0.775

GGT, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHE, cholinesterase; CK, creatinekinase; LDH, lactate dehydrogenase; CRP, C-reactive protein; MCV, mean cellular volume; MCH, mean cellular haemoglobin; MCHC, mean cellular haemoglobin concentration.

* Some samples are missing because of technical problems measurement; [§] Median with IQR in parentheses.

[1] $p < 0.05$ vs. week 0; [a] $p < 0.05$ vs. placebo at the same time point.

† Analyses of group differences in absolute changes from baseline (week 8 – week 0).

Table 8.16. Relative fatty acid composition (wt%) in plasma phospholipids at weeks 0 and 8, *DHAVEG study* (mean ± SD or median with IQR).

	DHA group (*n* = 55)		Placebo group (*n* = 53)	
	Week 0	Week 8	Week 0	Week 8
16:0[§]	27.95 (26.98 – 29.66)	28.15 (27.38 – 29.91)[a]	27.95 (26.40 – 29.20)	27.40 (26.38 – 29.58)
18:0[§]	12.03 (10.99 – 13.00)	12.08 (10.82 – 13.18)	12.40 (11.43 – 13.10)	12.23 (11.33 – 12.93)
18:1n-9	10.35 ± 1.42	9.07 ± 1.18[1,a]	10.33 ± 1.62	10.48 ± 1.43
20:3n-9[§]	0.14 (0.10 – 0.21)	0.08 (0.05 – 0.11)[1,a]	0.14 (0.10 – 0.20)	0.14 (0.12 – 0.18)
18:2n-6	22.01 ± 2.80	20.63 ± 2.82[1,a]	23.04 ± 3.23	22.91 ± 2.90
18:3n-6[§]	0.10 (0.07– 0.12)	0.06 (0.04 – 0.08) [1,a]	0.10 (0.08 – 0.13)	0.08 (0.07 – 0.12)
20:3n-6	3.49 ± 0.72	2.59 ± 0.60[1,a]	3.31 ± 0.87	3.34 ± 0.88
20:4n-6	8.93 ± 1.40	8.07 ± 1.42[1,a]	8.92 ± 1.66	8.99 ± 1.76
22:4n-6[§]	0.42 (0.35 – 0.50)	0.23 (0.20 – 0.28)[1,a]	0.40 (0.35 – 0.48)	0.40 (0.34 – 0.46)
22:5n-6[§]	0.34 (0.29 – 0.45)	0.66 (0.56 – 0.73)[1,a]	0.31 (0.24 – 0.45)	0.32 (0.24 – 0.44)
18:3n-3[§]	0.19 (0.14 – 0.24)	0.16 (0.11 – 0.21)	0.17 (0.14 – 0.24)	0.18 (0.13 – 0.26)
20:5n-3[§]	0.54 (0.42 – 0.64)	0.75 (0.62 – 0.87)[1,a]	0.52 (0.41 – 0.73)	0.48 (0.39 – 0.66) [1]
22:5n-3	0.90 ± 0.27	0.55 ± 0.18[1,a]	0.85 ± 0.26	0.85 ± 0.27
22:6n-3	2.84 ± 0.89	7.36 ± 1.61[1,a]	2.56 ± 0.74	2.52 ± 0.68
EPA + DHA	3.42 ± 0.91	8.13 ± 1.67[1,a]	3.13 ± 0.78	3.04 ± 0.69
∑n-6 FA	35.74 ± 2.15	32.62 ± 2.29[1,a]	36.49 ± 2.47	36.46 ± 2.27
∑n-3 FA[§]	4.61 (4.06 – 5.23)	8.84 (8.00 – 9.89)[1,a]	4.20 (3.75 – 4.85)	4.26 (3.79 – 4.70)
∑n-6/∑n-3 FA[§]	7.83 (6.87 – 8.91)	3.80 (3.11 – 4.15)[1,a]	8.55 (7.17 – 9.93)	8.32 (7.58 – 9.99)
∑n-6 LCPUFA	13.61 ± 1.74	11.92 ± 1.71[1,a]	13.35 ± 2.13	13.44 ± 1.98
∑n-3 LCPUFA	4.48 ± 0.94[a]	8.82 ± 1.66[1,a]	4.13 ± 0.78	4.03 ± 0.71
∑n-6/∑n-3 LCPUFA[§]	3.23 (2.82 – 3.64)	1.34 (1.18 – 1.56)[1,a]	3.31 (2.92 – 3.76)	3.29 (2.99 – 3.80)

IQR, interquartile range; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ∑n-6 FA, sum of n-6 fatty acids; ∑n-3 FA, sum of n-3 fatty acids; ∑n-6/∑n-3 FA, ratio of n-6 to n-3 fatty acids; ∑n-6 LCPUFA, sum of all long-chain n-6 fatty acids; ∑n-3 LCPUFA, sum of all long-chain n-3 fatty acids; ∑n-6/∑n-3 LCPUFA, ratio of n-6 to n-3 long-chain polyunsaturated fatty acids.

[§] Median with IQR in parentheses; [1] $p < 0.05$ vs. week 0; [a] $p < 0.05$ vs. placebo at the same time point.

Table 8.17. Relative fatty acid composition (wt%) in RBC phosphatidylcholine at weeks 0 and 8, *DHAVEG study* (mean ± SD or median with IQR).

	DHA group (*n* = 55)		Placebo group (*n* = 52)*	
	Week 0	Week 8	Week 0	Week 8
16:0[§]	35.59 (34.44 – 37.13)	36.26 (34.76 – 37.21)[1]	35.20 (34.26 – 37.35)	35.03 (34.20 – 36.68)[1]
18:0[§]	10.07 (8.71 – 10.90)	10.27 (9.25 – 10.99)[1]	10.13 (9.11 – 10.87)	10.33 (9.04 – 10.97)
18:1n-9	15.92 ± 1.48	15.45 ± 1.26[1,a]	15.99 ± 1.28	16.16 ± 1.30
20:3n-9[§]	0.08 (0.05 – 0.11)	0.04 (0.03 – 0.07)[1,a]	0.08 (0.06 – 0.11)	0.08 (0.06 – 0.11)
18:2n-6	21.09 ± 2.34	20.16 ± 2.34[1,a]	21.58 ± 2.21	21.43 ± 2.34
18:3n-6[§]	0.07 (0.06 – 0.09)	0.04 (0.03 – 0.05)[1,a]	0.07 (0.06 – 0.09)	0.06 (0.05 – 0.08)
20:3n-6	2.50 ± 0.52	1.96 ± 0.44[1,a]	2.45 ± 0.65	2.45 ± 0.65
20:4n-6	6.00 ± 1.10	5.49 ± 1.00[1,a]	5.97 ± 1.00	6.02 ± 1.09
22:4n-6[§]	0.31 (0.26 – 0.37)	0.22 (0.20 – 0.27)[1,a]	0.31 (0.25 – 0.34)	0.33 (0.26 – 0.35)[1]
22:5n-6[§]	0.16 (0.12 – 0.20)	0.33 (0.27 – 0.38)[1,a]	0.13 (0.11 – 0.21)	0.14 (0.11 – 0.21)
18:3n-3[§]	0.19 (0.15 – 0.24)	0.16 (0.12 – 0.20)[1]	0.18 (0.14 – 0.22)	0.18 (0.15 – 0.24)
20:5n-3[§]	0.31 (0.24 – 0.41)	0.43 (0.35 – 0.53)[1,a]	0.30 (0.23 – 0.41)	0.28 (0.22 – 0.36)
22:5n-3	0.52 ± 0.16	0.37 ± 0.10[1,a]	0.51 ± 0.14	0.51 ± 0.14
22:6n-3	1.38 ± 0.51	3.78 ± 0.95[1,a]	1.25 ± 0.41	1.23 ± 0.43
EPA + DHA[§]	1.58 (1.35 – 2.13)	4.10 (3.57 – 4.99)[1,a]	1.58 (1.22 – 1.91)	1.46 (1.23 – 1.90)
∑n-6 FA[§]	30.56 (29.00 – 32.19)	28.36 (27.57 – 29.69)[1,a]	30.91 (29.73 – 32.19)	30.33 (29.62 – 32.00)
∑n-3 FA	2.56 ± 0.62	4.88 ± 1.02[1,a]	2.41 ± 0.48	2.36 ± 0.47
∑n-6/∑n-3 FA[§]	12.73 (10.11 – 14.23)	5.84 (5.10 – 6.71)[1,a]	13.17 (11.38 – 15.36)	13.09 (11.17 – 14.71)
∑n-6 LCPUFA[§]	9.28 (8.26 – 10.52)	8.22 (7.20 – 9.43)[1,a]	9.02 (8.09 – 10.43)	9.27 (8.38 – 10.04)
∑n-3 LCPUFA	2.37 ± 0.60	4.71 ± 1.02[1,a]	2.20 ± 0.45	2.17 ± 0.45
∑n-6/∑n-3 LCPUFA[§]	4.23 (3.68 – 4.76)	1.82 (1.54 – 2.06)[1,a]	4.20 (3.85 – 4.77)	4.26 (3.95 – 4.91)

Abbreviations see Table 8.16.

* One sample was lost during preparation.
[§] Median with IQR in parentheses.
[1] $p < 0.05$ vs. week 0; [a] $p < 0.05$ vs. placebo at the same time point.

Table 8.18. Relative fatty acid composition (wt%) in RBC phosphatidylethanolamine at weeks 0 and 8, *DHAVEG study* (mean ± SD or median with IQR).

	DHA group (*n* = 55)		Placebo group (*n* = 52)*	
	Week 0	Week 8	Week 0	Week 8
16:0	15.97 ± 1.21	16.54 ± 1.21[1,a]	15.94 ± 1.27	15.79 ± 1.20
18:0	7.61 ± 0.71	7.61 ± 0.67	7.56 ± 0.47	7.71 ± 0.44[1]
18:1n-9	16.36 ± 1.56	16.12 ± 1.53[1]	16.70 ± 1.67	16.72 ± 1.61
20:3n-9	0.10 ± 0.04	0.08 ± 0.03[1,a]	0.10 ± 0.03	0.11 ± 0.04[1]
18:2n-6	6.55 ± 1.27	5.82 ± 1.16[1,a]	6.87 ± 1.19	6.88 ± 1.36
18:3n-6	0.11 ± 0.02	0.09 ± 0.02[1,a]	0.11 ± 0.02	0.11 ± 0.02
20:3n-6[§]	1.63 (1.41 – 1.87)	1.48 (1.29 – 1.62)[1,a]	1.57 (1.40 – 1.82)	1.60 (1.43 – 1.87)
20:4n-6	25.56 ± 1.84	23.77 ± 1.89[1,a]	25.49 ± 1.68	25.60 ± 1.65
22:4n-6	8.52 ± 1.40	6.57 ± 1.26[1,a]	8.45 ± 1.23	8.48 ± 1.20
22:5n-6	1.15 ± 0.30	1.67 ± 0.28	1.07 ± 0.33	1.07 ± 0.32
18:3n-3[§]	0.13 (0.11 – 0.16)	0.11 (0.08 – 0.14)[1,a]	0.13 (0.11 – 0.17)	0.14 (0.11 – 0.18)
20:5n-3[§]	0.71 (0.55 – 0.88)	0.81 (0.67 – 0.96)[1]	0.70 (0.59 – 0.89)	0.73 (0.60 – 0.89)
22:5n-3	5.12 ± 1.11	3.65 ± 0.85[1,a]	5.13 ± 1.02	5.15 ± 1.04
22:6n-3[§]	6.06 (4.83 – 8.13)	12.49 (10.97 – 13.39)[1,a]	5.72 (4.48 – 7.85)	5.32 (4.37 – 7.56)[1]
EPA + DHA[§]	7.03 (5.43 – 9.16)	13.45 (11.61 – 14.40)[1,a]	6.48 (5.22 – 8.62)	6.20 (5.00 – 8.56)[1]
∑n-6 FA	43.94 ± 2.85	39.76 ± 2.65[1,a]	44.04 ± 2.25	44.21 ± 1.93
∑n-3 FA	12.79 ± 2.30	16.93 ± 2.20[1,a]	12.37 ± 1.92	12.20 ± 1.98
∑n-6/∑n-3 FA[§]	3.44 (3.03 – 4.12)	2.36 (2.08 – 2.70)[1,a]	3.58 (3.12 – 4.15)	3.73 (3.18 – 4.08)[1]
∑n-6 LCPUFA[§]	38.35 (34.84 – 39.25)	33.88 (32.01 – 35.72)[1,a]	37.51 (36.34 – 38.34)	37.52 (35.91 – 38.64)
∑n-3 LCPUFA[§]	12.77 (11.04 – 14.13)	17.06 (15.41 – 18.27)[1,a]	12.15 (10.61 – 13.84)	11.77 (10.78 – 13.65)[1]
∑n-6/∑n-3 LCPUFA[§]	2.94 (2.62 – 3.57)	1.98 (1.79 – 2.31)[1,a]	3.06 (2.71 – 3.52)	3.21 (2.79 – 3.49)[1]

Abbreviations see Table 8.16.

* One sample was lost during preparation.
[§] Median with IQR in parentheses.
[1] $p < 0.05$ vs. week 0; [a] $p < 0.05$ vs. placebo at the same time point.

Table 8.19. Relative fatty acid composition (wt%) in RBC total lipids at weeks 0 and 8, *DHAVEG study* (mean ± SD or median with IQR).

	DHA group (*n* = 52)*		Placebo group (*n* = 51)*	
	Week 0	Week 8	Week 0	Week 8
16:0[§]	21.41 (20.88 – 21.94)	21.79 (21.14 – 22.26)[1,a]	21.25 (20.73 – 22.03)	20.98 (20.62 – 21.95)[1]
18:0	13.89 ± 0.67	13.99 ± 0.63[1]	13.88 ± 0.69	14.04 ± 0.68[1]
18:1n-9[§]	12.02 (11.63 – 12.90)	11.85 (11.42 – 12.40)[1,a]	12.32 (11.86 – 13.02)	12.51 (11.88 – 13.20)
20:3n-9[§]	0.07 (0.06 – 0.09)	0.05 (0.04 – 0.07)[1,a]	0.08 (0.05 – 0.09)	0.07 (0.06 – 0.09)
18:2n-6	10.18 ± 1.36	9.66 ± 1.42[1,a]	10.55 ± 1.18	10.56 ± 1.37
18:3n-6[§]	0.04 (0.03 – 0.05)	0.03 (0.02 – 0.03)[1,a]	0.04 (0.03 – 0.05)	0.04 (0.03 – 0.05)
20:3n-6[§]	1.82 (1.61 – 2.14)	1.53 (1.38 – 1.74)[1,a]	1.75 (1.54 – 2.15)	1.82 (1.53 – 2.08)
20:4n-6[§]	14.04 (13.27 – 14.67)	12.75 (12.19 – 13.26)[1,a]	14.09 (13.50 – 14.64)	14.26 (13.59 – 14.54)
22:4n-6	3.36 ± 0.60	2.58 ± 0.54[1,a]	3.31 ± 0.54	3.32 ± 0.51
22:5n-6	0.78 ± 0.20	1.07 ± 0.17[1,a]	0.72 ± 0.19	0.72 ± 0.19
18:3n-3[§]	0.12 (0.10 – 0.14)	0.11 (0.09 – 0.13)[1,a]	0.12 (0.11 – 0.15)	0.12 (0.10 – 0.15)
20:5n-3[§]	0.39 (0.32 – 0.49)	0.46 (0.37 – 0.53)[1,a]	0.38 (0.30 – 0.49)	0.39 (0.30 – 0.46)
22:5n-3	2.29 ± 0.47	1.66 ± 0.37[1,a]	2.32 ± 0.45	2.31 ± 0.43
22:6n-3[§]	4.26 (3.56 – 5.11)	8.03 (7.41 – 8.53)[1,a]	3.94 (3.42 – 5.14)	3.85 (3.21 – 4.88)[1]
EPA + DHA	4.84 ± 1.16	8.38 ± 1.31[1,a]	4.58 ± 1.03	4.43 ± 0.95[1]
∑n-6 FA	30.61 ± 1.64	27.98 ± 1.67[1,a]	30.85 ± 1.45	30.91 ± 1.52
∑n-3 FA	7.40 ± 1.17	10.28 ± 1.22[1,a]	7.18 ± 0.99	7.02 ± 0.89[1]
∑n-6/∑n-3 FA[§]	4.24 (3.60 – 4.85)	2.69 (2.42 – 3.03)[1,a]	4.35 (3.75 – 4.88)	4.39 (3.89 – 4.95)[1]
∑n-6 LCPUFA[§]	20.58 (19.70 – 21.34)	18.23 (17.49 – 18.93)[1,a]	20.51 (19.63 – 21.04)	20.44 (19.41 – 21.09)
∑n-3 LCPUFA	7.27 ± 1.16	10.17 ± 1.22[1,a]	7.04 ± 0.99	6.89 ± 0.88[1]
∑n-6/∑n-3 LCPUFA[§]	2.85 (2.51 – 3.33)	1.78 (1.63 – 1.99)[1,a]	2.97 (2.57 – 3.27)	3.03 (2.65 – 3.29)[1]

Abbreviations see Table 8.16.

* Some samples were lost during preparation.
[§] Median with IQR in parentheses.
[1] $p < 0.05$ vs. week 0; [a] $p < 0.05$ vs. placebo at the same time point.

Table 8.20. Spearman-Rho correlations between RBC total lipid fatty acids (wt%) and fatty acids from RBC PE, RBC PC and plasma PL at week 0 and week 8 in all subjects, *DHAVEG study* (*n* = 103).

	RBC PE		RBC PC		Plasma PL	
	Week 0	Week 8	Week 0	Week 8	Week 0	Week 8
16:0	0.491*	0.521*	0.785*	0.705*	0.745*	0.753*
18:1n-9	0.711*	0.664*	0.782*	0.704*	0.528*	0.515*
18:2n-6	0.896*	0.887*	0.858*	0.930*	0.759*	0.802*
20:3n-6	0.798*	0.791*	0.777*	0.757*	0.790*	0.781*
20:4n-6	0.700*	0.826*	0.615*	0.700*	0.581*	0.661*
22:4n-6	0.946*	0.965*	0.668*	0.821*	0.604*	0.758*
22:5n-6	0.973*	0.969*	0.905*	0.910*	0.899*	0.895*
18:3n-3	0.612*	0.672*	0.815*	0.827*	0.747*	0.743*
20:5n-3	0.939*	0.913*	0.857*	0.856*	0.830*	0.817*
22:5n-3	0.947*	0.961*	0.691*	0.755*	0.762*	0.851*
22:6n-3	0.954*	0.971*	0.921*	0.955*	0.921*	0.944*
EPA + DHA	0.956*	0.972*	0.900*	0.959*	0.911*	0.944*

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, phospholipids; RBC, red blood cells.

* Significant correlations between RBC total lipid fatty acids and fatty acids of the other lipid fractions ($p < 0.001$).

Table 8.21. Spearman-Rho correlations between percentage fatty acid changes from baseline in RBC total lipids or plasma phospholipids and percentage fatty acid changes in the other lipid fractions after 8-week supplementation with DHA, *DHAVEG study* (n = 52 – 55).

	RBC TL – Plasma PL	RBC TL – RBC PC	RBC TL – RBC PE	Plasma PL – RBC PC	Plasma PL – RBC PE
18:2n-6	0.746*	0.797*	0.666*	0.768*	0.595*
20:4n-6	0.580*	0.636*	0.628*	0.770*	ns
22:5n-6	0.750*	0.849*	0.965*	0.863*	0.705*
18:3n-3	0.823*	0.791*	0.531*	0.738*	0.278*
20:5n-3	0.849*	0.902*	0.754*	0.949*	0.525*
22:5n-3	0.603*	0.487*	0.834*	0.658*	0.412*
22:6n-3	0.814*	0.897*	0.941*	0.885*	0.720*
EPA + DHA	0.794*	0.898*	0.933*	0.861*	0.682*

DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ns, non-significant; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PL, phospholipids; RBC, red blood cells.

* Significant correlations between RBC total lipid fatty acids and fatty acids of the other lipid fractions ($p < 0.05$).

Table 8.22. BMI, blood pressure and heart rate at weeks 0 and 8, *FO/EPO study* (mean ± SD or median with IQR).

	FO/EPO group (*n* = 19)		Placebo group *(n* = 20)		
	Week 0	Week 8	Week 0	Week 8	*P†*
Weight (kg)	60.9 ± 7.0	61.0 ± 7.0	62.3 ± 6.5	62.0 ± 6.4	0.136
BMI (kg/m^2)	21.7 ± 1.9	21.7 ± 1.8	21.9 ± 2.0	21.9 ± 2.0	0.438
Systolic BP (mm Hg)	118 ± 13	120 ± 11	119 ± 11	120 ± 12	0.755
Diastolic BP (mm Hg)[§]	74 (67 – 78)	69 (67 – 77)[1]	71 (67 – 79)	73 (69 – 81)	0.126
Heart rate (beats/min)	74 ± 11	74 ± 10	74 ± 14	75 ± 14	0.710

BMI, body mass index; BP, blood pressure; IQR, interquartile range.

[§] Median with IQR in parentheses.

[1] $p < 0.05$ vs. week 0.

† Analyses of group differences in absolute changes from baseline (week 8 – week 0).

Table 8.23. Liver enzymes and full blood cell count at weeks 0 and 8, *FO/EPO study* (mean ± SD or median with IQR).

