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Macular carotenoids and long-chain ω-3 fatty acids in patients with age-related macular degeneration and the potential role of xanthophyll-rich food in disease prevention

Dissertation

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ALLES IST EINFACHER, ALS MAN DENKEN KANN,

ZUGLEICH VERSCHRÄNKTER, ALS ZU BEGREIFEN IST.

[Goethe, *Maximen*]

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Abbreviations

AA	Arachidonic acid
ABTS	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
AMD	Age-related macular degeneration
AREDS	Age-Related Eye Disease Study
BMI	Body mass index
CHC	Common household cooking
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
EPIC	European Investigation into Cancer and Nutrition
FAME	Fatty acid methyl ester
GC	Gas chromatography
HDL-C	High density lipoprotein cholesterol
HPLC	High pressure liquid chromatography
HPP	High pressure processing
HSS	Heat steam sterilisation
H-TEAC	Hydrophilic Trolox equivalent antioxidant capacity
LC-PUFA	Long-chain polyunsaturated fatty acids
LDL-C	Low density lipoprotein cholesterol
L-TEAC	Lipophilic Trolox equivalent antioxidant capacity
Lutega	Long term effects of lutein/zeaxanthin and ω-3 supplementation on optical density of AMD patients
Max OD	Maximal optical density
Mean OD	Mean optical density
MPOD	Macular pigment optical density
NS	Not significant
OD	Optical density of the macular pigment xanthophyll
ODU	Optical density units
RPE	Retinal pigment epithelium
SD	Standard deviation
Wk	Week

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1 Introduction

1.1 Age-related macular degeneration

The advances in health knowledge leading to prevention and treatment of infectious and non-infectious diseases resulted in large decreases in mortality over the last decades (CANNING 2011). The lengthened life expectancy accounts for an increase in age-associated diseases (LUNENFELD AND STRATTON 2013). Consequently, the prevalence of non-communicable diseases like diabetes, cancer, and cardiovascular diseases increases (UNITED NATIONS GENERAL ASSEMBLY 2011). This also applies to age-related macular degeneration (AMD) (VINGERLING *ET AL.* 1995). The chronic malady mostly affects people after 50 years of age and impairs the visual performance of the central field of vision (AUGOOD *ET AL.* 2006, BIRD *ET AL.* 1995). In developed countries, AMD is the primary cause of blindness and ranks third worldwide (RESNIKOFF *ET AL.* 2004). Until today, prevention and therapy strategies like medical or surgical treatments are limited.

The disease affects the human retina, which provides sensitive vision through the transduction of visible electromagnetic radiation into variations of photoreceptors´ membrane potential and the processing of the visual signals. As reviewed by BIRD (2010), the neural retina consists of ganglion cell layer, inner nuclear layer, outer nuclear layer (containing cell bodies and nuclei of rode and cone photoreceptors), and the photoreceptor outer segments, which separate the opsin-containing discs from the inner segments by a cilium abutting the retinal pigment epithelium (RPE) (**Figure 1**). The RPE is a monolayer of pigmented cells. It supports the visual function via transporting water, ions, and metabolic end products from subretinal space to the blood and by providing photoreceptors with oxygen and nutrients (glucose, retinol, fatty acids) (STRAUSS 2005). The RPE is located on Bruch´s membrane, which is the innermost layer of the choroid, specialised on metabolic exchange. Due to their function, rods (well-lit conditions) and cones (dim light conditions) are unevenly distributed across the retina. Densely packed cones in the central retina (macula) enable high acuity vision. The fovea (centre of the macula) is rod-free (MASLAND 2001). Towards the periphery of the retina the number of rods increases.

Figure 1: Structure of the human retina. (**a**) Schematic presentation of the human eye. (**b**) Cross-section of the human retina, showing its laminated structure, which consists of: (1) ganglion cell layer, (2) inner nuclear layer, (3) outer nuclear layer or photoreceptor layer, (4) photoreceptor outer segments, and (5) retinal pigment epithelium. (**c**) Higher resolution schematic of the retinal pigment epithelium and the Bruch´s membrane showing: (1) the photoreceptor outer pigment tips, (2) the retinal pigment epithelium cells, (3) Bruch´s membrane, and (4) choroidal capillaries. Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Genetics (WRIGHT *ET AL.* 2010), copyright 2010.

`The unique structural and functional organization of the vertebrate retina is finely adapted to the initial capture and processing of visual signals, but this organization makes is unusually vulnerable to dysfunction´ (WRIGHT ET AL. 2010, p 274). Two types of AMD are classified: The dry and the wet form (**Figure 2**). Accumulations of extracellular aggregates (drusen) in the area around the central retina are signs of

the early, asymptotic stage of this disease. The late stage of this non-exudative (dry) form of AMD is called geographic atrophy. Characteristic areas of degenerations of RPE cells and photoreceptors cause some degrees of visual impairment (AMBATI AND FOWLER 2012) and affects the majority of patients. Neovascularisation is typical for the advanced (wet) form of the disease and is mainly responsible for severe vision loss from AMD. The process of the disease is insidious and the dry form usually precedes the wet form. The loss of the central field of vision accounts for considerable restrictions and for a reduction in quality of life.

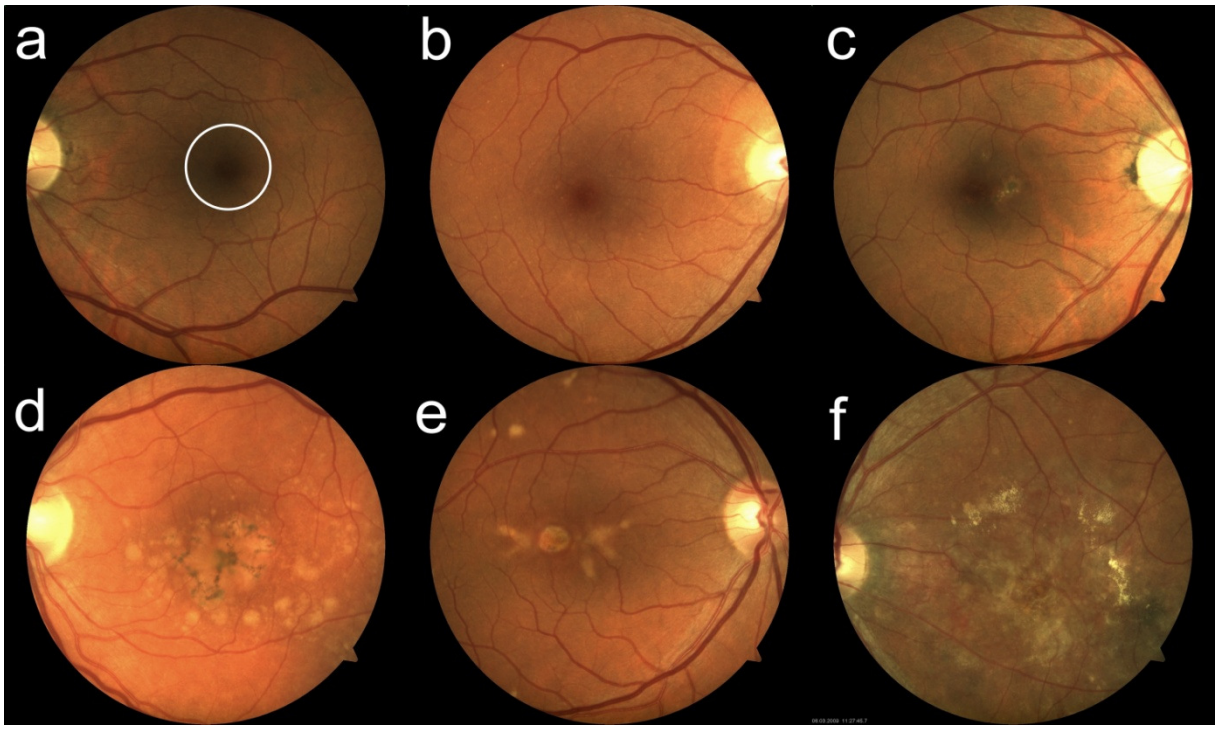

Figure 2: Photographs of the ocular fundus illustrating a healthy eyeground (**a**) and different stages and forms of age-related macular degeneration (**b-f**) after the age-related eye disease study (AREDS) classification system (AGE-RELATED EYE DISEASE STUDY RESEARCH GROUP 2001). (a) healthy (encircled macular pigment), (b) stage I with individual hard drusen, (c) stage II with small drusen and changes in retinal pigment epithelium, (d) stage III with large soft drusen and changes in retinal pigment epithelium, (e) stage IV with confluating drusen areas and central geographic atrophy, and (f) neovascularization.

