Aus der Klinik für Dermatologie und Venerologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Efficacy Of Docosahexaenoic Acid In Patients With Atopic Dermatitis

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für Felicitas

„Unser Wissen ist Stückwerk“
– 1. Korinther 13:9

Abstract

Docosahexaenoic acid (DHA) as found in fish oil has been shown to possess potent anti-inflammatory properties, including active resolution of inflammation through derivates like resolvins, protectins and maresins. This trial was conducted to evaluate effects of DHA on skin status of patients with atopic dermatitis (AD) and to examine possible physiological and biochemical effects in patients with AD.

Methods: Patients aged 18-40 were randomized (double-blind) into 2 groups: one received 4.9g/d of DHA, the other an isoenergetic mix of saturated short chain fatty acids as placebo, while continuing their skin care as usual (including topical corticoids). During a supplementation period of 8 weeks (January through March 2005) 3 examinations were performed: at baseline, after 4 weeks and at the end of supplementation period. An additional visit was scheduled after a 12-week wash-out phase. On every visit, skin status was evaluated by SCORAD. Additionally, skin physiology (pH, sebum, moisture and transepidermal water loss), blood samples (IgE, lipid metabolism), skin prick test and qualitiy of life (SF-36) were examined. Patients were instructed to keep a schematized diary recording their observations.

Results: Of the 54 Patients included in this trial, 47 completed the supplementation period. DHA was well tolerated and readily bioavailable through oral administration. SCORAD values showed significant improvements in both groups, no statistically relevant differences could be found between the groups. Of additional observed parameters, only lipid metabolism showed relevant differences: Triacylglycerides (TAG) and high-densitiy lipoprotein (HDL) values were better in the DHA group.

Conclusion: This trial examined the third-largest population of adult patients with AD on DHA-mediated skin status improvements. It was the highest dose of DHA supplemented so far (DHA/EPA-ratio about 7:1). Results show significant improvement in both treatment groups, however there was no statistically significant difference between the two groups. In this trial DHA is not more effective than short chain fatty acids in treating AD.

Keywords:
Docosahexaenoic acid, atopic dermatitis, treatment, supplementation

Zusammenfassung

Docosahexaensäure (DHA), die üblicherweise in Fischöl vorkommt, hat potente entzündungshemmende Eigenschaften. Unter anderem ist sie durch Derivatsubstanzen wie Resolvine, Protektine und Maresine aktiv an der Auflösung entzündlicher Zustände beteiligt. Diese Untersuchung wurde durchgeführt, um die Effekte von DHA auf den Hautzustand von Patienten mit Atopischer Dermatitis (AD) zu untersuchen, sowie mögliche physiologische und biochemische Auswirkungen in Patienten mit AD aufzuklären.

Methoden: Patienten im Alter von 18 bis 40 Jahren wurden in zwei Gruppen randomisiert. Eine Gruppe erhielt 4,9g DHA pro Tag, die andere eine isoenergetische Mischung aus kurzkettigen gesättigten Fettsäuren als Placebo. Die Hautpflege wurde mit den bisher verwendeten Produkten fortgesetzt (einschließlich Kortikoid-Präparate). Während eines Supplementationszeitraums von acht Wochen (Januar bis März 2005) wurden die Patienten dreimal untersucht: Zu Beginn, nach vier und nach acht Wochen. Eine zusätzliche Untersuchung wurde nach einer 12-wöchigen Auswasch-Phase durchgeführt. Bei jeder Vorstellung wurde der Hautzustand über den SCORAD erhoben. Zusätzlich wurden Hautphysiologie (pH, Sebum, Feuchtigkeit und transepidermaler Wasserverlust), Blut (IgE und Lipidstoffwechsel), Hautpricktest und die Lebensqualität (SF-36) untersucht. Die Patienten sollten in einem vorbereiteten Tagebuch ihre eigenen Beobachtungen eintragen.

Ergebnisse: 54 Patienten wurden in die Studie aufgenommen, von diesen beendeten 47 Patienten den gesamten Supplementationszeitraum. Die orale Aufnahme von DHA wurde gut vertragen und war sehr gut bioverfügbar. SCORAD-Werte zeigten signifikante Verbesserungen in beiden Gruppen, jedoch konnten keine statistisch signifikanten Unterschiede zwischen den Behandlungsgruppen festgestellt werden. Von den zusätzlich erhobenen Parametern zeigte nur der Lipidstoffwechsel relevante Unterschiede: Triacylglyceride (TAG) und die Werte für Lipoprotein hoher Dichte (HDL) waren besser unter DHA.

Zusammenfassung: Diese Studie untersuchte die drittgrößte Population von erwachsenen Patienten mit AD auf einen positiven Einfluss von DHA auf den Hautzustand. Es wurde die bisher höchste Dosis DHA verabreicht (DHA/EPA-Verhältnis ca. 7:1). Es wurden statistisch signifikante Verbesserungen in beiden Behandlungsgruppen festgestellt, die Studiengruppen können jedoch nicht statistisch signifikant voneinander unterschieden werden. DHA ist in der Behandlung von AD in dieser Studie nicht effektiver als kurzkettige Fettsäuren.

Schlagwörter:
Docosahexaensäure, Atopische Dermatitis, Behandlung, Supplementation

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1 Introduction

1.1 Definition and Treatment of Atopic Dermatitis

Atopy has been defined as "propensity to produce specific IgE antibodies upon exposure to allergens in the environment, with subsequent clinical symptoms" [1]. Atopic dermatitis (AD) is a chronic recurrent inflammatory skin disease, clinically characterized by eczema and itching, in which altered immune reactivity is a significant factor [2]. AD is also one of the most common skin diseases. Recent epidemiological findings point to an increase in prevalence and incidence over recent decades. Prevalence might be as high as 20% in children and 2% in adults [3].

Although there has been some improvement in the understanding of pathophysiological processes as well as treatment strategies, there remains much to be learned and efficient treatment of these patients can be difficult. Treatment often depends heavily on topical, or even systemic corticosteroid use, with well known side effects.

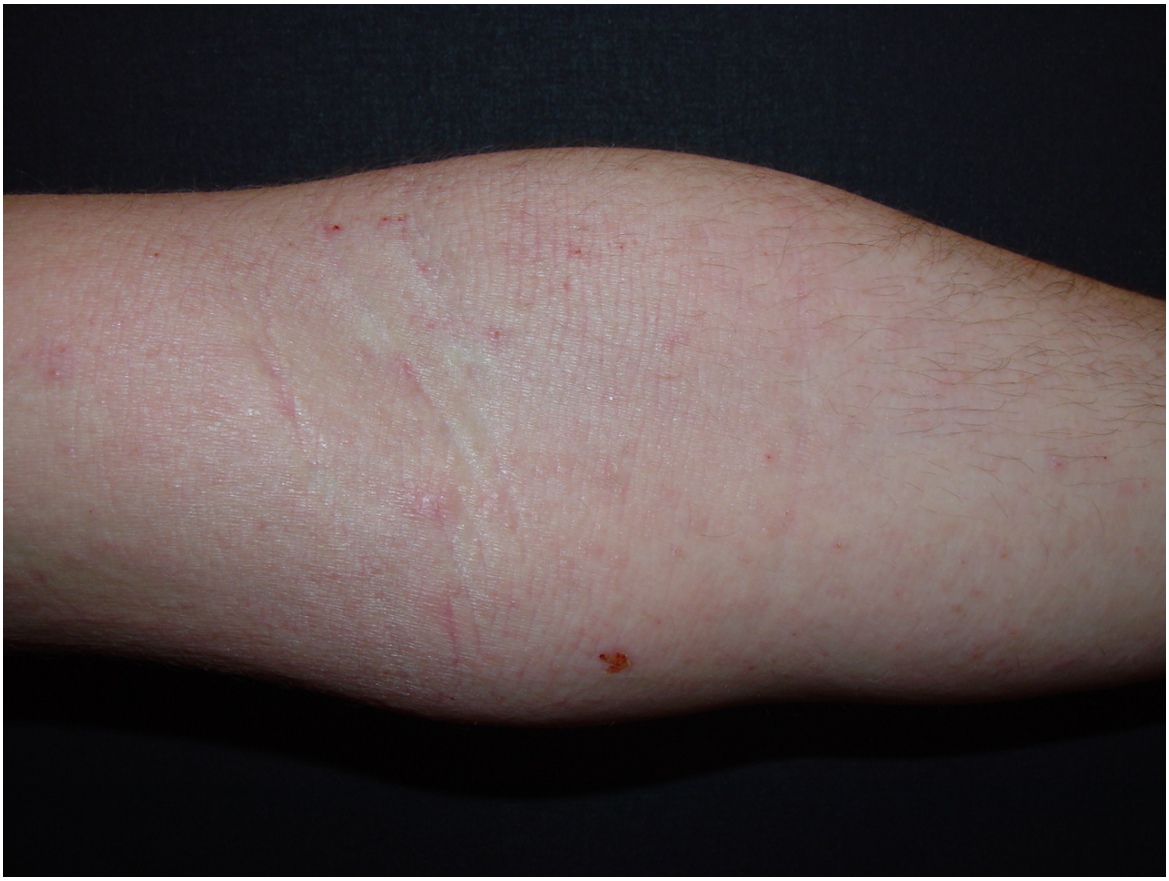
Ever since the discovery made by Brown et al. in 1937, who noticed a markedly changed fatty acid content in patients with atopic disease [4], there has been interest in finding a treatment strategy addressing this imbalance. The noted lack of arachidonic acid led to the hypothesis of a deficit in δ-6-desaturase being the cause of AD [5]. Research concentrated therefore consequently in counterbalancing this lack of ω-6 fatty acids (n-6-FA). However, results have been poor [6]. Recent findings now indicate a strong potential for beneficial effects through ω-3 fatty acids (n-3-FA) as found in fish oil. The following section gives a brief overview.

1.2 Immunoactive Fatty Acids In Fish Oil

Fish oil contains essential polyunsaturated fatty acids (PUFA) in high concentrations, namely eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These fatty acids are also referred to as n-3-FA, because of the characteristic double binding at the third carbon atom counted from the methylic end. Various immuno-modulatory qualities are attributed to them.

A key function seems to be competitive blocking of inflammatory mediator formation

Figure 1.1: Typical lesions of AD. This picture shows the right flexure of the arm with signs of eczema typical for AD. There is clearly visible erythema, papulation and lichenification. Note also the marks of excoriation due to scratching.



through arachidonic acid [7]. Inflammatory cell membrane usually contains much higher concentrations of arachidonic acid than of DHA or EPA. Arachidonic acid is therefore a preferred substrate for bioactive eicosanoid production through different cyclo- (COX) and lipoxygenases (LOX). The resulting 2-series prostaglandins and 4-series leukotrienes contribute to the pathologic inflammation in patients with AD. DHA and EPA are also substrates for both COX and LOX, but are transformed into less potent pro-inflammatory and even anti-inflammatory mediators. Long known in this context are 3-series thromboxanes and 5-series leukotrienes [7]. There is evidence that EPA via COX-2 transformation additionally forms a formerly unknown series of mediators, so-called e-series resolvins, showing anti-inflammatory properties [8]. Newer findings point toward DHA derived mediators as occupying a key position in this process. D-resolvins, protectins and the recently discovered maresins synthesized

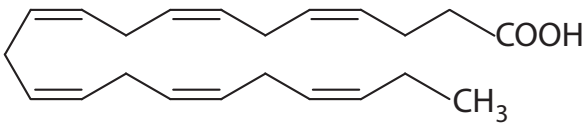


Figure 1.2: cis-4,7,10,13,16,19-Docosahexaenoic acid

from DHA display potent anti-inflammatory effects [9–11]. Resolution of inflammation was long thought to be a passive process mainly driven by dropping concentrations of pro-inflammatory mediators. With the discovery of resolvins, protectins and maresins, this process is now understood to be active and mediator controlled, with DHA and to a lesser extent also EPA being the centers of a whole array of inflammation resolving substances.

Apart from this, there are effects of fish oil modulating inflammation without changing eicosanoid metabolism. Suppressive actions on a series of pro-inflammatory cytokines like IL-1β, TNFα, IL-8 and IL-6 have been well documented. Such effects have been shown in vitro [12, 13], in animal models [14, 15] but also in healthy subjects [16–19].

By inhibiting expression of adhesion-molecules (like e-selectin, ICAM-1 and VCAM-1) DHA and EPA can diminish the migration of monocytes [20–22]. There are clues of potent n-3-FA effects conveyed at gene expression level. One finding indicates that mRNA of key enzymes in the metabolism of eicosanoids (COX-2 and 5-LOX) can be suppressed through n-3-FA [23]. Furthermore there is evidence for suppression of NF-κB [24–26], a nuclear factor regulating production of inflammatory mediators. It appears to prevent its activation by preventing the phosphorylation of IκB-α. Fish oil therefore contains, with its long chain n-3-FA, potent anti-inflammatory substances of potential clinical use.

1.3 Distribution and Bioavailability

Orally supplemented DHA and EPA are readily bioavailable and distributed throughout the body. Retinal tissue and cerebral cortex contain especially high concentrations of DHA [27]. Storage in adipose tissue is low, therefore continuous supply of these essential fatty acids (EFA) is needed [27]. Although theoretically there are pathways for interconversion of EPA into DHA and vice versa, this process does not produce large amounts of DHA [27].