	FO/EPO group (*n* = 19)		Placebo group (*n* = 20)		
	Week 0	Week 8	Week 0	Week 8	*P†*
GGT (U/l)[§]	14.0 (11.0 – 18.0)	12.0 (11.0 – 17.0)	12.0 (10.0 – 16.8)	12.5 (11.0 – 16.5)	0.216
ALT (U/l)[§]	21.0 (14.0 – 26.0)	19.0 (12.0 – 23.0)	18.0 (14.0 – 21.8)	17.5 (14.0 – 19.8)	0.872
AST (U/l)[§]	19.0 (18.0 – 29.0)	20.0 (19.0 – 22.0)	20.0 (17.0 – 23.5)	21.0 (18.3 – 23.0)	0.228
CHE (kU/l)	6.4 ± 1.6	6.3 ± 1.6	5.5 ± 1.2	5.8 ± 1.1[1]	0.001
Blood cell count					
Leucocytes (G/l)[§]	5.6 (5.0 – 6.3)	5.7 (5.2 – 6.8)	5.9 (5.4 – 6.7)	5.9 (5.3 – 6.7)	0.851
Erythrocytes (T/l)[§]	4.6 (4.4 – 4.8)	4.4 (4.2 – 4.8)	4.5 (4.3 – 4.8)	4.6 (4.3 – 4.9)	0.050
Haemoglobin (g/dl)	13.6 ± 0.7	13.4 ± 0.9	13.6 ± 0.9	13.9 ± 0.9	0.033
Haematocrit (%)	41.9 ± 2.5	40.0 ± 2.6[1]	41.3 ± 2.4	41.7 ± 2.8	0.010
MCV (fl)	90.9 ± 4.6	88.7 ± 5.7[1]	91.7 ± 2.9	90.8 ± 3.6	0.145
MCH (pg)	29.7 ± 1.7	29.8 ± 1.8	30.0 ± 1.0	30. 2 ± 1.1	0.744
MCHC (%)[§]	32.5 (32.2. – 32.7)	33.6 (32.6 – 34.3)[1]	32.7 (32.3. – 33.2)	33.3 (32.5 – 34.0)[1]	0.263
Platelets (G/l)	300 ± 59[a]	289 ± 71[a]	257 ± 50	246 ± 56	0.862

IQR, interquartile range; GGT, gamma-glutamyl transpeptidase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHE, cholinesterase; MCV, mean cellular volume; MCH, mean cellular haemoglobin; MCHC, mean cellular haemoglobin concentration.

[§] Median with IQR in parentheses.
[1] $p < 0.05$ vs. week 0; [a] $p < 0.05$ vs. placebo at the same time point.
† Analyses of group differences in absolute changes from baseline (week 8 – week 0).

Table 8.24. Relative fatty acid composition (wt%) of plasma total lipids at weeks 0, 4, 6 and 8, *FO/EPO study* (mean ± SD or median with IQR).

		FO/EPO (n = 19)				Placebo (n = 20)			
	T	Week 0	Week 4	Week 6	Week 8	Week 0	Week 4	Week 6	Week 8
20:3n-9	ln	-1.95 ± 0.33	-2.34 ± 0.42[1,a]	-2.34 ± 0.39[1,a]	-2.29 ± 0.08[1,a]	-1.93 ± 0.39	-2.03 ± 0.37	-1.98 ± 0.41	-2.03 ± 0.39
	*	0.142	0.096	0.096	0.101	0.144	0.131	0.138	0.131
18:2n-6	ln	3.24 ± 0.10	3.25 ± 0.10	3.26 ± 0.11	3.22 ± 0.13	3.28 ± 0.13	3.28 ± 0.11	3.29 ± 0.11	3.30 ± 0.13
	*	25.46	25.88	26.06	25.00	26.46	26.57	26.88	27.08
18:3n-6	ln	-1.05 ± 0.23	-0.658 ± 0.244[1,a]	-0.798 ± 0.235[1,a]	-0.686 ± 0.228[1,a]	-1.09 ± 0.48	-1.17 ± 0.28	-1.08 ± 0.33	-1.169 ± 0.329
	*	0.351	0.518	0.450	0.504	0.338	0.310	0.341	0.311
20:3n-6	-	2.00 ± 0.30[a]	2.25 ± 0.49[1,a]	2.20 ± 0.41[1,a]	2.27 ± 0.45[1,a]	1.76 ± 0.35	1.76 ± 0.36	1.78 ± 0.37	1.73 ± 0.35
20:4n-6	1/x	0.156 ± 0.034	0.155 ± 0.027	0.155 ± 0.035	0.161 ± 0.037	0.145 ± 0.020	0.148 ± 0.024	0.153 ± 0.022[1]	0.154 ± 0.019[1]
	*	6.42	6.44	6.45	6.20	6.90	6.74	6.52	6.49
22:4n-6	-	0.228 ± 0.061	0.194 ± 0.048[1]	0.179 ± 0.043[1,a]	0.190 ± 0.057[1]	0.231 ± 0.042	0.212 ± 0.034[1]	0.206 ± 0.037[1]	0.194 ± 0.038[1]
22:5n-6	-	0.267 ± 0.092	0.219 ± 0.073[1]	0.207 ± 0.074[1]	0.208 ± 0.079[1]	0.246 ± 0.068	0.237 ± 0.060	0.236 ± 0.057	0.218 ± 0.052[1]
18:3n-3	ln	-0.609 ± 0.310[a]	-0.726 ± 0.309	-0.751 ± 0.403	-0.802 ± 0.207[1]	-0.852 ± 0.302	-0.687 ± 0.285	-0.721 ± 0.248	-0.639 ± 0.302[1]
	*	0.503	0.484	0.472	0.440	0.427	0.503	0.486	0.528
20:5n-3[§]	-	0.510	0.679[1,a]	0.644[1,a]	0.686[1,a]	0.472	0.459	0.444	0.505[1]
		(0.388 – 0.684)	(0.524 – 0.783)	(0.598 – 0.747)	(0.548 – 0.811)	(0.329 – 0.521)	(0.377 – 0.551)	(0.349 – 0.671)	(0.428 – 0.599)
22:5n-3	-	0.392 ± 0.105	0.356 ± 0.072	0.350 ± 0.089	0.357 ± 0.081	0.355 ± 0.089	0.362 ± 0.075	0.350 ± 0.086	0.375 ± 0.092
22:6n-3	-	2.04 ± 0.43	3.12 ± 0.48[1,a]	3.25 ± 0.48[1,a]	3.14 ± 0.42[1,a]	1.87 ± 0.42	1.98 ± 0.60	1.92 ± 0.51	1.97 ± 0.61

IQR, interquartile range; T, transformation of the data; -, no transformation; ln, natural logarithm; 1/x, reciprocal value.

* Back transformed mean.
[§] Median with IQR in parentheses.
[1] Significantly different from baseline (Bonferroni-Holm adjusted significances).
[a] $p < 0.05$ vs. placebo at the same time point.

Table 8.25. Fatty acid sums (wt%) and ratios in plasma total lipids at weeks 0, 4, 6 and 8, *FO/EPO study* (mean ± SD or median with IQR).

		FO/EPO (*n* = 19)				Placebo (*n* = 20)			
	T	Week 0	Week 4	Week 6	Week 8	Week 0	Week 4	Week 6	Week 8
GLA + DGLA	-	2.36 ± 0.35	2.78 ± 0.55[1,a]	2.66 ± 0.45[1,a]	2.78 ± 0.49[1,a]	2.13 ± 0.41	2.08 ± 0.38	2.14 ± 0.40	2.06 ± 0.37
∑n-6 FA	-	35.40 ± 2.63	36.10 ± 2.68	36.31 ± 2.74	35.14 ± 3.56	36.57 ± 3.47	36.48 ± 2.86	36.53 ± 2.86	36.57 ± 3.36
∑n-3 FA	ln	1.30 ± 0.22[a]	1.56 ± 0.14[1,a]	1.58 ± 0.14[1,a]	1.56 ± 0.12[1,a]	1.16 ± 0.14	1.24 ± 0.21	1.20 ± 0.20	1.25 ± 0.23
	*	3.65	4.75	4.85	4.74	3.20	3.44	3.33	3.50
∑n-6/∑n-3 FA	-	9.94 ± 0.51[a]	7.65 ± 0.26[1,a]	7.55 ± 0.27[1,a]	7.46 ± 0.24[1,a]	11.57 ± 0.48	10.86 ± 0.53	11.21 ± 0.58	10.77 ± 0.60
∑n-6 LCPUFA	1/x	0.108 ± 0.017	0.107 ± 0.014	0.107 ± 0.018	0.109 ± 0.018	0.107 ± 0.013	0.108 ± 0.013	0.111 ± 0.013	0.113 ± 0.011[1]
	*	9.23	9.38	9.35	9.20	9.38	9.24	9.01	8.88
∑n-3 LCPUFA	ln	1.11 ± 0.24	1.43 ± 0.16[1,a]	1.46 ± 0.14[1,a]	1.44 ± 0.13[1,a]	0.99 ± 0.17	1.05 ± 0.24	1.02 ± 0.23	1.06 ± 0.25
	*	3.03	4.19	4.30	4.23	2.70	2.87	2.78	2.90
∑n-6/∑n-3 LCPUFA[§]	-	3.24	2.16[1,a]	2.08[1,a]	2.08[1,a]	3.53	3.27	3.38	3.27[1]
		(2.28 – 3.88)	1.99 – 2.54)	(1.85 – 2.54)	(1.94 – 2.43)	(3.19 – 3.89)	(3.04 – 3.71)	(2.72 – 3.84)	(2.48 – 3.85)

IQR, interquartile range; GLA, gamma-linolenic acid; DGLA, dihomo-gamma-linolenic acid; ∑n-6 FA, sum of n-6 fatty acids; ∑n-3 FA, sum of n-3 fatty acids; ∑n-6/∑n-3 FA, ratio of n-6 to n-3 fatty acids; ∑n-6 LCPUFA, sum of all long-chain n-6 fatty acids; ∑n-3 LCPUFA, sum of all long-chain n-3 fatty acids; ∑n-6/∑n-3 LCPUFA, ratio of n-6 to n-3 long-chain polyunsaturated fatty acids; T, transformation of the data; -, no transformation; ln, natural logarithm; 1/x, reciprocal value.

* Back transformed mean.
§ Median with IQR in parentheses.
1 Significantly different from baseline (Bonferroni-Holm adjusted significances).
a $p < 0.05$ vs. placebo at the same time point.

Table 8.26. Relative fatty acid composition (wt%) of plasma phospholipids at weeks 0, 4, 6 and 8, *FO/EPO study* (mean ± SD or median with IQR).

		FO/EPO (*n* = 19)				Placebo (*n* = 20)			
	T	Week 0	Week 4	Week 6	Week 8	Week 0	Week 4	Week 6	Week 8
20:3n-9	ln	-1.72 ± 0.29	-2.13 ± 0.40[1,a]	-2.17 ± 0.29[1,a]	-2.08 ± 0.35[1,a]	-1.76 ± 0.40	-1.82 ± 0.34	-1.78 ± 0.43	-1.81 ± 0.41
	*	0.179	0.119	0.114	0.125	0.172	0.163	0.169	0.164
18:2n-6	-	18.73 ± 1.87	18.08 ± 2.28	18.04 ± 2.51[a]	17.46 ± 2.29[a]	19.38 ± 2.42	19.49 ± 2.31	19.87 ± 2.37	20.02 ± 1.94
18:3n-6	1/x	11.19 ± 3.08	8.51 ± 2.49[1,a]	10.32 ± 3.16	8.49 ± 2.05[1,a]	11.52 ± 4.40	12.24 ± 3.45	11.34 ± 3.45	12.68 ± 3.75
	*	0.089	0.117	0.097	0.118	0.087	0.082	0.088	0.079
20:3n-6	-	3.50 ± 0.57[a]	3.89 ± 0.94[1,a]	3.77 ± 0.73[1,a]	3.98 ± 0.91[1,a]	3.04 ± 0.59	3.09 ± 0.72	3.09 ± 0.70	2.98 ± 0.63
20:4n-6	ln	2.20 ± 0.18	2.15 ± 0.16	2.16 ± 0.22	2.14 ± 0.20	2.26 ± 0.11	2.24 ± 0.13	2.19 ± 0.12[1]	2.18 ± 0.10[1]
	*	9.03	8.63	8.68	8.49	9.55	9.39	8.97	8.85
22:4n-6	-	0.369 ± 0.085	0.302 ± 0.077[1,a]	0.277 ± 0.065[1,a]	0.298 ± 0.092[1]	0.384 ± 0.077	0.348 ± 0.055[1]	0.339 ± 0.060[1]	0.317 ± 0.060[1]
22:5n-6	-	0.400 ± 0.138	0.312 ± 0.110[1]	0.291 ± 0.097[1,a]	0.300 ± 0.111[1]	0.377 ± 0.094	0.358 ± 0.081	0.354 ± 0.079	0.328 ± 0.070[1]
18:3n-3	ln	-1.53 ± 0.39[a]	-1.68 ± 0.36	-1.81 ± 0.44	-1.79 ± 0.28[1,a]	-1.81 ± 0.43	-1.62 ± 0.35	-1.65 ± 0.37	-1.56 ± 0.33
	*	0.216	0.186	0.164	0.168	0.163	0.198	0.191	0.210
20:5n-3[§]	-	0.591	0.724[1,a]	0.744[1,a]	0.767[1,a]	0.572	0.559	0.538	0.561
		(0.460 – 0.792)	(0.607 – 0.907)	(0.661 – 0.849)	(0.653 – 0.953)	(0.388 – 0.620)	(0.463 – 0.650)	(0.423 – 0.774)	(0.516 – 0.732)
22:5n-3	-	0.668 ± 0.157	0.590 ± 0.106[1]	0.576 ± 0.133[1]	0.600 ± 0.133[1]	0.606 ± 0.153	0.620 ± 0.130	0.603 ± 0.135	0.663 ± 0.141
22:6n-3[§]	-	3.09	4.99[1,a]	5.10[1,a]	5.03[1,a]	3.21	3.04	3.20	3.14
		(3.01 – 3.56)	(4.39 – 5.40)	(4.67 – 5.45)	(4.63 – 5.41)	(2.59 – 3.60)	(2.60 – 3.96)	(2.60 – 3.83)	(2.37 – 4.15)

IQR, interquartile range; T, transformation of the data; -, no transformation; ln, natural logarithm; 1/x, reciprocal value.

* Back transformed mean.
§ Median with IQR in parentheses.
1 Significantly different from baseline (Bonferroni-Holm adjusted significances).
a $p < 0.05$ vs. placebo at the same time point.

Table 8.27. Fatty acid sums (wt%) and ratios in plasma phospholipids at weeks 0, 4, 6 and 8, *FO/EPO study* (mean ± SD or median with IQR).

		FO/EPO (*n* = 19)				Placebo (*n* = 20)			
	T	Week 0	Week 4	Week 6	Week 8	Week 0	Week 4	Week 6	Week 8
GLA + DGLA	-	3.59 ± 0.58[a]	4.02 ± 0.94[1,a]	3.88 ± 0.73[1,a]	4.11 ± 0.90 [1,a]	3.14 ± 0.61	3.18 ± 0.72	3.19 ± 0.69	3.07 ± 0.63
∑n-6 FA[§]	-	32.63	31.35[a]	31.36[a]	31.88[1,a]	33.33	33.76	33.57	33.33
		(31.56 – 33.84)	(30.74 – 33.98)	(30.79 – 33.28)	(29.99 – 32.56)	(31.66 – 35.07)	(32.13 – 34.42)	(31.85 – 34.45)	(31.88 – 34.21)
∑n-3 FA	ln	1.61 ± 0.19	1.88 ± 0.13[1,a]	1.90 ± 0.14[1,a]	1.89 ± 0.11[1,a]	1.50 ± 0.16	1.57 ± 0.23	1.53 ± 0.22	1.56 ± 0.24
	*	5.00	6.54	6.67	6.65	4.49	4.79	4.62	4.77
∑n-6/∑n-3 FA	-	6.66 ± 0.29	4.92 ± 0.20[1,a]	4.82 ± 0.20[1,a]	4.72 ± 9.14[1,a]	7.51 ± 0.30	7.12 ± 0.35	7.38 ± 0.41	7.15 ± 0.41
∑n-6 LCPUFA[§]	-	13.61	13.28	13.51	13.60	13.55	14.28	13.00[1]	12.63[1]
		(12.33 – 15.70)	(12.56 – 15.06)	(12.10 – 14.19)	(11.87 – 15.08)	(13.08 – 14.41)	(12.25 – 14.98)	(12.43 – 14.08)	(12.09 – 13.53)
∑n-3 LCPUFA	2ln	0.435 ± 0.11	0.607 ± 0.08[1,a]	0.620 ± 0.08[1,a]	0.620 ± 0.06[1,a]	0.366 ± 0.12	0.402 ± 0.15	0.376 ± 0.17	0.395 ± 0.17
	*	4.69	6.26	6.41	6.42	4.23	4.46	4.29	4.41
∑n-6/∑n-3 LCPUFA	1/√	0.593 ± 0.020	0.685 ± 0.015[1,a]	0.696 ± 0.015[1,a]	0.694 ± 0.014[1,a]	0.559 ± 0.009	0.581 ± 0.013	0.580 ± 0.014	0.596 ± 0.017
	*	2.85	2.13	2.07	2.08	3.20	2.97	2.97	2.81

Abbreviations see Table 8.25. IQR, interquartile range; T, transformation of the data; -, no transformation; ln, natural logarithm; 2ln, double natural logarithm; √, square root.

* Back transformed mean.
[§] Median with IQR in parentheses.
[1] Significantly different from baseline (Bonferroni-Holm adjusted significances).
[a] $p < 0.05$ vs. placebo at the same time point.

Table 8.28. Relative fatty acid composition (wt%) of plasma cholesterol esters at weeks 0, 4, 6 and 8, *FO/EPO study* (mean ± SD).

		FO/EPO (*n* = 19)				Placebo (*n* = 20)			
	T	Week 0	Week 4	Week 6	Week 8	Week 0	Week 4	Week 6	Week 8
20:3n-9	ln	-2.59 ± 0.39	-2.91 ± 0.54[1]	-2.90 ± 0.57[1]	-2.82 ± 0.53[1]	-2.67 ± 0.42	-2.68 ± 0.46	-2.67 ± 0.53	-2.62 ± 0.53
	*	0.075	0.054	0.055	0.060	0.069	0.069	0.070	0.073
18:2n-6	-	48.45 ± 3.20	48.82 ± 4.23	48.47 ± 3.84	47.76 ± 4.25	50.16 ± 3.93	49.82 ± 3.35	49.69 ± 3.48	49.71 ± 3.82
18:3n-6	ln	-0.113 ± 0.214	0.219 ± 0.263[1,a]	0.078 ± 0.254[1,a]	0.223 ± 0.219[1,a]	-0.198 ± 0.444	-0.246 ± 0.277	-0.188 ± 0.338	-0.256 ± 0.305
	*	0.893	1.245	1.081	1.250	0.820	0.782	0.829	0.774
20:3n-6	x^2	0.783 ± 0.238[a]	1.03 ± 0.39[1,a]	1.11 ± 0.46[1,a]	1.12 ± 0.42[1,a]	0.621 ± 0.254	0.650 ± 0.309	0.638 ± 0.261	0.599 ± 0.248
	*	0.885	1.014	1.054	1.056	0.788	0.806	0.799	0.774
20:4n-6	ln	1.90 ± 0.22	1.92 ± 0.20	1.94 ± 0.25	1.91 ± 0.25	1.94 ± 0.15	1.94 ± 0.17	1.91 ± 0.16	1.89 ± 0.14
	*	6.69	6.85	6.99	6.77	6.98	6.98	6.74	6.63
22:4n-6									
22:5n-6	-	0.040 ± 0.017	0.032 ± 0.013[1]	0.032 ± 0.016[1]	0.030 ± 0.014[1]	0.037 ± 0.013	0.034 ± 0.011	0.036 ± 0.012	0.032 ± 0.008[1]
18:3n-3	-	0.693 ± 0.168[a]	0.592 ± 0.159[1]	0.526 ± 0.158[1]	0.539 ± 0.123[1]	0.553 ± 0.173	0.564 ± 0.127	0.580 ± 0.139	0.625 ± 0.172
20:5n-3	ln	-0.421 ± 0.483[a]	-0.233 ± 0.316[a]	-0.229 ± 0.167[a]	-0.203 ± 0.300[a]	-0.722 ± 0.373	-0.587 ± 0.385	-0.618 ± 0.381	-0.457 ± 0.409[1]
	*	0.656	0.792	0.795	0.816	0.486	0.556	0.539	0.633
22:5n-3									
22:6n-3	-	0.620 ± 0.096	0.938 ± 0.178[1,a]	1.02 ± 0.19[1,a]	0.979 ± 0.180[1,a]	0.581 ± 0.159	0.616 ± 0.186	0.614 ± 0.184	0.604 ± 0.198

T, transformation of the data; -, no transformation; ln, natural logarithm; x^2, squared.

* Back transformed mean.

[1] Significantly different from baseline (Bonferroni-Holm adjusted significances).

[a] $p < 0.05$ vs. placebo at the same time point.

Table 8.29. Fatty acid sums (wt%) and ratios in plasma cholesterol esters at weeks 0, 4, 6 and 8, *FO/EPO study* (mean ± SD or median with IQR).

