Retinal Hemodynamic Effects of Antioxidant Supplementation in an Endotoxin-Induced Model of Oxidative Stress in Humans

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PURPOSE. The Age-Related Eye Disease Study 1 (AREDS 1) has shown that nutritional supplementation with antioxidants and zinc modifies the natural course of AMD. It is presumed that the supplements exert their beneficial effects by ameliorating oxidative stress due to the scavenging of reactive oxygen species (ROS). We have shown in a human model that under oxidative stress induced by administration of lipopolysaccharide (LPS) the vasoconstrictor response of retinal vessels to oxygen breathing is diminished. This reduced vascular response to hyperoxia was previously shown to be normalized by the AREDS 1 supplements. In the present study, we tested the hypothesis that the response can also be restored by a different antioxidant formulation.

METHODS. This randomized, double-masked, placebo-controlled parallel group study included 40 healthy volunteers. On each study day, retinal red blood cell (RBC) flow and the reactivity of retinal RBC flow to hyperoxia were investigated in the absence and presence of 2 ng/kg LPS. Between the two study days, subjects received either the supplement or placebo for 14 days.

RESULTS. Before supplementation LPS reduced retinal arterial vasoconstriction (P < 0.001) and reactivity of retinal RBC flow (P = 0.03) in response to 100% oxygen breathing. Two weeks of supplementation did not affect baseline retinal RBC flow, but normalized the LPS-induced change in the response to hyperoxia. The arterial vasoconstrictor response during LPS and 100% oxygen breathing was 4.1 ± 1.0% after administration of placebo and 10.6 ± 0.9% after supplementation (P = 0.005). The response of RBC flow to 100% oxygen breathing during LPS was 52.2 ± 2.1% after administration of placebo and 59.5 ± 2.0% after supplementation (P = 0.033).

CONCLUSIONS. Our data show that the supplement used in the present study can normalize the response of retinal RBC flow to hyperoxia under LPS administration. This indicates that supplementation can prevent endothelial dysfunction induced by oxidative stress, which is assumed to play a role in the pathophysiology of AMD. (ClinicalTrials.gov number, NCT00914576.)

Keywords: nutritional supplementation, retinal blood flow regulation, oxygen, healthy subjects

The Age-Related Eye Disease Study 1 (AREDS) has shown that a combination of vitamin C, vitamin E, β-carotene, and zinc reduces the risk of developing advanced AMD1 by approximately 25% over a median of 6.3-years observation in high-risk patients. Since the results of this study were published concerns have been raised regarding the possible side effects of a high dose vitamin supplementation. In particular, there is evidence now that β-carotene increases the risk of lung cancer in smokers.2,3 Additionally, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) trial suggests an increased risk of prostate cancer in healthy men after high dose supplementation of vitamin E.4 These findings and the fact that the original AREDS formulation uses high dosages of vitamins C, E, and zinc, has stimulated the discussion on whether other formulations may reduce potential side effects and be therefore more beneficial for the patients, especially after long-term supplementation.

Since the publication of the promising results found in the AREDS 1, a large number of dietary supplements have been introduced, all aiming to reduce oxidative stress by the ROS scavenging properties of vitamins and related cofactors. Because of the recently reported side effects, these formulations often include reduced doses of vitamins E and C. In addition, a wide array of other dietary supplements is added by different manufacturers, based on their antiinflammatory and/ or antioxidative properties. However, given that an in vivo
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estimation of an antioxidative effect of vitamin combinations is difficult, for most of these supplements in vivo data are not available.

