Antioxidative Capacity of a Dietary Supplement on Retinal Hemodynamic Function in a Human Lipopolysaccharide (LPS) Model

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Purpose. Beneficial effects of dietary supplements in age-related macular degeneration (AMD) are related to antioxidative properties. In the Age-Related Eye Disease Study 1 (AREDS 1), a reduced progression to late stage AMD was found using vitamin C, E, zinc, and β-carotene. We showed previously that the AREDS 1 formulation restores the O_2-induced retinal vasoconstrictor response of retinal vessels in a human endotoxin (lipopolysaccharide [LPS]) model.

Methods. We hypothesized that the abnormal O_2-induced retinal red blood cell (RBC) flow response can be modulated by a different formulation (vitamin C, E, and zinc, lutein/zeaxanthin, selenium, taurine, Aronia extract, and omega-3 free fatty acids). A total of 43 healthy subjects was included in this randomized, double masked, placebo-controlled parallel group study. The reactivity of retinal arterial and venous diameter, RBC velocity, and flow to 100% O_2 breathing was investigated in the absence and presence of 2 ng/kg LPS. Between the two study days was a 14-day period of daily dietary supplement intake.

Results. The decrease in retinal arterial diameter, RBC velocity, and flow during 100% O_2 breathing was diminished significantly after LPS infusion. Dietary supplement intake for 14 days almost restored the response of retinal hemodynamic parameters to 100% O_2 after LPS administration. This effect was significant for retinal arterial diameter ($P = 0.03$ between groups), and RBC velocity and flow (each $P < 0.01$ between groups).

Conclusions. The present data indicate restoring of the RBC flow response to 100% O_2 after LPS administration. This is likely due to an amelioration of endothelial dysfunction resulting from oxidative stress, a factor involved in AMD pathophysiology. (ClinicalTrials.gov number, NCT00914576.)

Keywords: retinal hemodynamic function, retinal blood flow, dietary supplements, endotoxin model, young healthy subjects, red blood cell flow, white blood cell flow

In high-risk patients with early age-related macular degeneration (AMD) supplementation with vitamins and trace elements has become standard in clinical care. This is based to a large degree on the results of the Age-Related Eye Disease Study 1 (AREDS 1), which proved that a food supplement containing vitamin C, vitamin E, β-carotene, and zinc reduces the risk of developing late-stage AMD in high-risk patients by approximately 25% over a period of more than 6 years.[1] Given the high prevalence of AMD in the elderly and the enormous socioeconomic burden of the disease, this can be considered a landmark study. However, designing an interventional study to test the effects of food supplements is not easy. Complex considerations regarding the included supplements and their dosing must be done with emphasis on efficacy and safety. Most importantly, dose-finding studies that are standard in pharmacological drug development cannot be performed.

Since the results of AREDS 1 were published, a number of concerns regarding the included components and their dosing have been raised. High dose β-carotene increases the risk of lung cancer in smokers[2] and the dosing of zinc (80 mg) is above the tolerable upper intake level (UL) for adults of 40 mg/d.[3] In addition, basic and epidemiological studies indicated that reduced intake of lutein and zeaxanthin,[4] on one hand, and omega 3 free fatty acids, on the other hand,[5] may increase the risk of late stage AMD.

As such, the AREDS 2 was launched, and investigated as part of the primary randomization whether the addition of either lutein/zeaxanthin or omega-3 free fatty acids, or a combination of lutein/zeaxanthin and omega-3 free fatty acids exerts an additional effect to the AREDS 1 formulation. The study was negative in its primary analysis, but when the data for the 2 groups receiving lutein/zeaxanthin were pooled an additional risk reduction of approximately 10% was achieved, which was significant.[6] Part of the secondary randomization was to investigate whether β-carotene can be omitted and whether the dose of zinc can be reduced to 25 mg. In both cases no

Table 1. Comparison of the Food Supplement Retaron Administered in the Present Study and the AREDS 1 Formulation

	Daily Dose	
	Food Supplement Retaron	**AREDS 1 Formulation**
β-Carotene	–	15 mg
Lutein	10 mg	–
Zeaxanthin	2 mg	–
Vitamin C	100 mg	500 mg
Vitamin E	20 mg	267 mg
Zinc	10 mg	80 mg
Copper	–	2 mg
Selen	25 μg	–
Taurine	50 mg	–
Aronia extract	50 mg	–
Omega-3 free fatty acids, 250 mg DHA/30 mg EPA	500 mg	–

significant difference was observed, but it should be kept in mind that this part of the study was not performed as a noninferiority trial.

Very recent studies also indicate an increased risk of prostate cancer in healthy men after high dose (400 IU per day) supplementation of vitamin E.[7] Intake of vitamin C generally is considered safe, but high doses may increase the risk of kidney stones in men.[8] As a consequence of these studies, different supplementation products were launched containing the ingredients of the AREDS 1 and AREDS 2 formulations in modified dosing, but also including ginkgo biloba, resveratrol, flavonoids, taurine, aronia extract, or α-lipoic acid based on their antioxidative properties. For most of these food supplements very few in vivo data exist.