Depending on the form of the disease, diverse causes and mechanisms are discussed. However, the pathogenesis of AMD is not as well-defined as that of other

maladies (e.g. cancer) (AMBATI AND FOWLER 2012). As reviewed by AMBATI AND FOWLER (2012), toxic accumulations like lipofuscin in the RPE or between RPE and Bruch´s membrane are the molecular trait of dry AMD. Moreover, the authors held immunovascular processes responsible for neovascularisation. Finally, retinal degradation is due to photoreceptor cell death which may be a result of light damage, lipid oxidation, neuroinflammation, bioenergetic dysfunction and metabolic stress (WRIGHT *ET AL.* 2010).

The main risk factor for AMD is ageing, but a variety of other risk factors has been identified (AGE-RELATED EYE DISEASE STUDY RESEARCH GROUP 2000, CHAKRAVARTHY *ET AL.* 2010) including genetic polymorphisms (FRITSCHE *ET AL.* 2010, SEDDON *ET AL.* 2009), family history of AMD (KLAVER *ET AL.* 1998, KLEIN *ET AL.* 1994), gender (SMITH *ET AL.* 1997), cardiovascular risk factors like arterial hypertension (HOGG *ET AL.* 2008), atherosclerosis (TUÑÓN *ET AL.* 2009), estrogens (SMITH *ET AL.* 1997), sunlight exposure (FLETCHER *ET AL.* 2008), body mass index (SEDDON *ET AL.* 2003), smoking tobacco (CHAKRAVARTHY *ET AL.* 2007, CHRISTEN *ET AL.* 1996, SEDDON *ET AL.* 1996), and nutritional habits (AGTE AND TARWADI 2010, CHONG *ET AL.* 2008). Especially the last two factors can be influenced and can therefore be used for prevention strategies. Several studies showed that an unbalanced diet was associated with an increased risk of AMD (GOLDBERG *ET AL.* 1988, SEDDON *ET AL.* 1994). Preventive effects of various nutrients seem likely. Especially zinc, selenium, bioflavonoids, carotenoids, and vitamins A, C, and E may act protective (O'CONNELL *ET AL.* 2008).

1.2 Xanthophylls

The detection of the yellow spot (*macula lutea*) in the retina and the finding that it is solely composed of the xanthophylls lutein, zeaxanthin, and *meso*-zeaxanthin (BONE *ET AL.* 1985, LANDRUM AND BONE 2001) suggest a special role in visual performance. Lutein, zeaxanthin, and *meso*-zeaxanthin accumulate in the Henle fibre layer of the central retina (fovea) (SNODDERLY 1995) and are known as the `macular pigment´. Their presence in human tissues is completely of dietary origin because only higher plants, algae, bacteria, and fungi are able to synthesise carotenoids *de novo* (FRASER AND BRAMLEY 2004). Lutein and zeaxanthin are mainly found in dark green leafy vegetables but also in egg yolk and various fruits and herbs (MAIANI *ET AL.* 2009). Spinach, broccoli, lettuce, and eggs are the main contributors to lutein plus zeaxan-

thin intake in Europe (GRANADO *ET AL.* 2003). The carotenoid content in food depends among other things on storage and processing conditions. In European countries, the median dietary intake of lutein plus zeaxanthin varies between 1.56 mg/d (Republic of Ireland) and 3.25 mg/d (Spain) (MAIANI *ET AL.* 2009). *meso*-Zeaxanthin is usually not found in the human diet and it is absent in human plasma (BONE *ET AL.* 1993). Hence, the high concentrations of *meso*-zeaxanthin in the macula are attributed to its conversion from dietary lutein in the eye (KHACHIK *ET AL.* 2002).

The special characteristic of these oxygenated carotenoids is their spectral absorption profile based on the presence of conjugated double bonds in the polyene chain (**Figure 3**).

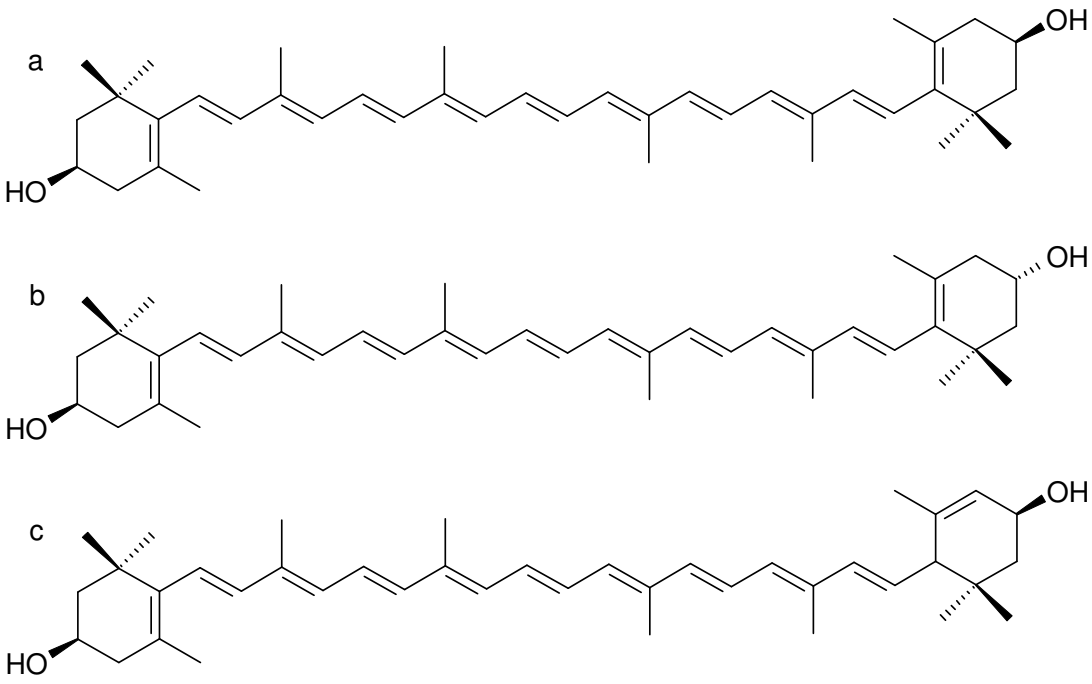

Figure 3: Xanthophylls found in the macula. (a) Zeaxanthin (3*R*,3´*R*-dihydroxy-β,β-carotene), (b) *meso*-zeaxanthin (3*R*,3´*S*-dihydroxy-β,β-carotene), and (c) lutein (3*R,*3´*R,*6´*R*-dihydroxy-β,ε-carotene).