1.4 Atopic Dermatitis and Fish Oil – Clinical Relevancy

Many patients hope for a treatment of AD with few adverse effects. It is not to be expected that supplementation of essential fatty acids could substitute conventional therapy with emollients and topical anti-inflammatory agents, but they might be able to lower the burden of the disease by stabilizing skin status and reduce the amount and frequency of corticoid application.

Better understanding of the anti-inflammatory properties of n-3-FA gives rise to an increasing interest in its possible therapeutic use. Now that initial studies have shown positive results in rheumatoid arthritis and psoriasis [28, 29], the use of fish oil for treating patients with AD should be examined in clinical studies.

Clinical trials conducted to date with n-3 supplementation showed contradictory results. After 12 weeks of oral treatment with fish oil, Bjørneboe et al. [30] observed statistically significant clinical improvement in some subscales in patients with AD as compared to treatment with olive oil. However these results were contradicted by Kunz et al. [31], Søyland et al. [32] and Berth-Jones et al. [33].

Most investigators have concentrated on n-6-FA [34–41]. There is very few data on n-3-FA in AD available and to date no trial at all has been conducted with DHA concentrations exceeding EPA concentrations. Also, so far no study has examined high dose supplementation of DHA. n-3-FA could widen the therapeutic spectrum for AD. In combination with other therapeutic modalities, it might result in a stabilisation of the disease with less relapses or longer remission phases or a diminished corticosteroid use.

1.5 Problem Formulation and Research Hypothesis

The major question addressed in this investigation was whether DHA might be beneficial as adjuvant treatment of AD, resulting in better skin status. To address this question, a prospective placebo-controlled supplementation in AD patients was performed and skin status (SCORAD) was assessed as main study outcome variable. Supplementation with DHA was set higher than in any trial conducted to date. Primary endpoint was a relevant difference in SCORAD score between treatment groups. Skin physiology, quality of life, atopy related parameters and lipid metabolism were examined on an explorational basis in order to gain insight into the physiological and biochemical effects of DHA in patients with AD.

2 Methods and Material

2.1 Participants

Male and female patients aged 18-40 attending the dermatology department for treatment of AD or responding to a newspaper advertisement were recruited. To avoid disparity in age distribution between treatment groups, participants were stratified according to sex and age in four groups (male/female, age up to 29/aged over 29) and randomized separately. Diagnosis was verified by a dermatologist, following the criteria of Hanifin and Rajka [42]. Written informed consent was obtained from all patients before launching the study.

Patients undergoing treatment with oral corticosteroids or other systemic immunomodulators were excluded from the study. Topical use of corticosteroids or other immunomodulators was acceptable. They were also asked whether they were suffering from metabolic or otherwise grave disease. All subjects included answered to the negative. Regular tan studio visits were an exclusion criterion to rule out the known positive effect of UV-light exposure. Patients were instructed to continue their normal skin-care as usual, including the use of topical preparations of corticosteroids by some patients. Exact documentation of these applications in a "patients diary" was emphasized, especially with corticosteroids. Table 2.1 gives an overview of in- and exclusion-criteria.

2.2 Concept of the Study

The study was approved by the ethics committee of the Charité – Universitätsmedizin Berlin and conducted according to European Good Clinical Practice (GCP) guidelines.

Participants entered a double-blind, randomized, placebo-controlled and prospective trial of two parallel groups. It was designed to examine the effects of DHA on the clinical appearance of AD. In order to provide sufficient time for fatty acids to accumulate and take effect, a period of eight weeks of supplementation was set. Participants were assessed four times, at baseline, at 4 and 8 weeks on treatment, and again after a 12

Table 2.1: Study protocol criteria.

Inclusion Criteria	Exclusion Criteria
age 18-40	oral corticosteroids, systemic immunomodulatory or immunosuppressive therapy in the past three months
confirmed atopic dermatitis	other systemic and chronic disorders
continued skin care	intensive UV light exposure (e.g. regular tan studio visits)
	pregnancy or lactation
	seafood allergies
	consumption of dietary supplements

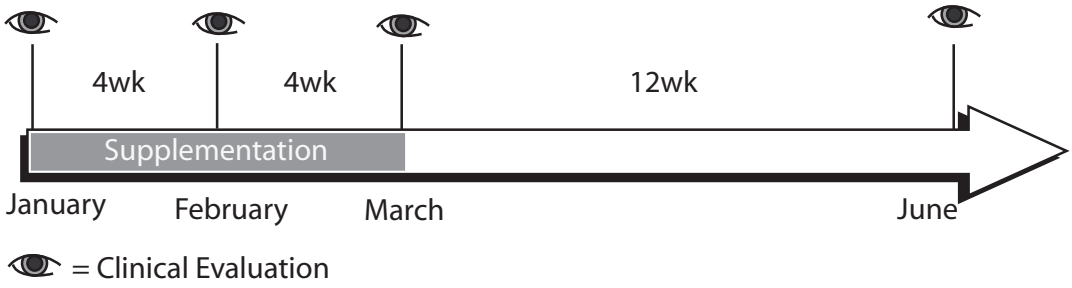


Figure 2.1: Trial time schedule

week wash out (week 20). Supplementation time was planned from January to March in order to minimize exposure to sunlight and allergies to pollen as interfering factors (see figure 2.1).

2.3 Treatment and Control

Treatment was allocated independently of evaluation using computer generated random numbers. Blinding was not broken for any patient until the trial and all data entry were completed. Treatments were supplied in identical neutral white plastic containers labelled with randomization codes and full name of the participant (see figure 2.2). Placebo capsules contained a mix of short, saturated fatty acids, commercially available through Croda International, East Yorkshire, UK (Estasan GT 8-60 3575). This was chosen with care as other commonly used “placebos” (like olive oil) have possible

immunomodulatory effects [43] and paraffin has uncomfortable laxative effects. For DHA-supplementation, a 70% DHA concentrate was used, also obtained through Croda International (Incromega DHA700E SR). It contains 700mg/g pure DHA (for composition details, see table 2.2).

Figure 2.2: Packaging of capsules: the name of the participant was made illegible.

Table 2.2: Contents of supplementation capsules

Incromega DHA700E SR		Estasan GT 8-60 3575	
DHA C22:6	min. 70%	Capronic acid C6:0	max. 2%
EPA C20:5	max. 10%	Caprylic acid C8:0	50.5%
Total n-3-FA	max. 80%	Capric acid C10:0	40.5%
		Lauric acid C12:0	max. 3%
		Myristic acid C14:0	max. 1%

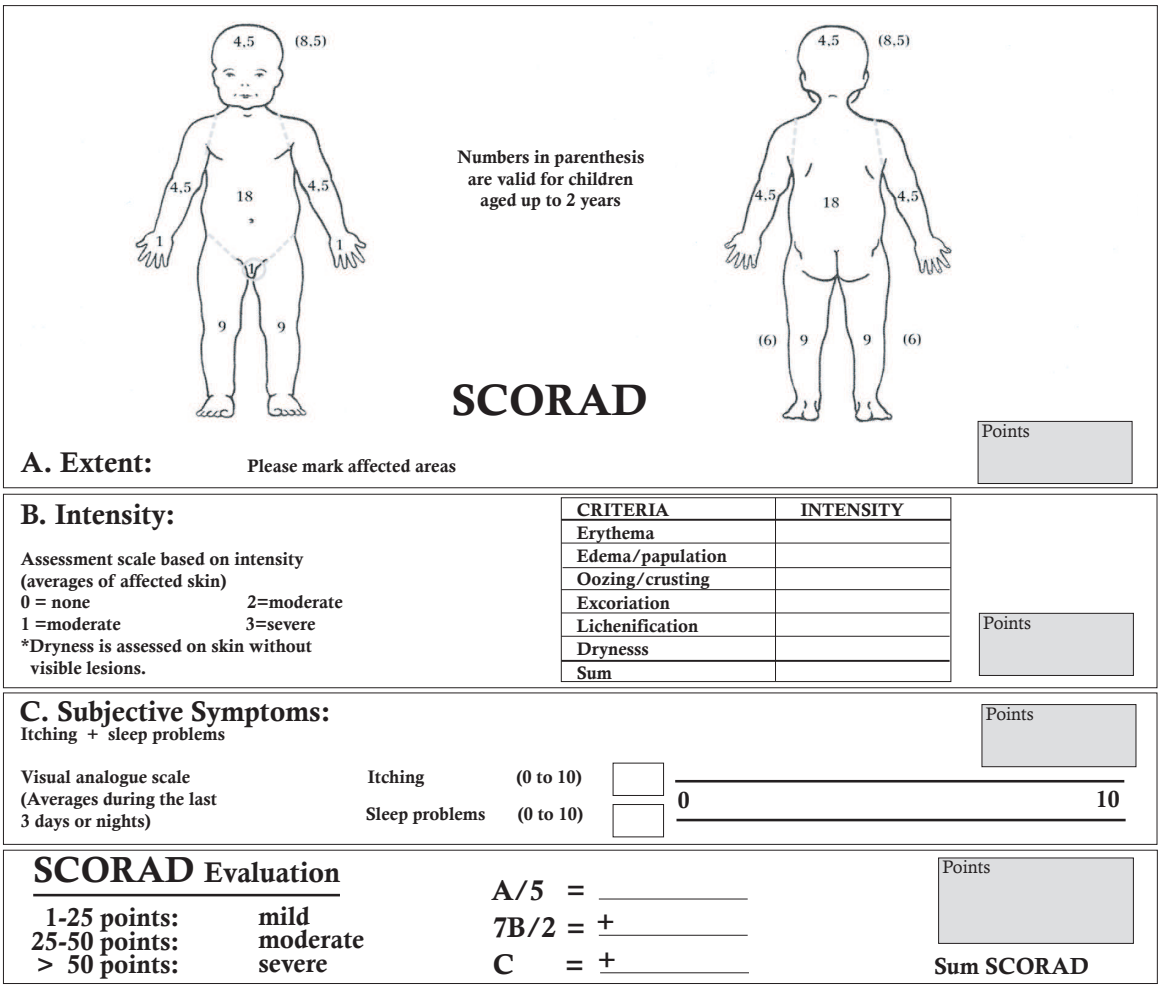
DHA and placebo treatments were packed and capsuled in matching soft gelatine through Norton Gelkaps GmbH (Falkenhagen). One capsule contained one gram of the respective FA mix. Consuming 7 capsules a day, patients in the fish oil arm ingested 4.9g of DHA and 0.7g EPA. A pattern of 2-3-2 (morning - noon - evening) was suggested as a daily regimen. Compliance was monitored on each visit controlling the amount of capsules in the package and by measuring fatty acid levels in patients' serum (see below). To simplify matters, treatment is referred to by the abbreviation of the main component: "DHA".

2.4 Assessments

2.4.1 SCORAD

Disease activity was assessed at each visit by using the SCORAD Index (SCOring Atopic Dermatitis). It was first proposed in 1993 [44]. Since then, this well validated scoring system has been used in many trials on AD. It is structured into physician-assessed objective (extent, intensity) and patient-rated subjective criteria (pruritus, sleep problems). Extent (A) is estimated in percentage of body surface area with visible AD, applying the rule of nines as known from basic burn assessment (maximum: 100). Intensity is assessed through six criteria: erythema, edema (or papulation), oozing (or

Figure 2.3: SCORAD score sheet used for documenting skin status in patients.



crusting), excoriation, lichenification and dryness are each evaluated on a 0-3 scale (0=none, 1=mild, 2=moderate, 3=severe). Dryness is assessed on skin without visible lesions. For the rest of the criteria, basis for intensity evaluation are averages of affected skin. Intensities add up to a maximum of 18 (B). The subjective symptoms are taken into account on a numeric analogue scale ranging from 0 to 10 (0=none, 10=worst imaginable), these are also added up (C, maximum 20). With these three values (A, B and C) the SCORAD is calculated applying the following formula:

$$SCORAD = A/5 + 7B/2 + C$$

Theoretical maximum of the SCORAD score is 103. The developers suggest the following interpretation: mild (1-25 points), moderate (25-50 points) and severe (> 50 points). The SCORAD was used as primary variable to assess the clinical state of the disease.

2.4.2 Erlangen Atopy Score

The Erlangen Atopy Score introduced by Diepgen et al. [45] was used to collect formalized data about minor atopic criteria and to confirm diagnosis of AD. It renders a result between 0 and 37 indicating the probability of atopic skin diathesis (ASD). A score of none to 3 points means that there is no evidence for ASD, with 4 to 9 points ASD is possible but not confirmed, 10 to 13 points make an ASD very probable and a score of more than 14 points is a clear indication of ASD. The Erlangen Atopy Score was just taken once at the beginning of the trial, as there is no change over time in this score. For a detailed listing of Erlangen Atopy Score items see Appendix A.

2.4.3 Skin-physiology

Skin-physiology was evaluated from the first follow-up examination onward. Two sites were chosen, one for apparently unaffected and one for heavily affected skin. These were taken note of and in the following visits care was taken to measure in the same places. Unaffected skin was defined as skin with no erythema, edema/papulation, oozing/crusting, excoriation, lichenification or palpable dry patches. The same investigator took the measurements on all study subjects. All devices used are manufactured by Courage + Khazaka electronic GmbH (Cologne, Germany).

Skin pH was measured with a glass electrode Skin-pH-meter® (PH 905). It was placed in a potassium hydroxide (KOH) solution between different measurements and

cleaned with distilled water before and after measurement.

Sebum values were evaluated with a photometric Sebumeter® (SM 815). It uses a special kind of tape, which becomes transparent in contact with the sebum surface. Transparency is then measured via photometry and expressed in $\mu g/cm^2$. The tape was changed at regular intervals and always when the measuring device required it.