In the present study we tested one of these food supplements in an in vivo model of oxidative stress in humans. In this model oxidative stress is caused by acute inflammation after systemic administration of lipopolysaccharide (LPS).[9] This is associated with vascular dysregulation in the human forearm[10–12] and an abnormal retinal vascular response to hyperoxia caused by endothelial dysfunction due to oxidative stress.[13]

We have shown previously that the AREDS-1 formulation (see Table 1) is capable of restoring the reduced response of retinal blood flow to 100% oxygen breathing after LPS administration.[14] In the present study, we hypothesized that the abnormal response to systemic hyperoxia after administration of LPS also can be restored by a 2-week supplementation containing the components of the AREDS 1 and AREDS 2 formulations, but in lower concentrations, as well as taurine and Aronia extract.

Methods

Study Population

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. A total of 43 healthy male subjects between 18 and 35 years, who signed written informed consent, was included. Before entering the study all subjects had to pass a screening examination that included physical examination, 12-lead electrocardiogram, hematological status (hemoglobin, hematocrit, red blood cell [RBC], mean cell hemoglobin [MCH], white blood cell [WBC], platelet count, activated partial thromboplastin time [APTT], and thrombin time), clinical chemistry (sodium, potassium, creatinine, glutamate pyruvate transferase [GPT], alanine aminotransferase [ALAT], γ-glytamyl transferase [γ-GT], total bilirubin, total protein), hepatitis B and C, and human immunodeficiency virus [HIV] serology, urinalysis (WBC, nitrite, pH, protein, glucose, ketones, urobilinogen, bilirubin, blood/hemoglobin), and a urine drug screening. Subjects were excluded if any abnormal value was found as part of the prestudy screening unless the investigators considered an abnormality to be clinically irrelevant. Other exclusion criteria were clinically relevant illness, blood donation, or intake of any medication, vitamin, or mineral supplement in the 3 weeks before the study. In addition, an eye exam was performed, which consisted of visual acuity, slit-lamp biomicroscopy, funduscopy, and measurement of IOP. Exclusion criteria were ametropia $\geq$ 3 diopters (D), anisometropia $\geq$ 3 D, or any signs of eye diseases. All subjects had to abstain from beverages containing alcohol or caffeine for 12 hours before each study day.

Protocol

The study followed a balanced, randomized (1:1), double-masked placebo-controlled parallel group design. Subjects who did not complete the study were replaced. The pupil of the subjects' study eye was dilated using mydriatic eye drops (Mydriatikum; AGEPHA, Vienna, Austria). A resting period of at least 20 minutes was scheduled to allow for normalization of blood pressure and heart rate. Thereafter, baseline values of all outcome parameters were taken. During all measurement cycles a predefined order of techniques was followed: laser Doppler velocimetry (LDV), Dynamic Vessel Analyzer (DVA), blue field entopic technique, and IOP assessment. Thereafter, an inhalation period of 100% oxygen (Messer Group GmbH, Vienna, Austria) was scheduled for 30 minutes. Measurements using LDV, DVA, and blue field entopic technique were repeated during the last 20 minutes of this breathing period. Blood pressure and pulse rate were obtained every 5 minutes during measurements of ocular hemodynamic parameters. Thereafter an LPS bolus was given intravenously at a dose of 2 ng/kg and the measurements were repeated in the same order 4 hours after LPS administration. Venous blood samples were drawn to determine C-reactive protein (CRP), malondialdehyde (MDA), and WBC count.

After LPS application, subjects were confined to bed rest throughout the remaining trial day except for approximately 60 minutes where measurements of ocular hemodynamics were performed. Safety parameters (blood pressure, pulse rate, body temperature) were obtained until the values had returned to baseline. Vital parameters (blood pressure, pulse rate) as well as tympanic temperature were monitored at 30-minute intervals. In the first 4 hours after LPS administration, 150 mL saline solution/h was infused intravenously. The subjects kept fasting during the study day until all outcome parameters were collected.

After completion of the first study day the participants were randomized in one of the two study groups (placebo/dietary supplement). The subjects received appropriate study medication and were instructed to take 1 capsule per day for two weeks without chewing or breaking the capsule. In addition the participants received a diary to document their study medication intake. The second trial day was performed 14 days after the first trial day. The schedule remained exactly the same as described for the first study day.

Food Supplement

The food supplement used in the present study (Retaron Kapseln; Ursapharm, Saarbrücken, Germany) contained vita-

min C, vitamin E, lutein, zeaxanthin, zinc, selenium, taurine, Aronia extract, and omega-3 free fatty acids (Table 1). To achieve double-masked conditions, placebo capsules were produced containing soy oil with identical appearance to active supplementation.