The absorption maximum is at wavelengths of about 460 nm. Before light reaches photoreceptors, RPE or choroid, it must pass through the *macula lutea*. Whereas UV radiation is effectively absorbed by cornea and lens, mainly blue light is filtered out by the macular pigment. About 60% of blue light at 460 nm is absorbed (SCHALCH *ET AL.* 2009). Hence, lutein and zeaxanthin act as an optical filter improving glare and en-

hancing contrast (STRINGHAM *ET AL.* 2010). Since even visible blue light (next to UV radiation) is high-energy, it is capable to induce photochemical damage by promoting the formation of reactive oxygen species within the highly aerobic retina (SCHALCH *ET AL.* 2009). Thus, the absorption of blue light reduces the generation of radicals. Besides optical mechanisms, xanthophylls also possess biological functions including lipophilic antioxidant activity (KRINSKY *ET AL.* 2003). Lutein and zeaxanthin are able to quench singlet oxygen and to act as chain breaking antioxidants (SNODDERLY 1995). Since oxidative stress causes photoreceptor cell death and retinal degeneration, a protective role of lutein and zeaxanthin seems plausible. This is further supported by the finding that patients with age-related maculopathies (including AMD) exhibit lower amounts of macular pigment xanthophylls than healthy persons (BONE *ET AL.* 2001, OBANA *ET AL.* 2008).

1.3 Fatty acids

The enhanced formation of radicals in the retina arises from high oxygen-consuming photoreceptors (RATTNER AND NATHANS 2006), light exposition, and from a high content of long-chain polyunsaturated fatty acids (LC-PUFA) in the disc membranes of photoreceptors (STRAUSS 2005). Omega-3 LC-PUFA like docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (**Figure 4**) appear to play a special role in visual performance and eye health. First, DHA is the main structure molecule of photoreceptors and is selectively concentrated in the disc membranes of the outer segment. It is assumed that DHA provides the essential membrane fluidity for photo transduction (JEFFREY *ET AL.* 2001). Secondly, ω-3 LC-PUFA promote survival of photoreceptors (ROTSTEIN *ET AL.* 1996) probably through an oxidative stress induced synthesis of neuroprotective mediators derived from DHA (BAZAN 2007).

Due to a lack of Δ12 and Δ15 desaturases, higher animals and humans are not able to create double bonds between the ninth carbon and the methyl end of the fatty acid. Therefore, precursors of DHA and EPA, linoleic and α-linolenic acid, are essential and required in diet. Since elongation and desaturation processes are inefficient, DHA and EPA are also termed as `essential fatty acids´ in humans (RUIZ-LOPEZ *ET AL.* 2012).

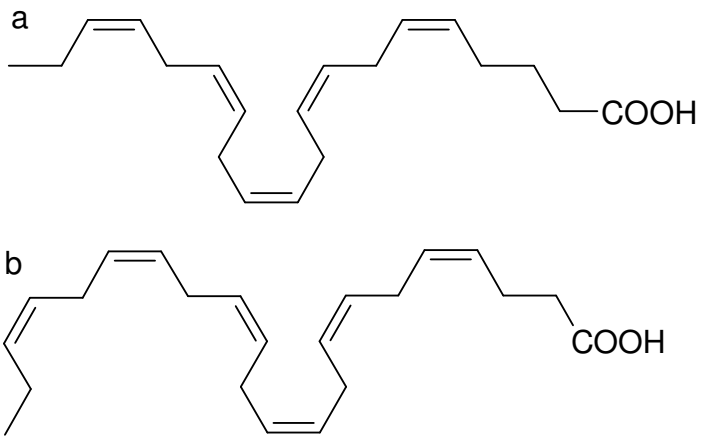

Figure 4: Long-chain ω-3 fatty acids. (a) Eicosapentaenoic acid (20:5 n3) and (b) Docosahexaenoic acid (22:6 n3).

The recommendations for total EPA and DHA intake vary between 200 and 500 mg/d (EUROPEAN FOOD SAFETY AUTHORITY 2009, MOZAFFARIAN AND WU 2012). In adults of European countries, the estimated average intake of EPA plus DHA varies between 80 and 420 mg/d (EUROPEAN FOOD SAFETY AUTHORITY 2009). The primary producer of ω-3 LC-PUFA is marine phytoplankton (BERGE AND BARNATHAN 2005). Hence, marine food chain makes mackerel, herring, and salmon to the most important dietary sources of ω-3 LC-PUFA (MOZAFFARIAN AND WU 2012, STROBEL *ET AL*. 2012).

2 Aims and Scope

Several studies reveal that the concentration of lutein and zeaxanthin in the *macula lutea* is inversely associated with the risk of AMD and/or its progression (AGE-RELATED EYE DISEASE STUDY RESEARCH GROUP 2007, BONE *ET AL.* 2000, CIULLA *ET AL.* 2001, CURRAN-CELENTANO *ET AL.* 2001, SABOUR-PICKETT *ET AL.* 2012). Additionally, ω-3 LC-PUFA may also protect against AMD (CHRISTEN *ET AL.* 2011, MERLE *ET AL.* 2011, TAN *ET AL.* 2009).

Hence, the following specific aims can be formulated for the present thesis:

Study 1: Investigation of the effects of a 12-month supplementation with lutein, zeaxanthin, and ω-3 LC-PUFA on xanthophylls and fatty acids in plasma, antioxidant capacity, and the optical density of the macular pigment of patients with non-exudative AMD.

Study 2: Investigation of the effects of an intervention with a non-purified, non-saponified oleaginous extract of kale on the concentrations of macular xanthophylls in plasma and macula of AMD patients during a 10-week study (2-week run-in, 4-week intervention, 4-week wash-out).

Examination 3: Investigation of the effects of food processing on the contents of carotenoids and chlorophylls in selected food with special regard to lutein and zeaxanthin.

In addition, the potential role of dietary xanthophylls in disease prevention is discussed.

3 Schedule of manuscripts

Manuscript I

Macular xanthophylls and ω-3 long-chain polyunsaturated fatty acids in age-related macular degeneration

Arnold C, Winter L, Fröhlich K, Jentsch S, Dawczynski J, Jahreis G, and Böhm V (2013): Macular xanthophylls and omega-3 long-chain polyunsaturated fatty acids in age-related macular degeneration: A randomised trial. *JAMA Ophthalmology* 131: 564-572.

Manuscript II

Age-related macular degeneration: Effects of a short-term intervention with an oleaginous kale extract - a pilot study.

Arnold C, Jentsch S, Dawczynski J, and Böhm V (2013): Age-related macular degeneration: Effects of a short-term intervention with an oleaginous kale extract - a pilot study. *Nutrition* 29: 1412-1417.

Manuscript III

Effects of food processing on the concentrations of xanthophylls and chlorophylls.

Arnold C, Schwarzenbolz U, and Böhm V

[Accepted for publication in *LWT - Food Science and Technology*]

3.1 Manuscript I

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4 Discussion

The parameters determined in studies 1 and 2 are summarised in the table below to give a short and informative overview. The composition of the supplements can be found in the respective manuscripts (study 1: chapter 3.1 and study 2: chapter 3.2).

Table 1: Overview of study parameters

		Study 1			Study 2	
		Placebo n=40	Group 1 n=50	Group 2 n=55	Placebo n=10	Verum n=10
Plasma						
	Lutein and zeaxanthin	✓	✓	✓	✓	✓
	Fatty acids	✓	✓	✓		
	Antioxidant capacity	✓	✓	✓		
MPOD						
	Volume	✓	✓	✓	✓	✓
	Area				✓	✓
	max OD				✓	✓
	mean OD				✓	✓

MPOD, optical density of the macular pigment xanthophyll; max OD, maximum optical density; mean OD, mean optical density.