In addition, several data including post hoc analysis of the AREDS 1 indicated that low intake of the macular pigments lutein and zeaxanthin as well as low intake of omega-3 free fatty acids is associated with increased risk for late-stage AMD. As such, the AREDS 2 was initiated, investigating in a complex design the additional effects of the supplementation of 10 mg lutein, 2 mg zeaxanthin, and 1 g omega-3 free fatty acids as well as the effect of eliminating β-carotene and lowering the zinc doses on the progression of AMD. Even though as many as 4205 patients were randomized the data were partially inclusive. Adding lutein and zeaxanthin, omega-3 free fatty acids, or both to the AREDS 1 formulation did not further reduce the risk of progression to advanced AMD in primary analyses. Secondary analysis, however, revealed a protective effect of lutein and zeaxanthin when both groups were pooled together with a hazard ratio of 0.91 (95% confidence interval [CI], 0.82–1.00) for progression to advanced AMD. Previous studies showed that beta carotene intake is associated with an increased risk of lung cancer in smokers. This finding was confirmed in the AREDS 2 trial in people assigned to one of the two groups receiving the AREDS formulation with beta carotene. In the AREDS 2 trial 91% of those developing lung cancer were former smokers. Long-term lutein and zeaxanthin supplementation were, however, not found to increase the risk of lung cancer, indicating an improved safety profile of these carotenoids for long-term supplementation. Concerning the zinc component, no statistically significant difference was found in terms of AMD progression between the low- and high-dose (25 vs. 80 mg). It needs, however, to be mentioned that according to the National Institutes of Health (NIH), the tolerable upper intake level for zinc in adults is 40 mg.

We have recently introduced an in vivo model of increased oxidative stress caused by acute inflammation, to test the effectiveness of antioxidants to restore impaired vascular function. Briefly, this model is based on the observation that after administration of lipopolysacharide (LPS) in humans the inflammatory response is associated with a diminished retinal vascular response to hyperoxia caused by endothelial dysfunction due to oxidative stress.

Using this model, we were able to show that the AREDS 1 formulation (Table 1) taken daily over 14 days restores the impaired response of retinal blood flow to hyperoxia during LPS administration. In the present study, we tested the hypothesis that the reduced response of retinal vascular reactivity to systemic hyperoxia during standardized experimental systemic inflammation can be restored by a 2-weeks supplementation with a combination of vitamin C, vitamin E, lutein, zeaxanthin, zinc, copper, selen, Ginkgo biloba, flavonoids, omega-3 free fatty acids, and alpha lipoic acid.

### METHODS

#### Subjects

The study protocol was approved by the Ethics Committee of the Medical University of Vienna and followed the guidelines set forth in the Declaration of Helsinki. This double-masked, placebo-controlled, parallel group study included 40 healthy male subjects between 18 and 35 years. All subjects signed written informed consent and passed a screening examination before the study days including physical examination, 12-lead electrocardiogram, hematological status (hemoglobin, hematocrit, red blood cell [RBC], mean corpuscular hemoglobin, white blood cell [WBC], platelet count, activated partial thromboplastin time, thrombin time), clinical chemistry (sodium, potassium, creatinine, glutamic-pyruvic transaminase, alanine aminotransferase, gamma-glutamyl transferase, total bilirubin, total protein), hepatitis B, C, and human immunodeficiency virus serology, urinalysis (WBC, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood/hemoglobin), urine drug screening, assessment of visual acuity, slit-lamp biomicroscopy, funduscopy, and measurement of IOP. Exclusion criteria were ametropia greater than or equal to 3 diopters (D), anisometropia greater than or equal to 3 D, other ocular abnormalities, and any clinically relevant illness, blood donation, or intake of a medication, including a vitamin or a mineral supplement in the 3 weeks prior to the study. Participants had to abstain from beverages containing alcohol or caffeine for 12 hours before each study day.