Dynamic Vessel Analyzer (DVA)

The diameters of retinal vessels were measured with the DVA (IMEDOS GmbH, Jena, Germany). The system was described in detail previously.[15] One major temporal retinal artery (D_{art}) and vein (D_{vein}) were measured within one to two disc diameters from the center of the optic disc. The system is based on a fundus camera (FF 450; Carl Zeiss Meditec AG, Jena, Germany) and uses a high-resolution digital video camera to record retinal vessel diameters continuously. A personal computer with analyzing software is attached and allows for monitoring of retinal vessels with excellent reproducibility and sensitivity.[16]

Laser-Doppler Velocimetry

A fundus camera–based system was used to measure retinal RBC velocity (LDV-5000; Oculix, Inc., Arbaz, Switzerland). To allow for the calculation of retinal blood flow through one particular vein, RBC velocity was measured at the same location as diameter measurements. In the present LDV system, laser light with a wavelength of 670 nm is scattered and reflected by moving erythrocytes leading to a frequency shift, which is proportional to the RBC velocity in the retinal vessel. The maximum Doppler shift corresponds to the centerline erythrocyte velocity (V_{max}).[17,18] Because the angle between the incident light and moving erythrocytes is not known, the Doppler shift power spectra are recorded simultaneously for two directions of the scattered light enabling absolute velocity measurements.[17] We showed recently that after determination of the absolute blood velocity, the angle of incidence can be calculated based on the data from both directions.[19] Only if the angle of incidence, as calculated from the two channels, is within 0.5 rad data were considered to be accurate,[20,21] otherwise they were not considered for analysis. From the data obtained at the two directions the angle of incidence was calculated as the mean of the two values. Based on this mean angle, the Doppler shifts f_1 and f_2 were recalculated and the difference in the Doppler Δf shift was recalculated as well. From this corrected Δf the absolute maximum RBC velocity was calculated (V_{max}). Compared to the previously published reproducibility data from our laboratory,[22] this procedure reduces variability (unpublished data). From V_{max}, mean RBC velocity in retinal vessels was calculated as RBC vel $= V_{max}/2$ assuming a parabolic velocity profile. Blood flow in the retinal vein under study was calculated as RBC flow = RBC vel*D_{vein}^2 *$\pi/4$.

Blue Field Entopic Technique

This technique is based on the blue field entopic phenomenon to investigate leukocyte dynamics in retinal perifoveal vessels (Bluefield Simulator; Oculix, Inc.).[23] When looking into blue light, one perceives many moving tiny corpuscles around an area of the center of the fovea, which represent WBCs.[24] In the blue field simulator, subjects must match their own entopic observation with the presented computer simulation. As such, the density of WBCs (WBCD) and mean velocity of WBCs (WBCV) are determined in five consecutive runs. The WBC flux (WBCF) is calculated as the product of WBCD*WBCV. During the prestudy screening visit, all subjects underwent training sessions to improve reproducibility. Only results varying less than 30% were considered accurate.

Measurement of IOP and Systemic Hemodynamics

The IOP was measured with a slit-lamp–mounted Goldmann applanation tonometer (Haag-Streit, Bern, Switzerland) after administration of oxybuprocaine hydrochloride combined with sodium fluorescein (Fluoresceine-Oxybuprocaine SDU Faure; Omnivision, Neuhausen, Germany). Systolic, diastolic, and mean arterial blood pressures (SBP, DBP, and MAP, respectively) were measured on the upper arm by an automated oscillometric device, pulse rate by a finger pulse oximeter (Infinity Delta; Dräger, Vienna, Austria). Ocular perfusion pressure (OPP) in the sitting position was calculated as OPP = 2/3*MAP − IOP.[25]

Blood Analysis

Leukocyte and thrombocyte counts, MDA, and CRP were measured with standard techniques at the central laboratory of the Medical University of Vienna.

Sample Size Calculation

For the sample size calculation the oxygen reactivity of RBC flow was taken as main outcome variable. The standard deviations of the measurements using LDV and DVA in our laboratory have been reported previously.[22] A total of 36 subjects was calculated to allow for detection of a minimum difference of 10% between the supplementation and placebo groups. Changes smaller than 10% were considered irrelevant. Allowing for a dropout rate of 10%, a total of 40 healthy subjects was included. The sample size calculation was based on an α-error of 0.05 and a β-error of 0.2 (2-tailed).

Statistical Analysis

Baseline values for both study days (days 1 and 15) were defined as the first value obtained before administration of either LPS or oxygen. The reactivity to 100% oxygen breathing was calculated for all retinal hemodynamic parameters. Reactivity of RBC flow to systemic hyperoxia was calculated as percent change in RBC flow = 100*(RBC flow O_2 − RBC $\text{flow}_{baseline}$)/RBC $\text{flow}_{baseline}$, and was selected as the main outcome variable. The other parameters were calculated accordingly. Data were analyzed using a repeated measures ANOVA model. Post hoc analysis was done using planned comparisons. Since oxygen reactivity of RBC flow was chosen as the only main outcome variable, no Bonferroni correction was used. A 2-tailed P value < 0.05 was considered the level of significance.

Results

In total, 43 male subjects were included in the present study, of whom 40 completed the study according to the protocol (age, 27.1 ± 4.6 years; mean ± SD). Two subjects withdrew consent after the first study day and one subject had flu-like symptoms on the first study day. In one subject in the supplementation group no adequate measurements of RBC velocity with LDV could be obtained and, therefore, this subject was excluded from the analysis. Hence, a total of 39 data sets was used for analysis.