4.1 Alteration of plasma xanthophyll concentrations in patients with age-related macular degeneration after supplementation with lutein and zeaxanthin

The alteration of plasma circulating macular xanthophylls following daily use of a supplement containing lutein and zeaxanthin was assessed by two human intervention studies (studies 1 and 2). The trials differed in terms of study duration, number of participants, as well as type and composition of supplements. Study 1 (entitled as Lutega) was conducted as a 12-month, randomised, double-blind, placebo-controlled, parallel clinical trial. One hundred seventy-two patients with non-exudative AMD were randomly assigned to one of three study groups: group 1 (receiving capsules providing 10 mg lutein, 1 mg zeaxanthin, 100 mg DHA, 30 mg EPA per day), group 2 (twice the dose of group 1), and placebo group. The study was completed by 145 patients according the trial protocol. Study 2 was also a randomised, double-blind, placebo-controlled, parallel clinical trial and lasted ten weeks for each patient (2-week run-in, 4-week intervention, 4-week wash-out). Twenty patients with non-exudative AMD were randomly assigned to one of two study groups receiving either a

beverage with an oleaginous extract of kale (providing 10 mg lutein and 3 mg zeaxanthin) or refined rapeseed oil (placebo).

Lutein and zeaxanthin concentrations in the plasma of AMD patients were analysed via normal-phase HPLC using an amino column. Astaxanthin served as internal standard. Given that astaxanthin is present in seafood (salmonids, arctic shrimp, and algae) and commercially available food supplements, the usefulness of astaxanthin as internal standard was a point of criticism in the peer-review process. The major counter-argument is that participants of studies 1 and 2 were instructed to abstain from dietary supplements. Subjects reporting a consumption of supplements were excluded from the study collective. In random checks, the concentrations of astaxanthin in the plasma of the participants were well below the limit of quantification. In addition, recent studies also failed to detect astaxanthin in human plasma samples prior to an intervention (CORAL-HINOSTROZA *ET AL.* 2004, ØSTERLIE *ET AL.* 2000). Besides that, astaxanthin is structurally related to lutein and zeaxanthin and therefore exhibits a similar behaviour against outside influences during sample preparation. Finally, astaxanthin was an appropriate internal standard for the current analyses.

At baseline, the groups within study 1 and within study 2 had comparable plasma concentrations of lutein and zeaxanthin. After four weeks of intervention, the plasma circulating macular xanthophylls increased significantly in both studies (capsules in study 1: 10 or 20 mg lutein plus 1 or 2 mg zeaxanthin and kale extract in study 2: 10 mg lutein plus 3 mg zeaxanthin). Due to the designs of the studies the following statements can be made: First, the concentrations of lutein and zeaxanthin in plasma of AMD patients reached a plateau after four weeks of intervention. A continued intake over one year did not lead to further alterations (study 1). Secondly, after four weeks of wash-out the circulating macular xanthophylls in plasma decreased significantly but did not reach the initial values again (study 2). Thirdly, an oleaginous extract of kale containing mainly esterified lutein (6.6 mg out of 10 mg) is as effective as the single dose of the supplement in study 1 (10 mg free lutein) to increase the concentrations of lutein in plasma in four weeks of intervention (study 1: from 0.22±0.15 to 0.60±0.32 µmol/L; study 2: from 0.34±0.11 to 1.18±0.34 µmol/L). Thus, the bioavailability of lutein esters is not reduced in comparison to free lutein. The results are confirmed by several studies conducted previously. It has been reported that esterifi-

cation does not impair the bioavailability of lutein in humans (BOWEN *ET AL.* 2002). THÜRMANN *ET AL.* (2005) showed by means of a human intervention study (19 healthy participants) that a supplementation with 4.1 mg lutein for 42 days increased plasma lutein concentrations approximately 3.5-fold. Unfortunately, the authors did not provide clear information whether free or esterified lutein was used. However, from the context it can be inferred that free lutein was utilized. In the present work, the concentrations of lutein in plasma increased approximately 2.7-fold in study 1 (free lutein) and 3.5-fold in study 2 (66% esterified lutein) after four weeks of intervention. Hence, lower doses of additional lutein like 4 mg seems to be an effective alternative to the common 10 mg lutein found in many commercially available supplements. The increase of lutein to plateau concentrations within four weeks (study 1) and the time of return to baseline after cessation of the supplementation between 20 and 50 days was found previously (BONE *ET AL.* 2003, LANDRUM *ET AL.* 1997, THÜRMANN *ET AL.* 2005). Finally, there is need of a continuous and adequate uptake of macular xanthophylls in order to increase and to maintain the circulating levels in human plasma.

4.2 Alteration of macular pigment optical density in patients with age-related macular degeneration after supplementation with lutein and zeaxanthin

The MPOD was detected with a fundus camera using a 1-wavelength reflection method. Four distribution parameters of the MPOD can be determined: maximal optical density (max OD), mean optical density (mean OD), volume, and area. The methodology is discussed controversially since intra-ocular scatter impairs reflectometric measurements (SCHWEITZER *ET AL.* 2010). Therefore, the developers of the method examined the alteration of ocular stray light with age and its influence on the calculated MPOD. The MPOD was corrected by an age-dependent term. In addition, the coefficient of variation is below 6% before stray-light correction. Within the Lutega study, alterations of the MPOD via 1-wavelength reflection method were successfully demonstrated in AMD patients (ARNOLD *ET AL.* 2013, DAWCZYNSKI *ET AL.* 2013). Hence, the method employed is objective and appropriate for the measurement of the MPOD.

At baseline, the volume of the macular pigment did not differ between the groups within study 1 and within study 2. In study 1, the volume was significantly elevated in both active treatments groups (single and double dose, capsules containing the

macular xanthophylls lutein and zeaxanthin in their unesterified form). This is in accordance with scientific literature (GARCIA-LAYANA *ET AL.* 2013, MURRAY *ET AL.* 2013, RICHER *ET AL.* 2007, SABOUR-PICKETT *ET AL.* 2012). In comparison to placebo group, the values of groups 1 and 2 were significantly higher during the entire study. In study 1, only the volume of the macular pigment was considered (**Table 1**). A second publication regarding the results of the Lutega study includes all measured distribution parameters. DAWCZYNSKI *ET AL.* (2013) showed, that all MPOD parameters increased significantly. Due to the intervention with an oleaginous extract of kale, a significant increase was observed for max OD, volume, and area. For example, the volume increased by about 12% following four weeks of intervention. Four weeks after cessation of the intervention eight percentage points of this increase remained. The alterations of the max OD and the area of the macular pigment xanthophyll were comparable to these results. The mean OD remained unchanged.

Taken together, studies 1 and 2 showed that the MPOD increased significantly and to the same extend (study 1: 1.31-fold, study 2: 1.13-fold) following a 4-week intervention with either capsules containing free lutein and zeaxanthin or a non-purified and non-saponified oleaginous extract of kale. In contrast to the circulating xanthophyll levels in plasma, a prolonged intake over one year led to a slight but continuous increase of the volume of the macular pigment (study 1). This might be a result of a sufficient concentration of lutein and zeaxanthin in plasma for xanthophyll accumulation in the retina. Briefly, the retina exhibits the highest concentration of lutein and zeaxanthin (0.1-1.0 mM) (LANDRUM *ET AL.* 1999) in comparison to other body tissues like liver (0.1-3.0 µM), kidney (0.037-2.1 µM), and lung (0.1-2.3 µM) (INSTITUTE OF MEDICINE 2000) . Hence, a selective retinal capture and storage of lutein and zeaxanthin is obvious (BONE *ET AL.* 1988, EVANS *ET AL.* 2013). The Pi isoform of glutathione S-transferase was identified as a human retinal zeaxanthin-binding protein (LOANE *ET AL.* 2008). MNL 64, which belongs to steroidonic acut regulatory domain protein family, acts as a lutein-binding protein (LI *ET AL.* 2011). These proteins facilitate the capture of lutein and zeaxanthin from the bloodstream to the macula and stabilise lipophilic xanthophylls in retinal cell structures (membranes, cytosol, cytoskeleton) (YEMELYANOV *ET AL.* 2001).