Moisture values were evaluated with the Corneometer® CM 825, in arbitrary units. In this device skin surface hydration is measured via capacitance, a method where skin products have little to no influence on measurements. The mean value out of 10 continuous measurements was considered the final data.

Transepidermal water loss (TEWL) was assessed as a last parameter, to allow an acclimatization period of at least 15 minutes. The time taken for the other parameters allowed the patient to settle a little and provided a calm environment with optimal surroundings for this measurement. A Tewameter® TW 300 device was used. Measurements were recorded as $g/h/m^2$ after the rate of TEWL had stabilized, usually after about 60 seconds.

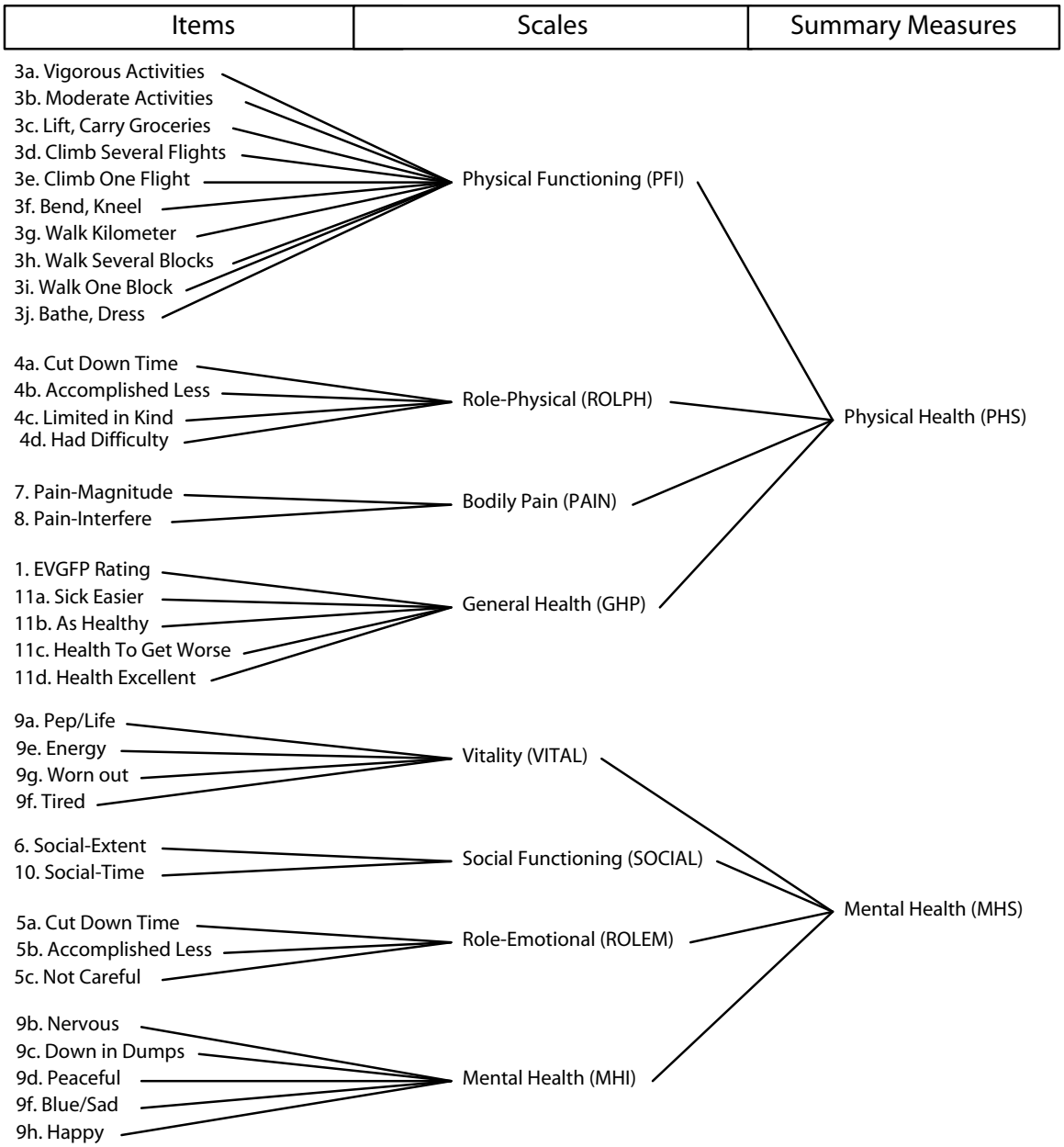
2.4.4 Quality of Life (SF-36®)

The standardized German translation of the SF-36® (Short Form Health Survey, 36 items) was used to evaluate quality of life. This extensively validated and valuable clinical tool contains 36 items that are used to calculate eight subscales (figure 2.4 illustrates this process). Each scale has a minimum of 0 and a maximum of 100 points, indicating worst (0) and best (100) possible results. The scales Physical Functioning Index (PFI), Role-physical Index (ROLPH), Bodily Pain Index (PAIN) and General Health Perceptions Index (GHP) are used to calculate a summary measure of physical health (PHS), Vitality Index (VITAL), Social Functioning Index (SOCIAL), Role-Emotional Index (ROLE) and Mental Health Index (MHI) are combined in a summary measure for mental health (MHS). SF-36® was taken in every visit, as it is very sensitive to change. For calculation of sub- and summary scores, an SPSS-program by Bullinger et al. [47] was used. In order to relate the results to normal values, data from the German Federal Health Survey (Bundesgesundheitssurvey, 1998) [48] was extracted and adapted according to age and sex structure of the trial population.

2.4.5 Patients Diary

Patients were given a diary with preprinted columns for each day of the supplementation period. Four items were to be rated each day on a numeric scale (1-10): itch, erythema,

Figure 2.4: SF-36® items, subscales and summary measures (adapted from [46]): This figure shows how items in the questionnaire add up to the subscales and summary measures of the SF-36®. For official wording of the items please refer to [46].



dryness and general impression. It had also sufficient space to accommodate remarks about important (and possibly disease influencing) events, notes on the medication they were on and eventual side effects of the capsules. Exact documentation of topical corticosteroid use was emphasized. The diary page layout can be found in Appendix B.

The first 10 days' entries were used to calculate a baseline-value (mean), the same was done with the last 10 days of supplementation in order to calculate an "end-of-treatment" value.

2.4.6 Weight and Body Fat

As fatty acid supplementation added an additional 7g of dietary fat per day (roughly 10% of recommended daily allowance), weight was monitored for all patients. An electronic scale ("Tanita Body Fat Monitor", made by Tanita, Tokyo, Japan) was used. In this scale, body fat is estimated by measuring bioelectrical impedance. As water conducts electric current quite well, while fat provides resistance to it, a fairly accurate measure for body composition can be calculated from this value [49]. Calculation was done through the internal algorithms of the scale, providing additionally the height of each subject.

2.4.7 Skin Prick Test

In order to evaluate the influence of DHA on IgE-dependent sensitivity, two skin prick tests (SPT) were conducted. In this test, a drop of allergen-containing liquid is "pricked" into the skin of the subject. First the forearm (in some cases the upper back) was cleaned with an alcoholic disinfection agent. Drops of allergen containing liquid were placed and marked by numbers. Using a sterile lancet, a small "prick" through the drops was made, breaking the skin barrier without penetrating the epidermis. Results were read after 20 minutes. During the first visit, participants were tested for various common allergens including a mixture of grass pollen, birch pollen, cat hair, mugwort, *Dermatophagoides pteronyssinus* and *farinae*, *Alternaria* and latex. In case of known anterior anaphylactic shock reactions these allergens were omitted. Controls were histamine (positive) and saline solution (negative). The allergen with the strongest reaction was noted, the resulting erythema and wheal were measured in millimeters along with the histamine-caused reaction. After finishing the supplementation only the strongest allergen was tested along with controls. Histamine control provided a baseline for individual reaction potential. For analysis and better comparability, results were thus calculated as relative to the histamine control (allergen/histamine ratio).

2.4.8 Blood Testing

Compliance and bioavailability were monitored by gas chromatographic examinations of fatty acid levels in patients' serum.[1] Clinical safety was assessed by laboratory parameters. Blood samples were obtained for hematology, liver function (ALT, GGT), lipid metabolism and immunologic tests (IgE, see following section). Parameters for hematology, liver and lipid metabolism were tested by the laboratory service of the Charité. Influence on lipid metabolism was evaluated by obtaining measures of total cholesterol (TC), HDL, and triacylglycerol (TAG). The LDL value was estimated by means of the Friedewald formula:

$$LDL = TC - HDL - (TAG/5)$$

2.4.9 IgE Determination

Total IgE, spontaneous IgE production and specific IgE values were specified in order to detect influences on production of IgE. Blood samples for IgE-analysis were taken before and after finishing the supplementation period. Specific IgE was determined for the strongest reacting allergen in the skin prick test by the Charité laboratory service by means of a radioallergosorbent test (RAST).

Total IgE was measured in patient serum, spontaneous IgE production was quantified in peripheral blood mononuclear cells (PBMC). PBMC were isolated from heparinized patient serum by means of density gradient centrifugation. Samples were layered onto a Ficoll®-sodium metrizoate gradient of specific density. After centrifugation (20min, 350g, 20°C), PBMC remain as white band above the Ficoll®-layer. This layer was transferred into 4°C cold phosphate-buffered saline (PBS) and centrifuged twice more to remove the last possible remnants of Ficoll® and thrombocytes. Before the last centrifugation an aliquot was removed for cell count (electronic cell counter CASY® Model TT). Dilution of cells was set to 212 cells/ml accordingly. Cells were then transfered to a 24 well tissue culture plate with 106 cells/well. PBMC were stimulated to produce IgE with purified anti-CD40 monoclonal antibody (1μg/mL, 82111, R&D, Wiesbaden, Germany) and IL-4 (5ng/mL, Immunotools, Friesoythe, Germany) and left to incubate for 10 days in an RPMI 1640 culture medium (Biochrom KG). L-glutamine (2mM), penicillin (100 U/mL), streptomycin (100 g/mL) and 10% heat-inactivated fetal calf serum (all Biochrom KG) were added. Atmosphere was held at 37°C, 100%

[1] Gas chromatographic examinations were kindly provided by work group members of Dr. Harald Renz; Department of Clinical Chemistry and Molecular Diagnostics – Central Laboratory – Marburg.

humidity and 5% CO_2. After incubation, the tissue culture plate was centrifuged (15min, 260g, 20°C), the supernatant was stored at -20°C.

Total IgE and spontaneous IgE production were determined in patient serum and supernatants by means of enzyme-linked immunosorbent assay (ELISA). ELISA is a very sensitive and widely used immunologic procedure to quantitatively detect proteins and other substances. Sandwich or capture ELISA applied in this investigation consisted in 5 working steps, illustrated in figure 2.5.

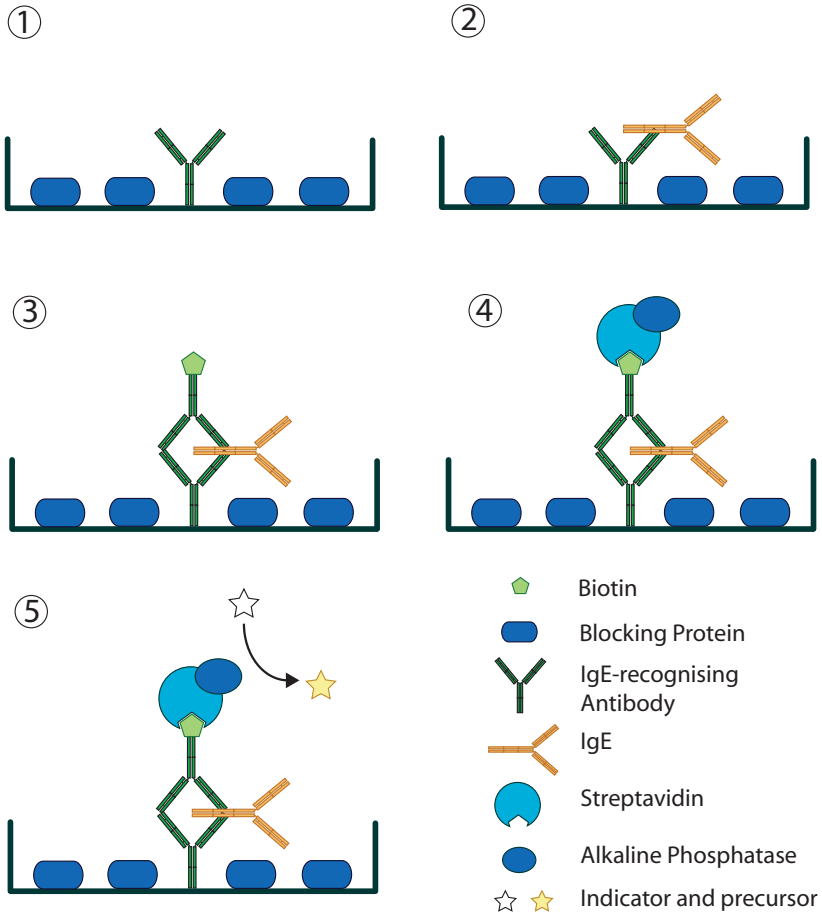


Figure 2.5: Working steps for ELISA: ① Coating and blocking: protein-binding 96-well microtiter plates were coated with antibodies specific for IgE (capture antibody). Non-specific binding sites were blocked with 2% bovine serum albumin (BSA). ② IgE-containing standards and samples were applied to the plate (standards and each sample twice for double testing and in a dilution series) see figure 2.6 for typical plate layout. Rinsing removed unbound IgE. ③ Biotinylated antibodies specific for IgE were applied, attaching to another site of the IgE molecules. ④ Alkaline phosphatase (AP) bound to streptavidin was added, streptavidin attaches strongly to biotin. Rinsing removed unbound streptavidin. ⑤ p-nitrophenylphosphate was applied as substrate for AP, rendering yellow p-nitrophenol. Absorbency at 405nm was measured in the wells to determine presence and quantity of antigen.