		FO/EPO (*n* = 19)				Placebo (*n* = 20)			
	T	Week 0	Week 4	Week 6	Week 8	Week 0	Week 4	Week 6	Week 8
GLA + DGLA	2ln	1.05 ± 0.06	1.13 ± 0.07[1,a]	1.11 ± 0.07[1,a]	1.14 ± 0.06[1,a]	1.02 ± 0.11	1.01 ± 0.08	1.02 ± 0.08	1.00 ± 0.08
	*	1.75	2.22	2.08	2.26	1.59	1.55	1.60	1.52
∑n-6 FA	x^2	3275 ± 304	3397 ± 429	3363 ± 371	3281 ± 463	3490 ± 427	3440 ± 335	3404 ± 366	3384 ± 436
	*	57.23	58.28	57.99	57.28	59.08	58.65	58.34	58.18
∑n-3 FA[§]	-	1.98[a]	2.44[1,a]	2.47[1,a]	2.41[1,a]	1.73	1.70	1.84	2.03
		(1.74 – 2.32)	(2.16 – 2.73)	(2.35 – 2.67)	(2.21 – 2.65)	(1.56 – 2.00)	(1.62 – 2.09)	(1.42 – 2.12)	(1.57 – 2.18)
∑n-6/∑n-3 FA	-	28.29 ± 1.55[a]	24.12 ± 0.83[1,a]	24.00 ± 0.75[1,a]	23.75 ± 0.98[1,a]	35.63 ± 2.18	32.99 ± 1.68	33.18 ± 1.94	30.96 ± 1.84
∑n-6 LCPUFA	ln	2.04 ± 0.20	2.07 ± 0.18	2.09 ± 0.23	2.07 ± 0.23	2.06 ± 0.15	2.06 ± 0.16	2.03 ± 0.15	2.01 ± 0.13
	*	7.66	7.93	8.11	7.92	7.84	7.86	7.61	7.48
∑n-3 LCPUFA	1/x	0.771 ± 0.194[a]	0.571 ± 0.121[1,a]	0.544 ± 0.097[1,a]	0.550 ± 0.101[1,a]	0.919 ± 0.242	0.850 ± 0.222	0.870 ± 0.263	0.820 ± 0.242
	*	1.30	1.75	1.84	1.82	1.09	1.18	1.15	1.22
∑n-6/∑n-3 LCPUFA	√	2.42 ± 0.11	2.12 ± 0.06[1,a]	2.10 ± 0.06[1,a]	2.09 ± 0.08[1,a]	2.66 ± 0.06	2.55 ± 0.06	2.54 ± 0.08	2.44 ± 0.07[1]
	*	5.88	4.50	4.42	4.38	7.06	6.51	6.44	5.95

Abbreviations see Table 8.25. IQR, interquartile range; T, transformation of the data; -, no transformation; 2ln, double natural logarithm (10*x); x^2, squared; ln, natural logarithm; 1/x, reciprocal value; √, square root.

* Back transformed mean.
[§] Median with IQR in parentheses.
[1] Significantly different from baseline (Bonferroni-Holm adjusted significances).
[a] $p < 0.05$ vs. placebo at the same time point.

Table 8.30. Relative fatty acid composition (wt%) of plasma triglycerides at weeks 0, 4, 6 and 8, *FO/EPO study* (mean ± SD or median with IQR).

		FO/EPO (*n* = 19)				Placebo (*n* = 20)			
	T	Week 0	Week 4	Week 6	Week 8	Week 0	Week 4	Week 6	Week 8
20:3n-9	ln	-1.67 ± 0.38	-1.91 ± 0.46[1]	-1.85 ± 0.40[1,a]	-1.85 ± 0.36[1]	-1.56 ± 0.33	-1.64 ± 0.43	-1.57 ± 0.44	-1.65 ± 0.38
	*	0.189	0.148	0.156	0.157	0.210	0.193	0.208	0.192
18:2n-6	ln	2.53 ± 0.19	2.61 ± 0.22	2.68 ± 0.20[1]	2.53 ± 0.19	2.59 ± 0.29	2.57 ± 0.23	2.60 ± 0.24	2.65 ± 0.28
	*	12.49	13.57	14.58	12.57	13.36	13.09	13.51	14.12
18:3n-6	1/√	1.95 ± 0.40	1.58 ± 0.28[1,a]	1.62 ± 0.25[1,a]	1.66 ± 0.34[1,a]	1.85 ± 0.51	2.06 ± 0.38	1.92 ± 0.43	2.01 ± 0.50
	*	0.263	0.402	0.381	0.361	0.293	0.236	0.271	0.248
20:3n-6	-	0.301 ± 0.79	0.400 ± 0.110[1,a]	0.376 ± 0.077[1,a]	0.378 ± 0.117[1,a]	0.278 ± 0.076	0.268 ± 0.092	0.274 ± 0.060	0.256 ± 0.059
20:4n-6	ln	0.151 ± 0.401	0.341 ± 0.287[1,a]	0.273 ± 0.337	0.248 ± 0.376	0.113 ± 0.259	0.098 ± 0.260	0.096 ± 0.322	0.046 ± 0.279
	*	1.16	1.41	1.31	1.28	1.12	1.10	1.10	1.05
22:4n-6	ln	-1.78 ± 0.36	-1.73 ± 0.31	-1.79 ± 0.31	-1.80 ± 0.36	-1.82 ± 0.26	-1.87 ± 0.20	-1.92 ± 0.24	-1.96 ± 0.24
	*	0.169	0.177	0.166	0.165	0.162	0.153	0.147	0.141
22:5n-6	√	0.444 ± 0.096	0.440 ± 0.097	0.429 ± 0.092	0.424 ± 0.101	0.415 ± 0.077	0.412 ± 0.086	0.418 ± 0.066	0.392 ± 0.076
	*	0.197	0.194	0.184	0.180	0.173	0.170	0.175	0.154
18:3n-3	1/x	1.19 ± 0.40[a]	1.30 ± 0.42	1.31 ± 0.63	1.42 ± 0.40	1.52 ± 0.55	1.19 ± 0.35[1]	1.26 ± 0.33[1]	1.19 ± 0.49[1]
	*	0.837	0.768	0.762	0.706	0.658	0.837	0.791	0.844
20:5n-3	ln	-2.06 ± 0.51	-1.65 ± 0.26[1,a]	-1.70 ± 0.25[1,a]	-1.70 ± 0.33[1,a]	-2.40 ± 0.56	-2.15 ± 0.51	-2.22 ± 0.59	-2.18 ± 0.56
	*	0.128	0.191	0.183	0.183	0.091	0.117	0.109	0.113
22:5n-3	ln	-1.60 ± 0.40	-1.54 ± 0.36	-1.49 ± 0.37[a]	-1.50 ± 0.32	-1.80 ± 0.44	-1.73 ± 0.27	-1.76 ± 0.43	-1.68 ± 0.51
	*	0.202	0.215	0.225	0.222	0.166	0.177	0.172	0.187
22:6n-3[§]	-	0.612	1.49[1,a]	1.42[1,a]	1.38[1,a]	0.520	0.473	0.574	0.527
		(0.421 – 0.761)	(1.12 – 2.04)	(1.11 – 1.79)	(1.08 – 1.76)	(0.301 – 0.607)	(0.327 – 0.747)	(0.373 – 0.683)	(0.322 – 0.698)

IQR, interquartile range; T, transformation of the data; ln, natural logarithm; √, square root; 1/x, reciprocal value.

* Back transformed mean.
[§] Median with IQR in parentheses.
[1] Significantly different from baseline (Bonferroni-Holm adjusted significances).
[a] $p < 0.05$ vs. placebo at the same time point.

Table 8.31. Fatty acid sums (wt%) and ratios in plasma triglycerides at weeks 0, 4, 6 and 8, *FO/EPO study* (mean ± SD).

		FO/EPO (*n* = 19)				Placebo (*n* = 20)			
	T	Week 0	Week 4	Week 6	Week 8	Week 0	Week 4	Week 6	Week 8
GLA + DGLA	ln	-0.555 ± 0.303	-0.214 ± 0.286[1,a]	-0.260 ± 0.164[1,a]	-0.281 ± 0.300[1,a]	-0.508 ± 0.349	-0.667 ± 0.272	-0.560 ± 0.251	-0.632 ± 0.361
	*	0.574	0.808	0.771	0.755	0.602	0.513	0.571	0.532
∑n-6 FA	-	15.16 ± 2.79	16.80 ± 3.63	17.62 ± 3.20[1]	15.50 ± 2.62	16.24 ± 4.18	15.65 ± 3.33	16.17 ± 3.2 6	16.78 ± 3.86
∑n-3 FA	1/x	0.509 ± 0.140[a]	0.366 ± 0.099[1,a]	0.355 ± 0.103[1,a]	0.383 ± 0.084[1,a]	0.645 ± 0.165	0.548 ± 0.152	0.564 ± 0.131	0.561 ± 0.224[1]
	*	1.96	2.73	2.82	2.61	1.55	1.82	1.77	1.78
∑n-6/∑n-3 FA	-	7.72 ± 0.64[a]	6.05 ± 0.41[1,a]	6.12 ± 0.38[1,a]	5.82 ± 0.27[1,a]	10.44 ± 0.80	8.39 ± 0.52[1]	9.19 ± 0.69	8.99 ± 0.59
∑n-6 LCPUFA	2ln	-0.410 ± 0.473	-0.189 ± 0.326[a]	-0.249 ± 0.316[a]	-0.301 ± 0.438[a]	-0.455 ± 0.342	-0.490 ± 0.344	-0.468 ± 0.322	-0.623 ± 0.529
	*	1.94	2.29	2.18	2.10	1.89	1.85	1.87	1.71
∑n-3 LCPUFA	ln	0.031 ± 0.395[a]	0.628 ± 0.355[1,a]	0.646 ± 0.277[1,a]	0.608 ± 0.274[1,a]	-0.231 ± 0.280	-0.102 ± 0.437	-0.104 ± 0.335	-0.106 ± 0.429
	*	1.03	1.87	1.91	1.84	0.79	0.90	0.90	0.90
∑n-6/∑n-3 LCPUFA	√	1.45 ± 0.08	1.15 ± 0.05[1,a]	1.11 ± 0.05[1,a]	1.11 ± 0.04[1,a]	1.58 ± 0.04	1.47 ± 0.05	1.48 ± 0.05	1.44 ± 0.05[1]
	*	2.11	1.32	1.23	1.24	2.48	2.17	2.18	2.07

Abbreviations see Table 8.25. T, transformation of the data; ln, natural logarithm; 1/x, reciprocal value; 2ln, double natural logarithm; √, square root.

* Back transformed mean.
[1] Significantly different from baseline (Bonferroni-Holm adjusted significances).
[a] $p < 0.05$ vs. placebo at the same time point.

Table 8.32. Spearman-Rho correlations between fatty acids (wt%) of plasma TL, PL, CE, and TG, *FO/EPO study* (*n* = 39/40).

	TL – CE	TL - PL	CE - PL	TL - TG	CE - TG	PL - TG
LA						
week 0	0.890*	0.850*	0.843*	0.731*	0.642*	0.677*
week 4	0.842*	0.864*	0.785*	ns	ns	ns
week 6	0.891*	0.817*	0.835*	0.575*	0.502*	0.359*
week 8	0.903*	0.845*	0.825*	0.689*	0.602*	0.462*
GLA						
week 0	0.941*	0.905*	0.886*	0.801*	0.648*	0.774*
week 4	0.937*	0.856*	0.742*	0.784*	0.585*	0.727*
week 6	0.941*	0.732*	0.648*	0.718*	0.495*	0.686*
week 8	0.936*	0.871*	0.794*	0.704*	0.479*	0.768*
ALA						
week 0	0.880*	0.896*	0.846*	0.822*	0.546*	0.645*
week 4	0.871*	0.818*	0.763*	0.868*	0.616*	0.659*
week 6	0.796*	0.772*	0.760*	0.930*	0.645*	0.597*
week 8	0.810*	0.789*	0.806*	0.869*	0.542*	0.514*
DGLA						
week 0	0.887*	0.954*	0.871*	0.663*	0.569*	0.694*
week 4	0.962*	0.959*	0.947*	0.843*	0.790*	0.803*
week 6	0.901*	0.958*	0.860*	0.731*	0.676*	0.722*
week 8	0.923*	0.972*	0.911*	0.707*	0.713*	0.682*
AA						
week 0	0.936*	0.940*	0.907*	0.805*	0.717*	0.722*
week 4	0.892*	0.893*	0.891*	0.477*	0.497*	0.376*
week 6	0.863*	0.926*	0.839*	0.784*	0.634*	0.621*
week 8	0.857*	0.875*	0.915*	0.702*	0.687*	0.623*
EPA						
week 0	0.924*	0.955*	0.887*	0.739*	0.563*	0.735*
week 4	0.955*	0.974*	0.948*	0.814*	0.762*	0.767*
week 6	0.912*	0.974*	0.905*	0.825*	0.751*	0.797*
week 8	0.936*	0.968*	0.929*	0.871*	0.778*	0.828*
DHA						
week 0	0.930*	0.960*	0.927*	0.755*	0.642*	0.682*
week 4	0.940*	0.960*	0.959*	0.902*	0.801*	0.810*
week 6	0.921*	0.958*	0.929*	0.927*	0.829*	0.852*
week 8	0.954*	0.951*	0.973*	0.921*	0.848*	0.867*

AA, arachidonic acid; ALA, alpha-linolenic acid; CE, cholesterol ester; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; LA, linoleic acid; PL, phospholipids, TG, triglycerides; TL, total lipids.

* $p < 0.05$; ns, non-significant.

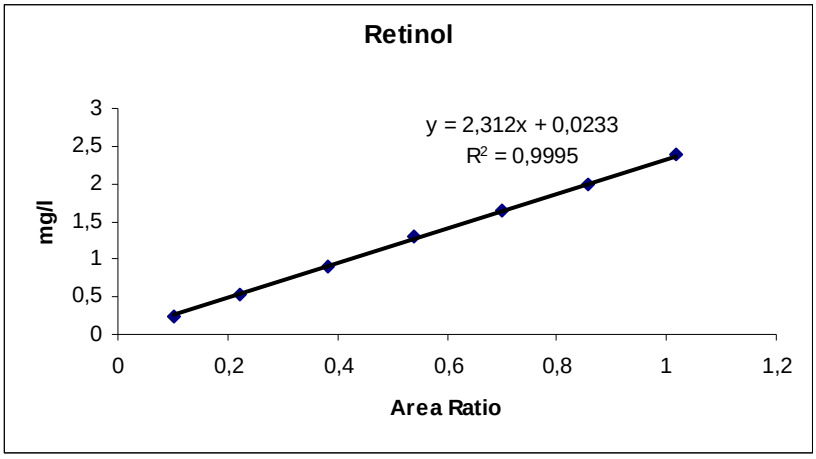
Table 8.33. Spearman-Rho correlations between plasma TL, PL, CE, and TG fatty acid changes from baseline with FO/EPO supplementation, *FO/EPO study* (wt%/wt%, *n* = 19).

	TL – CE	TL - PL	CE - PL	TL - TG	CE - TG	PL - TG
GLA						
week 4 - 0	0.914*	0.682*	0.547*	ns	ns	0.481*
week 6 - 0	0.942*	0.882*	0.732*	0.542*	ns	0.761*
week 8 - 0	0.879*	0.937*	0.809*	0.647*	ns	0.640*
DGLA						
week 4 - 0	0.689*	0.877*	0.611*	0.637*	ns	0.672*
week 6 - 0	0.561*	0.911*	0.544*	0.589*	ns	0.712*
week 8 - 0	0.868*	0.865*	0.730*	0.658*	0.630*	0.730*
AA						
week 4 - 0	0.805*	0.947*	0.840*	ns	ns	ns
week 6 - 0	0.805*	0.867*	0.847*	ns	ns	ns
week 8 - 0	0.758*	0.816*	0.898*	0.479*	ns	ns
EPA						
week 4 - 0	0.968*	0.977*	0.933*	0.660*	0.540*	0.709*
week 6 - 0	0.879*	0.921*	0.907*	0.639*	0.474*	0.554*
week 8 - 0	0.921*	0.888*	0.835*	ns	ns	0.521*
DHA						
week 4 - 0	0.846*	0.895*	0.904*	0.858*	0.663*	0.702*
week 6 - 0	0.905*	0.947*	0.821*	0.740*	0.725*	0.588*
week 8 - 0	0.853*	0.911*	0.889*	0.851*	0.649*	0.761*

AA, arachidonic acid; ALA, alpha-linolenic acid; CE, cholesterol ester; DGLA, dihomo-gamma-linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GLA, gamma-linolenic acid; LA, linoleic acid; PL, phospholipids, TG, triglycerides; TL, total lipids.

* $p < 0.05$; ns, non-significant.

8.2 Figures



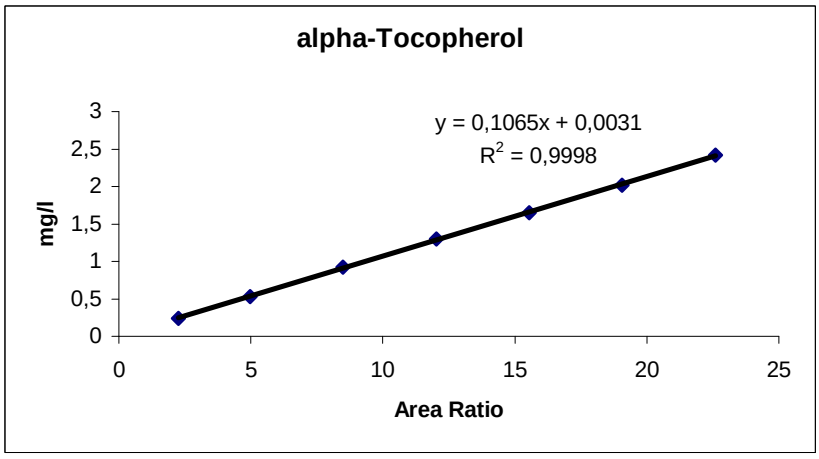


Figure 8.1. Seven-point standard curves for retinol and alpha-tocopherol, *DHAVEG study.*

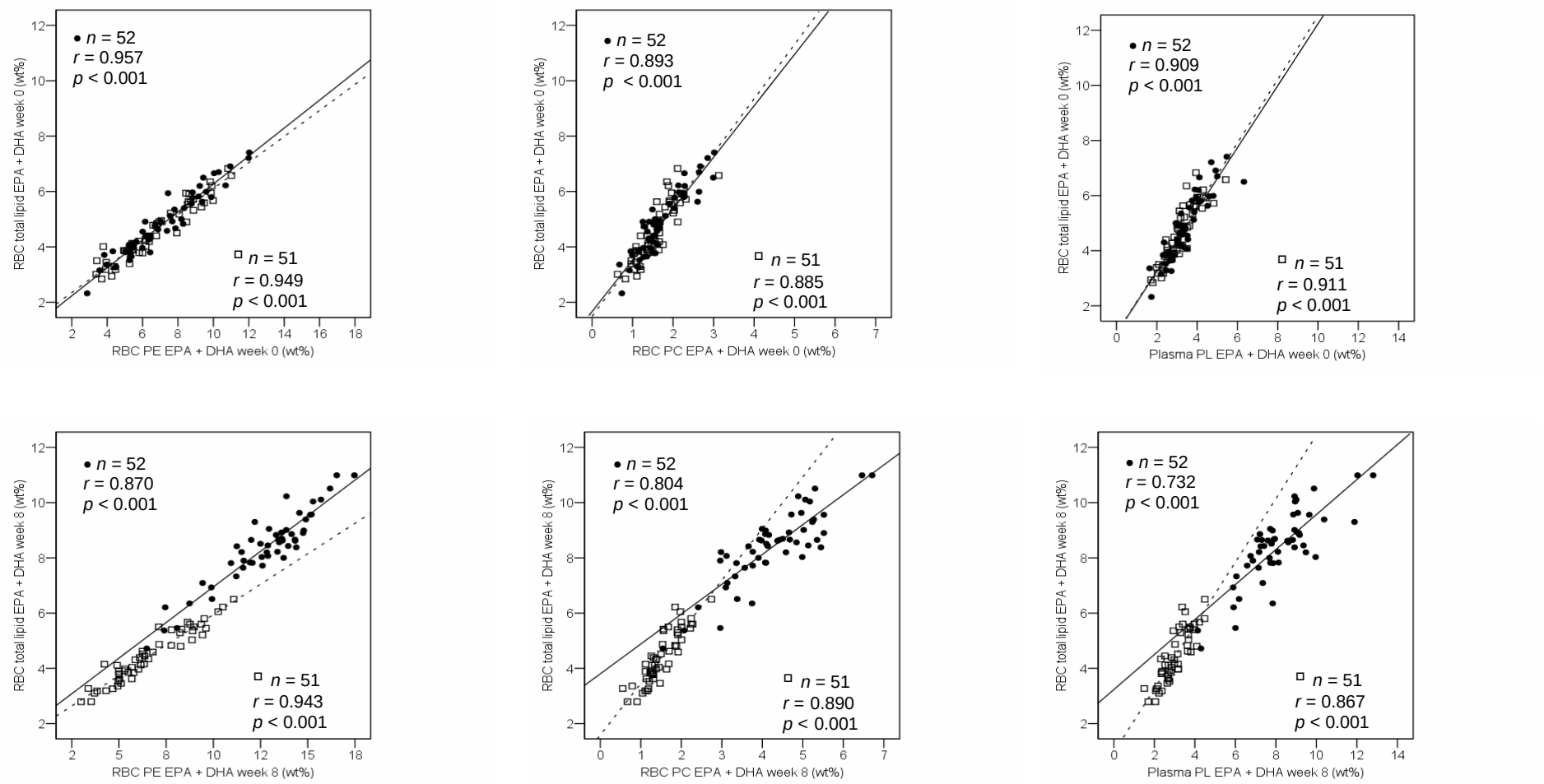


Figure 8.2. Correlations between EPA + DHA in RBC total lipids (wt%) vs. EPA + DHA in RBC PE, PC and plasma PL (Spearman-Rho correlation), *DHAVEG study*.

□ and dashed line, placebo group; • and solid line, DHA group.