The 4-week wash-out period in study 2 resulted in a significant decline of the MPOD (area, volume, and max OD). Thus far, several studies demonstrated that the MPOD remains stable after intervention with macular carotenoids ceases, even when plasma circulating xanthophyll concentrations declined (LANDRUM *ET AL.* 1997, THURNHAM 2007). The significant decrease of max OD, area, and volume of the MPOD in study 2 give a first cautious hint to a more dynamic distribution of xanthophylls in the macula. The discrepancy to other published studies is maybe due to different techniques and methodologies employed. For that reason, further clinical controlled trials with larger study collectives and different methodologies of macular pigment measurement are urgently required to verify the findings of the present work.

4.3 Effects of a supplementation with long-chain ω-3 fatty acids on the profile of fatty acids in plasma of patients with age-related macular degeneration

In contrast to the quantitative determination of macular xanthophylls in plasma, ω-3 LC-PUFA were expressed as the percentage of the total peak area of all fatty acid methyl esters (%FAME). The major limitation of study 1 is the absence of study groups ingesting only ω-3 LC-PUFA or only macular xanthophylls to examine single and additive effects. However, intervention with the fixed combination of ω-3 LC-PUFA and macular xanthophylls in group 1 (study 1) resulted in a significant decrease of arachidonic acid, whereas EPA, DHA, and the sum of ω-3 fatty acids increased significantly. The ingestion of the double dose in group 2 was furthermore potent to increase the sums of polyunsaturated fatty acids and to decrease the sums of saturated and monounsaturated fatty acids significantly. No alterations were observed in placebo group. After 12 months of intervention, patients of group 2 exhibited significantly higher EPA and DHA circulating levels than patients in group 1 or placebo group. The changes in group 1 did not reach statistical significance in comparison to placebo group. In contrast to other studies, the amounts of ω-3 LC-PUFA tested in study 1 are relatively low (e.g. 60 mg EPA+200 mg DHA (double dose) *vs.* 700 mg EPA+400 mg DHA (DAWCZYNSKI *ET AL.* 2009) and 440 mg EPA+310 mg DHA (DAWCZYNSKI *ET AL.* 2012)). Despite the differences in concentrations and time of intervention, changes in fatty acid profile regarding arachidonic acid and DHA were comparable with the results of DAWCZYNSKI *ET AL.* (2009). Since AMD is associated with atherogenesis (TUÑÓN *ET AL.* 2009) and inflammation (WHITCUP *ET AL.* 2013), the

alterations to a more anti-atherogenic and anti-inflammatory fatty acid profile in both treatment groups are considered to be beneficial.

As reviewed by LAWRENSON AND EVANS (2012), current available evidence does not support an increased intake of ω-3 LC-PUFA for primary and secondary prevention of AMD. This was confirmed by AREDS 2 (AGE-RELATED EYE DISEASE STUDY RESEARCH GROUP 2013). The authors showed by the means of a multi-centre, randomised, double-masked, placebo-controlled study including 4203 patients at risk for progression to advanced AMD, that supplementation with 350 mg DHA and 650 mg EPA did not significantly reduce the progression to advanced AMD. However, some evidence arises from animal and observational studies showing an inverse association between the dietary intake of ω-3 LC-PUFA and the risk of developing or progression to advanced AMD (CHRISTEN *ET AL.* 2011, KOTO *ET AL.* 2007, SANGIOVANNI *ET AL.* 2009, TAN *ET AL.* 2009, TUO *ET AL.* 2009). Finally, the role of ω-3 LC-PUFA in retina has been intensively studied (KISHAN *ET AL.* 2011, SANGIOVANNI AND CHEW 2005) and beneficial effects of an increased intake seem to be plausible. Nonetheless, they are not supported by the available data from randomised clinical trials and consequently recommendations remain speculative.

4.4 Alteration of antioxidant capacity in plasma of patients with age-related macular degeneration after supplementation with lutein, zeaxanthin, vitamins C, and E

In study 1, the antioxidant capacity was analysed in plasma of 30 randomly selected AMD patients (10 per group). A lipophilic and a hydrophilic version of the Trolox equivalent antioxidant capacity assay and the photochemiluminescence assay (lipophilic) were conducted to test the influence of the intervention on the antioxidant capacity in plasma. In principal, antioxidant capacity assays are classified as assays based on hydrogen atom transfer or electron transfer (HUANG *ET AL.* 2005). The TEAC assay belongs to the class of electron transfer assays, whereas the photochemiluminescence assay depends on hydrogen atom transfer reactions (HUANG *ET AL.* 2005). Hence, the present work covers both mechanisms. Antioxidant capacity assays are often a target of criticism. The main issue in this context is the measurement of a sum parameter and the fact that values of different assays are hardly comparable due to different mechanistic principals. For the standardisation of the relative contribution of

the values obtained in different assays, the calculation of a weighted mean has been developed (MÜLLER *ET AL.* 2011). However, antioxidant capacity assays are appropriate to show alterations in plasma following nutritional interventions either by comparing baseline and *post*-intervention values in one group or by the comparison of different study groups. The major limitation of the current analysis is the reduced number of participants included (30 out of 145). However, representativeness for the whole study collective is assumed.

On the one hand the interventions in study 1 did not lead to changes in the hydrophilic antioxidant capacity in the plasma of AMD patients, which is based on the composition of the administered supplement. Besides 60 mg (group 1) and 120 mg (group 2) of vitamin C the supplement did not contain further hydrophilic antioxidants. The constant hydrophilic antioxidant capacity may be a result of a sufficient supply of vitamin C in Germany (SCHULZE *ET AL.* 2001) and the renal excretion of excessive doses. On the other hand, the intervention increased the lipophilic antioxidant capacity in group 1 and group 2 significantly due to the presence of lutein, zeaxanthin, and vitamin E in the supplement. However, antioxidant capacity assays are not potent to provide information about the contribution of the single substances on the antioxidative status. As reported by SHEN *ET AL.* (2012), AMD is associated with a lower antioxidative status, basing upon the comparison of healthy and diseased subjects. With respect to the lipophilic antioxidant capacity, the probable beneficial effect of an adequate antioxidative status may arise from higher amounts of carotenoids in plasma and thus likely in macula. In addition, substances contributing to the antioxidative capacity may provide further metabolic functions relevant to AMD including neuroprotective effects of tocopherols (FRANK *ET AL.* 2012) and vitamers like β-carotene.

4.5 Nutritional suggestions regarding age-related macular degeneration

The findings obtained in studies 1 and 2 arise the question whether a modified diet might be a favourable alternative to supplements for persons high at risk for developing AMD and/or progression to advanced AMD. Commercially available supplements mainly contain 6 to 10 mg of lutein plus about 1 to 2 mg zeaxanthin. These doses are comparable to those administered in studies 1 and 2. Furthermore supplements containing *meso*-zeaxanthin are available. However, there are rich natural sources of lutein like eggs (0.4-1.3 mg/100 g egg yolk), dark green leafy vegetables including

spinach (5.9-7.9 mg/100 g fresh matter) and kale (4.8-11.5 mg/100 g fresh matter) (MAIANI *ET AL.* 2009). An exotic plant providing remarkable amounts of zeaxanthin is Chinese wolfberry (82 mg/100 g dried berries) (WELLER AND BREITHAUPT 2003).
Since common xanthophyll-rich foods like spinach and kale are often consumed in processed forms, examination 3 (manuscript III) of the present work dealt with the effects of three food processing techniques on the contents of lutein and zeaxanthin in selected xanthophyll-rich foods. The effects of heat steam sterilisation (HSS), high pressure processing (HPP), and common household cooking (CHC) were tested. The analyses showed that HSS decreased the concentrations of lutein in kale, parsley, and dill significantly. Due to an improved extractability from food matrix after HSS and a higher stability of zeaxanthin against thermal treatment, the concentrations of zeaxanthin were significantly increased (BEHSNILIAN *ET AL.* 2006, MAYER-MIEBACH AND BEHSNILIAN 2007). In sum, HSS resulted in a significant reduction of the xanthophyll concentrations (lutein plus zeaxanthin) in spinach, parsley, and dill. In contrast, treatment via HPP did not reduce the concentrations of lutein and zeaxanthin in a xanthophyll-rich food sample (spinach puree). Hence it is a good alternative to HSS for the industrial preservation of xanthophyll-rich food.