Effect of LPS and 100% Oxygen Breathing on Systemic Parameters

In Table 2, baseline parameters for both study groups are presented. No difference in the LPS-induced rise in body temperature was observed between the two study groups ($P =$ 0.81). A total of 14 days intake of supplementation did not have

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TABLE 2. Body Temperature (BT), Systemic Hemodynamic Parameters, Leukocyte Count (LC), Platelet Count (PC), CRP, MDA on Days 1 and 15 at Baseline and 4 Hours After LPS Administration

	Placebo, $n = 20$						Supplementation, $n = 19$					
	26.6 ± 1.2						27.7 ± 0.8					
	Day 1			Day 15			Day 1			Day 15		
Age, y	Baseline	LPS	*P* Value	Baseline	LPS	*P* Value	Baseline	LPS	*P* Value	Baseline	LPS	*P* Value
BT, °C	35.8 ± 0.1	37.5 ± 0.1	<0.001	35.8 ± 0.1	37.4 ± 0.1	<0.001	35.8 ± 0.1	37.5 ± 0.1	<0.001	35.7 ± 0.1	37.4 ± 0.1	<0.001
PR, min^{-1}	66.0 ± 3.1	87.4 ± 2.9	<0.001	59.9 ± 2.1	83.1 ± 3.0	<0.001	62.6 ± 3.3	89.3 ± 2.9	<0.001	63.8 ± 1.8	88.8 ± 3.2	<0.001
SBP, mm Hg	117.9 ± 2.3	117.8 ± 1.8	0.95	119.4 ± 1.9	120.1 ± 2.6	0.84	119.0 ± 2.3	118.4 ± 2.3	0.87	119.0 ± 1.7	118.4 ± 1.8	0.80
DBP, mm Hg	67.4 ± 2.7	52.4 ± 1.7	<0.001	67.8 ± 1.5	54.7 ± 1.3	<0.001	64.1 ± 2.9	53.4 ± 2.3	0.01	64.4 ± 2.4	56.8 ± 1.8	0.02
MAP, mm Hg	84.9 ± 2.1	72.7 ± 1.3	<0.001	84.8 ± 1.7	75.0 ± 1.7	<0.001	84.0 ± 2.5	74.6 ± 1.8	<0.01	82.7 ± 2.1	78.5 ± 1.9	0.14
LC, G/l	5.2 ± 0.2	7.8 ± 0.4	<0.001	5.3 ± 0.2	9.0 ± 0.4	<0.001	5.4 ± 0.3	8.8 ± 0.5	<0.001	5.7 ± 0.2	10.1 ± 0.5	<0.001
PC, G/l	190 ± 7	159 ± 6	<0.01	205 ± 9	168 ± 8	<0.01	212 ± 10	181 ± 10	0.04	220 ± 11	184 ± 9	0.02
MDA, ng/mL	66.0 ± 7.0	75.6 ± 4.4	0.25	69.5 ± 7.7	82.4 ± 6.2	0.20	61.3 ± 6.7	69.2 ± 6.1	0.39	64.1 ± 8.7	71.5 ± 8.5	0.54
CRP, mg/dL	0.08 ± 0.02	0.70 ± 0.05	<0.001	0.06 ± 0.01	0.62 ± 0.06	<0.001	0.04 ± 0.00	0.70 ± 0.05	<0.001	0.08 ± 0.02	0.60 ± 0.04	<0.001

Measurements were taken before administering 100% oxygen (except CRP, which was measured 8 hours after administration). Baseline measurements were the first measurements on each study day before any intervention (LPS, oxygen) was done. At day 15 "Baseline" represents data after 14 days of supplementation/placebo intake. Data are presented as mean ± SEM. PR, pulse rate.

any effect on the rise in body temperature ($P = 0.80$ between study groups on day 15). At 8 hours after LPS administration, body temperature always had returned to baseline.

The LPS increased pulse rate on both study days, but no difference was observed between the two groups on either of the two study days ($P = 0.26$ on day 1 and $P = 0.72$ on day 15 between groups). A decrease in MAP was seen 4 hours after LPS administration, but again, there was no difference between the two study groups ($P = 0.38$ on day 1 and $P = 0.07$ on day 15). The LPS induced a significant increase in leukocyte count and a decrease in platelet count on both study days ($P < 0.01$ each). However, no difference was observed between the study groups (leukocyte count, $P = 0.92$; platelet count, $P = 0.85$) and intake of the supplement did not have any influence on the LPS-induced changes in leukocyte and platelet counts (leukocyte count, $P = 0.19$; platelet count, $P = 0.98$). The LPS had no effect on the antioxidative capacity of the blood as assessed by determination of MDA and this response was not altered by 14 days intake of the supplement ($P = 0.46$ between groups). A pronounced increase in CRP after LPS infusion was seen on both study days ($P < 0.01$ each) and supplementation did not alter this systemic inflammatory response ($P = 0.58$). Breathing of 100% oxygen did not induce any effects on either blood pressure or pulse rate.

Effect of LPS on Retinal Hemodynamic Parameters

At baseline, D_{vein} ($P = 0.007$), RBC velocity ($P = 0.04$), and RBC flow ($P = 0.009$) were higher in the supplementation group than in the placebo group. The response of retinal hemodynamic parameters to LPS administration is presented in Table 3. The LPS had no significant effect on D_{vein} and D_{art} before 100% oxygen provocation in both groups on either of the study days. Also, supplementation did not have an effect on this response ($P = 0.67$ for D_{vein} and $P = 0.36$ for D_{art} between groups). The LPS did not induce a significant increase in RBC velocity before 100% oxygen provocation on both study days in either of the two groups ($P = 0.14$ between groups on study day 15). No significant change in RBC flow was seen after LPS administration, except for a significant increase on study day 1 in the placebo group ($P = 0.04$), which probably was a chance finding. Again, supplementation did not alter this response ($P = 0.92$ between groups). Results for D_{vein}, D_{art}, RBC velocity, and RBC flow are presented in Figure 1.