### Protocol

The protocol followed our previous study with the AREDS formulation and was performed in a double-masked placebo-controlled parallel group design. Subjects who did not complete the study were replaced. This was done using block randomization with three 1:1 blocks consisting of 20:20, 2:2, and 4:4 subjects, respectively. After instillation of one drop of tropicamide (Mydriatikum AGEPHA, Vienna, Austria) into the study eye a resting period of at least 20 minutes was scheduled. Retinal RBC flow was assessed by combining measurements of retinal vessel diameters using the Dynamic Vessel Analyzer (DVA; IMEDOS GmbH, Jena, Germany) and RBC velocity using laser-Doppler velocimetry (LDV; LDV-5000; Oculix, Inc., Arbaz, Switzerland). Retinal WBC flux was measured with the blue field entoptic system (blue-field simulator; Oculix, Inc.). Additionally to these variables, baseline systemic hemodynamic parameters and IOP were taken. The reactivity of retinal hemodynamic parameters to systemic hyperoxia was investigated during breathing of 100% oxygen (gases for human use; AGA, Vienna, Austria). For this purpose an oxygen breathing period of 30 minutes was scheduled and retinal hemodynamic measurements were started 10 minutes after the start of inhalation. Breathing of pure oxygen leads to retinal vasoconstriction and a pronounced reduction in retinal blood flow.

### Table 1. Composition of the Food Supplement Vitamac Administered in the Present Study

<table>
<thead>
<tr>
<th></th>
<th>Morning Capsules</th>
<th>Evening Capsules</th>
<th>Daily Dose</th>
<th>AREDS 1 Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lutein</td>
<td>12 mg</td>
<td>-</td>
<td>12 mg</td>
<td>-</td>
</tr>
<tr>
<td>Zeaxanthin</td>
<td>-</td>
<td>5 mg</td>
<td>5 mg</td>
<td>-</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>300 mg</td>
<td>-</td>
<td>300 mg</td>
<td>500 mg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>60 mg</td>
<td>60 mg</td>
<td>60 mg</td>
<td>400 IU</td>
</tr>
<tr>
<td>Zinc</td>
<td>10 mg</td>
<td>10 mg</td>
<td>10 mg</td>
<td>80 mg</td>
</tr>
<tr>
<td>Copper</td>
<td>-</td>
<td>1 mg</td>
<td>1 mg</td>
<td>2 mg</td>
</tr>
<tr>
<td>Selen</td>
<td>-</td>
<td>20 µg</td>
<td>20 µg</td>
<td>-</td>
</tr>
<tr>
<td>Ginkgo biloba</td>
<td>10 mg</td>
<td>10 mg</td>
<td>20 mg</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>25 mg</td>
<td>25 mg</td>
<td>50 mg</td>
<td>-</td>
</tr>
<tr>
<td>Omega-3 free fatty acids (&gt;50%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>DHA/EPA</td>
<td>300 mg</td>
<td>-</td>
<td>300 mg</td>
<td>-</td>
</tr>
<tr>
<td>Alpha-lipoic acid</td>
<td>-</td>
<td>150 mg</td>
<td>150 mg</td>
<td>-</td>
</tr>
<tr>
<td>Beta-carotene</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>15 mg</td>
</tr>
</tbody>
</table>

The ingredients are divided into morning and evening capsules. DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid.

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**Retinal Effects of Antioxidants During Oxidative Stress**

**Methods**

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Thereafter intravenous infusion of a bolus containing 2 ng/kg bodyweight Escherichia coli endotoxin (US Standard Reference Endotoxin; NIH-CC, Bethesda, MD, USA) also known as LPS was used on each study day as a standardized model of systemic inflammation and oxidative stress. Lipopolysaccharide is a cell wall component of gram-negative bacteria and a major mediator in the pathogenesis of septic shock. All measurements including the hyperoxia stimulus were repeated 4 hours after administration of LPS.

After the first study day subjects were randomly assigned to take either the supplement (n = 20) or matching placebo (n = 20) for 14 days. Thereafter, a second study day with the same measurements and procedures as described for day 1 was performed.

**Food Supplement**

Vitamac (Croma Pharma GmbH, Korneuburg, Austria) was taken as a supplement by 20 subjects. The supplement contains vitamin C, vitamin E, lutein, zeaxanthin, zinc, copper, selen, G. biloba, flavonoids, omega-3 free fatty acids, and alpha-lipoic acid. The ingredients were divided into morning and evening doses as specified in Table 1. For blinding placebo capsules were produced with similar appearance to supplementation and consisting of lactose and wheat starch.