Given that spinach is one of the main contributors to xanthophyll intake in Europe (GRANADO *ET AL.* 2003) and that it is mainly prepared at private households, the contents of lutein and zeaxanthin after CHC were determined. Cooked spinach contained 7.8 mg lutein and 1.1 mg zeaxanthin per 100 g fresh matter. Thus, a typical serving size of spinach (150 g) provides approximately 13.5 mg macular xanthophylls. The *in vitro* bioavailability of lutein from cooked green leafy vegetables ranges between 10 and 72% (CHANDRIKA *ET AL.* 2010) and it is considerably higher than that of β-carotene (12 to 43%) (CHANDRIKA *ET AL.* 2010, VAN HET HOF *ET AL.* 1999). Conclusively, absorption of 1.2 to 8.4 mg lutein per spinach serving can be assumed. VAN HET HOF *ET AL.* (1999) conducted a human intervention study with 54 healthy subjects consuming either a high-vegetable diet (490 g/d), a low-vegetable diet (130 g/d), or a supplement (6 mg β-carotene and 9 mg lutein per day). Based on the change of plasma lutein concentrations (0.041 µmol/L per mg dietary lutein and 0.062 µmol/L per mg supplemental lutein, respectively), the authors showed that the relative bioavailability of dietary lutein is 67% in comparison to supplemental lutein. The increased bioavailability of supplemental lutein might be due to the presence of oil as

carrier substance in the supplement. Interestingly, lutein from egg yolk was more bioavailable than pure lutein from supplements, lutein-ester supplements, and spinach in human subjects (CHUNG *ET AL.* 2004, THURNHAM 2007). Since lutein in egg yolk is located in a lipid matrix, it is more easily released than lutein from the protein complexes found in chloroplasts of leafy vegetables (BURNS-WHITMORE *ET AL.* 2010, TYSSANDIER *ET AL.* 2003). Insofar, several xanthophyll-rich foods are available and exhibit an acceptable bioavailability. As discussed in chapter 4.1, lower doses of additional lutein like 4 mg per day seems to be an effective alternative to the common 10 mg lutein found in many commercially available supplements. Therefore, xanthophyll-rich foods can replace the use of supplements to a large extend.

But indeed, the current practice of eye care professionals from the United Kingdom is to recommend nutritional supplements to patients at risk of progression to advanced AMD (LAWRENSON AND EVANS 2013). The authors found that the advice to eat a plenty of green leafy vegetables and to increase the intake of marine fish to twice a week is mainly given to people at risk to develop AMD. Hence, AMD is linked to nutritional advices. LAWRENSON AND EVANS (2013) pointed out that eye professionals should consider whether their advices are supported by the recent available evidence. Due to a systematic review and meta-analysis of CHONG *ET AL.* (2007), the evidence for primary prevention via dietary and supplemental antioxidants of early AMD is insufficient. In contrast, evidence for secondary prevention comes from AREDS (EVANS AND LAWRENSON 2012, WONG *ET AL.* 2011). The study group demonstrated a 25% reduction in the 5-year progression to advanced AMD by the use of a supplement containing extraordinary high doses of vitamin A (as β-carotene, 28.6 IU), zinc (69.6 mg), copper (1.6 mg), vitamins E (400 IU), and C (452 mg) (AGE-RELATED EYE DISEASE STUDY RESEARCH GROUP 2001). In concordance, EVANS AND LAWRENSON (2012) reviewed that AMD patients may benefit from a supplementation with antioxidant vitamins and minerals through a delay in progression. However, AUGUSTIN AND SCHMIDT-ERFURTH (2002) criticised that the AREDS initially intended to test the effects of lutein (not β-carotene) on the progression of AMD. Only organisational and economic reasons have led to the incorporation of β-carotene into the supplement. Besides that, they criticised the statistical procedure. Finally, AUGUSTIN AND SCHMIDT-ERFURTH (2002) agreed that the study supports the finding, that AMD is

associated with oxidative processes but they recommended to interpret the AREDS results with caution.

Moreover, `there is no evidence to date that the general population should take antioxidant vitamin and mineral supplements to prevent or delay the onset of AMD´ (EVANS AND HENSHAW 2008, p 2). The authors raise awareness of the possible harmful effects of vitamin supplements and required a systematic review on the harms of vitamin supplements. Particularly, smokers should abstain from supplements containing β-carotene because of the potentially increased risk of lung and stomach cancer (DRUESNE-PECOLLO *ET AL.* 2010). Similarly, in AREDS 2 more lung cancer cases occurred in former smokers achieving β-carotene (AGE-RELATED EYE DISEASE STUDY RESEARCH GROUP 2013). The study group of this multicenter, randomised, double-masked, placebo-controlled trial concluded that lutein and zeaxanthin are suitable substitutes for β-carotene in the AREDS formula. Initially, AREDS 2 was conducted to investigate the effects of a modified AREDS formula on the risk of developing advanced AMD. The 4203 participants were randomly assigned to achieve lutein plus zeaxanthin (10+2 mg), DHA plus EPA (350+650 mg), a combination of both, or placebo in addition to the original AREDS formulation. The primary analyses showed that the addition of lutein and zeaxanthin, DHA and EPA, or both combined did not result in a further reduction of AMD progression compared to AREDS 1 (AGE-RELATED EYE DISEASE STUDY RESEARCH GROUP 2001, 2013).

In fact, the results of different studies are conflicting. Several research groups reported that elevated lutein and zeaxanthin concentrations in diet and thus in plasma and macula may provide beneficial effects for persons with or at risk for AMD (BUCHELI *ET AL.* 2011, DELCOURT *ET AL.* 2006, JOHNSON *ET AL.* 2008, SEDDON *ET AL.* 1994, SNELLEN *ET AL.* 2002, ZEIMER *ET AL.* 2009). In contrast, other studies did not support the findings (FLETCHER *ET AL.* 2008, FLOOD *ET AL.* 2002, MARES-PERLMAN *ET AL.* 1995, MARES-PERLMAN *ET AL.* 1996, SANDERS *ET AL.* 1993). It is noticeable that particularly observational studies did not find relationships between the intake of xanthophylls and the risk of developing AMD or progression to advanced AMD. This is in contrast to the results of numerous randomised, controlled intervention studies and might be due to the character of the two study types. Since the validity of observational studies is lower than that of randomised, controlled intervention studies, beneficial effects of lutein and zeaxanthin are assumed. Even if no risk assessment can

be derived from the Lutega study, it reveals that AMD patients benefit from an elevated intake of lutein and zeaxanthin by an improved visual acuity (DAWCZYNSKI *ET AL.* 2013). This is in concordance with RICHER *ET AL.* (2011) and MURRAY *ET AL.* (2013). According to LAWRENSON AND EVANS (2012), an increased intake of ω-3 LC-PUFA for the prevention of AMD is not supported by the available randomised trials. Contrary, WEIKEL *ET AL.* (2012) found that `a significant body of observational epidemiological data indicates that increased consumption of long-chain omega-3 fatty acids (EPA, DHA) reduces risk for neovascular as well as early AMD´ (p 333).