Standard	Standard	Sample 1	Sample 1	Sample 2	Sample 2	Sample 3	Sample 3	Sample 4	Sample 4	Sample 5	Sample 5
10.000 U/ml	10.000 U/ml	1:100	1:100	1:100	1:100	1:100	1:100	1:100	1:100	1:100	1:100
5.000 U/ml	5.000 U/ml	1:200	1:200	1:200	1:200	1:200	1:200	1:200	1:200	1:200	1:200
2.500 U/ml	2.500 U/ml	1:400	1:400	1:400	1:400	1:400	1:400	1:400	1:400	1:400	1:400
1.250 U/ml	1.250 U/ml	1:800	1:800	1:800	1:800	1:800	1:800	1:800	1:800	1:800	1:800
0.625 U/ml	0.625 U/ml	1:1,600	1:1,600	1:1,600	1:1,600	1:1,600	1:1,600	1:1,600	1:1,600	1:1,600	1:1,600
0.313 U/ml	0.313 U/ml	1:3,200	1:3,200	1:3,200	1:3,200	1:3,200	1:3,200	1:3,200	1:3,200	1:3,200	1:3,200
0.156 U/ml	0.156 U/ml	1:6,400	1:6,400	1:6,400	1:6,400	1:6,400	1:6,400	1:6,400	1:6,400	1:6,400	1:6,400
0.000 U/ml	0.000 U/ml	1:12,800	1:12,800	1:12,800	1:12,800	1:12,800	1:12,800	1:12,800	1:12,800	1:12,800	1:12,800

Figure 2.6: Microtiter plate layout: Spontaneous IgE was determined using sandwich/capture-ELISA. Microtiter plates were prepared in this layout. See section 2.4.9 for ELISA description and working steps.

2.5 Statistical Analysis

R 2.9.2 (The R Foundation for Statistical Computing, Vienna, Austria) and the SPSS 16.0.1 for Mac Standard Package (SPSS Inc., Chicago, USA) were used for statistical analyses. Normal distribution of a variable was assumed if there was only one maximum in the histogram and skewness was within the range of $]-1;1[$. Normally distributed variables were tested with Student's t-Test. To compare means of successive measurements in one group the Paired Samples t-Test was used. To determine statistical relevancy of differences between two groups the Independent-Samples t-Test was applied. Alternatively, a non-parametric method was used for non-normally, but similarly distributed results. In this case, the Mann-Whitney U-Test was applied to independent samples while paired samples were tested with Wilcoxon's Test. Difference in SCORAD score at the end of treatment was defined as primary endpoint. The trial was designed so that a difference in SCORAD of 8 Points could be detected with 80% power using the t-Test for independent samples. Population size

was calculated according to the formula:

$$n = \frac{(2\sigma^2)(z_{1-\alpha/2} + z_{1-\beta})^2}{D^2}$$

Where σ is the estimated SD[2], z indicates a standard normal distribution, α refers to the probability of a type I error ($\alpha = 0.05$) and β represents the probability of a type II error ($\beta = 0.2$). D is the clinical relevant difference ($D = 8$). This shows that a population of 25 subjects per group is needed to achieve the desired power. The main outcome variable (SCORAD) was analyzed on an intention-to-treat basis (ITT), including the last available data for each participant who returned at least once after receiving treatment. Missing data was imputed using linear regression. Additionally, per-protocol analysis was performed for the main study criterion. Explorative data was analysed as available, regardless of adherence to protocol and without imputing data. A significance level of $p \leqslant 0.05^{*}$ was chosen for the main outcome variable. Values below this limit would be considered significant, for $p \leqslant 0.01^{**}$ very significant and from $p \leqslant 0.001^{***}$ onward highly significant. Differences that do not reach significance would be termed as a not significant trend if $p \leqslant 0.1^{\circ}$. For explorational variables, p-values are termed "low" if $p \leqslant 0.1^{\circ}$, and "very low" for $p \leqslant 0.05^{*}$, to clearly distinguish them from the main outcome variable[3]. Confidence intervals are given at 95% (CI95%).

[2]SD of SCORAD score was estimated to be about 10 using data of patients attending the dermatology department of the Charité. As outcome is a difference of two SCORAD scores, σ is calculated by the formula $\sigma = SD\sqrt{2(1-\rho)}$, where ρ is the correlation coefficient when the two measurements are plotted against each other. If the correlation coefficient is not lower than 0.5, the standard deviation of the differences will be equal to σ (getting smaller as the correlation increases). Correlation is very unlikely to be lower than 0.5 as the same subjects are evaluated over time, so σ can be approximated quite well by the estimated SD.

[3]Conducting 20 tests with a 5% type I error results in a 65% chance of having one false positive. That is why explorational variables cannot have the same statistical value as the predefined main outcome variable. This difference is expressed by using "low p-value" instead of "trend" and "very low p-value" instead of "significant".

3 Results

3.1 Participants

A total of 72 participants was screened between October 2004 and January 2005. Of these, 13 patients did not meet inclusion criteria and were therefore excluded from the trial, resulting in a trial population of 59 Patients. Randomization assigned 31 patients

Table 3.1: Baseline characteristics of the study population. Values are mean ± SD if normally distributed or median (1^{st} – 3^{rd} quartile) if otherwise.

Variable	Placebo (n=26)	DHA (n=28)
General		
Sex: male/female	8/18	10/18
Age (years)	26.4 ± 6.9	27.6 ± 6.4
Height (cm)	172.5 ± 8.3	173.5 ± 11.2
Weight (kg)	68.0 ± 13.1	73.1 ± 19.8
Bloodpressure (mmHg)	119/72 (±10/±10)	126/78 (±15/±10)
Disease activity		
SCORAD	37.6 ± 12.9	37.8 ± 11.9
Erlangen Score	20.0 ± 4.3	19.5 ± 5.4
Total IgE (U/ml)	303 (138–657)	258 (20-556)
Lipid metabolism		
Total Cholesterin (mg/dl)	178.4 ± 28.8	179.4 ± 39.1
Trigylcerides (mg/dl)	81.0 ± 44.8	83.4 ± 41.2
HDL (mg/dl)	60.7 ± 16.2	58.0 ± 12.8
LDL (mg/dl)	104.3 ± 31.5	102.1 ± 31.8
Quality of life (SF-36)		
Mental health	45.6 ± 12.1	47.4 ± 9.9
Physical health	53.0 ± 6.9	50.9 ± 7.3

to the DHA group and 28 to receive placebo treatment. Five Patients withdrew from the study without any assessment and without study medication, leaving an evaluable population of 54 patients (DHA 28, placebo 26). During the further course of the trial 7 patients withdrew before completing supplementation, 4 of these without any further assessment besides baseline. Reasons included: hospitalization (for other cause than AD), personal reasons, inability to swallow capsules and withdrawal of consent. This

left an evaluable total of 50 with at least one assessment during supplementation and a total of 47 participants concluding supplementation period according to protocol. Final assessment was attended by 43 patients. Figure 3.1 summarises the flow of patients through the study. None of the participants withdrew for adverse effects. The two treatment groups were well matched with respect to all baseline characteristics (see table 3.1). Most patients showed a very clear ASD in the Erlangen Score and SCORAD showed moderate disease activity on average.

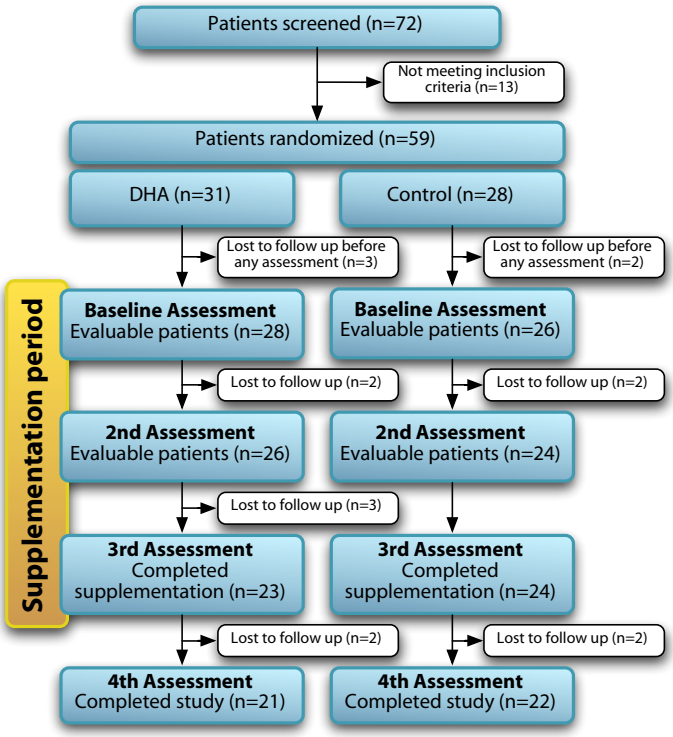


Figure 3.1: Flow chart of trial participants

Table 3.2: Reasons for withdrawals during treatment

	DHA	Placebo
Private reasons	2	0
Withdrawn consent	2	1
Inability to swallow capsules	0	1
Hospitalization (not for AD)	1	0
Total	5	2

3.2 Efficacy on Skin Status

Table 3.3 shows mean SCORAD scores. In order to perform intention-to-treat analysis, three values in the DHA group had to be imputed by using linear regression. Mean SCORAD score decreased by 5.5 points from 37.8 (SD = ± 9.9) to 32.3 (SD = ± 11.8) in the DHA-group ($p = 0.015^{*}$, CI95%: 1.2 to 9.8) while falling by 4.4 points from 37.6 (SD = ± 12.9) to 33.2 (SD = ± 12.5) in the placebo group at the end of treatment ($p = 0.048^{*}$, CI95%: 0.03 to 8.9). Student's t-Test shows no statistically relevant difference between the groups at the end of the supplementation period ($p = 0.79$). CI95% of differences is -5.2 to 7.0 at baseline and -6.0 to 7.8 after the intervention. Per-protocol analysis, i.e. only including cases true to protocol, shows patients who continued to supplement DHA as specified, while not changing their normal skin care habits, show very significantly better skin status as compared to baseline ($p = 0.009^{**}$, CI95%: 1.7 to 10.7). Protocol violations included total discontinuation of topical anti-inflammatory treatment with corticoids with resulting exacerbation (2 cases in the DHA group: "I forgot to get new cream") or intense sun exposure during trial phase (1 case in the placebo group: "My skin is great since that vacation in Egypt"). Change in the placebo group as compared to baseline is not significant, showing a trend toward improvement ($p = 0.09^{\circ}$, CI95%: -0.5 to 6.9). There is no statistically relevant difference between the two groups after supplementation ($p = 0.15$). CI95% of difference is -5.0 to 8.5 at baseline and -1.8 to 11.4 at 4 weeks of treatment. After the wash-out phase there are neither significant changes compared to the end of supplementation, nor are there significant differences between treatment groups.

Table 3.3: Effects of DHA on skin status. Values are mean ± SD. "ITT" indicates analysis based on intention to treat, "PPA" refers to per-protocol analysis. Asterisks (*) are used to show different levels of statistical significance of differences compared to baseline. * indicates $p \leqslant 0.05$, ** indicates $p \leqslant 0.01$ and *** is used for $p \leqslant 0.001$. $^{\circ}$ shows a non-significant trend, $p \leqslant 0.1$.

	SCORAD (ITT)		SCORAD (PPA)	
Visit	Placebo (n=24)	DHA (n=26)	Placebo (n=23)	DHA (n=21)
January	37.6 ± 12.9	37.8 ± 11.9	36.8 ± 12.5	35.1 ± 9.4
February	33.4 ± 12.1	35.8 ± 14.2	33.3 ± 12.3	31.1 ± 10.9
March	33.2*± 12.5	32.3*± 11.8	33.6 ± 12.6	28.8**± 8.8
June	30.0 ± 13.0	33.8 ± 13.6	29.6 ± 13.2	29.4 ± 9.8

An overview over skin-physiology parameters is given in table 3.4. As none of

these variables are normally distributed, p-values were calculated applying the Mann-Whitney U- or Wilcoxon-Test (see section 2.5). All variables show improvement after the supplementation period, especially pH and moisture-values (comparing end of supplementation values with those in June). TEWL for less affected skin in DHA subjects shows a reduction in water loss median values by 5.6 g/h/m^2 ($p = 0.016^*$), in heavily affected skin, improvement shows low p-values ($p = 0.099^\circ$). With placebo, change in less affected skin (0.9 g/h/m^2) is not statistically relevant ($p = 0.35$), p-values for improvement in heavily affected skin are very low ($p = 0.004^*$). TEWL for skin of normal apearance showed correlation with SCORAD values (Spearman's $\rho = 0.3$, $p = 0.048^*$ in February and $\rho = 0.5$, $p < 0.0001^*$ in March). Decrease in moisture values for heavily affected skin in placebo-treated subjects show very low p-values ($p = 0.002^*$) toward the end of supplementation when compared to baseline.