**Dynamic Vessel Analyzer (DVA)**

The diameters of one major temporal retinal artery (D_{art}) and vein (D_{vein}) within 1 to 2 disc diameters from the center of the optic disc were measured in mydriasis using the commercially available DVA system (IMEDOS GmbH, Jean, Germany). This system comprises a fundus camera (FF 450; Carl Zeiss Meditec AG, Jena, Germany), a high-resolution digital video camera, and a personal computer with analyzing software (all provided by IMEDOS GmbH). For the determination of retinal vessel diameters recorded images are digitized and analyzed in real-time with a frequency of 50 Hz. The system provides excellent reproducibility and sensitivity. After selection of the measurement location the DVA is able to follow the vessels during movements within the measurement window.

**Laser-Doppler Velocimetry**

For measurement of retinal RBC velocity we used a fundus camera–based system (LDV-5000; Oculix, Inc.). Measurements were performed in retinal veins at the same locations as diameter measurements. The principle of LDV is based on the optical Doppler effect. Laser light of a single-mode laser diode with a wavelength of 670 nm is scattered and reflected by moving erythrocytes leading to a broadened and shifted optical Doppler effect. Laser light of a single-mode laser diode with a wavelength of 670 nm is scattered and reflected by moving erythrocytes leading to a broadened and shifted optical Doppler effect. Laser light of a single-mode laser diode with a wavelength of 670 nm is scattered and reflected by moving erythrocytes leading to a broadened and shifted optical Doppler effect.

The system (blue-field simulator; Oculix, Inc.) uses the blue field entoptic phenomenon in order to investigate the leukocyte dynamics in retinal perifoveal vessels. The phenomenon is best observable at a wavelength of 450 nm and shows many tiny corpuscles around an area of the center of the fovea, which result from different absorption spectra of RBCs and WBCs. Subjects were asked to match their own leukocyte dynamics in retinal perifoveal vessels.

**Measurement of IOP and Systemic Hemodynamics**

Intracocular pressure was measured before and after DVA measurements with a slit-lamp mounted Goldmann applanation tonometer (Haag-Streit, Bern, Switzerland). Before each measurement two drops of oxybuprocain hydrochloride (Fluoresceine-Oxybuprocaine SDFaure; Omnivision, Neuhäusen, Germany) combined with sodium fluorescein were instilled for local anesthesia. Systolic, diastolic, and mean arterial blood pressures (SBP, DBP, MAP) were repeatedly measured before and after DVA measurements on the upper arm by an automated oscillometric device (Infinity Delta; Dräger, Vienna, Austria). The same device automatically recorded pulse rate by a finger pulse oxymeter. Ocular perfusion pressure in the sitting position was calculated as ocular perfusion pressure (OPP) = \( \frac{1}{3} \times MAP - IOP \).

**Blood Analysis**

Leukocyte and thrombocyte counts as well as C-reactive protein (CRP) were measured with standard techniques at the central laboratory of the Medical University of Vienna.

**Sample Size Calculation**

The sample size was based on oxygen reactivity of RBC flow as main outcome variable. It was calculated according to the variability of retinal blood flow measurements using LDV and DVA in our laboratory, which has been published previously. Based on this assumption, a sample size of 36 subjects was calculated to allow for detection of a minimum difference of
Since oxygen reactivity of RBC flow was chosen as the only value less than 0.05 was considered the level of significance. A repeated measures ANOVA model was applied to detect statistically significant changes. Changes in main outcome variables smaller than 10% were considered irrelevant.

10% in oxygen reactivity between groups. Changes in main outcome variables smaller than 10% were considered irrelevant. In order to allow for a dropout rate of 10% a total of 40 healthy subjects were included. This sample size calculation was based on an α-error of 0.05 and a β-error of 0.2 (two-tailed).