Finally, the potential effects of dietary and supplemental macular xanthophylls and ω-3 LC-PUFA in AMD prevention have a physiological and an epidemiological rationale but remains to be verified for explicit recommendations in clinical practice. Conclusively, it is difficult to give precise nutritional advices. However, a modified diet regarding AMD consists of foods rich in lutein and zeaxanthin (e.g. eggs, spinach, kale), vitamins E (e.g. nuts, wheat germ oil) and C (e.g. fennel, citrus fruits), β-carotene (e.g. bell pepper, carrots), as well as ω-3 LC-PUFA (herring, mackerel). Finally, a practicable nutritional concept for the prevention of AMD should be adapted to individual habits, disorders, physical impairments, and socio-economic factors. For individuals with problems to ingest adequate levels of the above mentioned nutrients by their diet, the intake of supplements should be considered. However, the lower costs and the less excessive amounts of potential harmful antioxidants are the most important advantages of a modified diet in comparison to supplements.

4.6 Conclusion

The present work investigated the effects of macular carotenoids and ω-3 LC-PUFA in patients with AMD and discussed the potential role of xanthophyll-rich food in disease prevention. Due to the studies that have been conducted, the following conclusion can be drawn. First, the intake of macular xanthophylls either from capsules containing free lutein and zeaxanthin or a non-purified and non-saponified oleaginous extract of kale increased the concentrations of lutein and zeaxanthin in plasma of AMD patients. Secondly, the plasma circulating xanthophylls reached a plateau after four weeks of intervention (study 1) and a continued intake over one year did not lead to further alterations. Thirdly, a 4-week wash-out period led to a significant decline of lutein and zeaxanthin in plasma (study 2). Fourthly, an oleaginous extract of kale is

as effective as the supplement in study 1 to increase the concentrations of lutein in plasma in four weeks of intervention. Fifthly, following a 4-week intervention with lutein and zeaxanthin, the MPOD was elevated in studies 1 and 2, whereas a prolonged intake over one year (study 1) led to a slight but continuous increase of the volume of the macular pigment. Comparable to the plasma xanthophyll concentrations, a 4-week wash-out period resulted in a decline of the MPOD (study 2). Sixthly, intervention with the fixed combination of ω-3 LC-PUFA and macular xanthophylls in study 1 resulted in a decrease of arachidonic acid, whereas EPA, DHA, and the sum of ω-3 fatty acids increased in plasma. The intake of the double dose (group 2) was furthermore potent to increase the sums of polyunsaturated fatty acids and to decrease the sums of saturated and monounsaturated fatty acids significantly. Seventhly, the interventions with the supplement capsules in study 1 did not lead to changes in the hydrophilic antioxidant capacity in the plasma of AMD patients. However, the intervention increased the lipophilic antioxidant capacity significantly. Regarding the contents of xanthophylls in diet, it was shown that HPP is a good alternative to HSS for the industrial processing of xanthophyll-rich food. Furthermore, CHC preserves the concentrations of lutein and zeaxanthin in food and a typical serving size of cooked green leafy vegetables provides considerable amounts of lutein and zeaxanthin. Hence, xanthophyll-rich foods can replace the use of supplements to a large extend and are advantageous due to the potential harmful effects of supplemental antioxidants.

5 Summary

Background

Age-related macular degeneration (AMD) is the primary cause of blindness in developed countries and mostly affects people after 50 years of age. Due to the lengthened life expectancy the cases of AMD will increase steadily. Several studies reveal that the intake of dietary or supplemental lutein and zeaxanthin and thus the concentrations in plasma and macula are inversely associated with the risk of AMD and/or its progression. Additionally, long-chain omega-3 fatty acids (ω-3 LC-PUFA) may also be protective.

Objectives

The aims of the present thesis were to investigate the effects of lutein, zeaxanthin, and ω-3 LC-PUFA on plasma circulating xanthophylls and fatty acids, the antioxidant capacity in plasma, and the optical density of the macular pigment (MPOD) in patients with non-exudative AMD. Furthermore, the influence of three different food processing techniques on the contents of carotenoids in selected food was determined.

Methods

Study 1 was a randomised, double-blind, placebo-controlled, parallel trial and was conducted for 12 months. A total of 172 AMD patients were randomly divided into three study groups: placebo group, group 1 (10 mg lutein, 1 mg zeaxanthin, 100 mg docosahexaenoic acid (DHA), 30 mg eicosapentaenoic acid (EPA)), and group 2 (twice the dose of group 1). The main outcome measures were: plasma xanthophyll concentrations and fatty acid profile, hydrophilic and lipophilic antioxidant capacity and the volume of the macular pigment.

Study 2 was a randomised, double-blind, and parallel trial and lasted ten weeks for each participant (2-week run-in, 4-week intervention, 4-week wash-out). Twenty AMD patients were randomly divided into two groups. All participants consumed daily 50 mL of a beverage either enriched with an oleaginous extract of kale (10 mg lutein, 3 mg zeaxanthin) or refined rapeseed oil (placebo). The main outcome measures were the plasma xanthophyll concentrations and four distribution parameters of the macular pigment.

Examination 3 determined the effects of heat steam sterilisation (HSS), high pressure processing (HPP), and common household cooking (CHC) on the contents of carotenoids and chlorophylls in selected xanthophyll-rich foods. For HSS, the samples (kale, parsley, and dill) were heated in an autoclave for 5, 10, 15, and 20 min at 121°C. A spinach puree was treated by means of a high pressure pilot plant at 200, 400, and 600 MPa for 5, 10, and 40 min at room temperature. For CHC, frozen leafy spinach was cooked according the instructions of the manufacturer.

Results

The intake of macular xanthophylls either from capsules containing free lutein and zeaxanthin or a non-purified and non-saponified oleaginous extract of kale increased the concentrations of lutein and zeaxanthin in plasma of AMD patients and reached a plateau after four weeks of intervention (study 1). Cessation of the intervention (4-week wash-out) resulted in a decline of lutein and zeaxanthin in plasma (study 2). Moreover, an oleaginous extract of kale was as effective as the supplement to increase the concentrations of lutein in plasma after four weeks of intervention. The MPOD was elevated in studies 1 and 2. Study 2 stands out due to the significant decline of the MPOD after intervention ceases. Furthermore, intervention with the fixed combination of ω-3 LC-PUFA and macular xanthophylls in study 1 decreased the amounts of arachidonic acid in plasma of AMD patients, whereas the amounts of EPA, DHA, and the sum of ω-3 fatty acids increased. The intake of the double dose (group 2) additionally increased the sums of polyunsaturated fatty acids and decreased the sums of saturated and monounsaturated fatty acids. The hydrophilic antioxidant capacity in the plasma of AMD patients remained stable following the interventions in study 1 but the lipophilic antioxidant capacity increased. Regarding the contents of xanthophylls in preserved food, the contents of lutein in kale, parsley, and dill were decreased by HSS, whereas the contents remained stable after HPP and CHC in spinach.