Corneometer values for heavily affected skin were statistically different between the two treatment groups ($p = 0.037^*$) on the first measurement in February. Apart from this, there are no statistically detectable differences between treatment groups concerning any skin physiological parameter.

Table 3.4: Effects of DHA on skin physiology. Values are median (1st quartile - 3rd quartile). AU refers to arbitrary units given by the measuring device. Mann-Whitney U or Wilcoxon Tests were used to determine p-values. Asterisks (*) are used to show p-values of differences compared to baseline. * indicates $p \leqslant 0.05$, $^\circ$ is used for $p \leqslant 0.1$.

	Less affected skin		Heavily affected skin	
	Placebo (n=22)	DHA (n=20)	Placebo (n=22)	DHA (n=20)
TEWL (g/h/m^2)				
February	15.1 (12.6 - 20.4)	20.1 (11.5 - 35.5)	54.0 (24.8 - 69.5)	48.9 (30.2 - 62.6)
March	14.0 (10.5 - 18.2)	14.5*(13.3 - 17.8)	32.6*(27.5 - 49.3)	33.1°(25.2 - 44.0)
June	13.7 (10.1 - 18.4)	15.0 (11.5 - 18.6)	41.6 (24.4 - 60.9)	29.6 (23.4 - 47.9)
pH				
Febuary	5.5 (5.4 - 6.2)	5.6 (5.3 - 5.9)	5.7 (5.4 - 5.9)	5.6 (5.3 - 6.0)
March	5.7 (5.4 - 6.2)	5.8 (5.4 - 6.0)	5.6 (5.2 - 5.9)	5.6 (5.3 - 5.9)
June	5.3*(4.9 - 5.6)	5.1*(4.6 - 5.8)	5.2*(5.0 - 5.4)	5.0*(4.8 - 5.7)
Sebum (µg/cm^2)				
February	1.0 (1.0 - 5.5)	1.0 (1.0 - 7.0)	2.0 (0.5 - 10.5)	2.0 (1.0 - 4.3)
March	1.5 (0.0 - 6.0)	1.0 (1.0 - 6.5)	1.5 (1.0 - 8.0)	1.0 (0.0 - 7.5)
June	1.5 (0.0 - 15.0)	3.0 (0.0 - 10.3)	1.5 (0.0 - 7.0)	2.5 (0.0 - 5.0)
Moisture (AU)				
Febuary	34.5 (30.6 - 40.8)	30.3 (25.2 - 44.1)	29.7 (19.7 - 40.8)	20.9 (14.4 - 34.5)
March	32.5 (26.1 - 43.7)	31.7 (26.1 - 36.2)	21.3*(15.3 - 31.5)	20.3 (14.9 - 26.0)
June	67.5*(51.5 - 74.0)	65.0*(46.1 - 78.8)	60.5*(44.0 - 70.0)	49.8*(34.0 - 73.0)

3.3 IgE and Skin Sensitization

DHA group wheal and erythema sizes as measured in prick testing for allergens showed considerable decrease. Allergen/histamine wheal ratio in the DHA group improved by 0.36 mm/mm, showing low p-values ($p = 0.072^{\circ}$). Changes compared to baseline noticed in erythema of DHA-treated patients (0.87 mm/mm) show very low p-values ($p = 0.001^{*}$). In the placebo group, a 0.1 mm/mm change in wheal ratio is measurable (not statistically relevant). Erythema ratio decreased by 0.46 mm/mm, low p-values could be observed for this change ($p = 0.075^{\circ}$). Comparing treatment groups, there are no statistically relevant differences ($p = 0.95$ for wheal, $p = 0.83$ for erythema).

Table 3.5: Effects of DHA on prick test results. Values are presented as relative to the histamine-control (value/control). Values are mean ± SD [mm/mm]. Asterisks (*) are used to show p-values of differences compared to baseline. * indicates $p \leqslant 0.05$, ° is used for $p \leqslant 0.1$.

	Wheal		Erythema	
	Placebo (n=21)	DHA (n=20)	Placebo (n=21)	DHA (n=20)
January	1.59 ± 1.00	1.87 ± 0.78	1.67 ± 1.11	2.04 ± 1.04
March	1.49 ± 0.74	1.51°± 0.63	1.21°± 0.70	1.17*± 0.37

IgE values are shown in table 3.6. Statistically relevant differences were observed neither at baseline nor after the supplementation period. There is a general tendency toward lower IgE values and lower IgE production in both groups. In both stimulated IgE-production ($p = 0.002^{*}$) and specific IgE ($p = 0.026^{*}$), p-values are very low for changes within the DHA group, although placebo treatment p-values are also low ($p = 0.067^{\circ}$ for stimulated IgE-response and $p = 0.052^{\circ}$ for specific IgE).

3.4 Quality of Life

Quality of life as measured by SF-36® shows no measurable correlation on any sub- or summary scale to clinical observations through SCORAD (scatterplots not shown).

The mental health index (MHI) improved remarkably in both groups, with very low p-values ($p = 0.008^{*}$) in the placebo group and low p-values in the DHA group ($p = 0.069^{\circ}$). Of the other sub-scales, only vitality (VITAL) in the DHA-group showed improvement with very low p-values ($p = 0.015^{*}$). Low p-values could be made out for role physical (ROLPH) and the social functioning index (SOCIAL). There was a considerable deviation from population average values in the DHA group regarding the

Table 3.6: Effects of DHA on total IgE and IgE production. Values are median (1st quartile - 3rd quartile) and given in [U/ml]. Mann-Whitney U or Wilcoxon Tests were used to determine p-values. Asterisks (*) are used to show p-values of differences compared to baseline. * indicates $p \leqslant 0.05$, ° is used for $p \leqslant 0.1$.

	Placebo (n=24)	DHA (n=23)
Total IgE		
January	303.8 (137.5 - 657.4)	258.0 (19.5 - 555.8)
March	265.1 (143.2 - 722.6)	239.1 (66.2 - 630.1)
Spontaneous IgE Production		
January	2.8 (0.8 - 4.5)	3.3 (0.9 - 8.2)
March	1.7°(0.4 - 2.8)	1.1*(0.7 - 2.6)
Specific IgE		
January	11.0 (1.9 - 41.8)	10.0 (3.1 - 34.6)
March	9.9°(1.8 - 44.1)	8.5*(2.1 - 36.7)

general health perceptions (GHP) scale. Also, ROLPH and MHI, after the intervention, seemed slightly above average within this group. Figure 3.2 displays the results as profiles against the national average corrected for age and sex. No statistically relevant differences between the two groups could be determined after the supplementation.

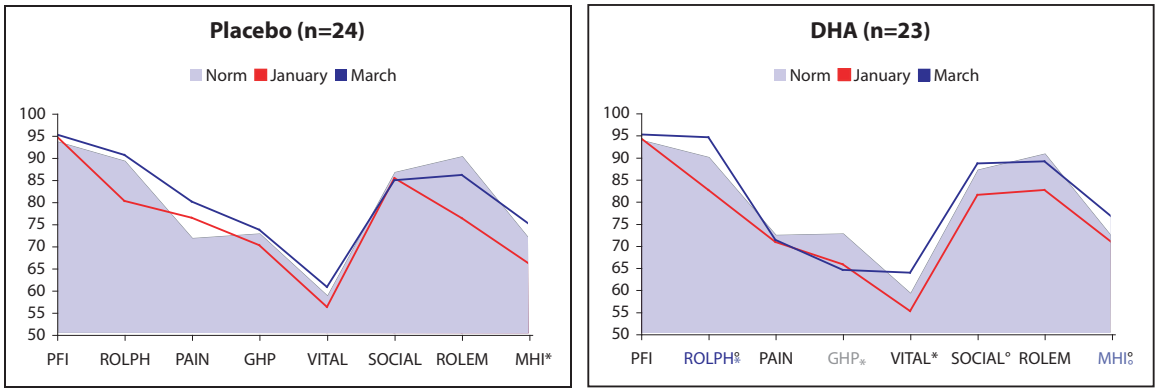


Figure 3.2: SF-36® in placebo and DHA group. Notable differences compared to normal values are indicated in the corresponding color of the dataset (red for January and blue for March, gray for both). Asterisks (*) are used to show p-values of differences compared to baseline. * indicates $p \leqslant 0.05$, ° is used for $p \leqslant 0.1$.

A total of 46 patients returned their diaries (23 in each group). Analysis of data in patients diaries showed probable correlation with the SCORAD-scales used to assess patients skin status (see figure 3.4). However besides a small, statistically irrelevant improvement in means, no changes could be made out between the two treatment

groups or within groups comparing self-assessment before and after treatment. Figure 3.3 gives an overview of the mean scores of the items patients were evaluating. Patients' use of topical corticosteroids was also examined. Thirteen patients in the placebo group and 14 patients in the DHA group applied corticosteroids during the trial phase. There was a statistically relevant difference between treatment groups at baseline, so no evaluation of possible treatment effects could be performed (data not shown).

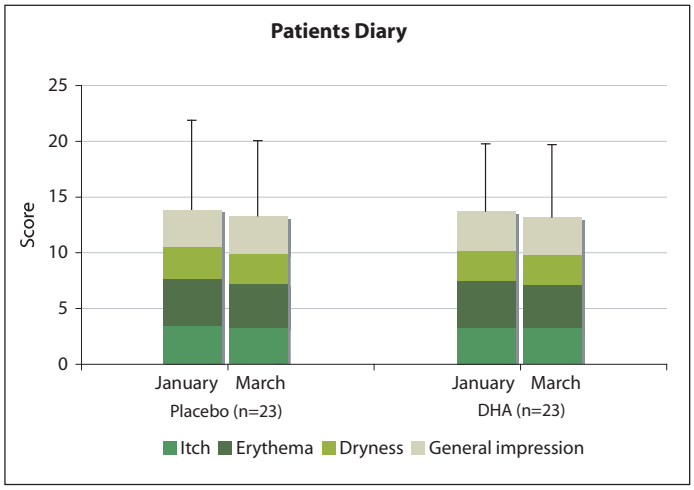


Figure 3.3: Patients diary summary: Bars represent scale summaries with each subscale marked by a different color. Hair-lines indicate SD for the summary score.

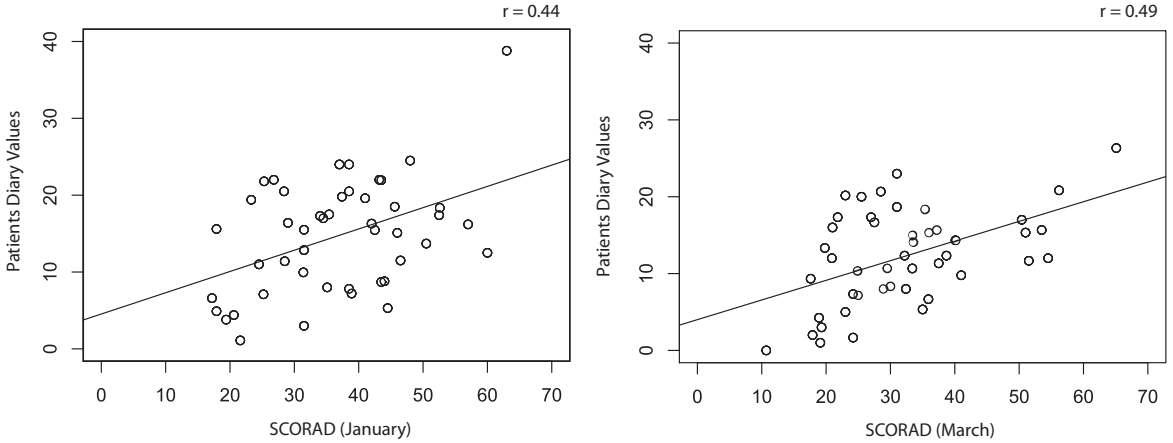


Figure 3.4: Scatterplots showing the correlation between SCORAD and patients' diary scores. Each circle represents a patient. X-axis value is SCORAD, y-axis shows the sum of all diary scores.

3.5 Safety Parameters

Blood pressure, routine blood cell counts as well as parameters of hepatotoxicity (ALT, GGT) in all patients remained stable throughout the study. Statistically relevant differences between the two groups were observed neither at the beginning, nor at the end of the supplementation period. Weight in DHA patients increased slightly by 0.9kg ($p = 0.013^{*}$) which equals a weight gain of 1.3%. There were no measurable statistically relevant changes in body fat distribution.

3.6 Lipid Metabolism

Parameters of lipometabolism showed considerable changes. Under placebo a decrease of total cholesterin by 10 (mg/dl) ($p = 0.035^{*}$) was observed. HDL cholesterin also dropped ($p = 0.022^{*}$), as well as LDL ($p = 0.032^{*}$). TAG increased slightly without statistical relevance. Patients given DHA showed low p-values for increase in HDL cholesterin ($p = 0.079^{\circ}$) and very low p-values for change in triglycerol values ($p = 0.016^{*}$). Difference between the two groups regarding TAG has a low p-value ($p = 0.066^{\circ}$, CI95%: -1.8 to 56.6). Difference in HDL also showed a low p-value: by the end of the supplementation period the t-Test renders $p = 0.057^{\circ}$, CI95%: -17.5 to 1.5 for HDL. These results are summarized in table 3.7.