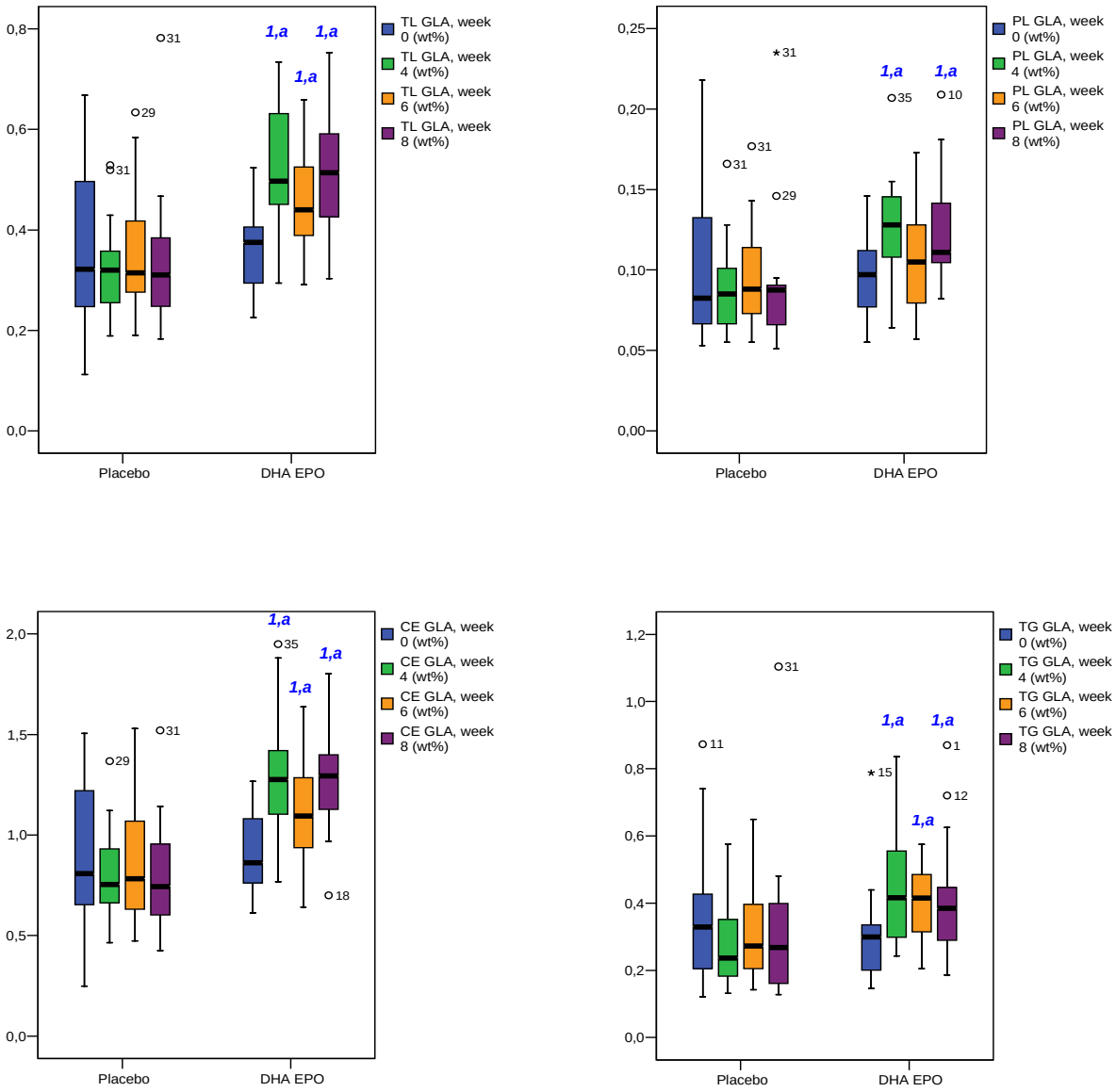


Figure 8.3. GLA (wt%) in plasma TL, PL, CE, and TG at weeks 0, 4, 6 and 8, *FO/EPO study* (placebo: *n* = 20; FO/EPO: *n* = 19).

Bottom and top edges are located at 25th and 75th percentiles, centre horizontal line is drawn at the median, whiskers mark the data points nearest to the 1.5 interquartile ranges, outliers are shown as points together with subject code.
CE, cholesterol esters; GLA, gamma-linolenic acid; PL, phospholipids; TG, triglycerides; TL, total lipids.

[1] Significantly different from baseline (Bonferroni-Holm adjusted significances);
[a] $p < 0.05$ vs. placebo at the same time point.

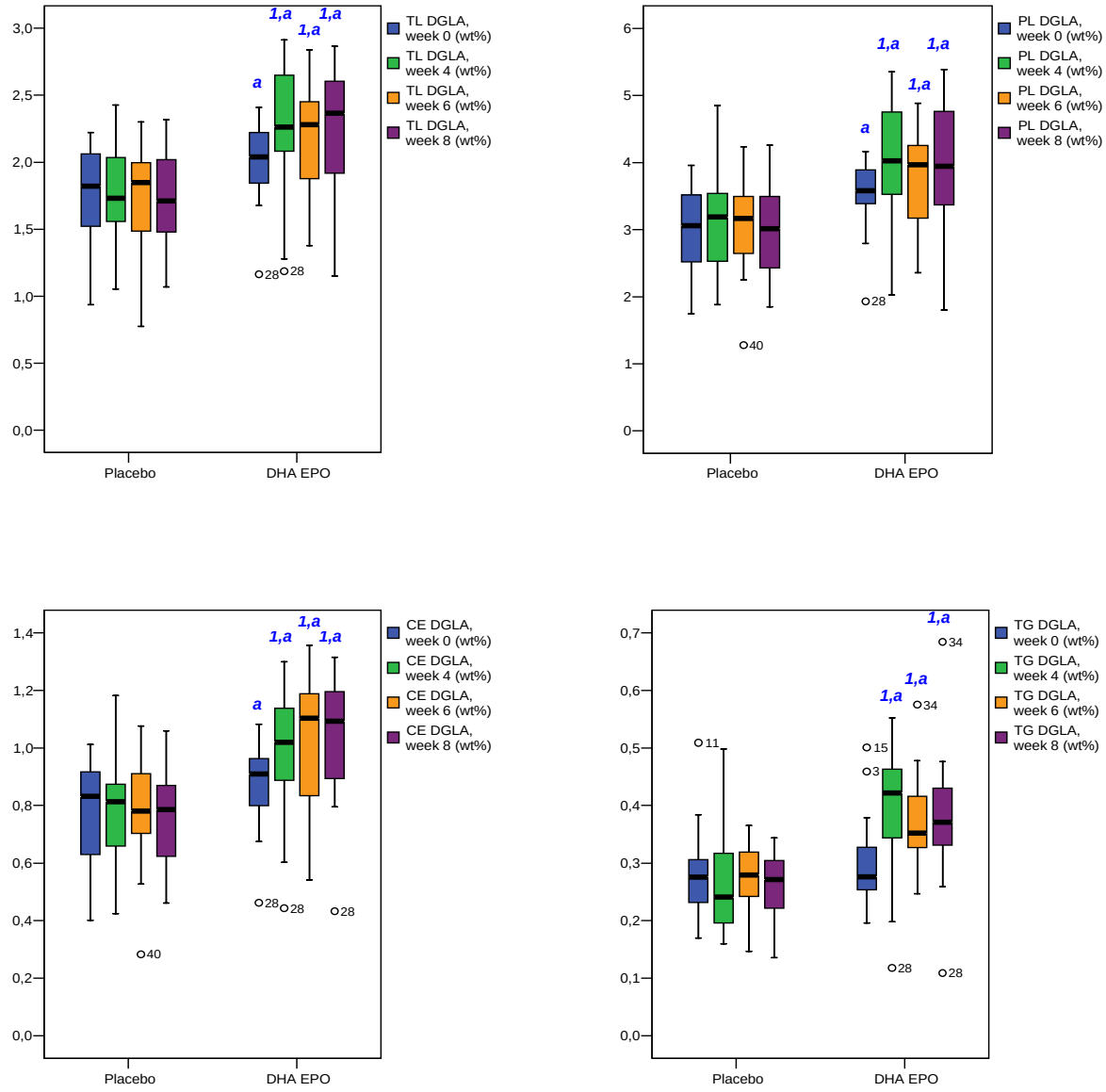


Figure 8.4. DGLA (wt%) in plasma TL, PL, CE, and TG at weeks 0, 4, 6 and 8, *FO/EPO study* (placebo: *n* = 20; FO/EPO: *n* = 19).

Bottom and top edges are located at 25th and 75th percentiles, centre horizontal line is drawn at the median, whiskers mark the data points nearest to the 1.5 interquartile ranges, outliers are shown as points together with subject code.
CE, cholesterol esters; DGLA, dihomo-gamma-linolenic acid; PL, phospholipids; TG, triglycerides; TL, total lipids.

[1] Significantly different from baseline (Bonferroni-Holm adjusted significances);
[a] $p < 0.05$ vs. placebo at the same time point.

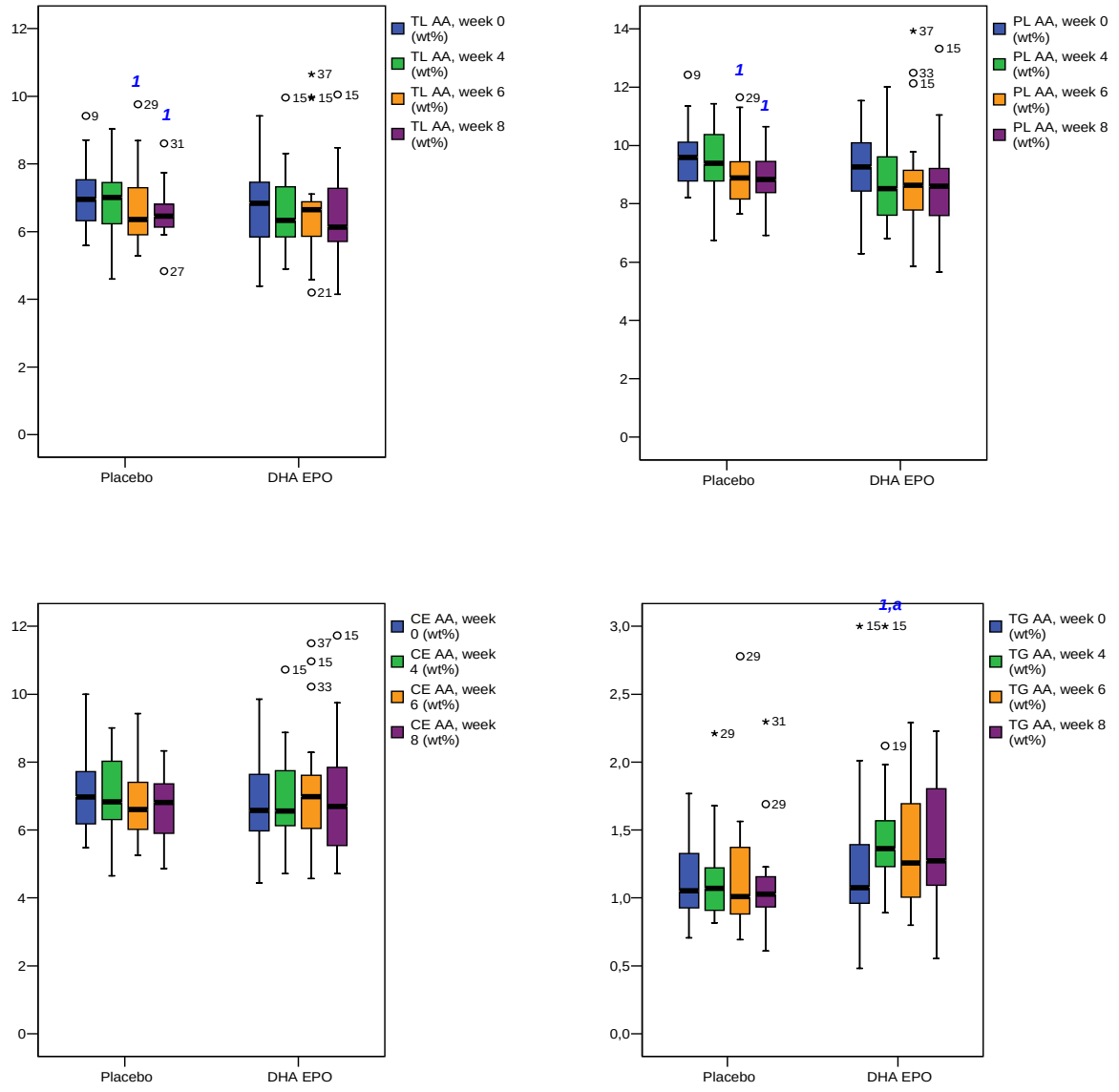


Figure 8.5. Arachidonic acid (wt%) in plasma TL, PL, CE, and TG at weeks 0, 4, 6 and 8, *FO/EPO study* (placebo: *n* = 20; FO/EPO: *n* = 19).

Bottom and top edges are located at 25th and 75th percentiles, centre horizontal line is drawn at the median, whiskers mark the data points nearest to the 1.5 interquartile ranges, outliers are shown as points together with subject code.
CE, cholesterol esters; PL, phospholipids; TG, triglycerides; TL, total lipids.

[1] Significantly different from baseline (Bonferroni-Holm adjusted significances);
[a] $p < 0.05$ vs. placebo at the same time point.

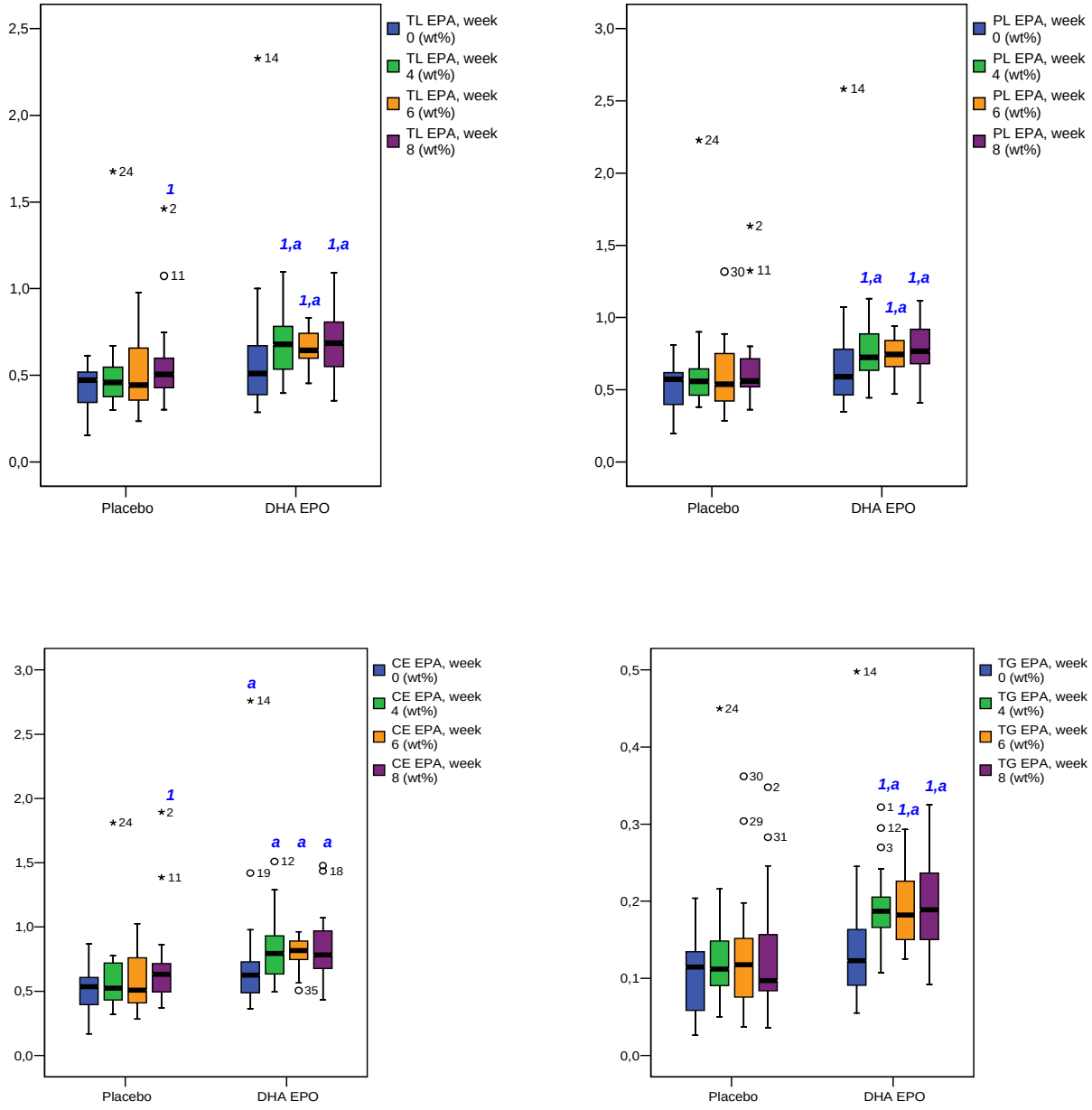


Figure 8.6. EPA (wt%) in plasma TL, PL, CE, and TG at weeks 0, 4, 6 and 8, *FO/EPO study* (placebo: *n* = 20; FO/EPO: *n* = 19).

Bottom and top edges are located at 25th and 75th percentiles, centre horizontal line is drawn at the median, whiskers mark the data points nearest to the 1.5 interquartile ranges, outliers are shown as points together with subject code.
CE, cholesterol esters; EPA, eicosapentaenoic acid; PL, phospholipids; TG, triglycerides; TL, total lipids.

[1] Significantly different from baseline (Bonferroni-Holm adjusted significances);
[a] $p < 0.05$ vs. placebo at the same time point.

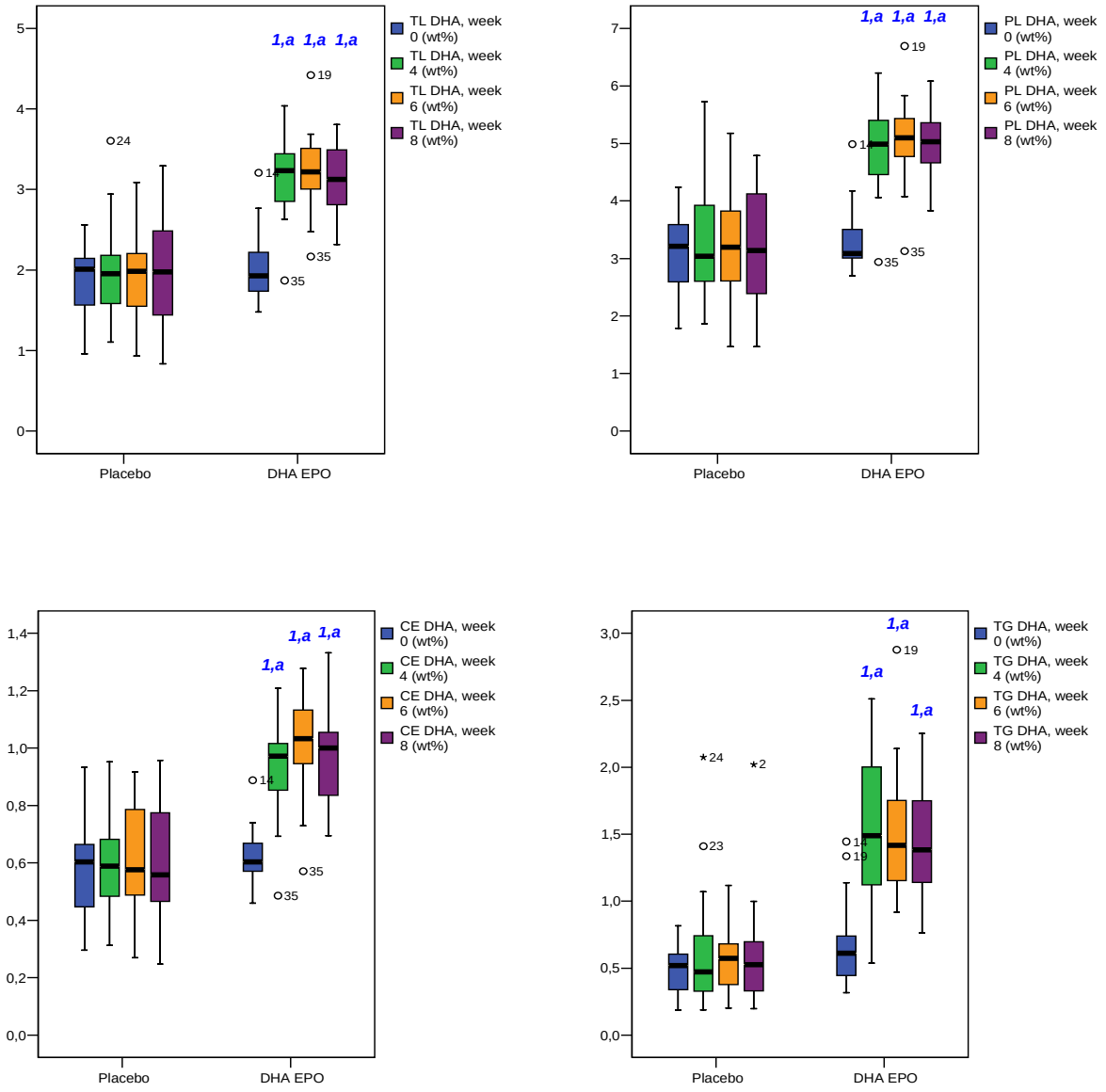


Figure 8.7. DHA (wt%) in plasma TL, PL, CE, and TG at weeks 0, 4, 6 and 8, *FO/EPO study* (placebo: *n* = 20; FO/EPO: *n* = 19).

Bottom and top edges are located at 25th and 75th percentiles, centre horizontal line is drawn at the median, whiskers mark the data points nearest to the 1.5 interquartile ranges, outliers are shown as points together with subject code.
CE, cholesterol esters; DHA, docosahexaenoic acid; PL, phospholipids; TG, triglycerides; TL, total lipids.

[1] Significantly different from baseline (Bonferroni-Holm adjusted significances);
[a] $p < 0.05$ vs. placebo at the same time point.