Conclusions

The intake of macular xanthophylls either from capsules containing pure lutein and zeaxanthin or a non-purified and non-saponified oleaginous extract of kale increased the concentrations of lutein and zeaxanthin in plasma and the MPOD of patients with non-exudative AMD. With regard to the decline of MPOD after cessation of the intervention in study 2, the distribution of xanthophylls in the macula is more dynamic than originally assumed. Additionally, the lipophilic antioxidant capacity was elevated following the intervention in study 1. Furthermore it was shown that HPP is an alternative to HSS for the industrial processing of xanthophyll-rich food. Since common household cooking preserves the contents of lutein and zeaxanthin, a typical serving size of cooked green leafy vegetables provides considerable amounts of lutein and zeaxanthin. Hence, xanthophyll-rich foods can replace the use of supplements to a large extend and are advantageous due to the potential harmful effects of supplemental antioxidants.

Zusammenfassung

Hintergrund

In den Industrieländern ist die altersbezogene Makuladegeneration (AMD) die häufigste Ursache für schwere Sehbehinderungen im Alter und betrifft vorwiegend Menschen jenseits des 50. Lebensjahres. Aufgrund der steigenden Lebenserwartung nimmt die Zahl der Betroffenen stetig zu. Studien zeigen, dass die Aufnahme der Xanthophylle Lutein und Zeaxanthin und damit deren Konzentrationen im Plasma und in der Makula invers mit dem AMD-Risiko assoziiert sind. Außerdem werden protektive Effekte von langkettigen ω-3 Fettsäuren (ω-3 LC-PUFA) hinsichtlich des AMD-Risikos diskutiert.

Zielstellung

Die vorliegende Dissertation untersuchte die Effekte einer zusätzlichen Aufnahme von Lutein, Zeaxanthin und ω-3 LC-PUFA bei Patienten mit nichtexsudativer AMD. Dazu wurden die Konzentrationen von Lutein, Zeaxanthin und ω-3 LC-PUFA sowie die antioxidative Kapazität im Plasma ermittelt. Zusätzlich wurde der Einfluss der Supplementation auf die optische Dichte des makulären Pigmentes (MPOD) bestimmt. Ein Hauptziel war der Vergleich zwischen der Wirkung eines Supplement mit frei vorliegenden Xanthophyllen und einem öligen Grünkohlextrakt mit hauptsächlich verestert vorliegenden Xanthophyllen. Außerdem wurde der Einfluss von drei verschiedenen Verarbeitungstechniken auf die Carotinoidgehalte in ausgewählten Lebensmitteln untersucht.

Methoden

Studie 1 war eine zwölfmonatige, randomisierte, doppelblinde, placebokontrollierte Studie im Paralleldesign, an der 172 Patienten mit nichtexsudativer AMD teilnahmen. Die Teilnehmer wurden zufällig auf drei Studiengruppen verteilt: Placebo, Gruppe 1 (10 mg Lutein, 1 mg Zeaxanthin, 100 mg Docosahexaensäure (DHA), 30 mg Eicosapentaensäure (EPA)) oder Gruppe 2 (doppelte Dosis von Gruppe 1). Neben den Konzentrationen von Lutein und Zeaxanthin wurden das Fettsäurespektrum sowie die hydrophile und die lipophile antioxidative Kapazität im Plasma und die optische Dichte des makulären Pigmentes (MPOD) bestimmt.
Studie 2 war eine randomisierte, doppelblinde, placebokontrollierte Studie im Paralleldesign. Zwanzig Patienten mit nichtexsudativer AMD wurden für die zehnwöchige Studie (zwei Wochen Run-in, vier Wochen Intervention, vier Wochen Washout) rekrutiert und zufällig auf zwei Gruppen verteilt. Alle Patienten tranken täglich 50 mL eines Getränkes, welches entweder mit einem öligen Grünkohlextrakt (Verum) oder raffiniertem Rapsöl (Placebo) angereichert wurde. In dieser Studie wurden sowohl die Konzentrationen von Lutein und Zeaxanthin im Plasma als auch die MPOD bestimmt.

Weiterhin wurde der Einfluss von Dampfsterilisation, Hochdruckbehandlung und haushaltsüblicher Speisenzubereitung auf die Carotinoidgehalte im Lebensmittel untersucht. Für die Dampfsterilisation wurden ausgewählte xanthophyllreiche Lebensmittel (Grünkohl, Petersilie und Dill) für 5, 10, 15 und 20 min bei 121 °C in einem Autoklaven behandelt. Außerdem wurde ein Spinatpüree bei 200, 400 und 600 MPa für 5, 10 und 40 min bei Raumtemperatur hochdruckbehandelt, daneben wurde tiefgekühlter Blattspinat nach Packungsanleitung zubereitet.

Ergebnisse

Die zusätzliche Aufnahme von Lutein und Zeaxanthin über ein Supplement oder einen öligen Grünkohlextrakt steigerte die Konzentrationen beider Xanthophylle im Plasma von AMD-Patienten signifikant, wobei nach vierwöchiger Zufuhr ein Plateau erreicht wurde (Studie 1). Nach Beendigung der Supplementation (vier Wochen Washout) sanken die Lutein- und Zeaxanthinkonzentrationen im Plasma signifikant ab (Studie 2). Eine vierwöchige Intervention mit dem Supplement oder dem Grünkohlextrakt führte zu vergleichbaren Anstiegen der Xanthophyllkonzentrationen im Plasma. In den Studien 1 und 2 wurde die MPOD signifikant gesteigert, wobei die MPOD nach Absetzen des Grünkohlextraktes wieder absank (Studie 2). Ferner führte die Intervention in Studie 1 zum Absinken des Gehaltes an Arachidonsäure und zum Anstieg der Gehalte an EPA, DHA und der Summe der ω-3-Fettsäuren im Plasma der AMD-Patienten. Die Aufnahme der doppelten Dosis (Studie 1, Gruppe 2) steigerte zudem die Summe der mehrfach ungesättigten Fettsäuren und senkte sowohl die Summe der gesättigten als auch die Summe der einfach ungesättigten Fettsäuren im Plasma. Die hydrophile antioxidative Kapazität im Plasma der AMD-Patienten wurde durch die Intervention in Studie 1 nicht moduliert, während die lipophile antioxidative Kapazität signifikant anstieg. Bezüglich der Xanthophyllgehalte in Lebensmitteln wurde gezeigt, dass die Dampfsterilisation im Gegensatz zur Hochdruckbehandlung die Luteinkonzentration signifikant reduzierte. Die Zubereitung von Tiefkühlblattspinat laut Packungsanleitung führte zu einer geringen Abnahme des Luteingehaltes.

Schlussfolgerung

Die Aufnahme eines Supplementes mit freiem Lutein und Zeaxanthin oder eines nicht aufgereinigten und nicht verseiften, öligen Grünkohlextraktes erhöht sowohl die Konzentrationen von Lutein und Zeaxanthin in Plasma als auch die MPOD von Patienten mit nichtexsudativer AMD. Aufgrund des Rückganges der MPOD nach Beendigung der Intervention in Studie 2 verhält sich das Makulapigment gegenüber Ernährungsmodulationen wahrscheinlich dynamischer als ursprünglich angenommen. Weiterhin wurde gezeigt, dass die industrielle Hochdruckbehandlung hinsichtlich der Xanthophyllgehalte eine Alternative zur Dampf-

sterilisation ist. Da eine nach Packungsanleitung zubereitete Portion Blattspinat (150 g) 13,5 mg Lutein plus Zeaxanthin liefert, können xanthophyllreiche Lebensmittel Nahrungsergänzungsmittel weitestgehend ersetzen. Aufgrund der möglichen unerwünschten Wirkungen von antioxidantienreichen Supplementen sollten xanthophyllreiche Lebensmittel bevorzugt werden.

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Erklärungen

Hiermit erkläre ich, dass…

mir die geltende Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena bekannt ist.

ich die vorliegende Arbeit selbstständig angefertigt habe.

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Jena, August 2013

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Christin Arnold

Wissenschaftliche Publikationen

Referierte Zeitschriften, Sammelbände und Übersichten

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