Table 3.7: Lipid metabolism parameters: Values are given in [mg/dl] and as mean ± SD. Asterisks (*) are used to show p-values of differences compared to baseline. * indicates $p \leqslant 0.05$, $^{\circ}$ is used for $p \leqslant 0.1$. Low p-values for differences between treatment groups are indicated on arrows between table columns.

	Placebo (n=24)		DHA (n=23)
Cholesterin			
January	178.4 ± 38.8		173.7 ± 35.6
March	168.6*± 34.1		176.3 ± 40.3
TAG			
January	81.0 ± 44.8		83.4 ± 41.2
March	92.4 ± 66.4	$\overset{\circ}{\leftrightarrow}$	65.0*± 20.9
HDL			
January	58.0 ± 12.8		59.5± 14.3
March	54.9*± 13.2	$\overset{\circ}{\leftrightarrow}$	62.9°± 18.8
LDL			
January	104.3± 31.5		98.1 ± 30.8
March	95.4*± 25.1		100.4 ± 36.8

3.7 Tolerance and Compliance

Both treatments were generally well tolerated, though some minor side effects were noted in both groups. Table 3.8 gives an overview, listing all adverse events reported. Patients given DHA complained more about eructations, sometimes with a fishy smell to them ($p = 0.006^*$, Odds ratio: 5.3). No relevant changes in any of the haematological or biochemical parameters monitored for safety were noted. In no patient did any haematological or biochemical monitoring reveal changes causing clinical concern.

Gas chromatographic examinations[1] in the serum of patients before and after supplementation reveal more serum n-3 fatty acids in the DHA-group (very low p-value). The n-6/n-3 ratio is also lower than in the short chain fatty acid group (very low p-value, data not shown).

Table 3.8: Adverse effects reported by more than one participant: percentage (absolute number) of participants reporting

Event	Placebo (n=26)	DHA (n=24)
Eructation	8% (2)	42%*(10)
Upper respiratory tract infection	27% (7)	30% (7)
Headache	19% (5)	21% (5)
Acne	4% (1)	4% (1)
Slight digestive tract symptoms	15% (4)	8% (2)
Fatigue	4% (1)	4% (1)
Other infections	12% (3)	–
New rash	8% (2)	–

[1] Gas chromatographic examinations were kindly provided by work group members of Dr. Harald Renz; Department of Clinical Chemistry and Molecular Diagnostics – Central Laboratory – Marburg.

4 Discussion

4.1 Influence of DHA On Skin Status

In this study clinical efficacy of high-dose DHA supplementation was evaluated in AD patients in a placebo-controlled prospective trial. Although a significant decrease of the severity of AD was observed in the DHA-group, the main study criterion was not achieved as SCORAD values show no statistically relevant differences between verum and placebo groups after supplementation.

Until now, seven other studies have been conducted evaluating fish oil supplementation in patients with AD [30–33, 50–52]. Four of these had less than 40 participants [30, 31, 50, 52] and one had structural flaws [51]. Berth-Jones et al. [33] found no effect of fish oil when supplementing comparatively low concentrations of FA (204mg EPA/d, 132mg DHA/d). On the other hand, Mayser et al. [50] infused high concentrations and saw statistically relevant effects, and assumed an advantage of fish oil administration. Interestingly, it was the only trial where the ratio DHA/EPA was greater than 1 (Exact value: 1.01), with concentrations of DHA being a 30-fold of what was used by Berth-Jones et al. In the current trial, an exceptionally high amount of DHA was used, with an DHA/EPA ratio of about 7. Therefore, treatment is referred to as "DHA" although there is a small fraction of EPA involved.

As in the case of observations by Søyland et al. [32], this trial showed improvements in skin status upon fish oil supplementation. Levels of serum fatty acids show very good bio-availability and compliance in participants (data not shown). With the differences between treatment groups being not statistically relevant, no evidence could be found for an advantage of DHA supplementation over placebo. The trial was sufficiently powered to detect clinically relevant differences, but as confidence intervals show, such differences are very unlikely. In fact, risk of type I error (rejecting the null hypothesis when the null hypothesis is true) is 79% for the intention-to-treat analysis, and when analyzing per-protocol this chance is still too high for clinical standards (15%).

Could it be possible that the short chain fatty acids used as a "placebo" have themselves immunologic effects? A carefully conducted MEDLINE research found no conclusive evidence. Capric acid (40.5% of control treatment) was found to enhance

IL-8 production in Caco-2 cells in one study [53], while another showed caprylic acid (50.5% of control treatment) to inhibit IL-8 gene transcription in the same cell line of epithelial colorectal cells [54]. No evidence for immunological effects of capronic, lauric or myristic acid could be identified. IL-8 is an important interleukin in inflammation processes, so a possible anti-inflammatory effect especially through the main component of the "placebo"-treatment cannot categorically be ruled out. Due to the contrary nature of the components' effect, neutralization of stimulating and inhibiting influences is also a possibility.

On the other hand, observed effect sizes could very well be explained through placebo effect itself. In this trial besides the "true placebo effect", several mechanisms could be contributing to the overall perceived placebo effect: It is well known that chronic, relapsing and remitting diseases with subjective symptoms like pain or itching (as is the case with AD) respond especially well to placebo treatment [55]. Clinician-patient interaction might be another factor, as well as the natural history (relapsing and remitting), regression towards the mean and social desirability (i.e. the patient "wants" to report improvement)[56]. These factors provide a very reasonable basis for the significant changes in clinical appearance observed in both groups.

TEWL reflects the barrier function of the skin and is greater in areas of more clinically involved disease [57]. Studies have shown a positive correlation between clinical features and barrier function as measured by TEWL [58–60]. Likewise, in this trial TEWL showed good correlation to SCORAD values in normal-appearing skin. This correlation is not seen for heavily affected skin. As expected, values are higher in these areas, indicating a more disturbed barrier function. Changes are similar in numbers, and statistical analysis shows improvement in both groups: in less affected skin for DHA, and heavily affected skin in placebo group. As groups are not statistically different at any point, no relevant advantage for DHA can be seen. The general improvement seen after the wash-out phase could be attributed to positive climate effects like temperature and UV-light during this period of the year.

pH seems only slightly, if at all, affected by AD [58, 61]. This concurs with the findings in this study showing no differences in pH of affected and less affected skin. Summer values are more acidic, reflecting the more balanced skin status seen at this examination. No evidence for a change of pH through DHA could be seen in this trial.

Sebum values were found to be lower in patients with AD in clinically uninvolved skin compared to healthy controls [61, 62]. No correlation between Sebum values and clinical features could be observed in this study. No evidence for effects of DHA on

Sebum values could be gathered.

Decreased stratum corneum hydration as measured by conductance is seen in dry and scaly skin, but also in clinically normal skin of AD patients [60, 61]. A large multicenter study found very dry skin was characterized by corneometer units (arbitrary units, AU) below 30, dry skin between 30 and 40 AU and normal skin higher than 40 AU [63]. Seen from this perspective corneometer values show lack of hydration during supplementation even in clinically normal-appearing skin. Values in June show considerably higher stratum corneum hydratation. An explanation for this observation could be exposure to very dry air of artificially heated rooms during winter. No trials could be found examining the effects of dietary DHA on skin water content. In this study skin conductance remained constant over the supplementation period in DHA subjects, while showing a loss of hydratation with very low p-values in heavily affected skin of placebo patients. First measurements in February show relevant differences, so groups don't seem really well matched regarding this parameter and are therefore not comparable in this aspect.

Measurements of biophysical parameters in general confirm the overall positive clinical development as observed by the SCORAD evaluation and show no differences between treatment groups.

4.2 Influence of DHA On Quality of Life

SF-36® has been used in various trials to evaluate health related quality of life in atopic patients [64, 65]. Most studies show the relationship between disease severity and quality of life, demonstrating the considerable burden of disease of AD.

Data adapted according to age and sex from the German Federal Health Survey (Bundesgesundheitssurvey, 1998) [48] are displayed to show population averages. Compared to this, general health perception (GHP) at baseline is lower than average in the DHA group (very low p-value). Toward the end of treatment there is no statistically relevant difference concerning this sub-scale. Role Physical Index (ROLPH) rises from a little lower than average to above average in the DHA group (very low p-value). This scale shows the extent to which state of health affects work or other daily activities, for example accomplishing less than usual, being limited in patients' choice of activities or having difficulties while performing them [47]. Apart from the ROLPH scale DHA treatment sees improvements with very low or low p-values for 3 more indexes (VITAL, SOCIAL, MHI). There is evidence for a connection between positive mood and DHA

intake [66]. In fact, DHA is most important for brain function and development [67]. This could be a physiological basis for positive changes seen in quality of life of DHA patients during the supplementation period. However, differences between the treatment groups do not show p-values low enough to support this hypothesis. As the MHI-scale in the placebo group seems to demonstrate, changes in quality of life can be due to general mood swings inherent to seasonal changes more than being directly affected through treatment.

Patients' diary entries show no statistically relevant differences either compared to baseline or between treatment groups. This might be due to difficulties inherent to the numeric scale. Although the concept was explained thoroughly to participants, findings indicate that very few made full use of the scales' total range. Mean standard deviation for all scales reached only a value of 1.1 which evidences a strong conservative factor overlying patients' self assessment. In fact, this number evidences that 95% of self-evaluation was kept within a range of little more than 4 digits. Maybe the method of day to day evaluation led to underestimation of gradual improvement. Scatterplots in figure 3.4 also seem to suggest that a considerable number of patients estimated their skin status slightly worse than the physician evaluated it by the end of the supplementation period (see the "cloud" of dots at the lower end in the scatterplot for March). At baseline this difference in assessment is not so obvious. Correlation with SCORAD values is expected, as both evaluate the same condition and SCORAD scores contain a considerable subjective component.

4.3 Influence of DHA On Lipid Metabolism

Comparing and analyzing 65 studies on the influences of marine n-3-FA on serum lipoproteins humans Harris et al. found well-established effects: While total cholesterol is not materially affected, LDL tends to rise by 5-10% and HDL by 1-3%. Most affected are TAG concentrations, which show decreases of about 25-30% [68].

This is well in line with results seen in this trial. In percentages, DHA increased total cholesterin by 1.5%, HDL by 6%, LDL by 2% and decreased TAG by 9%. TAG decrease is less than that seen by Harris et al., the reason for this observation could be the fairly low TAG-values at baseline, as higher baseline values see a stronger effect with marine n-3-FA: Average baseline in the studies considered in the review was 220 mg/dl, while subjects in this trial averaged 82 mg/dl (see results of studies cited by [68]). The trend to slightly higher HDL-values with DHA corresponds well with known

effects of marine PUFA. Very low p-values for HDL-decrease in the control group could indicate negative effects of increased saturated fatty acid intake on HDL. Lower cholesterin levels are seen at the expense of a worse HDL/LDL ratio. Total cholesterin and LDL decreased significantly in the Placebo group, nevertheless HDL/LDL-ratio is better in the DHA group (Placebo: 0.56, DHA: 0.63). Low p-values in favor of the DHA group confirm TAG reducing and HDL raising effect seen in other studies. Mechanism of action for this effect remains unclear to date and should be considered in future research.

4.4 Influence On Atopy

SPT serves to prove immediate-type specific sensitization. Crosslinking of mast cell bound IgE through SPT allergens leads to the release of mast cell granules containing histamine. Histamine dilates local small vessels and increases their permeability, resulting in local edema (wheal) and erythema. Strong reaction in SPT corresponds to high serological IgE values [69]. High values of EPA in red blood cells were found to be associated with a decreased risk of allergic sensitization [70]. No trials have been conducted so far examining specifically effects of DHA on IgE values or skin reactivity. Several trials examined the effects of fish oil supplementation during pregnancy and nursing to prevent atopy [71, 72]. A recent meta-analysis however failed to identify any clear benefits associated with n-3-FA supplementation for the prevention of atopic diseases [73].

As to be expected, IgE values and SPT results show similar patterns towards a general improvement. Although pronounced change could be seen especially in the DHA group, no differences of statistical importance between treatment groups could be determined. Therefore, placebo effects cannot be ruled out as possible explanation. In this trial no evidence could be gathered for DHA to be superior to placebo in modulating atopy.

4.5 Conclusion

Contrary to the evidence from in-vitro and in-vivo findings proving convincingly the theoretical anti-inflammatory properties, fish oil fails to demonstrate clinically relevant effects in this trial. Methodically the trial was sufficiently powered, examining the third largest population of adult patients with AD on PUFA mediated skin

improvement. With 4.9g/day it was the highest dose of PUFA and the highest dose of DHA supplemented so far (DHA/EPA-ratio about 7:1). Patients showed good compliance with notably higher serum levels of DHA. There is no conclusive evidence for relevant immunologic effects mediated by the short chain fatty acid mix used as placebo treatment, although conflicting influence on IL-8 production is discussed. This leaves a very weak clinical effect of the examined PUFA as the most likely explanation.

Considering previous studies, it seems unlikely that larger study populations or higher PUFA concentrations would yield any different results. Judging by the results of this trial and its precedents, fish oil cannot be recommended for improving skin status in patients with AD. The question of whether fish-oil supplementation can stabilize skin status leading to less topical corticosteroid use could not be addressed in this trial and might be worth further research.

4.6 Summary

Docosahexaenoic acid (DHA) as found in fish oil has been shown to possess potent anti-inflammatory properties, including active resolution of inflammation through derivates like resolvins, protectins and maresins. This trial was conducted in order to evaluate effects of DHA on skin status of patients with atopic dermatitis (AD) and to examine possible physiological and biochemical effects in patients with AD.