The LPS did not significantly alter WBC velocity on either of the two study days. In contrast, on both study days a significant increase in WBC density and WBC flow was observed ($P < 0.001$ each). A total of 14 days of supplementation did not have an effect on this pronounced increase ($P = 0.29$ for WBC density and $P = 0.24$ for WBC flow between groups). Figure 2 illustrates these results graphically.

Retinal Hemodynamic Response to 100% Oxygen Breathing

Inhalation of 100% oxygen induced a pronounced decrease in all retinal hemodynamic parameters on study day 1 before administration of LPS (Table 4). After intravenous infusion of LPS on the first study day, the reduction in D_{art}, RBC velocity, RBC flow, and WBC density was significantly blunted in both study groups (Figs. 3–5). A total of 14 days of intake of placebo did not alter this response. In contrast, intake of the dietary supplement almost normalized the response of retinal hemodynamic parameters during hyperoxia and LPS administration. This effect was significant for D_{art} ($P = 0.03$ between groups), RBC velocity, and RBC flow (each $P < 0.01$ between groups), as well as WBC velocity ($P = 0.03$ between groups). In contrast, dietary supplementation did not have an effect on the response

Table 3. Reactivity of Retinal Hemodynamic Parameters to LPS Before 100% Oxygen Breathing on Days 1 and 15

	Day 1					Day 15				
	Placebo, $n = 20$	*P* Value vs. Baseline	Supplementation, $n = 19$	*P* Value vs. Baseline	*P* Value Between Groups	Placebo, $n = 20$	*P* Value vs. Baseline	Supplementation, $n = 19$	*P* Value vs. Baseline	*P* Value Between Groups
D_{vein}	4.4 ± 1.3	0.07	2.6 ± 1.2	0.24	0.46	4.0 ± 1.0	0.09	4.2 ± 1.2	0.12	0.67
D_{art}	3.9 ± 1.2	0.11	5.9 ± 1.6	0.08	0.33	4.0 ± 1.6	0.17	2.3 ± 1.3	0.37	0.36
RBC vel	5.0 ± 2.8	0.13	4.9 ± 1.8	0.06	0.68	5.2 ± 2.5	0.11	0.4 ± 1.9	0.88	0.14
RBC flow	15.3 ± 4.4	0.04	10.7 ± 2.7	0.04	0.99	13.9 ± 3.4	0.09	9.6 ± 3.7	0.25	0.92
WBC vel	11.1 ± 5.7	0.35	24.5 ± 14.0	0.46	0.42	3.5 ± 4.8	0.54	8.2 ± 4.5	0.37	0.57
WBC dens	73.1 ± 9.8	<0.001	98.5 ± 11.2	<0.001	0.17	75.3 ± 8.6	<0.001	107.2 ± 11.8	<0.001	0.29
WBC flow	97.6 ± 19.1	<0.001	150.1 ± 34.8	<0.001	0.21	81.6 ± 12.6	<0.001	127.4 ± 18.7	<0.001	0.24

Data are presented as % changes ± SEM.

of D_{vein} ($P = 0.28$ between groups), WBC density ($P = 0.45$ between groups), and WBC flow ($P = 0.70$ between groups).

Discussion

In the present study, we tested the hypothesis that a dietary supplement that differs from the original AREDS 1 formulation is capable of restoring the abnormal retinal vasoconstrictor response to 100% oxygen breathing in a human LPS model. This response most likely is caused by endothelial dysfunction induced by oxidative stress. Indeed, a 14-day intake of the used formulation almost normalized this response. We have shown previously that the AREDS 1 formulation[14] as well as another vitamin supplement[20] also are capable of modifying this LPS-induced effect.

The food supplement studied in the present work differs considerably from the formulations used in AREDS 1 and AREDS 2. Vitamins C and E are included in much lower doses. The dietary supplement contains the macular carotenoids lutein and zeaxanthin in the same dosage as used in AREDS 2, but does not contain β-carotene. Evidence has accumulated that lutein and zeaxanthin have potent radical scavenger as well as antioxidative properties,[4] and, indeed, secondary analysis of AREDS 2 indicates that supplementation is beneficial in AMD.[6] In addition, omega-3 free fatty acids at half of the dose tested in AREDS 2 are part of the dietary supplement used in our study, with more than 50% docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Omega-3 free fatty acids have a key role in determining the permeability, fluidity, thickness, and lipid phase of photoreceptor membranes.[26] In addition, DHA is the precursor for neuroprotectin D1, a potent neuroprotectant,[27] but the results of AREDS 2 for omega-3 free fatty acids were negative.[6] Moreover, zinc was used at an 8-fold reduced dose compared to AREDS 1 and even 2.5-fold reduced compared to the lower dose tested in AREDS 2.