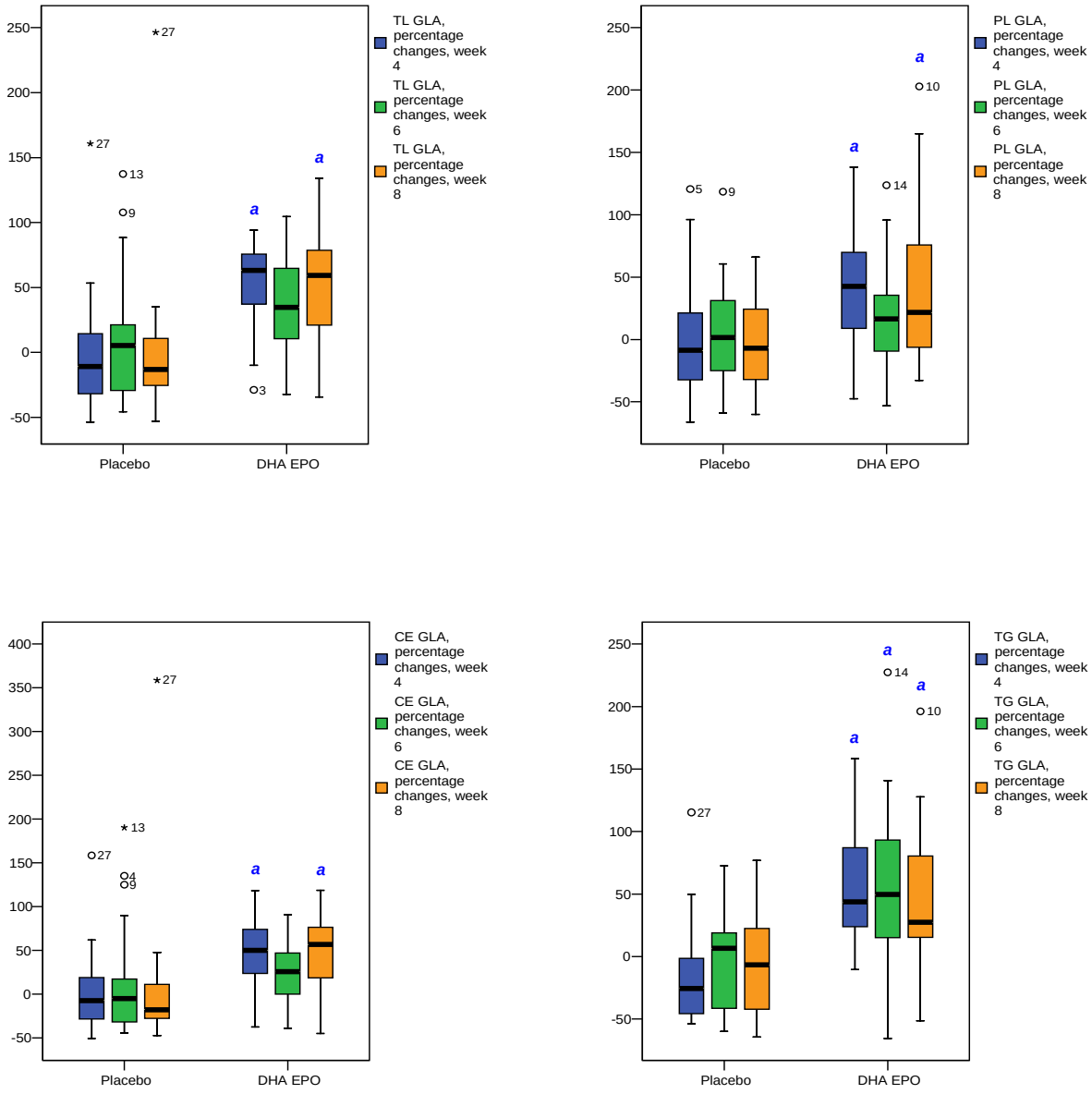


Figure 8.8. Percentage changes from baseline in relative GLA levels at weeks 4, 6, and 8, *FO/EPO study* (placebo: *n* = 20; FO/EPO: *n* = 19).

Bottom and top edges are located at 25th and 75th percentiles, centre horizontal line is drawn at the median, whiskers mark the data points nearest to the 1.5 interquartile ranges, outliers are shown as points together with subject code.
CE, cholesterol esters; GLA, gamma-linolenic acid; PL, phospholipids; TG, triglycerides; TL, total lipids.
[a] $p < 0.05$ vs. placebo at the same time point.

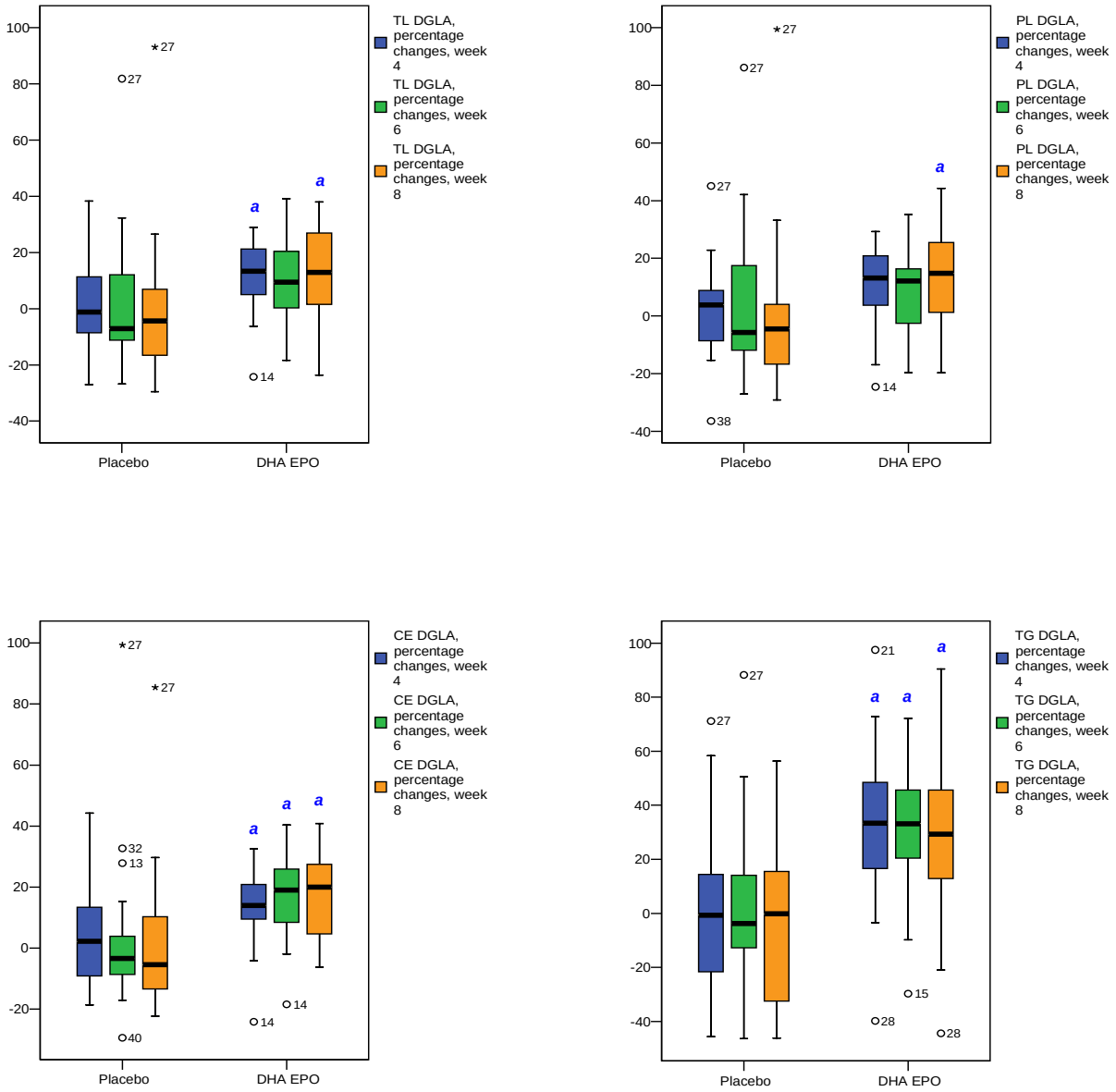


Figure 8.9. Percentage changes from baseline in relative DGLA levels at weeks 4, 6, and 8, *FO/EPO study* (placebo: *n* = 20; FO/EPO: *n* = 19).

Bottom and top edges are located at 25th and 75th percentiles, centre horizontal line is drawn at the median, whiskers mark the data points nearest to the 1.5 interquartile ranges, outliers are shown as points together with subject code.
CE, cholesterol esters; DGLA, dihomo-gamma-linolenic acid; PL, phospholipids; TG, triglycerides; TL, total lipids.
[a] $p < 0.05$ vs. placebo at the same time point.

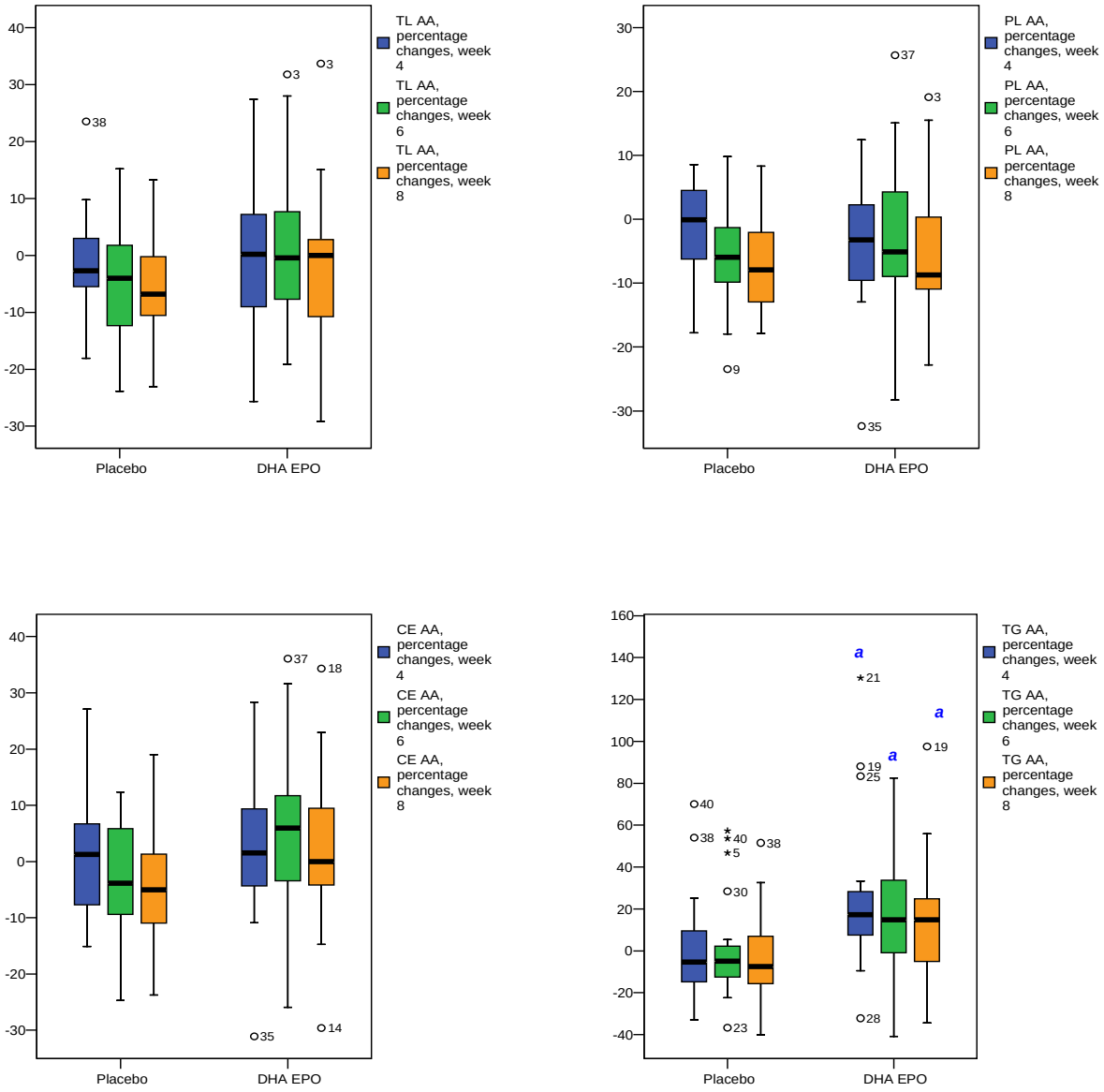


Figure 8.10. Percentage changes from baseline in relative AA levels at weeks 4, 6, and 8, *FO/EPO study* (placebo: *n* = 20; FO/EPO: *n* = 19).

Bottom and top edges are located at 25th and 75th percentiles, centre horizontal line is drawn at the median, whiskers mark the data points nearest to the 1.5 interquartile ranges, outliers are shown as points together with subject code.
CE, cholesterol esters; AA, arachidonic acid; PL, phospholipids; TG, triglycerides; TL, total lipids.
[a] $p < 0.05$ vs. placebo at the same time point.

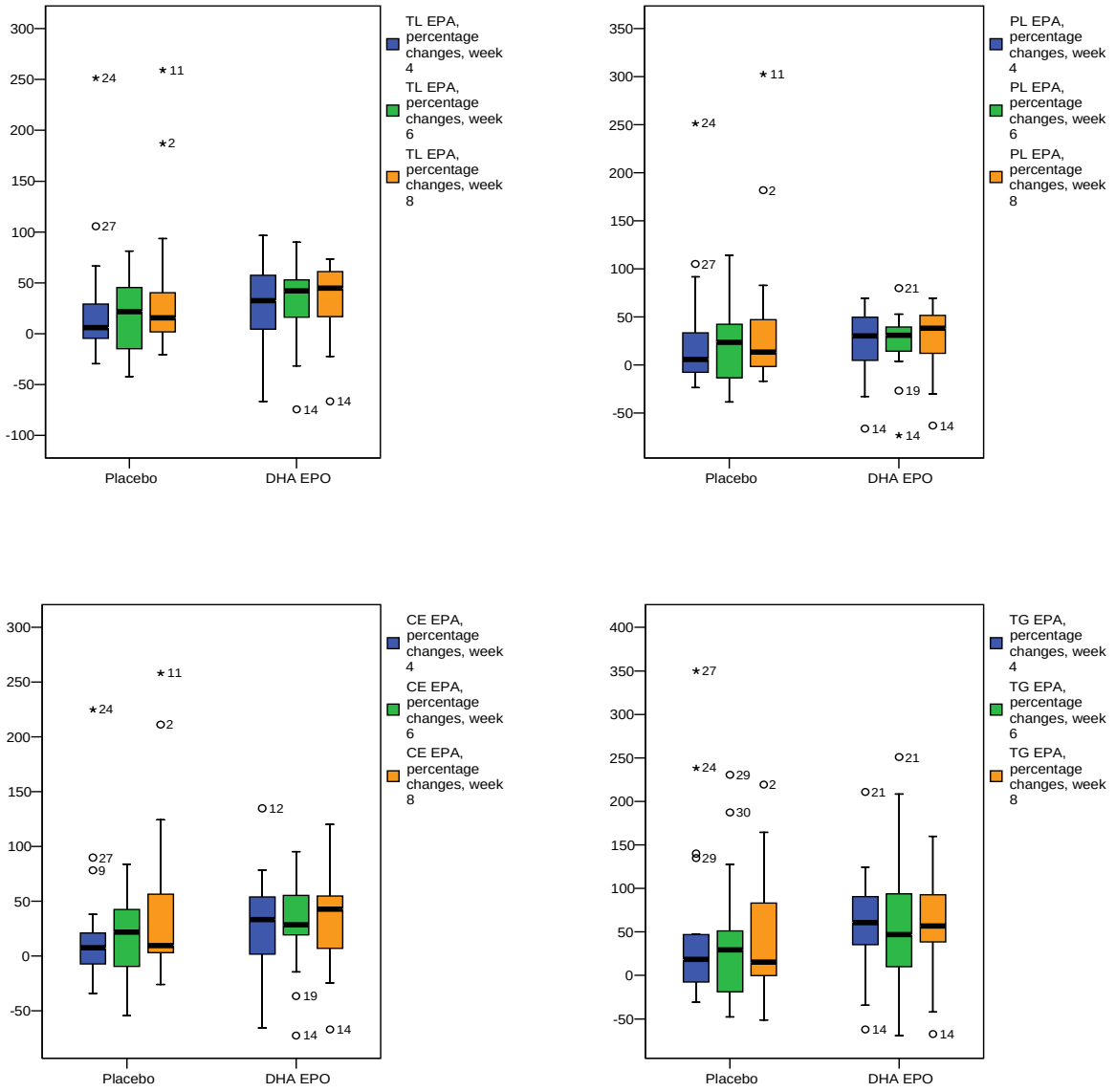


Figure 8.11. Percentage changes from baseline in relative EPA levels at weeks 4, 6, and 8, *FO/EPO study* (placebo: *n* = 20; FO/EPO: *n* = 19).

Bottom and top edges are located at 25th and 75th percentiles, centre horizontal line is drawn at the median, whiskers mark the data points nearest to the 1.5 interquartile ranges, outliers are shown as points together with subject code.
CE, cholesterol esters; EPA, eicosapentaenoic acid; PL, phospholipids; TG, triglycerides; TL, total lipids.

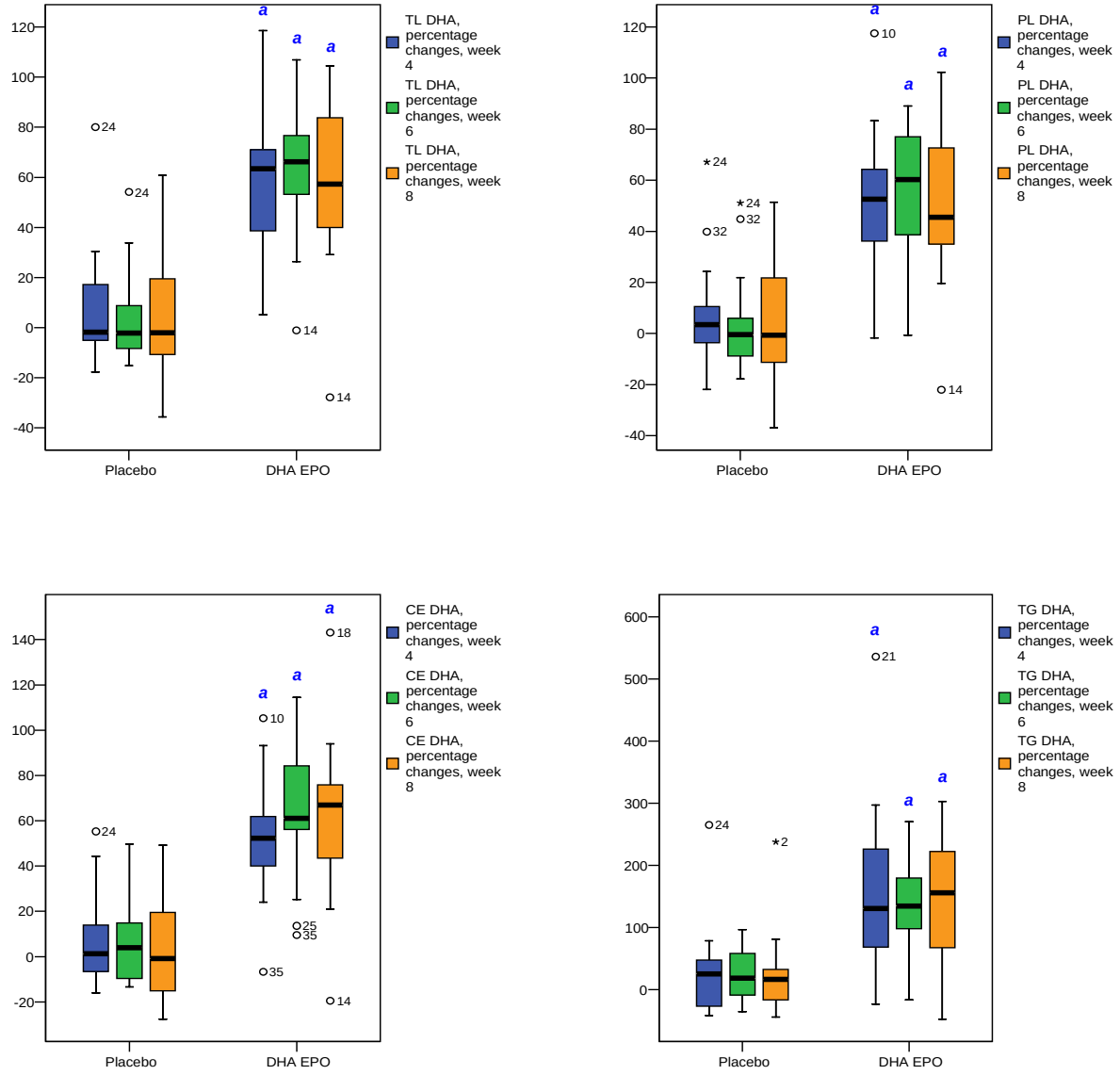


Figure 8.12. Percentage changes from baseline in relative DHA levels at weeks 4, 6, and 8, *FO/EPO study* (placebo: *n* = 20; FO/EPO: *n* = 19).

Bottom and top edges are located at 25th and 75th percentiles, centre horizontal line is drawn at the median, whiskers mark the data points nearest to the 1.5 interquartile ranges, outliers are shown as points together with subject code.
CE, cholesterol esters; DHA, docosahexaenoic acid; PL, phospholipids; TG, triglycerides; TL, total lipids.
[a] $p < 0.05$ vs. placebo at the same time point.

8.3 Used information and documentation materials

8.3.1 DHAVEG study

Poster for subject recruiting, DHAVEG study

Vegetarier/-innen gesucht

zur Teilnahme an Ernährungsstudie

Voraussetzungen:

- mindestens 18 Jahre
- normalgewichtig
- vegane bzw. ovo-lacto-vegetarische Ernährung seit mindestens 1 Jahr
- weniger als eine Fischmahlzeit pro Monat während des letzten Jahres

Aufwand:

- Tägliche Einnahme von 4 Algenölkapseln (rein pflanzlich!) mit Omega-3 Fettsäuren über 8 Wochen zusätzlich zur normalen Ernährung
- eine Untersuchung mit Nüchternblutentnahme sowie das Führen eines Ernährungsprotokolls zu Beginn und am Ende der Studie

Aufwandsentschädigung: 200 Euro

Bei Interesse bitte melden bei:
Dipl. oec.troph. Julia Geppert
Stoffwechsellabor, Dr. von Haunersches Kinderspital
Tel: 089-5160-3486
Email: jgeppert@med.uni-muenchen.de

Invitation to participate in the study, DHAVEG study

Dipl. oec.troph. Julia Geppert
Stoffwechsellabor
Dr. von Haunersches Kinderspital

Lindwurmstr. 4
D-80337 München
Tel: 089-5160-3486
Email: jgeppert@med.uni-muenchen.de

Stoffwechsellabor • Dr. von Haunersches Kinderspital • Lindwurmstr. 4 • D-80337 München

Einladung zur Teilnahme an der Vegetarierstudie

München, den

Sehr geehrter,

vorab möchte ich mich auch im Namen unserer Arbeitsgruppe für Ihr Interesse an unserer Studie bedanken.