Patients aged 18-40 were randomized double-blind into 2 groups: one received 4.9g/d of DHA, the other a mix of saturated short chain fatty acids as placebo, while continuing their skin care as usual (including topical corticoids). During a supplementation period of 8 weeks (January through March 2005) 3 examinations were performed: at baseline, after 4 weeks and at the end of supplementation period. An additional visit was scheduled after a 12-week wash-out phase. On every visit, skin status was evaluated by SCORAD. Additionally, skin physiology (pH, sebum, moisture and transepidermal water loss), blood samples (IgE, lipid metabolism), skin prick test and quality of life (SF-36) were examined. Patients were instructed to keep a schematized diary recording their observations.

Of the 54 Patients included in this trial, 47 completed the supplementation period. DHA was well tolerated and readily bioavailable through oral administration. SCORAD values showed significant improvements in both groups, no statistically relevant differences could be found between the groups. Of additional observed parameters, only lipid metabolism showed relevant differences: triacylglycerol (TAG) and high

density lipoprotein (HDL) values were better in the DHA group.

This trial examined the third-largest population of adult patients with AD on DHA-mediated skin status improvements. It was the highest dose of DHA supplemented so far (DHA/EPA-ratio about 7:1). Results show significant improvement in both treatment groups, however there was no statistically significant difference between the two groups. In this trial, DHA is not more effective than short chain fatty acids in treating AD.

Bibliography

[1] Staffan Ahlstedt and Clare S Murray. In vitro diagnosis of allergy: how to interpret IgE antibody results in clinical practice. *Prim Care Respir J*, 15(4):228–36, Aug 2006. doi: 10.1016/j.pcrj.2006.05.004.

[2] Nancy Wasserbauer and Mark Ballow. Atopic dermatitis. *Am J Med*, 122(2):121–125, Feb 2009. ISSN 1555-7162 (Electronic). doi: 10.1016/j.amjmed.2008.09.014.

[3] Peck Y Ong and Donald Y M Leung. Immune dysregulation in atopic dermatitis. *Curr Allergy Asthma Rep*, 6(5):384–389, Sep 2006. ISSN 1529-7322 (Print).

[4] WR Brown and AE Hansen. Arachidonic acid and linoleic acid of the serum in normal and eczematous human subjects. *Proc Soc Exp Biol Med*, 36:113–17, 1937.

[5] M. S. Manku, D. F. Horrobin, N. Morse, V. Kyte, K. Jenkins, S. Wright, and J. L. Burton. Reduced levels of prostaglandin precursors in the blood of atopic patients: defective delta-6-desaturase function as a biochemical basis for atopy. *Prostaglandins Leukot Med*, 9(6):615–28, 1982.

[6] C. J. van Gool, M. P. Zeegers, and C. Thijs. Oral essential fatty acid supplementation in atopic dermatitis-a meta-analysis of placebo-controlled trials. *Br J Dermatol*, 150(4):728–40, 2004.

[7] P. C. Calder. Polyunsaturated fatty acids and inflammation. *Biochem Soc Trans*, 33(Pt 2):423–7, 2005.

[8] C. N. Serhan, C. B. Clish, J. Brannon, S. P. Colgan, K. Gronert, and N. Chiang. Anti-microinflammatory lipid signals generated from dietary n-3 fatty acids via cyclooxygenase-2 and transcellular processing: a novel mechanism for NSAID and n-3 PUFA therapeutic actions. *J Physiol Pharmacol*, 51(4 Pt 1):643–54, 2000.

[9] S. Hong, K. Gronert, P. R. Devchand, R. L. Moussignac, and C. N. Serhan. Novel docosatrienes and 17S-resolvins generated from docosahexaenoic acid in murine brain, human blood, and glial cells. Autacoids in anti-inflammation. *J Biol Chem*, 278(17):14677–87, 2003.

[10] Charles N Serhan, Rong Yang, Kimberly Martinod, Kie Kasuga, Padmini S Pillai, Timothy F Porter, Sungwhan F Oh, and Matthew Spite. Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J Exp Med*, 206(1):15–23, Jan 2009. doi: 10.1084/jem.20081880.

[11] C N Serhan and N Chiang. Endogenous pro-resolving and anti-inflammatory lipid mediators: a new pharmacologic genus. *Br J Pharmacol*, 153 Suppl 1:S200–15, Mar 2008. doi: 10.1038/sj.bjp.0707489.

[12] A. J. Chu, M. A. Walton, J. K. Prasad, and A. Seto. Blockade by polyunsaturated n-3 fatty acids of endotoxin-induced monocytic tissue factor activation is mediated by the depressed receptor expression in THP-1 cells. *J Surg Res*, 87(2):217–24, 1999.

[13] T. R. Billiar, P. E. Bankey, B. A. Svingen, R. D. Curran, M. A. West, R. T. Holman, R. L. Simmons, and F. B. Cerra. Fatty acid intake and kupffer cell function: fish oil alters eicosanoid and monokine production to endotoxin stimulation. *Surgery*, 104(2):343–9, 1988.

[14] S. Sadeghi, F. A. Wallace, and P. C. Calder. Dietary lipids modify the cytokine response to bacterial lipopolysaccharide in mice. *Immunology*, 96(3):404–10, 1999.

[15] P. Yaqoob and P. Calder. Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. *Cell Immunol*, 163(1):120–8, 1995.

[16] FA Wallace, EA Miles, and PC Calder. Comparison of the effects of linseed oil and different doses of fish oil on mononuclear cell function in healthy human subjects. *British Journal of Nutrition*, 89(5):679–89, 2003.

[17] T. Trebble, N. K. Arden, M. A. Stroud, S. A. Wootton, G. C. Burdge, E. A. Miles, A. B. Ballinger, R. L. Thompson, and P. C. Calder. Inhibition of tumour necrosis factor-alpha and interleukin 6 production by mononuclear cells following dietary fish-oil supplementation in healthy men and response to antioxidant co-supplementation. *Br J Nutr*, 90(2):405–12, 2003.

[18] R. Abbate, A. M. Gori, F. Martini, T. Brunelli, M. Filippini, I. Francalanci, R. Paniccia, D. Prisco, G. F. Gensini, and G. G. Neri Serneri. n-3 PUFA supplementation, monocyte PCA expression and interleukin-6 production. *Prostaglandins Leukot Essent Fatty Acids*, 54(6):439–44, 1996.

[19] S. N. Meydani, S. Endres, M. M. Woods, B. R. Goldin, C. Soo, A. Morrill-Labrode, C. A. Dinarello, and S. L. Gorbach. Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. *J Nutr*, 121(4):547–55, 1991.

[20] E. A. Miles, F. A. Wallace, and P. C. Calder. Dietary fish oil reduces intercellular adhesion molecule 1 and scavenger receptor expression on murine macrophages. *Atherosclerosis*, 152(1):43–50, 2000.

[21] E. A. Miles, F. Thies, F. A. Wallace, J. R. Powell, T. L. Hurst, E. A. Newsholme, and P. C. Calder. Influence of age and dietary fish oil on plasma soluble adhesion molecule concentrations. *Clin Sci (Lond)*, 100(1):91–100, 2001.

[22] R. De Caterina and P. Libby. Control of endothelial leukocyte adhesion molecules by fatty acids. *Lipids*, 31 Suppl:S57–63, 1996.

[23] C. L. Curtis, S. G. Rees, C. B. Little, C. R. Flannery, C. E. Hughes, C. Wilson, C. M. Dent, I. G. Otterness, J. L. Harwood, and B. Caterson. Pathologic indicators of degradation and inflammation in human osteoarthritic cartilage are abrogated by exposure to n-3 fatty acids. *Arthritis Rheum*, 46(6):1544–53, 2002.

[24] Y. Zhao, S. Joshi-Barve, S. Barve, and L. H. Chen. Eicosapentaenoic acid prevents LPS-induced TNF-alpha expression by preventing NF-kappaB activation. *J Am Coll Nutr*, 23(1):71–8, 2004.

[25] J. A. Ross, A. G. Moses, and K. C. Fearon. The anti-catabolic effects of n-3 fatty acids. *Curr Opin Clin Nutr Metab Care*, 2(3):219–26, 1999.

[26] C. J. Lo, K. C. Chiu, M. Fu, R. Lo, and S. Helton. Fish oil decreases macrophage tumor necrosis factor gene transcription by altering the NF kappa B activity. *J Surg Res*, 82(2):216–21, 1999.

[27] Linda M Arterburn, Eileen Bailey Hall, and Harry Oken. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *Am J Clin Nutr*, 83(6 Suppl):1467S–1476S, Jun 2006.

[28] P. C. Calder and R. B. Zurier. Polyunsaturated fatty acids and rheumatoid arthritis. *Curr Opin Clin Nutr Metab Care*, 4(2):115–21, 2001.

[29] V. A. Ziboh, K. A. Cohen, C. N. Ellis, C. Miller, T. A. Hamilton, K. Kragballe, C. R. Hydrick, and J. J. Voorhees. Effects of dietary supplementation of fish oil on neutrophil and epidermal fatty acids. Modulation of clinical course of psoriatic subjects. *Arch Dermatol*, 122(11):1277–82, 1986.

[30] A. Bjørneboe, E. Søyland, G. E. Bjørneboe, G. Rajka, and C. A. Drevon. Effect of dietary supplementation with eicosapentaenoic acid in the treatment of atopic dermatitis. *Br J Dermatol*, 117(4):463–9, 1987.

[31] B Kunz, J Ring, and O Braun-Falco. Eicosapentaenoic acid (EPA) treatment in atopic eczema (AE): a prospective double-blind trial. *J Allergy Clin Immunol*, 83: 196, 1989.

[32] E. Søyland, J. Funk, G. Rajka, M. Sandberg, P. Thune, L. Rustad, S. Helland, K. Middelfart, S. Odu, E. S. Falk, K. Solvoll, G. E. A. Bjørneboe, and C. A. Drevon. Dietary supplementation with very long-chain n-3 fatty acids in patients with atopic dermatitis. A double-blind, multicentre study. *Br J Dermatol*, 130(6): 757–764, 1994.

[33] J. Berth-Jones and R. A. Graham-Brown. Placebo-controlled trial of essential fatty acid supplementation in atopic dermatitis. *Lancet*, 341(8860):1557–60, 1993.

[34] J T Bamford, R W Gibson, and C M Renier. Atopic eczema unresponsive to evening primrose oil (linoleic and gamma-linolenic acids). *J Am Acad Dermatol*, 13(6):959–965, 1985 Dec. ISSN 0190-9622 (Print).

[35] A Bordoni, P L Biagi, M Masi, G Ricci, C Fanelli, A Patrizi, and E Ceccolini. Evening primrose oil (efamol) in the treatment of children with atopic eczema. *Drugs Exp Clin Res*, 14(4):291–297, 1988. ISSN 0378-6501 (Print).

[36] B. M. Henz, S. Jablonska, P. C. van de Kerkhof, G. Stingl, M. Blaszczyk, P. G. Vandervalk, R. Veenhuizen, R. Muggli, and D. Raederstorff. Double-blind, multicentre analysis of the efficacy of borage oil in patients with atopic eczema. *Br J Dermatol*, 140(4):685–8, 1999.

[37] P F Morse, D F Horrobin, M S Manku, J C Stewart, R Allen, S Littlewood, S Wright, J Burton, D J Gould, and P J Holt. Meta-analysis of placebo-controlled studies of the efficacy of epogam in the treatment of atopic eczema. Relationship between plasma essential fatty acid changes and clinical response. *Br J Dermatol*, 121(1):75–90, 1989 Jul. ISSN 0007-0963 (Print).

[38] M Schalin-Karrila, L Mattila, C T Jansen, and P Uotila. Evening primrose oil in the treatment of atopic eczema: effect on clinical status, plasma phospholipid fatty acids and circulating blood prostaglandins. *Br J Dermatol*, 117(1):11–19, 1987 Jul. ISSN 0007-0963 (Print).

[39] Swapan Senapati, Sabyasachi Banerjee, and Dwijendra Nath Gangopadhyay. Evening primrose oil is effective in atopic dermatitis: a randomized placebo-controlled trial. *Indian J Dermatol Venereol Leprol*, 74(5):447–452, 2008 Sep-Oct. ISSN 0973-3922 (Electronic).

[40] A. Takwale, E. Tan, S. Agarwal, G. Barclay, I. Ahmed, K. Hotchkiss, J. R. Thompson, T. Chapman, and J. Berth-Jones. Efficacy and tolerability of borage oil in adults and children with atopic eczema: randomised, double blind, placebo controlled, parallel group trial. *Bmj*, 327(7428):1385, 2003.

[41] S Wright and J L Burton. Oral evening-primrose-seed oil improves atopic eczema. *Lancet*, 2(8308):1120–1122, 1982 Nov 20. ISSN 0140-6736 (Print).

[42] J.M. Hanifin and G Rajka. Diagnostic features of atopic dermatits. *Acta Dermatol Venerol*, pages 44–47, 1980.

[43] P. Yaqoob. Monounsaturated fats and immune function. *Braz J Med Biol Res*, 31 (4):453–65, 1998.

[44] Severity scoring of atopic dermatitis: the SCORAD index. Consensus Report of the European Task Force on Atopic Dermatitis. *Dermatology*, 186(1):23–31, 1993.

[45] T. L. Diepgen, M. Fartasch, and O. P. Hornstein. Evaluation and relevance of atopic basic and minor features in patients with atopic dermatitis and in the general population. *Acta Derm Venereol Suppl (Stockh)*, 144:50–4, 1989.