The dietary supplement, however, also contained two antioxidants that were not part of the spectrum tested in AREDS 1 and 2. Taurine is a well-known antioxidant that is found in high concentrations in animals. The antioxidant properties of taurine are considered to be mediated mainly by

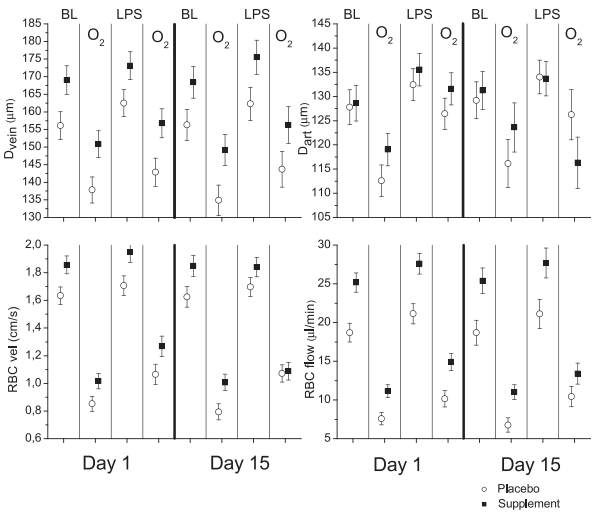


Figure 1. Response of D_{ar} and D_{vein} as well as RBC velocity (RBC vel) and RBC flow to LPS administration and 100% oxygen provocation. Data are shown separately for the placebo ($n = 20$) and supplementation ($n = 19$) groups on both study days. Data are shown as means ± SEM.

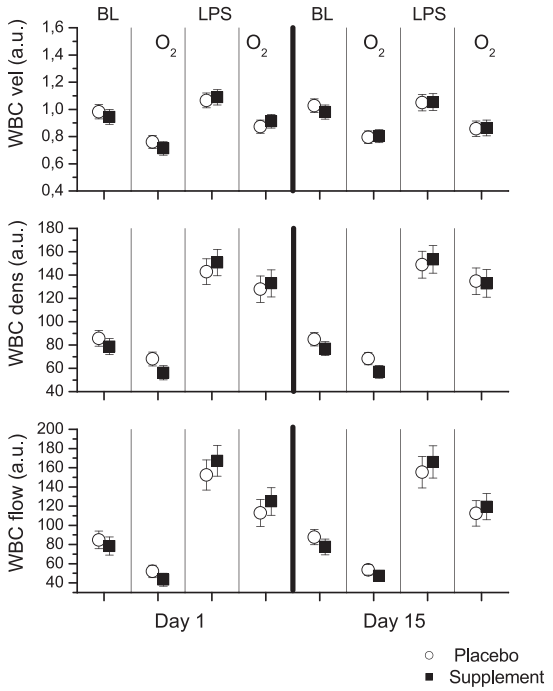


Figure 2. Response of WBC velocity (WBC vel), WBC density (WBC dens), and WBC flow (WBC flow) to LPS administration and 100% oxygen provocation in arbitrary units (a.u.). Data are separately shown for the placebo ($n = 20$) and supplementation ($n = 19$) groups on both study days at baseline (BL) and after 100% oxygen (O_2) as well as LPS administration. Data are shown as means ± SEM.

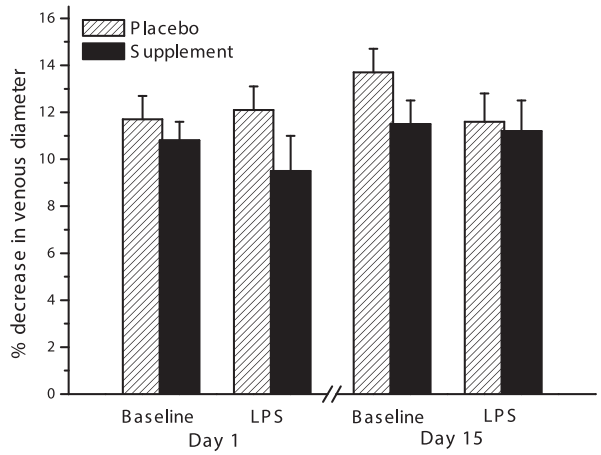
Table 4. Oxygen Reactivity (%) of Retinal Hemodynamic Parameters on Days 1 and 15 at Baseline and After LPS Administration