In diesem Schreiben finden Sie fünf Formulare als Anlage. Zum einen den **Aufklärungsbogen** mit detaillierten Informationen zur Studie. Zweitens ein **Merkblatt**, in dem der Studienablauf beschrieben wird. Bitte lesen Sie sich alles in Ruhe durch. Teilen Sie mir umgehend mit, wenn Sie sich gegen die Teilnahme an der Studie entschieden haben! Ansonsten melde ich mich dann demnächst bei Ihnen zur Abklärung weiterer Fragen sowie der Termine von Eingangs- und Abschlussuntersuchung.
Als drittes finden Sie die **Einverständniserklärung**. Bringen Sie diese unterschrieben zur Eingangsuntersuchung mit. Als vierte Anlage ist ein **Fragebogen** beigefügt. Bitte füllen Sie diesen aus und bringen ihn ebenfalls zur Eingangsuntersuchung mit. Als fünftes finden Sie ein **Ernährungsprotokoll** mit Anleitung. Bitte führen Sie dieses an drei aufeinanderfolgenden Tagen (davon einer ein Samstag oder Sonntag!) und bringen Sie dieses ausgefüllt zur Eingangsuntersuchung mit!

Zusammenfassend ist folgendes für die Eingangsuntersuchung wichtig:

- ✓ **Nüchtern kommen (keine Nahrungsaufnahme in den letzten 10 Stunden)!**
- ✓ **Einverständniserklärung unterschrieben mitbringen!**
- ✓ **Fragebogen ausgefüllt mitbringen!**
- ✓ **Ernährungsprotokoll ausgefüllt mitbringen!**
- ✓ **Falls Sie eine Brille oder Kontaktlinsen tragen, bringen Sie diese bitte unbedingt zu den Untersuchungen mit!**

Mit freundlichen Grüßen

Einfluss von n-3 Fettsäuren aus Algenöl auf den Lipidstoffwechsel (DHAVEG)

J. Geppert, H. Demmelmair, B. Koletzko

Stoffwechsellabor der Kinderklinik im Dr. von Haunerschen Kinderspital der LMU

(Leiter: Prof. Dr. B. Koletzko)

Lindwurmstr. 4, 80337 München, Tel: 089-5160-7767

Sehr geehrter Studienteilnehmer, sehr geehrte Studienteilnehmerin,

als Vegetarier ernähren Sie sich bewußt. Es ist allgemein akzeptiert, dass sich eine fleischlose Ernährung günstig auf das Risiko für viele Krankheiten auswirkt. Wenn Sie allerdings auch auf Seefisch verzichten, können Ihrer Ernährung die wichtigen langkettigen n-3 Fettsäuren fehlen. Sie beeinflussen einige Risikofaktoren für Herz-Kreislauf-Erkrankungen und sind maßgebliche Bestandteile von Zellmembranen, vor allem in Nervengewebe. Wir wollen in unserer Studie die Auswirkung eines neuen pflanzlichen Öls, das aus Algen gewonnen wird, auf diese Punkte untersuchen. Es besteht fast zur Hälfte aus Docosahexaensäure, der wichtigsten langkettigen n-3 Fettsäure. Wenn Sie seit mehr als drei Monaten keine Medikamente einnehmen, die den Lipidstoffwechsel beeinflussen, und sich seit mehr als einem Jahr vegetarisch oder vegan ernähren, können Sie an unserer Studie teilnehmen.

Das Algenöl wird dabei mit Olivenöl, das sich auch eher günstig auf die Blutfette auswirkt, verglichen.

Als Studienteilnehmer bekommen sie nach dem Zufallsprinzip von uns Kapseln mit dem einen oder dem anderen Öl. In jeder Kapsel (aus pflanzlicher Stärke hergestellt) sind 0,5 g Fett enthalten, und sie müssen über 8 Wochen jeweils 4 Kapseln täglich zu sich nehmen. Da die Kapseln als Nahrungsergänzung gedacht sind, brauchen Sie Ihre gewohnte Ernährung nicht umzustellen. Da wir diese aber dokumentieren müssen, bitten wir sie zu Beginn und zum Ende der Studie jeweils für drei Tage aufzuschreiben, was sie an diesen Tagen an Speisen und Getränken zu sich genommen haben.

Um den Effekt der Fettkapseln beurteilen zu können, werden sie vor und nach der Einnahme untersucht, wobei auch jeweils ca. 30 ml Blut abgenommen werden. Anhand der Blutproben wird festgestellt, ob sich das von Ihnen eingenommene Präparat auf den Gehalt an langkettigen n-3 Fettsäuren und fettlöslichen Vitaminen in Ihrem Blut, die Blutfettwerte (u.a. Triglyceride, Cholesterin), Leberwerte und hämatologischen Parameter auswirkt. Die neurologische Untersuchung zeigt die Reizleitungsgeschwindigkeit in Ihrem Nervensystem an. Dabei werden auf Sie entweder optische oder sensorische Reize ausgeübt und über Elektroden, die auf Ihre Kopfhaut geklebt werden, das Ankommen der Signale im Gehirn erfasst.

Aufklärungsbogen DHAVEG-Studie

Zur Auswertung der Ergebnisse benötigen wir einige Informationen von Ihnen (Größe, Gewicht, Geburtsdatum, Medikamente, vorliegende Erkrankungen).

Unerwünschte Nebenwirkungen sind nicht zu erwarten, so dass als Risiko nur unwahrscheinliche, sehr seltene Komplikationen durch die Blutabnahmen eintreten könnten, z.B. Bluterguss, Venenentzündung.

Selbstverständlich können Sie jederzeit ohne Angabe von Gründen die Teilnahme an der Studie abbrechen.

Für den Fall einer studienbedingten Gesundheitsschädigung sind Sie bei der GERLING-Versicherung versichert. Der Abschluss dieser Versicherung beruht auf gesetzlicher Verpflichtung und nicht darauf, dass wir den Eintritt einer Schädigung erwarten. Die Versicherung tritt nicht für Schäden ein, die nur mittelbar mit der Studie zusammenhängen, wie zum Beispiel Wegeunfälle.

Um den Versicherungsschutz nicht zu gefährden, müssen Sie folgende Regeln beachten:

- Halten Sie sich genau an die Anweisungen des Studienteams.
- Unterziehen Sie sich während der Dauer der Studie einer medizinischen Behandlung nur im Einvernehmen mit dem Studienteam. Dies gilt selbstverständlich nicht für Notfälle, allerdings sollten Sie in diesem Fall baldmöglichst den Studienbetreuer informieren.
- Zeigen Sie jede Gesundheitsschädigung, die als Folge der Studie aufgetreten sein könnte, sofort dem Versicherer (GERLING Industrie Deutschland GmbH, NL-S, Ganghoferstr. 39, 80339 München, Tel: 089-2107-527), Versicherungsscheinnummer: 70-005607766-3 an.

Alle persönlichen Daten werden streng vertraulich behandelt und nicht weitergegeben, sondern nur für die Studie gespeichert. Nach Abschluss der Studie erfolgt die Auswertung der Daten in anonymisierter Form.

Als Entschädigung für Ihre Bemühungen im Zusammenhang mit der Studie erhalten Sie nach vollständigem Abschluss der Studienteilnahme 200,-€. Bitte haben Sie Verständnis dafür, dass wir eine Aufwandsentschädigung nur an Teilnehmer auszahlen können, welche die Studie dem Plan entsprechend zu Ende führen und die erforderlichen Angaben machen.

Falls Sie noch weitere Fragen haben stehen wir Ihnen gerne zur Verfügung.

Mit freundlichen Grüßen

Prof. Dr. med. B. Koletzko Dr. H. Demmelmair Dipl. oec.troph. J. Geppert

Informed consent of the subjects, DHAVEG study

Einfluss von n-3 Fettsäuren aus Algenöl auf den Lipidstoffwechsel (DHAVEG-Studie)

Stoffwechsellabor der Kinderklinik im Dr. von Haunerschen Kinderspital der LMU
(Leiter: Prof. Dr. B. Koletzko)
Lindwurmstr. 4, 80337 München, Tel: 089-5160-3486

Einverständniserklärung

Ich bin mit der Teilnahme an der oben genannten Studie entsprechend dem Inhalt des Aufklärungsbogens einverstanden. Alle aufgetretenen Fragen konnten besprochen werden.

Name: _____ Vorname: _____

Anschrift: _____

_____

_____

Bankverbindung (für die Überweisung des Probandenhonorars):

Kontoinhaber: _____

Kontonummer: _____

BLZ: _____

Bank: _____

_____ _____

Ort, Datum Unterschrift

Merkblatt für die Teilnehmer

Titel der Studie

Einfluss von n-3 Fettsäuren aus Microalgenöl auf den Lipidstoffwechsel

Studienablauf
Vor Aufnahme eines Probanden in die Studie findet telefonisch die Überprüfung der Ein- und Ausschlusskriterien statt. Den geeigneten Personen werden dann per Post oder Email ein Aufklärungsbogen, ein Merkblatt, ein Ernährungsprotokoll, ein Fragebogen und die Einverständniserklärung zugeschickt. Mit Hilfe des **Fragebogens** werden die Krankheitsgeschichte, Einnahme von Medikamenten und Supplementen, Ernährungsgewohnheiten etc. genauer abgefragt. Vor der Eingangsuntersuchung füllen Sie bitte an drei aufeinanderfolgenden Tagen (einer davon ein Samstag oder Sonntag) das beiliegende **Ernährungsprotokoll** aus. Dieses Protokoll soll eine allgemeine Übersicht über die tägliche Aufnahme an Nährstoffen geben. Die Ergebnisse sind notwendig, um die gemessenen Parameter genau interpretieren zu können.

Der Termin der Eingangs- sowie Abschlussuntersuchung wird telefonisch oder per Email noch mit Ihnen abgesprochen. Bitte bringen Sie hierzu den ausgefüllten Fragebogen, das Ernährungsprotokoll und die unterschriebene **Einverständniserklärung** mit. Der Termin der **Eingangsuntersuchung** soll zwischen 7 Uhr und 9 Uhr morgens liegen, um eine Blutentnahme im nüchternen Zustand (10 Stunden nach der letzten Nahrungsaufnahme) zu gewährleisten. Der Zeitaufwand beträgt etwa 45 Minuten. Es werden dabei folgende Untersuchungen durchgeführt:

- Kurze Befragung
- Abnahme einer Nüchternblutprobe (ca. 30 ml)
- Bestimmung von Körperlänge und Gewicht
- Messung von Blutdruck und Puls
- Zwei einfache, schmerzfreie, neurologische Untersuchungen

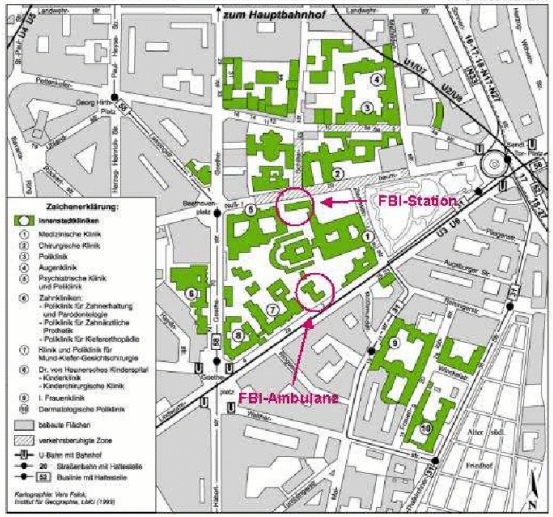
Treffpunkt für Eingangs- und Abschlussuntersuchung
Eingangshalle der Ambulanz des Friedrich-Baur-Instituts (FBI-Ambulanz)
Ziemssenstraße 1a
80336 München

Wegbeschreibung
Die Ambulanz des Friedrich-Baur-Instituts befindet sich im Innenhof der Medizinischen Klinik (rotes, neues Gebäude). Es ist bequem mit öffentlichen Verkehrsmitteln zu erreichen:

Per Bus/Tram/U-Bahn:
Haltestelle *Goetheplatz* (Gehstrecke ca. 300 m): Bus Linie 58, U-Bahn U3, U6.
Haltestelle *Sendlinger Tor* (Gehstrecke ca. 500 m): Bus Linien 56, 31, Tram Linien 16, 17, 18, 27, U-Bahn U1, U2, U3, U6.
Lindwurmstraße entlanggehen, in die Ziemssenstraße abbiegen, links durch Toreinfahrt dem Schild FBI folgen.

Mit der Deutschen Bahn:
München Hauptbahnhof, Ausgang Bayerstraße (Südseite), über die gegenüberliegende Goethestraße, ca. 800 m weiter in Richtung Süden, Eingang über Goethestraße 69 gegenüber Zahnklinik.

Lageplan



Nach der Eingangsuntersuchung werden die Studienkapseln ausgegeben. Bitte bewahren Sie diese im Kühlschrank und vor Sonne geschützt auf. Ab dem Tag der Eingangsuntersuchung verzehren Sie **8 Wochen lang täglich vier Kapseln zusätzlich zur normalen Ernährung**. Empfohlen wird die Einnahme mit kaltem Wasser zu den Mahlzeiten, z.B. eine Kapsel morgens, zwei mittags und eine abends. Bitte ändern Sie während der gesamten Studie Ihre normalen Gewohnheiten (Ernährung, körperliche Aktivität, etc.) nicht!

Für den Studienzeitraum wird ein **einfaches Protokoll** ausgegeben, in dem Sie besondere Vorkommnisse (z.B. Erkrankungen, Brechreiz, Aufstoßen, Durchfall, Blutungsereignisse, Arztbesuche) festhalten sollen. Für diese Zeit bekommen Sie auch eine Telefonnummer, unter der ein Arzt zu erreichen ist. Sie werden nach etwa zwei, vier und sechs Wochen angerufen, um eventuell auftretende Fragen zu klären. Weiterhin wird beim letzten Anruf der Termin für die Abschlussuntersuchung bestätigt. Kurz vorher wird Ihnen das **zweite Ernährungsprotokoll** zugeschickt, das Sie wieder an drei aufeinanderfolgenden Tagen (mit einem Samstag oder Sonntag) kurz vor der Abschlussuntersuchung führen sollen.

Die **Abschlussuntersuchung** nach der Studienperiode wird wieder morgens durchgeführt, um sicherzustellen, dass eine Nüchternblutprobe gewonnen werden kann. Der Termin kann bis zu 60 Tage nach Studienbeginn liegen, dann muss allerdings auch die Studienmedikation länger eingenommen werden. Zu diesem Termin geben Sie auch Ihr Ereignisprotokoll, das zweite Ernährungsprotokoll und die nicht verbrauchten Kapseln ab. Sie werden gewogen, und es wird wieder eine kurze Befragung und Untersuchung wie zu Studienbeginn durchgeführt.

Leaflet with study informations, DHAVEG study (continuation)

Übersicht über den Studienablauf

Woche	-1	0	1	2	3	4	5	6	7	8
3-Tage-Ernährungs-protokoll	✓								✓	
Untersuchung		✓								✓
Einnahme der Kapseln		✓ (Aus-gabe)	✓	✓	✓	✓	✓	✓	✓	✓ (Rück-gabe der Reste)
Ereignisprotokoll		✓	✓	✓	✓	✓	✓	✓	✓	✓

Und noch ein paar Hinweise:

- Bitte nehmen Sie während der Dauer dieser Studie sowie 30 Tage vorher an keiner anderen Studie teil.
- Auch eine Blutspende darf 4 Wochen vor Beginn der Studie und während der Studie nicht geleistet werden.
- Nehmen Sie vor und während der Studie bitte keine weiteren Supplemente mit Omega–3 Fettsäuren ein.
- Falls Sie eine Brille oder Kontaktlinsen tragen, bringen Sie diese bitte unbedingt zu den Untersuchungen mit!
- Bitte benutzen am Morgen der Untersuchungen kein Haargel oder ähnliches, da es sonst Probleme mit der Leitung der Elektroden bei den neurologischen Untersuchungen geben kann!

Probandenfragebogen

Stoffwechsellabor, Dr. von Haunersches Kinderspital, Lindwurmstr. 4, 80337 München, Tel.: 089-5160-3486

Alle Angaben unterliegen der Schweigepflicht.

Biometrische Daten

Name: ..... **Probandennummer:** .....

Geschlecht: ☐ **männlich** ☐ **weiblich** **Geburtsdatum:** .....

Größe: ..... **Gewicht:** .....

War Ihr Gewicht in den letzten 3 Monaten weitgehend konstant? ja nein

Schwangerschaft / Stillzeit (nur für weibliche Probanden)

Sind Sie möglicherweise schwanger? ja nein weiß nicht

Haben Sie in den letzten 3 Monaten gestillt? ja nein

Zigaretten

Rauchen Sie? ja nein

wenn ja, seit wann und wie viele Zigaretten pro Tag? .....

.....

Körperliche Aktivität

Leistungssport? ja nein

wenn ja, welchen und wie oft? .....

.....

Ausgleichssport? ja nein

wenn ja welchen und wie oft? .....

.....

körperliche Arbeit? ja nein

wenn ja, welche und wie oft? .....

.....

Bewegung allgemein? .....

.....

.....

Ernährungsgewohnheiten

Wie häufig verzehren Sie üblicherweise ...

Bitte geben Sie als Portionsgröße gewöhnliche Maßeinheiten wie Scheiben, Stück, Gläser, Esslöffel, etc. an!

	Portionsgröße	0x/Tag	1x/Tag	2x/Tag	3x/Tag	4x/Tag	5x/Tag
Obst							
Gemüse							
	Portionsgröße	**0x/Jahr**	**1-3x/Jahr**	**3-6x/Jahr**	**7-9x/Jahr**	**10-12x/Jahr**	**> 1x/Monat**
Mageren Fisch (z.B. Kabeljau, Scholle, Seelachs)							
Mittelfetten Fisch (z.B. Seehecht, Rotbarsch)							
Fetten Fisch (z.B. Lachs, Thunfisch, Hering, Makrele)							
	Portionsgröße	**täglich**	**Mehrmals/ Woche**	**1x/Woche**	**Mehrmals/ Monat**	**1x/Monat**	**nie**
Butter, Schmalz							
Palm-, Kokosfett							
Mayonnaise Sorte?							
Margarine Sorte?							
Sonnenblumen-, Distel-, Maiskeim-, Kürbis-, Traubenkernöl							
Oliven-, Erdnussöl							
Soja-, Raps-, Weizenkeim-, Walnussöl							
Leinöl							
Müsli							
Haferflocken							
Weißbrot, Toast, weiße Brötchen, Baguette							
Roggenbrot, Roggenbrötchen							
Vollkornbrot, Vollkornbrötchen							
Sojateigwaren							
Tofu							
Sojafleisch, Sojabratlinge							
Sojasoße							
Sojamilch							
Hülsenfrüchte							

Name: _____

	Portions-größe	täglich	Mehrmals/ Woche	1x/Woche	Mehrmals/ Monat	1x/Monat	nie
Kartoffeln, Kartoffelprodukte							
Reis geschält							
Naturreis							
Teigwaren							
Vollkornteigwaren							
Pistazien, Hasel-, Wal-, Paranüsse							
Mandeln, Cashew-, Macadamianüsse							
Erdnüsse							
Ölsamen (u.a. Sonnenblumen-, Kürbiskerne)							
Kuchen							
Backwaren, Teilchen							
Kekse							
Vollkornkekse							
Milcheis							
Schokolade, Pralinen, Schokoriegel							
Bonbons, Weingummi							
Marzipan							
Weichkäse							
Schnittkäse							
Quark, Frischkäse							
Eier, Eiprodukte							
Bier							
Rotwein							
Weißwein							
Sekt							
Likör							
Schnaps, Branntwein							
Ich koche selbst.							
Ich esse in der Mensa / im Restaurant.							
Ich esse Fastfood.							

Checkliste „Gesundheit“

Hatten Sie in den letzten 3 Monaten ernsthafte Erkrankungen oder operative Eingriffe?

ja nein **wenn ja, welche?** .....

.....

Haben oder hatten Sie früher Allergien oder Unverträglichkeiten?

ja nein **wenn ja, welche?** .....

.....

Haben oder hatten Sie früher eine der folgenden Erkrankungen?

Diabetes mellitus .....

Schilddrüsenerkrankungen (wenn ja, welche und seit wann?) .....

Hohe Blutfette (wenn ja, welche, seit wann und wie hoch?) .....

Bluthochdruck (wenn ja, seit wann und wie hoch?) .....

Störung der Blutgerinnung (wenn ja, welche und seit wann?) .....

Magen-/Darmerkrankungen (wenn ja, welche und wann?) .....

Nierenerkrankungen (wenn ja, welche und seit wann?) .....

Leber-/Gallenerkrankungen (wenn ja, welche und seit wann?) .....

Sonstige wichtige Erkrankungen (wenn ja, welche und seit wann?) .....

.....

.....

Medikamenten-/Supplementeinnahme

Medikamenteneinnahme (wenn ja, welche, seit wann und wie oft?) .....

.....

.....

Einnahme von Supplementen, z.B. Vitamine, Mineralstoffe, n-3 Fettsäuren (wenn ja, welche, seit wann und wie oft?) .....

.....

.....