**Statistical Analysis**

Reactivity of retinal hemodynamic parameters to 100% oxygen breathing was calculated. Reactivity of RBC flow to systemic hyperoxia was calculated as a percent change in RBC flow = 100 * (RBC flow_{baseline} - RBC flow_{O_2}). RBC flow_{baseline} was selected as the main outcome variable. The other parameters were calculated accordingly. A repeated measures ANOVA model was applied to detect statistically significant changes. Post hoc analysis was done using planned comparisons. A P value less than 0.05 was considered the level of significance. Since oxygen reactivity of RBC flow was chosen as the only main outcome variable, no Bonferroni correction was used.

**RESULTS**

Five subjects did not finish the study and were replaced according to the block randomization. In two subjects, no adequate measurements of RBC velocity with LDV could be obtained and these subjects were excluded from analysis. As such a total of 38 subjects were finally included for analysis, 21 in the supplementation group and 17 in the placebo group.

Baseline parameters of both study groups are shown in Table 2. As expected, administration of LPS increased body temperature in both groups, but there was no difference in this response (P = 0.36). After a period of 8 hours, however, body temperature had returned to baseline. Intraocular pressure did not change throughout the experiments (P = 0.80). Administration of LPS increased pulse rate, but no difference was seen between the two study days (P = 0.62). Systemic MAP and OPP were slightly reduced after LPS in both groups (P < 0.01 each). No difference was, however, observed in MAP (P = 0.08) or OPP (P = 0.31) between the two groups. On both study days LPS induced a pronounced increase in leucocyte counts and a decrease in platelet counts (P < 0.001 each), which was comparable between the two study days (leucocyte count: P = 0.86; platelet count: P = 0.28). C-reactive protein increased significantly after administration of LPS on both study days (P < 0.001). This effect was not altered after intake of the supplement (P = 0.06).

**Effect of LPS on Hemodynamic Parameters**

Baseline values for the retinal hemodynamic parameters are presented in Table 3. No significant differences were found between the two study groups. Lipopolysaccharide increased D_{art} (P < 0.001) and D_{ven} (P < 0.001) before the oxygen provocation (Fig. 1). This LPS-induced increase was not altered after administration of LPS administration. Data are separately shown for the placebo (n = 17) and the supplementation (n = 21) group on both study days. Data are shown as means ± SEM.

**TABLE 2.** Subject Characteristics and Parameters on Both Study Days Under Baseline Conditions and 4 Hours After Administration of LPS

<table>
<thead>
<tr>
<th></th>
<th>Placebo, n = 17</th>
<th>Supplementation, n = 21</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 1</td>
<td>Day 15</td>
</tr>
<tr>
<td>Age, y</td>
<td>24.5 ± 0.6</td>
<td>24.5 ± 0.6</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BT, °C</td>
<td>35.8 ± 0.1</td>
<td>37.2 ± 0.2</td>
</tr>
<tr>
<td>IOP, mm Hg</td>
<td>13.9 ± 0.7</td>
<td>14.4 ± 0.6</td>
</tr>
<tr>
<td>PR, min⁻¹</td>
<td>61.9 ± 2.7</td>
<td>85.6 ± 3.0</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>86.5 ± 1.9</td>
<td>78.4 ± 2.4</td>
</tr>
<tr>
<td>OPP, mm Hg</td>
<td>42.0 ± 1.5</td>
<td>38.9 ± 1.9</td>
</tr>
<tr>
<td>LC, g/L</td>
<td>5.6 ± 0.4</td>
<td>7.9 ± 0.5</td>
</tr>
<tr>
<td>PC, g/L</td>
<td>250 ± 10</td>
<td>187 ± 8</td>
</tr>
<tr>
<td>CRP, mg/dL</td>
<td>0.09 ± 0.03</td>
<td>0.21 ± 0.04</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM. BT, body temperature; PR, pulse rate; LC, leucocyte count; PC, platelet count.