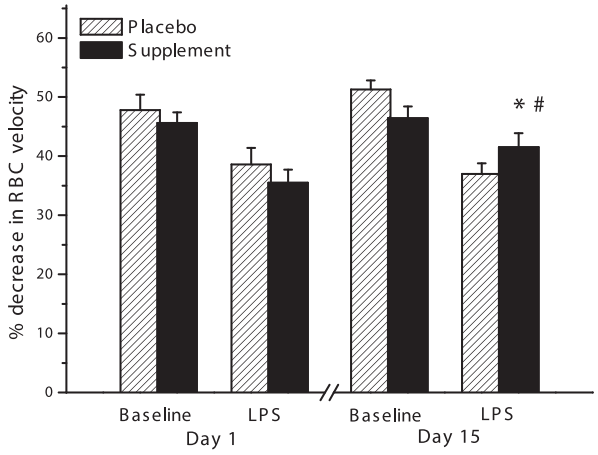
	Placebo, $n = 20$					Supplementation, $n = 19$				
	Day 1		Day 15		*P*	Day 1		Day 15		*P*
	Baseline	LPS	Baseline	LPS	Value	Baseline	LPS	Baseline	LPS	Value
D_{vein}	−11.7 ± 4.5	−12.1 ± 4.3	−13.7 ± 4.3	−11.6 ± 5.2	0.60	−10.8 ± 3.3	−9.5 ± 6.5	−11.5 ± 4.2	−11.2 ± 5.7	0.62
D_{art}	−11.6 ± 5.3	−4.5 ± 4.1	−9.9 ± 5.7	−5.8 ± 4.3	0.52	−7.5 ± 4.3	−2.7 ± 7.8	−5.9 ± 5.3	−13.2 ± 9.0	0.004
RBC vel	−47.8 ± 11.8	−38.6 ± 12.7	−51.3 ± 6.9	−37.0 ± 8.0	0.75	−45.6 ± 8.0	−35.5 ± 9.5	−46.4 ± 8.7	−41.5 ± 10.5	<0.01
RBC flow	−58.9 ± 10.2	−52.5 ± 10.3	−63.6 ± 7.0	−50.4 ± 10.0	0.75	−56.6 ± 7.1	−46.5 ± 12.8	−57.9 ± 8.2	−53.3 ± 11.9	0.60
WBC vel	−22.7 ± 13.7	−18.2 ± 11.2	−22.7 ± 12.3	−18.3 ± 12.2	0.61	−23.0 ± 16.3	−15.9 ± 8.7	−17.7 ± 10.9	−18.7 ± 8.8	0.10
WBC dens	−20.4 ± 12.1	−12.5 ± 10.3	−20.1 ± 10.8	−10.3 ± 5.4	0.85	−29.6 ± 9.8	−12.2 ± 8.0	−27.1 ± 9.5	−14.0 ± 10.6	1.0
WBC flow	−37.8 ± 16.4	−28.3 ± 13.4	−38.6 ± 11.7	−26.9 ± 9.7	1.0	−45.5 ± 14.8	−26.1 ± 10.8	−40.2 ± 10.1	−29.9 ± 12.0	0.92

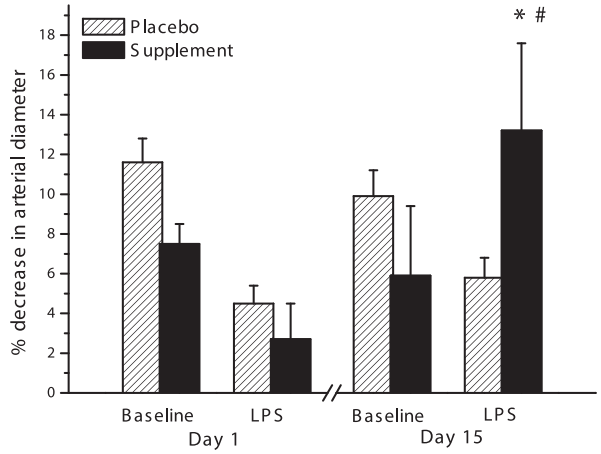
Data are presented as mean % changes ± SEM.

decreasing rates of MDA formation from unsaturated membrane lipids.[28] In the present study, however, no effect on MDA was seen after 14 days of intake. In the retina the importance of taurine has been reported a long time ago, because it appears to be the key constituent of the free amino acid pool in photoreceptors,[29] and taurine deficiency results in retinal degeneration and blindness.[30] In bovine photoreceptor cell membranes, taurine provides antioxidative properties[31] and a relatively recent study (The Taurine, Omega-3 Fatty Acids, Zinc, Antioxidant, Lutein [TOZAL] study[32]) found an increase in visual acuity in patients with dry AMD treated with a food supplement containing taurine.

The fruit of the aronia contains high levels of polyphenol compounds that are potent radical scavengers. It contains







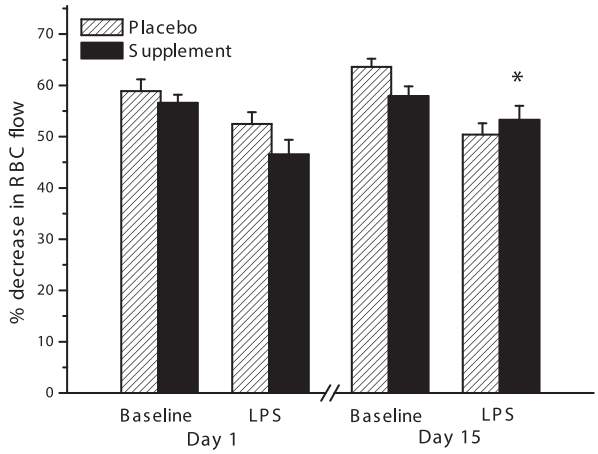


Figure 3. Percentage decrease of D_{vein} and D_{art} to 100% oxygen breathing. Data are shown separately for the placebo ($n = 20$) and supplementation ($n = 19$) groups on both study days during baseline condition and after LPS infusion. Data are shown as means ± SEM. *Significant changes compared to placebo. #Significant changes compared to study day 1.

Figure 4. Percentage decrease of RBC velocity and RBC flow to 100% oxygen breathing. Data are separately shown for the placebo ($n = 19$) and supplementation ($n = 20$) groups on both study days during baseline condition or after LPS infusion. Data are shown as means ± SEM. *Significant changes compared to placebo. #Significant changes compared to study day 1.