[46] SF-36.org. A community for measuring health outcomes using sf tools, accessed 02/25/2010, 12:26 at http://www.sf-36.org/tools/sf36.shtml.

[47] Monika Bullinger and Inge Kirchberger. *SF-36 Fragebogen zum Gesundheitszustand (Handanweisung)*. Hogrefe-Verlag, Göttingen, 1998.

[48] U Ellert and B M Bellach. The SF-36 in the federal health survey–description of a current normal sample. *Gesundheitswesen*, 61 Spec No:S184–90, Dec 1999. ISSN 0941-3790 (Print).

[49] Mahshid Dehghan and Anwar T Merchant. Is bioelectrical impedance accurate for use in large epidemiological studies? *Nutr J*, 7:26, 2008. ISSN 1475-2891 (Electronic). doi: 10.1186/1475-2891-7-26.

[50] P. Mayser, K. Mayer, M. Mahloudjian, S. Benzing, H. J. Kramer, W. B. Schill, W. Seeger, and F. Grimminger. A double-blind, randomized, placebo-controlled trial of n-3 versus n-6 fatty acid-based lipid infusion in atopic dermatitis. *JPEN J Parenter Enteral Nutr*, 26(3):151–8, 2002.

[51] A. Gimenez-Arnau, C. Barranco, M. Alberola, C. Wale, S. Serrano, M. R. Buchanan, and J. G. Camarasa. Effects of linoleic acid supplements on atopic dermatitis. *Adv Exp Med Biol*, 433:285–9, 1997.

[52] W. Meigel, T. Dettke, E. M. Meigel, and U. Lenze. [additional oral therapy of atopic dermatitis with unsaturated fatty acids]. *Z Hautkr*, 62 Suppl 1:100–3, 1987.

[53] S Tanaka, O Saitoh, K Tabata, R Matsuse, K Kojima, K Sugi, K Nakagawa, M Kayazawa, T Teranishi, K Uchida, I Hirata, and K Katsu. Medium-chain fatty acids stimulate interleukin-8 production in Caco-2 cells with different mechanisms from long-chain fatty acids. *J Gastroenterol Hepatol*, 16(7):748–54, Jul 2001.

[54] Aihiro Hoshimoto, Yasuo Suzuki, Tatsuro Katsuno, Hiroshi Nakajima, and Yasushi Saito. Caprylic acid and medium-chain triglycerides inhibit IL-8 gene transcription in Caco-2 cells: comparison with the potent histone deacetylase inhibitor trichostatin A. *Br J Pharmacol*, 136(2):280–6, May 2002. doi: 10.1038/sj.bjp.0704719.

[55] Daniel O Clegg, Domenic J Reda, Crystal L Harris, Marguerite A Klein, James R O'Dell, Michele M Hooper, John D Bradley, Clifton O Bingham, 3rd, Michael H Weisman, Christopher G Jackson, Nancy E Lane, John J Cush, Larry W Moreland, H Ralph Schumacher, Jr, Chester V Oddis, Frederick Wolfe, Jerry A Molitor, David E Yocum, Thomas J Schnitzer, Daniel E Furst, Allen D Sawitzke, Helen Shi, Kenneth D Brandt, Roland W Moskowitz, and H James Williams. Glucosamine, chondroitin sulfate, and the two in combination for painful knee osteoarthritis. *N Engl J Med*, 354(8):795–808, Feb 2006. doi: 10.1056/NEJMoa052771.

[56] Edzard Ernst. Placebo: new insights into an old enigma. *Drug Discov Today*, 12 (9-10):413–8, May 2007. doi: 10.1016/j.drudis.2007.03.007.

[57] Jeffrey L Sugarman. The epidermal barrier in atopic dermatitis. *Semin Cutan Med Surg*, 27(2):108–14, Jun 2008. doi: 10.1016/j.sder.2008.04.005.

[58] Do-Won Kim, Jee-Young Park, Gun-Yoen Na, Seok-Jong Lee, and Weon-Ju Lee. Correlation of clinical features and skin barrier function in adolescent and adult patients with atopic dermatitis. *Int J Dermatol*, 45(6):698–701, Jun 2006. doi: 10.1111/j.1365-4632.2005.02644.x.

[59] Jayanta Gupta, Emilie Grube, Mark B Ericksen, Michelle D Stevenson, Anne W Lucky, Anita P Sheth, Amal H Assa'ad, and Gurjit K Khurana Hershey. Intrinsically defective skin barrier function in children with atopic dermatitis correlates with disease severity. *J Allergy Clin Immunol*, 121(3):725–730.e2, Mar 2008. doi: 10.1016/j.jaci.2007.12.1161.

[60] S Seidenari and G Giusti. Objective assessment of the skin of children affected by atopic dermatitis: a study of pH, capacitance and TEWL in eczematous and clinically uninvolved skin. *Acta Derm Venereol*, 75(6):429–33, Nov 1995.

[61] A Firooz, F Gorouhi, P Davari, M Atarod, S Hekmat, M Rashighi-Firoozabadi, and A Solhpour. Comparison of hydration, sebum and pH values in clinically normal skin of patients with atopic dermatitis and healthy controls. *Clin Exp Dermatol*, 32(3):321–2, May 2007. doi: 10.1111/j.1365-2230.2007.02364.x.

[62] Paul-Gunther Sator, Jolanta B Schmidt, and Herbert Hönigsmann. Comparison of epidermal hydration and skin surface lipids in healthy individuals and in patients with atopic dermatitis. *J Am Acad Dermatol*, 48(3):352–8, Mar 2003. doi: 10.1067/mjd.2003.105.

[63] U Heinrich, U Koop, M-C Leneveu-Duchemin, K Osterrieder, S Bielfeldt, C Chkarnat, J Degwert, D Häntschel, S Jaspers, H-P Nissen, M Rohr, G Schneider, and H Tronnier. Multicentre comparison of skin hydration in terms of physical-, physiological- and product-dependent parameters by the capacitive method (Corneometer CM 825). *Int J Cosmet Sci*, 25(1-2):45–53, Apr 2003. doi: 10.1046/j.1467-2494.2003.00172.x.

[64] Silvana Coghi, Maria Cecilia Bortoletto, S A P Sampaio, Heitor Franco de Andrade Junior, and Valeria Aoki. Quality of life is severely compromised in adult patients with atopic dermatitis in brazil, especially due to mental components. *Clinics (Sao Paulo)*, 62(3):235–42, Jun 2007.

[65] E A Holm, H C Wulf, H Stegmann, and G B E Jemec. Life quality assessment among patients with atopic eczema. *Br J Dermatol*, 154(4):719–25, Apr 2006. doi: 10.1111/j.1365-2133.2005.07050.x.

[66] Gordon Parker, Neville A Gibson, Heather Brotchie, Gabriella Heruc, Anne-Marie Rees, and Dusan Hadzi-Pavlovic. Omega-3 fatty acids and mood disorders. *Am J Psychiatry*, 163(6):969–78, Jun 2006. doi: 10.1176/appi.ajp.163.6.969.

[67] F. V. Velzing-Aarts, F. R. van der Klis, F. P. van der Dijs, C. M. van Beusekom, H. Landman, J. J. Capello, and F. A. Muskiet. Effect of three low-dose fish oil supplements, administered during pregnancy, on neonatal long-chain polyunsaturated fatty acid status at birth. *Prostaglandins Leukot Essent Fatty Acids*, 65(1): 51–7, 2001.

[68] W S Harris. n-3 fatty acids and serum lipoproteins: human studies. *Am J Clin Nutr*, 65(5 Suppl):1645S–1654S, May 1997.

[69] G Schuetze, K Storm van's Gravesande, S Sparhold, T Frischer, and J Kuehr. Comparison between serial skin-prick tests and specific serum immunoglobulin E to mite allergens. *Pediatr Allergy Immunol*, 10(2):138–42, May 1999.

[70] S Hoff, H Seiler, J Heinrich, I Kompauer, A Nieters, N Becker, G Nagel, K Gedrich, G Karg, G Wolfram, and J Linseisen. Allergic sensitisation and allergic rhinitis are associated with n-3 polyunsaturated fatty acids in the diet and in red blood cell membranes. *Eur J Clin Nutr*, 59(9):1071–80, Sep 2005. doi: 10.1038/sj.ejcn.1602213.

[71] Guy B Marks, Seema Mihrshahi, Andrew S Kemp, Euan R Tovey, Karen Webb, Catarina Almqvist, Rosario D Ampon, Daniel Crisafulli, Elena G Belousova, Craig M Mellis, Jennifer K Peat, and Stephen R Leeder. Prevention of asthma during the first 5 years of life: a randomized controlled trial. *J Allergy Clin Immunol*, 118(1):53–61, Jul 2006. doi: 10.1016/j.jaci.2006.04.004.

[72] Janet A Dunstan and Susan L Prescott. Does fish oil supplementation in pregnancy reduce the risk of allergic disease in infants? *Curr Opin Allergy Clin Immunol*, 5 (3):215–21, Jun 2005.

[73] C Anandan, U Nurmatov, and A Sheikh. Omega 3 and 6 oils for primary prevention of allergic disease: systematic review and meta-analysis. *Allergy*, 64(6):840–8, Jun 2009. doi: 10.1111/j.1398-9995.2009.02042.x.

Appendix A

Erlangen Atopy Score: 0-3 points = no atopic skin diathesis; 4-9 points = atopic diathesis possible; 10-13 points = atopic skin diathesis probable; more than 14 points = atopic skin diathesis

Item	Max. points
Atopic family history (1st degree relatives)	
Eczema	2
Rhinitis/Asthma	1
Atopic patient history	
Flexural exzema	1
Rhinitis/Conjunctivitis	1
Allergic asthma	1
Infantile eczema	3
Itching while sweating	3
Textile intolerance	1
Metal intolerance	1
Atopic minimal forms	
Xerosis (dry skin)	3
Ear rhagades	2
Dyshydrosis	2
Nipple eczema	2
Perlèche (stomatitis angularis)	1
Pulpitis sicca	1
Atopic stigmata	
Hyperlinearity of the palms	2
Hertoghe's sign	2
White dermographism	3
"Dirty neck" – dry neck skin	2
Laboratory values	
IgE over 150 U/ml	1
IgE over 400 U/ml	2
	Max. total: 37

Appendix B

Patients diary: one sample page of the diary patients were given to report self assessment of their symptoms.

Pat-Initialen: □□ Codierung: □□□□□

Patiententagebuch für den Zeitraum:

vom: □□.□□.□□ (Montag) bis: □□.□□.□□ (Sonntag)

Wochentag	**Montag**	**Dienstag**	**Mittwoch**	**Donner-stag**	**Freitag**	**Samstag**	**Sonntag**
Symptome	**Stärke des Auftretens der Symptome (0 – gar nicht; 10 – sehr stark)**						
Juckreiz							
Hautrötung							
Trockenheit							
Hautgesamt-zustand							
Sonstige **gesundheit-liche Probleme** (bitte genau angeben, z.B. Kopfschmerz							
Medikamente	**Häufigkeit der Anwendungen an diesem Tag**						
eventuelle Nebenwir-kungen der Kapseln (z.B. Aufstoßen)							
sonstige Bemerkungen, Besonderheiten							

List of Abbreviations

AD	Atopic dermatitis
ALT	Alanine Transaminase
AP	Alkaline Phosphatase
ASD	Atopic Skin Diathesis
BSA	Bovine Serum Albumin
CI95%	Confidence Interval at 95%
COX	Cyclooxigenase
DHA	Docosahexaenoic acid
ELISA	Enzyme-Linked ImmunoSorbent Assay
EPA	Eicosapentaenoic acid
FA	Fatty Acid
GCP	Good Clinical Practice
GGT	Gamma-Glutamyltransferase
HDL	High density lipoprotein
IκB-α	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
ICAM	Inter Cell Adhesion Molecule
Ig	Immunoglobulin, Subtypes A, D, E, G, M
IL	Interleukin
LDL	Low Density Lipoprotein
LOX	Lipoxygenase
mRNA	Messenger Ribonucleic Acid
n-3-FA	ω-3 Fatty Acids

n-6-FA	ω-6 Fatty Acids
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate-buffered saline
PUFA	Polyunsaturated fatty acids
RAST	Radioallergosorbent Test
SF-36®	Short Form Health Survey with 36 items
SPSS	Statistical Package for the Social Sciences
SPT	Skin Prick Test
TAG	Triacylglycerol
TC	Total cholesterol
TEWL	Transepidermal Water Loss
TNF	Tumor Necrosis Factor
VCAM	Vascular Cell Adhesion Molecule

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Lebenslauf

Mein Lebenslauf wird aus Gründen des Datenschutzes in der elektronischen Version meiner Arbeit nicht veröffentlicht.

Publikationsliste

Koch C, Dölle S, **Metzger M**, Rasche C, Jungclas H, Rühl R, Renz H, Worm M: Docosahexaenoic acid (DHA) supplementations in atopic eczema: a randomized double-blind, controlled trial. Br J Dermatol. 2008 Apr; 158 (4): 786-92. Epub 2008 Jan 30

Erklärung

„Ich, Johannes Manuel Metzger, erkläre, dass ich die vorgelegte Dissertation mit dem Thema: „Efficacy Of Docosahexaenoic Acid In Patients With Atopic Dermatitis“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

Berlin, den 16. Februar 2011

J. Manuel Metzger