Sonstiges

Nehmen Sie zur Zeit an einer anderen Studie teil? ja nein

Waren Sie in den letzten 4 Wochen Blut spenden? ja nein

Wie wird das 3-Tage-Schätzprotokoll ausgefüllt?

Allgemeiner Hinweis

- Bitte notieren Sie an drei aufeinanderfolgenden Tagen (einer davon ein Samstag oder Sonntag) vor der Eingangs- bzw. Abschlussuntersuchung **was** und **wieviel** Sie gerade essen und trinken. Notieren Sie das Datum der Tage.

Bitte essen und trinken Sie an diesen Tagen genauso wie immer !!!

Wie soll notiert werden?

- **Tragen Sie bitte grundsätzlich alles ein, was Sie verzehren**, d.h. alle Speisen und Getränke (auch Wasser, Milch und Zucker für den Kaffee oder Tee), Süßigkeiten und andere Kleinigkeiten zwischendurch und auch Präparate (z.B. Vitamin-, Mineralstoffpräparate, Stärkungsmittel) sowie Medikamente.
- Suchen Sie ein verzehrtes Lebensmittel in der entsprechenden **Lebensmittelgruppe** (z.B. Marmelade in der Gruppe *Brotbelag*).
- Falls verlangt, benennen Sie bitte Sorte bzw. Fettgehalt genauer.
- Schätzen Sie die **Menge** bitte gut ab und machen Sie in der Spalte **Anzahl** an dem entsprechenden Tag (1, 2 oder 3) entweder für jede Portion einen Strich oder notieren Sie eine Ziffer (z.B. für 3 Scheiben Graubrot entweder 3 Striche oder eine „3" eintragen).
- Für die einzelnen Lebensmittel sind jeweils die gewöhnlichen **Maßeinheiten** angegeben. So wird Brot in *Scheiben* angegeben, Kuchen in *Stück*, Kaffee in *Kaffeetassen* und Getränke in *Gläsern* oder *Tassen*. Ändern Sie die Mengenbezeichnungen nicht!
  Hat man eine **kleinere Portion** als angegeben gegessen bzw. getrunken (z.B. ½ Portion Nudeln oder ¼ Apfel) trägt man einfach ½ oder ¼ ein.
- Gerichte/Lebensmittel, die Sie **nicht in der Liste** finden können, tragen Sie bitte unter *Sonstiges* in der jeweiligen Lebensmittelgruppe oder unter *Lebensmittel, die nicht aufgeführt sind* mit möglichst genauer Portionsangabe ein.

Tipps

- Notieren Sie am besten **während oder direkt nach der Mahlzeit.**
- Am einfachsten ist es, wenn man den **Fragebogen immer dabei** hat, auch im Restaurant, bei Verwandten oder Freunden. So kann nichts vergessen werden.
- **Am Abend sollte man noch einmal über den vergangenen Tag nachdenken** - am besten mit der Liste in der Hand - ob etwas vergessen wurde und sollte das dann noch eintragen.

Name: _____

Datum: _____

Verzehr über drei Tage

Brot • Brötchen			**Anzahl**		
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Graubrot		Scheibe 45 g			
Weißbrot		Scheibe 35 g			
Toastbrot		Scheibe 20 g			
Vollkornbrot		Scheibe 50 g			
Knäckebrot		Scheibe 10 g			
Zwieback		Scheibe 10 g			
Hefezopf		Scheibe 45 g			
Normales Brötchen		Stück 45 g			
Vollkornbrötchen		Stück 55 g			
Croissant		Stück 50 g			
Sonstiges		....			

Brotbelag • Butter • Margarine			**Anzahl**		
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Butter		TL 5 g			
Margarine	Sorte:	TL 5 g			
Margarine halbfett	Sorte:	TL 5 g			
Marmelade, Konfitüre, Gelee		EL 20 g			
Honig		EL 20 g			
Nuss-Nougat-Creme		EL 20 g			
Vegetarischer Brotaufstrich	Sorte:	EL 30 g			
Frischkäse	Fettgehalt (% i. Tr.)	EL 30 g			
Quark	Fettgehalt (% i. Tr.)	EL 20 g			
Schmelzkäse	Fettgehalt (% i. Tr.)	Port. 30 g			
Schnittkäse	Fettgehalt (% i. Tr.)	Scheibe 30 g			
Weichkäse	Fettgehalt (% i. Tr.)	Scheibe 30 g			
Sonstiges		....			

Frühstücksallerlei			**Anzahl**		
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Gekochtes Ei		Stück 55 g			
Cornflakes trocken		EL 4 g			
Cornflakes trocken	gezuckert/geröstet	EL 6 g			
Haferflocken trocken		EL 10 g			
Müsli trocken		EL 15 g			
Sonstiges		....			

3-day dietary record, DHAVEG study (continuation 1)

Name: _____

Datum: _____

Gemüse • Salate			Anzahl		
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Blattsalat ohne Dressing	Sorte:	Portion 50 g			
Rohkost / Salatgemüse ohne Dressing	Sorte:	Portion 50 g			
Mangold, Spinat	Blattgemüse Sorte:	Portion 200 g			
Grüne Bohnen		Portion 200 g			
Aubergine, Gurke, Paprika, Tomate, Zucchini, ...	Fruchtgemüse Sorte:	Portion 200 g			
Gemüsemais		Portion 200 g			
Blumenkohl, Broccoli, Kohl (Rot-, Grün-, Weiß-), Kohlrabi, Rosenkohl, Wirsing,...	Kohlgemüse Sorte:	Portion 200 g			
Sauerkraut		Portion 150 g			
Fenchel, Lauch, Spargel,...	Sprossengemüse Sorte:	Portion 200 g			
Möhre, Radieschen, Rettich, Rote Bete, Rüben, Sellerie, Schwarzwurzel,...	Wurzel- und Knollengemüse Sorte:	Portion 200 g			
Pilze		Portion 120 g			
Zwiebel		Stücke 30 g			
Küchenkräuter	Sorte:	EL 1g			
Gewürzgurken		Portion 50 g			
Sonstiges		....			

Hülsenfrüchte		Anzahl		
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Weiße / Rote Bohnen gegart	Portion 200 g			
Erbsen gegart	Portion 200 g			
Linsen gegart	Portion 200 g			
Sonstiges	....			

Beilagen (Kartoffeln, Nudeln, Reis,...)		Anzahl		
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Salzkartoffeln	Portion 200 g			
Pellkartoffeln	Portion 200 g			
Bratkartoffeln	Portion 200 g			
Kartoffelbrei	Portion 200 g			
Kartoffelknödel	Stück 100 g			
Kartoffelsalat	Portion 250 g			
Pommes Frites	Portion 200 g			
weißer Reis gekocht	Portion 180 g			
Natur-Reis gekocht	Portion 180 g			
Nudeln eifrei gekocht	Portion 180 g			

Milch • Milchprodukte • Sojaprodukte		Anzahl			
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Buttermilch		Glas 200 g			
Joghurt natur	Fettarm (1,5 % Fett)	Becher 150 g			
Joghurt natur	Vollfett (3,5 % Fett)	Becher 150 g			
Joghurt mit Frucht	Fettarm (1,5 % Fett)	Becher 150 g			
Joghurt mit Frucht	Vollfett (3,5 % Fett)	Becher 150 g			
Milch	Fettarm (1,5 % Fett)	Glas 200 g			
Milch	Vollfett (3,5 % Fett)	Glas 200 g			
Kakao/Trinkschokolade	Fettgehalt (%)	Glas 200 g			
Sojamilch	Sorte:	Glas 200 g			
Tofu	Sorte:	Portion 100 g			
Sahne	Fettgehalt (%)	EL 10 g			
Kondensmilch	Fettgehalt (%)	Portion 12 g			
Sonstiges	Fettgehalt (%)	....			

Obst		Anzahl			
		Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Brombeere, Erdbeere, Himbeere, Johannisbeere, Heidelbeere	Beerenobst Sorte:.....	Portion 125 g			
Weintrauben		Portion 150 g			
Apfel, Birne, Quitte,...	Kernobst Sorte:.....	Portion 150 g			
Aprikosen, Kirschen, Mirabellen, Pflaumen, Pfirsich,...	Steinobst Sorte:	Portion 150 g			
Banane		Stück 120 g			
Ananas, Kiwi, Mango, Maracuja,...	Südfrüchte Sorte:	Portion 150 g			
Grapefruit, Mandarine, Orange, Zitrone, ...	Zitrusfrüchte Sorte:.....	Portion 150 g			
Rosinen, Trockenobst	Sorte:	Portion 50 g			
Kompott, Obstkonserven	Sorte:	Portion 150 g			
Sonstiges		....			

3-day dietary record, DHAVEG study (continuation 2)

Beilagen (Kartoffeln, Nudeln, Reis,...)		Einheit	Anzahl Tag 1	Tag 2	Tag 3
Eiernudeln	gekocht	Portion 180 g			
Vollkornnudeln eifrei	gekocht	Portion 180 g			
Vollkornnudeln mit Ei	gekocht	Portion 180 g			
Spätzle	gekocht	Portion 200 g			
Sonstiges		.....			
.....		.....			
.....		.....			

Soßen • Fette • Öle		Einheit	Anzahl Tag 1	Tag 2	Tag 3
Salatsoße	Sorte:	Portion 20 g			
	.....				
Bechamelsoße		Portion 75 g			
Käsesoße		Portion 75 g			
Tomatensoße		Portion 75 g			
Tomatenketchup		Portion 20 g			
Tomatenmark		TL 6 g			
Senf		TL 6 g			
Mayonnaise	Sorte:	EL 12 g			
Kokosfett, Butterschmalz	Sorte:	EL 10 g			
Pflanzenöl	Sorte:	EL 10 g			
Sonstiges		.....			
.....		.....			
.....		.....			

Suppen • Eintöpfe	Einheit	Anzahl Tag 1	Tag 2	Tag 3
als Vorsuppe				
Suppe klar	Portion 200 g			
Suppe gebunden	Portion 200 g			
Cremesuppe	Portion 200 g			
als Hauptgericht				
Gemüsesuppe	Portion 400 g			
Kartoffelsuppe	Portion 400 g			
Linseneintopf	Portion 400 g			
Sonstiges	.....			
.....	.....			
.....	.....			

Fertig- und Schnellgerichte	Einheit	Anzahl Tag 1	Tag 2	Tag 3
Vegetarische Pizza	Portion 370 g			
Spaghetti in Tomatensoße	Portion 250 g			
Gemüselasagne	Portion 250 g			
Pfannkuchen	Portion 180 g			
Sonstiges	.....			
.....	.....			
.....	.....			
.....	.....			

Name: _____

Datum: _____

Dessert • Kuchen • Gebäck • Süßes	Einheit	Anzahl Tag 1	Tag 2	Tag 3
Pudding	Portion 150 g			
Eiscreme	Kugel 50 g			
Obstkuchen	Stück 120 g			
Cremetorte	Stück 150 g			
Rührkuchen	Stück 60 g			
Plätzchen, Kekse	Stück 10 g			
Schokolade	Riegel 20 g			
Praline	Stück 10 g			
Bonbon, Hartkaramelle	Stück 3 g			
Fruchtgummi	Portion 50 g			
Zucker	TL 5 g			
Sonstiges	.....			
.....	.....			
.....	.....			
.....	.....			

Knabbereien • Nüsse		Einheit	Anzahl Tag 1	Tag 2	Tag 3
Nüsse	Sorte:	EL 20 g			
Erdnüsse gesalzen		Tasse 100 g			
Erdnussflips		Tasse 50 g			
Chips		Tasse 30 g			
Salzstangen		Portion 30 g			
Sonstiges		.....			
.....		.....			
.....		.....			
.....		.....			

Wasser • Säfte • alkoholfreie Getränke		Einheit	Anzahl Tag 1	Tag 2	Tag 3
Mineralwasser		Glas 200 g			
Leitungswasser		Glas 200 g			
Fruchtsaft, 100 % Frucht	Sorte:	Glas 200 g			
Fruchtnektar	Sorte:	Glas 200 g			
Gemüsesaft	Sorte:	Glas 200 g			
Multivitaminsaft	Sorte:	Glas 200 g			
Limonade	Sorte:	Glas 200 g			
Colagetränke	Sorte:	Glas 200 g			
Diätgetränke (mit Süßstoff)	Sorte:	Glas 200 g			
Sonstiges		.....			
.....		.....			
.....		.....			
.....		.....			

3-day dietary record, DHAVEG study (continuation 3)

Kaffee • Tee			**Anzahl**	
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Kaffee (koffeinhaltig)	Tasse 150 g			
Kaffee (entkoffeiniert)	Tasse 150 g			
Malzkaffee / Zichorien-kaffee	Tasse 150 g			
Schwarzer Tee	Tasse 150 g			
Kräutertee, Früchtetee	Tasse 150 g			
Sonstiges	....			
Alkoholische Getränke			**Anzahl**	
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**
Bier	Glas 330 g			
Bier alkoholfrei	Glas 330 g			
Weizenbier	Glas 500 g			
Weißwein	Glas 200 g			
Rotwein	Glas 200 g			
Sekt	Glas 100 g			
Likör	Glas 40 g			
Schnaps, Branntwein	Glas 20 g			
Sonstiges	....			
Lebensmittel, die nicht aufgeführt sind			**Anzahl**	
	Einheit	**Tag 1**	**Tag 2**	**Tag 3**

Record of side effects, signs of illness and intake of medication, DHAVEG study

Ereignisprotokoll

Probandennummer:

Ihre Termine zur Blutentnahme:

Woche 0: Woche 8:

Bitte halten Sie während der Studie besondere Vorkommnisse (z.B. Erkrankungen, Brechreiz, Aufstoßen, Durchfall, Blutungsereignisse, Arztbesuche, Reisen) fest!

Datum	besonderes Ereignis

Investigation protocol at week 0, DHAVEG study

Einfluss von n-3 Fettsäuren aus Algenöl auf den Lipidstoffwechsel (DHAVEG)

Eingangsuntersuchung

Datum: _____ **Probandennummer:** _____

Name: _____ **Geburtsdatum:** _____

Erhaltene Unterlagen

- **Einverständniserklärung** ☐ **ja** ☐ **nein**
- **Fragebogen** ☐ **ja** ☐ **nein**
- **Ernährungsprotokoll** ☐ **ja** ☐ **nein**

Befragung

Haben Sie heute morgen schon etwas gegessen oder getrunken? ☐ **ja** ☐ **nein**
Wenn ja, was? _____

Sind Sie im Moment krank? ☐ **ja** ☐ **nein**
Wenn ja, was? _____

Haben Sie in den letzten Tagen Medikamente eingenommen? ☐ **ja** ☐ **nein**
Wenn ja, welche? _____

Sind Sie möglicherweise schwanger? ☐ **ja** ☐ **nein**

Wann war Ihr letzter Fischverzehr? _____

Haben Sie in letzter Zeit Supplemente mit Omega-3 Fettsäuren genommen oder mit Omega-3 Fettsäuren angereicherte Lebensmittel verzehrt? ☐ **ja** ☐ **nein**
Wenn ja, was? _____

Messparameter

Größe: _____ **Gewicht:** _____

Blutdruck: _____ **Puls:** _____

Einfluss von n-3 Fettsäuren aus Algenöl auf den Lipidstoffwechsel (DHAVEG)

Abschlussuntersuchung

Datum: _____ **Probandennummer:** _____

Name: _____ **Geburtsdatum:** _____

Erhaltene Unterlagen

- **Ereignisprotokoll** ☐ **ja** ☐ **nein**
- **Ernährungsprotokoll** ☐ **ja** ☐ **nein**

Befragung allgemein

Haben Sie heute morgen schon etwas gegessen oder getrunken? ☐ **ja** ☐ **nein**
Wenn ja, was? _____

Sind Sie im Moment krank? ☐ **ja** ☐ **nein**
Wenn ja, was? _____

Haben Sie in den letzten Tagen Medikamente eingenommen? ☐ **ja** ☐ **nein**
Wenn ja, welche? _____

Haben Sie während der Studie Fisch oder Meeresfrüchte verzehrt? ☐ **ja** ☐ **nein**
Wenn ja, wann und was? _____
_____

Haben Sie während der Studie weitere Supplemente mit Omega-3 Fettsäuren genommen oder mit Omega-3 Fettsäuren angereicherte Lebensmittel verzehrt? ☐ **ja** ☐ **nein**
Wenn ja, was und wie oft? _____

Wie viele Kapseln haben Sie nicht verzehrt? Warum?
_____
_____

Befragung zu Kapseln

Wie war die Verträglichkeit der Kapseln? Nebenwirkungen?
- ☐ Aufstoßen
- ☐ Übelkeit
- ☐ Durchfall
- ☐ Bauchschmerzen
- ☐ Blutungen
- ☐ Sonstiges _____
_____
_____

Wie war der Geschmack/Geruch?
- ☐ kein Geschmack/Geruch
- ☐ unangenehmer Geschmack/Geruch nach
_____

Wie waren die Kapseln zu schlucken?
- ☐ gut
- ☐ geht so, weil _____
- ☐ schlecht, weil _____

Messparameter

Gewicht: _____ **Blutdruck:** _____ **Puls:** _____

8.3.2 FO/EPO study

Poster for subject recruiting, FO/EPO study

Weibliche Probanden gesucht

zur Teilnahme an Ernährungsstudie (Mai-August 2005)

Voraussetzungen:

- Frauen zwischen 18 und 40 Jahren
- normalgewichtig
- gesund
- keine Vegetarier

Aufwand:

- Tägliche Einnahme eines Fettsäuresupplements (bestehend aus Nachtkerzen- und Fischöl bzw. ein Placebo, 6 Kapseln pro Tag) über 8 Wochen zusätzlich zur normalen Ernährung
- Nüchternblutentnahme vor der Studie und nach 4, 6 und 8 Wochen

Aufwandsentschädigung: 100 Euro (sowie Blutbild und Leberwerte)

Bei Interesse bitte melden bei:
Dipl. oec.troph. Julia Geppert
Labor für Stoffwechsel- und Ernährungsforschung, Dr. von Haunersches Kinderspital
Tel: 089-5160-7767
Email: Julia.Geppert@med.uni-muenchen.de

Invitation to participate in the study, FO/EPO study

Dipl. oec.troph. Julia Geppert
Labor für Stoffwechsel- und Ernährungsforschung
Dr. von Haunersches Kinderspital

Lindwurmstr. 4
D-80337 München
Tel: 089-5160-7767
Fax: 089-5160-3487
Email: jgeppert@med.uni-muenchen.de

Stoffwechsellabor • Dr. von Haunersches Kinderspital • Lindwurmstr. 4 • D-80337 München

Einladung zur Teilnahme an der EFANATAL-Pilotstudie

München,

Sehr geehrte

in diesem Schreiben finden Sie drei Formulare als Anlage. Zum einen den **Aufklärungsbogen** mit detaillierten Informationen zur Studie. Zweitens ein **Merkblatt**, in dem der Studienablauf beschrieben wird. Bitte lesen Sie sich alles in Ruhe durch. Teilen Sie mir umgehend mit, wenn Sie sich gegen die Teilnahme an der Studie entschieden haben! Ansonsten melde ich mich dann demnächst bei Ihnen zur Abklärung weiterer Fragen sowie der Termine für die Blutentnahmen. Als drittes finden Sie die **Einverständniserklärung**. Bringen Sie diese unterschrieben zur Eingangsuntersuchung mit.
Zusammenfassend ist folgendes für die Eingangsuntersuchung wichtig:

- ✓ **Nüchtern kommen (keine Nahrungsaufnahme in den letzten 10 Stunden, Wasser ist in geringen Mengen erlaubt)!**
- ✓ **Einverständniserklärung unterschrieben mitbringen!**

Mit freundlichen Grüßen

Anlage

Auswirkungen eines Fettsäuresupplements auf den Fettsäurestatus nicht-schwangerer Frauen (EFANATAL-Pilotstudie)

J. Geppert, H. Demmelmair, B. Koletzko
Labor für Stoffwechsel- und Ernährungsforschung
Dr. von Haunersches Kinderspital der LMU
Lindwurmstr. 4, 80337 München, Tel: 089-5160-7767

Sehr geehrte Studienteilnehmerin,

essentielle Fettsäuren können vom Körper nicht produziert werden und müssen deshalb mit der Nahrung aufgenommen werden. Essentielle Fettsäuren und ihre langkettigen, hochungesättigten Metabolite (LCPUFA) sind in nennenswerten Mengen in Seefischen, pflanzlichen Ölen und Getreidekeimölen (z. B. Distelöl, Sesamöl, Leinöl, Weizenkeimöl, etc.) enthalten. Sie sind unverzichtbar für die menschliche Entwicklung und optimale Gesundheit. Studien zeigten, dass die LCPUFA-Verfügbarkeit die Entwicklung des kindlichen Sehvermögens sowie komplexer neurologischer Funktionen beeinflusst. Deshalb wird besonders während einer Schwangerschaft und in den ersten Monaten nach der Geburt eine optimale Versorgung von Mutter und Kind mit essentiellen Fettsäuren/LCPUFA angestrebt.

Wir wollen in dieser Pilotstudie die Auswirkung einer neuen Fettsäuremischung, die aus Pflanzen- und Fischöl besteht, auf die Fettsäurezusammensetzung im Blut bei nicht-schwangeren Frauen untersuchen. Wenn sich in dieser Studie ein günstiger Einfluss auf den Fettsäurestatus feststellen lässt, soll eine weitere Studie an schwangeren und stillenden Frauen sowie ihren Kindern durchgeführt werden.

Die zu untersuchende Mischung mit essentiellen Fettsäuren/LCPUFA wird dabei mit einer Mischung pflanzlicher Öle verglichen, dessen Fettsäurezusammensetzung der üblichen Ernährung entspricht. Als Studienteilnehmerin bekommen Sie nach dem Zufallsprinzip von uns Kapseln mit dem einen oder dem anderen Öl. In jeder Kapsel sind 0,5 g Fett enthalten, und Sie müssen über 8 Wochen jeweils 6 Kapseln täglich zu sich nehmen (jeweils 3 x 2 Kapseln oder 2 x 3 Kapseln zu den Mahlzeiten). Da die Kapseln als Nahrungsergänzung gedacht sind, brauchen Sie Ihre gewohnte Ernährung nicht umzustellen.