**TABLE 3.** Baseline Values of Retinal Hemodynamic Variables

<table>
<thead>
<tr>
<th></th>
<th>Placebo, n = 17</th>
<th>Supplementation, n = 21</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>D_{ven}, μm</td>
<td>153.5 ± 4.8</td>
<td>152.6 ± 3.0</td>
<td>0.88</td>
</tr>
<tr>
<td>D_{art}, μm</td>
<td>127.3 ± 4.4</td>
<td>125.3 ± 3.2</td>
<td>0.45</td>
</tr>
<tr>
<td>RBC velocity, cm/s</td>
<td>1.43 ± 0.09</td>
<td>1.50 ± 0.07</td>
<td>0.55</td>
</tr>
<tr>
<td>RBC flow, L/min*</td>
<td>17.1 ± 2.2</td>
<td>17.0 ± 1.4</td>
<td>1.00</td>
</tr>
<tr>
<td>WBC velocity, a.u.</td>
<td>1.01 ± 0.09</td>
<td>1.08 ± 0.07</td>
<td>0.54</td>
</tr>
<tr>
<td>WBC density, a.u.</td>
<td>92.8 ± 8.8</td>
<td>94.1 ± 8.5</td>
<td>0.91</td>
</tr>
<tr>
<td>WBC flux, a.u.</td>
<td>96.4 ± 15.0</td>
<td>101.1 ± 10.6</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Data are presented as means ± SEM. a.u., arbitrary units.

* Blood flow through one specific vein and not total retinal blood flow.

**FIGURE 1.** Response of D_{art} and D_{ven} as well as RBC velocity, and RBC flow to LPS administration. Data are separately shown for the placebo (n = 17) and the supplementation (n = 21) group on both study days. Data are shown as means ± SEM.
after 2 weeks of supplementation ($D_{art}$: $P = 0.38$, $D_{vein}$: $P = 0.14$). Administration of LPS did not increase RBC velocity ($P = 0.06$). Red blood cell flow increased significantly after LPS ($P < 0.001$), an effect that was not altered by supplementation ($P = 0.53$). The hemodynamic effect of LPS on blue field entoptic parameters is shown in Figure 2. Endotoxemia did not change WBC velocity ($P = 0.52$). As expected, LPS increased WBC density and WBC flux ($P < 0.001$ each) due to pronounced increase in circulating leukocytes. This increase was, however, not altered after the intake of the supplement (WBC density: $P = 0.93$; WBC flux: $P = 0.84$). Supplementation did not change any of the retinal or systemic hemodynamic parameters (data not shown).

Hemodynamic Response to Oxygen Breathing

A pronounced decrease in all retinal hemodynamic parameters was induced by 100% oxygen breathing (Fig. 3; $P < 0.001$ each), but did not affect systemic hemodynamic parameters or IOP (Table 2). Administration of LPS on the first study day significantly blunted the decrease of most retinal parameters in response to hyperoxia. (Figs. 3–5, $P < 0.05$ each). Retinal venous vasoconstriction was, however, not diminished after LPS ($P = 0.37$). After 14 days of placebo intake the response of retinal hemodynamic parameters to LPS was not altered. By contrast, the response was almost normalized after 14 days of supplementation. This effect was significant for the main outcome parameter RBC flow ($P = 0.033$). The effect was also significant for $D_{art}$ ($P = 0.005$), RBC velocity ($P < 0.001$), WBC density ($P = 0.002$), and WBC flux ($P = 0.001$), but was not significant for WBC velocity ($P = 0.09$).

DISCUSSION

The data of the present study show that the chosen supplement restores the retinal vasoconstrictor response to hyperoxia during LPS infusion. This indicates that the formulation used in the current experiment prevents the LPS induced endothelial dysfunction comparable with the AREDS formulation used in our recent experiment. To the best of our knowledge the dietary supplement used in the present trial is the only one except the AREDS formulation for which antioxidative properties have been shown for the human retina.