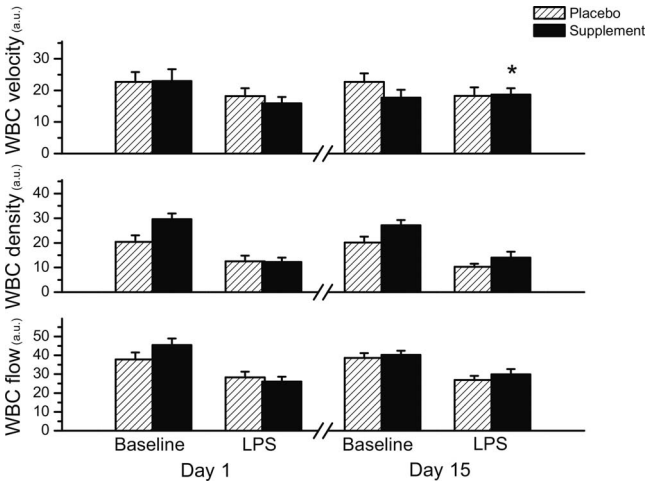


FIGURE 5. Percentage decrease of WBC velocity, WBC density, and WBC flux to 100% oxygen breathing. Data are separately shown for the placebo ($n = 19$) and supplementation ($n = 20$) groups on both study days during baseline condition or after LPS infusion. Data are shown as means ± SEM. *Significant changes compared to placebo.

high levels of cyanidin 3-O-beta-glucoside, which was highly effective in scavenging the 2,2-diphenyl-1-picrylhydrazyl (DPPH) stable radical and partially suppressed ethanol-induced gastric mucosal damage.[33] Aronia extract exerted anti-inflammatory effects in an LPS-induced uveitis model in rats mediated by blockade of nitric oxide synthase-2 and cyclooxygenase 2.[34] Evidence also has accumulated that anthocyanin-enhanced extracts can induce vasoactive effects. In the porcine coronary artery aronia extract produced endothelium-dependent relaxation and protected from reactive oxygen species.[35]

The present study raises the question whether results comparable to AREDS 1 and AREDS 2 in AMD also can be obtained with food supplements that contain antioxidants in lower concentrations or with different components. This is an important question given that safety issues may not be elucidated completely as mentioned above. Answering the question, however, is extremely difficult, because it would require a noninferiority trial with very large sample size.

The human LPS model used in the present study is used widely to study inflammation and oxidative stress, as it provides reproducible effects and allows for easy dosing.[36] In vascular research the model is used because it leads to endothelial dysfunction due to oxidative stress, which, in turn, impairs regulation of vascular tone in response to exogenous stimuli.[10–12] This also is the case in the retinal circulation, where the vasoconstrictor response of retinal vessels to 100% oxygen, which partially depends on endothelin-1,[37,38] is reduced after administration of LPS.[9,13] The response of retinal blood flow to 100% oxygen breathing in healthy subjects is well compatible with previously published data.[39–42]

A number of limitations must be considered when discussing the data of the present study. Except for MDA, no other markers of systemic oxidation or endothelial function were measured. However, to which degree such markers would reflect the situation at the retina is unclear. When comparing the results of the present study to the results of previous studies performed in our laboratory,[9,13,14,20] some differences in the amount of the retinal hemodynamic responses can be observed. This may have several reasons. On one hand, different charges of LPS were used that may have different potency.[43] On the other hand, we already have noticed in our previous studies that the intraindividual variability in the responses is less than the interindividual variability, which also could account for the differences between cohorts. This also highlights the need for longitudinal study designs as also used in the present trial. At baseline D_{vein}, RBC velocity and RBC flow were higher in the supplementation group than in the placebo group. The reason is, however, not related to the selection of the target vein. Rather differences in the individual retinal angioarchitecture are responsible for this result, as only one large vein was chosen for the measurements.

It is obvious that the LPS model does not reflect the etiology of AMD, but is a short-term inflammatory model available in humans. In this respect it is, however, interesting that complement factor H polymorphisms have been linked to vascular disease in several studies. The homozygous C allele (CC) of rs1061170, a single nucleotide polymorphism strongly associated with AMD, also is linked to the risk of mortality in Finnish nonagenarians.[44] The same group showed that in young healthy male subjects the interaction between CRP haplotypes and CC allele of rs1061170 is associated with increased carotid artery stiffness.[45] In complement factor H–deficient mice, a progressive deposition of C3 and C3b on choroidal and retinal vessels is observed, inducing endothelial dysfunction.[46] This is in good agreement with recent data from our group indicating that young healthy carriers of the CC allele of rs1061170 show abnormal choroidal blood flow regulation.[47] Considering the chronic low-grade inflammation present in AMD,[48] the LPS-model seems to be a suitable approach to study the effect of dietary supplementation on the retinal hemodynamic function.

In conclusion, the data of the present study indicated that intake of a food supplement containing the macula carotinoids lutein and zeaxanthin, omega-3-fatty acids, vitamins C and E, trace elements zinc and selenium, extract from Aronia and taurine, and with it differing from the ones used in the AREDS studies, reverses LPS-induced changes in retinal vascular reactivity. This indicates that the present formulation is capable of exerting antioxidative properties in the retina in vivo. Further studies are required to investigate whether this effect is associated with an improvement of clinical signs and symptoms in patients with AMD.

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