Um den Effekt der Fettkapseln beurteilen zu können, wird Ihnen vor der Studie (Woche 0) und nach 4, 6 und 8 Wochen jeweils etwa 7 ml Blut abgenommen. Anhand der Blutproben wird festgestellt, ob wie sich das von Ihnen eingenommene Präparat auf den Gehalt an essentiellen Fettsäuren und LCPUFA im Blut auswirkt.

Zur Auswertung der Ergebnisse benötigen wir einige Informationen von Ihnen (Größe, Gewicht, Blutdruck, Geburtsdatum, Medikamente, vorliegende Erkrankungen).

Unerwünschte Nebenwirkungen sind nicht zu erwarten, so dass als Risiko nur unwahrscheinliche, sehr seltene Komplikationen durch die Blutabnahmen eintreten könnten, z.B. Bluterguss, Venenentzündung.

Selbstverständlich können Sie jederzeit ohne Angabe von Gründen die Teilnahme an der Studie abbrechen. Für die vollständige Dokumentation der Studienergebnisse ist es allerdings erforderlich, dass bereits gesammelte Informationen und Blutproben ausgewertet und in pseudonymisierter Form gespeichert werden.

Für den Fall einer studienbedingten Gesundheitsschädigung sind Sie bei der GERLING-Versicherung versichert. Der Abschluss dieser Versicherung beruht auf gesetzlicher Verpflichtung und nicht darauf, dass wir den Eintritt einer Schädigung erwarten. Die Versicherung tritt nicht für Schäden ein, die nur mittelbar mit der Studie zusammenhängen, wie zum Beispiel Wegeunfälle.

Um den Versicherungsschutz nicht zu gefährden, müssen Sie folgende Regeln beachten:

- Halten Sie sich genau an die Anweisungen des Studienteams.
- Unterziehen Sie sich während der Dauer der Studie einer medizinischen Behandlung nur im Einvernehmen mit dem Studienteam. Dies gilt selbstverständlich nicht für Notfälle, allerdings sollten Sie in diesem Fall baldmöglichst den Studienbetreuer informieren.
- Zeigen Sie jede Gesundheitsschädigung, die als Folge der Studie aufgetreten sein könnte, sofort dem Versicherer (GERLING Vertrieb Deutschland GmbH, Ganghoferstr. 39, 80339 München, Tel: 089-2107-451, Herr Michael Kloiber), Versicherungsscheinnummer: 70-005834022-0 an.

Alle persönlichen Daten werden streng vertraulich behandelt und nicht weitergegeben, sondern nur für die Studie gespeichert. Nach Abschluss der Studie erfolgt die Auswertung der Daten in pseudonymisierter Form.

Als Entschädigung für Ihre Bemühungen im Zusammenhang mit der Studie erhalten Sie nach vollständigem Abschluss der Studienteilnahme 100,-€. Bitte haben Sie Verständnis dafür, dass wir eine Aufwandsentschädigung nur an Teilnehmer auszahlen können, welche die Studie dem Plan entsprechend zu Ende führen und die erforderlichen Angaben machen.

Falls Sie noch weitere Fragen haben stehen wir Ihnen gerne zur Verfügung.

Mit freundlichen Grüßen

Prof. Dr. med. B. Koletzko Dr. H. Demmelmair Dipl. oec.troph. J. Geppert

Informed consent of the subjects, FO/EPO study

Auswirkungen eines Fettsäuresupplements auf den Fettsäurestatus nicht-schwangerer Frauen (EFANATAL-Pilotstudie)

Labor für Stoffwechsel- und Ernährungsforschung
Dr. von Haunersches Kinderspital der LMU
(Leiter: Prof. Dr. med. Berthold Koletzko)
Lindwurmstr. 4, 80337 München, Tel: 089-5160-7767

Einverständniserklärung

Ich bin mit der Teilnahme an der oben genannten Studie entsprechend dem Inhalt des Aufklärungsbogens einverstanden. Alle aufgetretenen Fragen konnten besprochen werden.

Name: _____ Vorname: _____

Anschrift: _____

_____

_____

Bankverbindung (für die Überweisung des Probandenhonorars):

Kontoinhaber: _____

Kontonummer: _____

BLZ: _____

Bank: _____

_____ _____

Ort, Datum Unterschrift

Merkblatt für die Teilnehmer

Titel der Studie

Auswirkungen eines Fettsäuresupplements auf den Fettsäurestatus nicht-schwangerer Frauen

Studienablauf

Vor Aufnahme eines Probanden in die Studie findet telefonisch/per Email die Überprüfung der Ein- und Ausschlusskriterien mittels Fragebogen statt. Den geeigneten Personen werden dann per Post oder Email der **Aufklärungsbogen**, ein **Merkblatt zum Studienablauf** sowie die **Einverständniserklärung** zugeschickt. Die Studie wird von etwa Mitte/Ende Mai bis August 2005 durchgeführt. Die Termine der Blutentnahmen (Woche 0, 4, 6 und 8) werden telefonisch oder per Email mit Ihnen abgesprochen.

Bitte bringen Sie zur **ersten Blutentnahme (Woche 0)** die unterschriebene **Einverständniserklärung** mit. Die Termine der Blutentnahmen sollten zwischen 7 Uhr und 10 Uhr morgens liegen, um eine Blutentnahme im nüchternen Zustand (10 Stunden nach der letzten Nahrungsaufnahme) zu gewährleisten. Der Zeitaufwand beträgt etwa 10 Minuten. Es werden bei der Eingangsuntersuchung folgende Untersuchungen durchgeführt:

- Kurze Befragung
- Bestimmung von Körperlänge und Gewicht
- Abnahme einer Nüchternblutprobe (ca. 7 ml)
- Blutdruckmessung

Nach 4, 6 und 8 Wochen findet jeweils nur eine Nüchternblutentnahme (jeweils 5 ml) statt (Zeitaufwand etwa 5 Minuten).

Treffpunkt für alle Untersuchungen

Eingangshalle Dr. von Haunersches Kinderspital (am Aquarium)
Lindwurmstraße 4
80337 München

Wegbeschreibung

Das Dr. von Haunersche Kinderspital befindet sich in der Lindwurmstraße 4, Nähe Goetheplatz. Es ist bequem mit öffentlichen Verkehrsmitteln zu erreichen:

Per Bus/Tram/U-Bahn:
Haltestelle *Goetheplatz* (Gehstrecke ca. 50 m): Bus Linie 58, U-Bahn U3, U6.
Haltestelle *Sendlinger Tor* (Gehstrecke ca. 500 m): Bus Linien 56, 31, Tram Linien 16, 17, 18, 27, U-Bahn U1, U2, U3, U6.

Mit der Deutschen Bahn:
München Hauptbahnhof, Ausgang Bayerstraße (Südseite), über die gegenüberliegende Goethestraße, ca. 800 m weiter in Richtung Süden, am Goetheplatz links in die Lindwurmstraße.

Nach der Eingangsuntersuchung werden die **Studienkapseln** ausgegeben. Bitte bewahren Sie diese bei Raumtemperatur und vor Sonne geschützt auf. Ab dem Tag der Eingangsuntersuchung verzehren Sie 8 Wochen lang täglich 6 Kapseln zusätzlich zur normalen Ernährung.

- Nehmen Sie täglich 3 x 2 Kapseln oder 2 x 3 Kapseln mit kaltem Wasser zu den Mahlzeiten ein!
- Am Morgen der Blutentnahmen (Woche 4, 6 und 8) bitte keine Kapseln einnehmen!
- Bitte ändern Sie während der gesamten Studie Ihre normalen Gewohnheiten (Ernährung, körperliche Aktivität, etc.) nicht!

Für den Studienzeitraum wird ein **einfaches Protokoll** ausgegeben, in dem Sie besondere Vorkommnisse (z.B. Erkrankungen, Übelkeit, Aufstoßen, Durchfall, Blutungsereignisse, Arztbesuche) festhalten sollen.

Bei der **letzten Blutentnahme (Woche 8)** geben Sie Ihr Ereignisprotokoll und die nicht verbrauchten Kapseln ab.

Übersicht über den Studienablauf

Woche	0	1	2	3	4	5	6	7	8
Blutentnahme	✓				✓		✓		✓
Einnahme der Kapseln	✓ (Ausgabe)	✓	✓	✓	✓	✓	✓	✓	✓ (Rückgabe der Reste)
Ereignisprotokoll	✓	✓	✓	✓	✓	✓	✓	✓	✓

Und noch ein paar Hinweise:

- Eine Blutspende sollte 4 Wochen vor Beginn der Studie und während der Studie nicht geleistet werden.
- Nehmen Sie vor und während der Studie bitte keine weiteren Fettsäure-haltigen Supplemente ein.

Probandenfragebogen

Alle Angaben unterliegen der Schweigepflicht.

Name: ..... **Vorname:** .....

Geburtsdatum: .....

Adresse: .....

Telefon: ..... **Email:** .....

Größe: ..... **Gewicht:** ..... **BMI:** .....
(wird von uns ausgefüllt)

Sind Sie möglicherweise schwanger? ja nein weiß nicht

Stillen Sie im Moment? ja nein

Rauchen Sie? ja nein wenn ja, wie viele Zigaretten pro Tag? .....

Trinken Sie Alkohol? ja nein wenn ja, wie viele Gläser pro Woche?.....

Ernährungsgewohnheiten:

Haben Sie sich während der letzten 3 Monate vegetarisch ernährt? ja nein

Essen Sie mehr als 2x pro Woche Fisch? ja nein

Checkliste „Gesundheit“:

Diabetes mellitus (wenn ja, seit wann?) .....

Schilddrüsenerkrankungen (wenn ja, welche und seit wann?) .....

Hohe Blutfette (wenn ja, welche, seit wann und wie hoch?) .....

Störung der Blutgerinnung (wenn ja, welche und seit wann?) .....

Blutarmut (wenn ja, seit wann?) .....

Bluthochdruck (wenn ja, seit wann und wie hoch?) .....

Magen-/Darmerkrankungen (wenn ja, welche und wann?) .....

Nierenerkrankungen (wenn ja, welche und seit wann?) .....

Leber-/Gallenerkrankungen (wenn ja, welche und seit wann?)

Neurologische Erkrankungen (wenn ja, welche und seit wann?) .....

Operationen (wenn ja, welche und wann?) .....

Allergien/Unverträglichkeiten (wenn ja, welche und seit wann?) .....

Sonstige wichtige Erkrankungen (wenn ja, welche und seit wann?) .....

Medikamenten-/Supplementeinnahme:

Medikamenteneinnahme (wenn ja, welche seit wann und wie oft?) .....
.....

Einnahme von Supplementen, die essentielle oder langkettige Fettsäuren enthalten? (wenn ja, welche, seit wann und wie oft?) .....
.....

Einnahme von anderen Supplementen, z.B. Vitamine oder Mineralstoffe (wenn ja, welche, seit wann und wie oft?) .....
.....

Haben Sie in den letzten 2 Monaten an einer anderen Studie teilgenommen?

ja nein wenn ja, wann und was war das? .....

Record of side effects, signs of illness and intake of medication, FO/EPO study

Ereignisprotokoll

Probandennummer:

Ihre Termine zur Blutentnahme:

Woche 0: Woche 4: Woche 6: Woche 8:

Bitte notieren Sie während der Studie besondere Vorkommnisse
(z.B. Erkrankungen, Übelkeit, Aufstoßen, Durchfall, Arztbesuche, Reisen)!

Datum	besonderes Ereignis

Investigation protocol at week 0, FO/EPO study

Woche 0

Datum: _____ **Probandennummer:** _____

Erhaltene Unterlagen

- **Einverständniserklärung** ☐ **ja** ☐ **nein**

Befragung

Haben Sie heute morgen schon etwas gegessen oder getrunken? ☐ **ja** ☐ **nein**
Wenn ja, was? _____

Sind Sie im Moment krank? ☐ **ja** ☐ **nein**
Wenn ja, was? _____

Haben Sie in den letzten Tagen Medikamente eingenommen? ☐ **ja** ☐ **nein**
Wenn ja, welche? _____

Haben Sie in letzter Zeit Fettsäure-haltige Supplemente verzehrt? ☐ **ja** ☐ **nein**
Wenn ja, welche? _____

Messparameter

Größe: _____ **Gewicht:** _____

Blutdruck: _____ **Puls:** _____

Investigation protocol at weeks 4 and 6, FO/EPO study

EFANATAL-Pilotstudie

Woche 4

Datum: _____ **Probandennummer:** _____

Befragung

Haben Sie heute morgen schon etwas gegessen oder getrunken?

☐ **ja** ☐ **nein**
Wenn ja, was? _____

Woche 6

Datum: _____ **Probandennummer:** _____

Befragung

Haben Sie heute morgen schon etwas gegessen oder getrunken?

☐ **ja** ☐ **nein**
Wenn ja, was? _____

Woche 8

Datum: _____ **Probandennummer:** _____

Erhaltene Unterlagen

- **Ereignisprotokoll** ☐ **ja** ☐ **nein**

Befragung allgemein

Haben Sie heute morgen schon etwas gegessen oder getrunken? ☐ **ja** ☐ **nein**
Wenn ja, was? _____

Sind Sie im Moment krank? ☐ **ja** ☐ **nein**
Wenn ja, was? _____

Haben Sie in den letzten Tagen Medikamente eingenommen? ☐ **ja** ☐ **nein**
Wenn ja, welche? _____

Haben Sie während der Studie weitere Supplemente mit Fettsäuren genommen? ☐ **ja** ☐ **nein**
Wenn ja, was und wie oft? _____

Kapselrest: _____ **Anzahl nicht verzehrter Kapseln:** _____

Messparameter

Gewicht: _____

Blutdruck: _____ **Puls:** _____

Befragung zu Kapseln

Wie war die Verträglichkeit der Kapseln? Nebenwirkungen?
- ☐ Aufstoßen
- ☐ Übelkeit
- ☐ Durchfall
- ☐ Bauchschmerzen
- ☐ Blutungen
- ☐ Sonstiges _____

Wie waren die Kapseln zu schlucken?
- ☐ gut
- ☐ geht so, weil _____
- ☐ schlecht, weil _____

Hatten Sie Ihrer Meinung nach die EFA/LCPUFA-Mischung oder Placebokapseln?
- ☐ Verum
- ☐ Placebo

Bermerkungen:

8.4 Publications and presentations

8.4.1 Publications and work in progress

2007
- Geppert J, Kraft V, Demmelmair H, Mueller-Felber W, Koletzko B. Effects of DHA supplementation on visual and somatosensory evoked potentials in healthy adult vegetarians (manuscript in preparation).
- Geppert J, Demmelmair H, Hornstra G, Koletzko B. Co-supplementation of healthy women with evening primrose oil and fish oil increases docosahexaenoic acid, γ-linolenic acid and dihomo-γ-linolenic acid levels without reducing arachidonic acid concentrations (submitted).

2006
- Geppert J, Kraft V, Demmelmair H, Koletzko B. Microalgal docosahexaenoic acid decreases plasma triacylglycerol in normolipidaemic vegetarians: a randomised trial. Br J Nutr 2006, 95(4), 779-786.

2005
- Geppert J, Kraft V, Demmelmair H, Koletzko B. Docosahexaenoic Acid Supplementation in Vegetarians Effectively Increases Omega-3 Index: a Randomized Trial. Lipids 2005, 40(8), 807-814.

8.4.2 Presentations at conferences and summer schools

2006
- Geppert J, Demmelmair H, Hornstra G, Koletzko B. Co-supplementation with evening primrose oil and fish oil does not lower arachidonic acid status and increases the dihomo-gamma-linolenic acid status in women of childbearing age. 7th Congress of the International Society for the Study of Fatty Acids and Lipids (ISSFAL), Cairns, Australia (poster).

2005
- Kraus-Etschmann S, Shadid R, Geppert J, Koletzko B, Berger M, Chiolero R. Cytokine pattern after parenteral fish oil supplementation in patients after abdominal aorta aneurysm surgery. 27th Congress of the European Society of Clinical Nutrition and Metabolism (ESPEN), Brussels, Belgium (poster).

- Geppert J, Kraft V, Demmelmair H, Mueller-Felber W, Koletzko B. Effects of docosahexaenoic acid on fatty acids, plasma lipids and evoked potentials in vegetarians. Nutrition Summer School of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN), Athens, Greece (oral presentation).

2004

- Geppert J, Koletzko B.
  Nährstoffversorgung von Münchener Ovo-Lakto-Vegetarierinnen. 41st Congress of the German Nutrition Society (DGE), Freising, Germany (oral presentation).
- Geppert, J, Koletzko B.
  Zufuhr an essentiellen Fettsäuren bei ovo-lakto-vegetarischer Ernährung. Congress of the German Society of Nutritional Medicine (DGEM), Ernährung 2004, Munich, Germany (poster).
- Geppert J, Kraft V, Demmelmair H, Koletzko B.
  Effekte einer Supplementierung mit Docosahexaensäure-reichem Mikroalgenöl auf den Lipidstoffwechsel bei Vegetariern. Congress of the German Society of Nutritional Medicine (DGEM), Ernährung 2004, Munich, Germany (poster).
- Geppert J, Kraft V, Demmelmair H, Koletzko B.
  Effects of docosahexaenoic acid rich microalgae oil on fatty acids and plasma lipids in vegetarians. 6th Congress of the International Society for the Study of Fatty Acids and Lipids (ISSFAL), Brighton, UK (oral presentation).

8.5 Acknowledgements

My last remaining task is to acknowledge all people who have contributed to the work described in this thesis. First of all I would like to thank my doctoral adviser, Prof. Dr. med. Berthold Koletzko, who gave me the opportunity to do this dissertation. I am obliged to him for the opportunities to travel and to introduce me to other leading scientists in the field of nutrition research. I would also like to express a special thank to Dr. Hans Demmelmair for his untiring support.

Furthermore I would like to thank the whole “Stoffwechsellabor” team for their helpfulness, encouragement and for creating such a pleasant working atmosphere!

I acknowledge the excellent technical assistance of Monika Rachl, Ismihan Gökce and the team of Anna-Maria Prause and wish to thank the participating subjects for their commitment to the studies.

Concerning *DHAVEG study*, I would like to thank Nutrinova GmbH (Frankfurt am Main) for providing microalgae oil and placebo capsules and for financial support. Thanks to Veronika Kraft for taking blood samples and for the reliable collaboration in conducting the study.
Concerning *FO/EPO study*, I would like to thank EFAMOL Ltd. (Brackenholme, Selby, North Yorkshire, UK) for providing the FO/EPO and placebo capsules and for financial support. Thanks to Jeannette Franke and Dr. Hiromichi Shoji for taking blood samples, measuring blood pressure and performing anthropometrical measurements. I would like to express special thanks to Prof. em. Gerard Hornstra and Dr. Reto Muggli for their helpful advices.

8.6 Curriculum Vitae

Dipl. oec.troph. Julia Geppert
Evelten 7, 45478 Mülheim an der Ruhr, Germany
Julia.Geppert@gmx.de

PERSONAL DETAILS

Date and Place of Birth	21st July 1977 in Mülheim a.d. Ruhr, Germany
Nationality	German
Marital status	Single

EMPLOYMENT HISTORY

since 02/2007 **Research assistant**

Institute of Brain Chemistry and Human Nutrition,
London Metropolitan University, London, UK.

Responsibilities:

- maintenance and management of laboratory instruments,
- analyses of fatty acids using thin layer chromatography and gas chromatography,
- dietary assessment,
- data analyses and preparation of manuscripts,
- training of students.

06/2006 - 11/2006 **Graduate assistant**

Division of Metabolism and Nutrition,
Dr. von Hauner Children's Hospital,
Ludwig Maximilians University, Munich, Germany.

Responsibilities:

- contribution to the publication of the "Guidelines Parenteral Nutrition" (German Society for Nutritional Medicine, DGEM).

05/2003 - 12/2005 **Research associate**

Division of Metabolism and Nutrition,
Dr. von Hauner Children's Hospital,
Ludwig Maximilians University, Munich, Germany.

Responsibilities:

- conducting clinical studies,
- dietary assessment,
- analyses of fatty acids using thin layer chromatography and gas chromatography, analyses of vitamin A and E using high performance liquid chromatography,
- data analyses and preparation of manuscripts,
- presentations at conferences.

07/2002 - 08/2002 **Student assistant**

German Aerospace Centre, Institute of Aerospace Medicine, Cologne-Porz, Germany.

Responsibilities:

- contribution to the "STBR-IP-Study" (Short-Term Bedrest - Integrated Physiology Study), 3rd phase: preparation of study meals.

EDUCATION AND QUALIFICATIONS

05/2003 - 01/2007 **Doctoral thesis**

Evaluation of two supplementation strategies to improve long-chain n-3 fatty acid status in healthy adults.

Division of Metabolism and Nutrition,
Dr. von Hauner Children's Hospital,
Ludwig Maximilians University, Munich, Germany.

Supervisors: Dr. Hans Demmelmair,
Prof. Dr. Berthold Koletzko.

08/2002 **Diploma in nutritional science and home economics**

Rheinische Friedrich Wilhelms University, Bonn, Germany.

Grade: 1.1, with distinction.

05/2001 - 07/2002 **Diploma thesis**

Short-term effects of high soy supplementation on bone markers in young female adults.

Department of Nutrition Science,
Rheinische Friedrich Wilhelms University, Bonn, Germany.

Supervisors: PD Dr. Armin Zittermann, Prof. Dr. Peter Stehle.

10/1996 - 08/2002 **University studies**

Nutritional science and home economics
(emphasis on nutrition science),

Rheinische Friedrich Wilhelms University, Bonn, Germany.

06/1996 **Abitur**

Secondary school "Gymnasium Broich", Mülheim a. d. Ruhr, Germany (emphasis on mathematics and biology).

London, 27th March 2007