Administration of bacterial endotoxin, or LPS, is one of the most widely used models to induce inflammation and oxidative stress in animal as well as in human subjects. Because LPS is available highly purified and can easily be dosed, it induces well described and reproducible physiological effects in the subjects under study. As such, administration of LPS leads to endothelial dysfunction caused by an increase in reactive oxygen species and oxidative stress, which in turn leads to impaired regulation of vascular tone in response to exogenous stimuli. We could demonstrate in prior experiments that the vasoconstrictor response of retinal vessels to hyperoxia is reduced under states of LPS-induced inflammation in humans. Interestingly, this LPS effect can be reversed by co-administration of antioxidants as, for example, the AREDS 1 formulation, which normalizes the retinal vasoconstrictor response to hyperoxia in the LPS model.
Our data indicates that this also holds true for the formulation used in the current experiment. A 14 days, administration of the formulation specified in Table 1 is able to restore the vascular response of retinal vessels to hyperoxia. This indicates that the antioxidant combination used in the current study antagonizes endothelial dysfunction caused by the inflammation model and restores vascular function, comparable with the formulation used in the AREDS.\textsuperscript{10} Additionally, the data are in good accordance with our previous data on the retinal hemodynamic effects of LPS.\textsuperscript{9,10,29} Interestingly, LDV data show less variability than in these previous studies, which is most likely related to the new algorithm used in the present study. Further reductions in variability may be achieved by using Doppler optical coherence tomography technologies.\textsuperscript{19,30–35}

Obviously, the present study cannot answer which of the administered components are responsible for the effects seen. Vitamins E and C are radical scavengers and involved in several enzymatic processes. Lutein and zeaxanthin, the natural components of the macular pigment, have been proposed as dietary supplements for AMD, because they absorb blue light and scavenge reactive oxygen species.\textsuperscript{36} Several studies have shown that supplementation with lutein is capable of increasing macular pigment optical density\textsuperscript{37–40} and secondary analysis of the AREDS 2 indicates a protective effect in AMD.\textsuperscript{7} Importantly, lutein has been shown to have antiinflammatory effects in an LPS-induced model of ocular inflammation in rats\textsuperscript{41} and reduces inflammation and retinal gliosis in an ischemia-reperfusion model.\textsuperscript{42}

Omega-3 free fatty acids were intensively discussed in the treatment of AMD, because of the strong association between low intake and AMD.\textsuperscript{43} The data of the AREDS 2 do not support that omega-3 free fatty acids lead to an additional risk reduction to the AREDS 1 formulation.\textsuperscript{7} Supplementation with omega-3 free fatty acids may, however, decrease the risk of cardiovascular events.\textsuperscript{44}

The supplement used in the present study also includes components for which only circumstantial evidence for a protective effect in eye disease has been provided. \textit{G. biloba}, an extract from tree leaves containing flavonoid glycosides and terpenoids, was shown to exert neuroprotective effects in a rat glaucoma model.\textsuperscript{45} Furthermore, a small pilot study indicates a favorable effect on visual fields in patients with normal-tension glaucoma.\textsuperscript{46} In addition, some,\textsuperscript{47,48} but not all, studies\textsuperscript{49} indicate that \textit{G. biloba} increases ocular blood flow. Flavonoids have been implicated in the treatment of ocular disease because of their antioxidative properties.\textsuperscript{50} A small, randomized, placebo-controlled parallel group study has recently shown that a combination of flavonoids with \textit{Centella asiatica} and \textit{Melilotus} preserves visual sensitivity in patients with macular edema,\textsuperscript{51} a result that needs to be confirmed in larger-scale trials. Alpha-lipoic acid is a cofactor in the mitochondrial
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dehydrogenase complexes that catalyze the oxidative decarboxylation of z-keto acids and has potent radical scavenger properties. It has been shown to protect the retina in an ischemia-reperfusion model, as well as in diabetic mice.

In conclusion this study shows that 14-days supplementation with a combination of vitamin C, vitamin E, lutein, zeaxanthin, zinc, copper, selen, G. biloba, flavonoids, and alpha-lipoic acid reverses LPS-induced alterations in the reactivity of the retinal vasculature. Although the present data do not replace a clinical outcome study, our results indicate that dietary supplementation with the formulation used in the current study is capable to reduce oxidative stress and restores vascular function in the human retina.

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