

Increased Lipid Peroxidation in Trained Men After 2 Weeks of Antioxidant Supplementation

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Purpose: To assess the effects of an encapsulated antioxidant concentrate (EAC) and exercise on lipid peroxidation (LIPOX) and the plasma antioxidant enzyme glutathione peroxidase (Pl-GPx). **Methods:** Eight trained male cyclists ($VO_{2max} > 55 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) participated in this randomized, placebo-controlled, double-blinded, cross-over study and undertook 4 cycle-ergometer bouts: 2 moderate exercise bouts over 90 min at 45% of individual VO_{2max} and 2 strenuous exercise bouts at 75% of individual VO_{2max} for 30 min. The first 2 exercise tests—1 moderate and 1 strenuous—were conducted after 4 weeks wash-out and after 12 and 14 days of EAC (107 IU vitamin E, 450 mg vitamin C, 36 mg β -carotene, 100 μg selenium) or placebo treatment. After another 4 weeks wash-out, participants were given the opposite capsule treatment and repeated the 2 exercise tests. Physical exercise training was equal across the whole study period, and nutrition was standardized by a menu plan the week before the tests. Blood was collected before exercise, immediately postexercise, and 30 min and 60 min after each test. Plasma samples were analyzed for LIPOX marker malondialdehyde (MDA) and the antioxidant enzyme pl-GPx. **Results:** MDA concentrations were significantly increased after EAC supplementation at rest before exercise and after moderate exercise ($p < .05$). MDA concentrations showed no differences between treatments after strenuous exercise ($p > .1$). Pl-GPx concentrations decreased at all time points of measurement after EAC treatment ($p < .05$). **Conclusions:** The EAC induced an increase of LIPOX as indicated by MDA and decreased pl-GPx concentrations pre- and postexercise.

Keywords: malondialdehyde, glutathione peroxidase, athletes, strenuous exercise

Dietary intake of vitamins C and E and β -carotene is an exogenous contribution to support the body's antioxidant system. Together with several endogenous antioxidant compounds—for example, the enzymatic antioxidants glutathione

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peroxidases (GPx) and superoxide dismutases—these vitamins help protect the body against oxidative stress in its cells and tissues (Hamilton, 2007).

Physical exercise enhances an organism's oxygen consumption (VO_2), particularly by heart and skeletal muscle. The increase in VO_2 is associated with a rise in the production of reactive oxygen and nitrogen species (RONS). Even in trained skeletal muscles, exercise of high intensity and long duration can overwhelm the antioxidant system with RONS generation, leading to a condition called oxidative stress (Powers, DeRuisseau, Quindry, & Hamilton, 2004).

Frequent targets of RONS attacks are polyunsaturated fatty acids from cell membranes. They are oxidized through a series of reactions that are collectively called lipid peroxidation (LIPOX; Radak, Taylor, Ohno, & Goto, 2001).

Antioxidant supplementation with vitamins C and E, sometimes in combination with β -carotene and/or selenium, can decrease pre- and postexercise concentrations of the LIPOX marker malondialdehyde (MDA; Goldfarb, Bloomer, & McKenzie, 2005; Kanter, Nolte, & Holloszy, 1993). On the other hand, it has also been reported that these vitamin supplements had no significant effects on pre- and postexercise MDA concentrations (Bloomer, Goldfarb, & McKenzie, 2006; Bryant, Ryder, Martino, Kim, & Craig, 2003; Schröder, Navarro, Mora, Galiano, & Tramullas, 2001).

In this study, we investigated a group of trained male cyclists who use a particular encapsulated antioxidant concentrate (EAC) in their daily life, containing vitamins C and E and β -carotene + selenium. Aside from the scientific interest, it was of practical relevance to observe the effect of this supplement on LIPOX in combination with cycling exercise of different intensities. We proofed bioavailability of the supplements' vitamins and estimated the influence of this EAC on concentrations of plasma glutathione peroxidase (pl-GPx). This enzyme is a member of the selenocysteine-containing GPx family. It is regarded as an important antioxidant enzyme in plasma and as a valuable marker for vascular oxidative stress (Rush & Sandiford, 2003).

Methods

Participants

Eight healthy trained men (maximum oxygen uptake [$\text{VO}_{2\text{max}}$] > 55 ml · kg⁻¹ · min⁻¹), nonsmokers, volunteered to participate in this study. Each participant was informed to perform no hard physical training 3 days before the exercise tests and to document diet, sleep time, and well-being the week before each test. Exclusion criteria were use of tobacco products, chronic or excessive alcohol consumption, recent surgery or illness, and use of drugs or dietary supplements in the 4 weeks before the study. After explanation of all experimental procedures, all participants signed informed consent before participation in this study. The protocol of the study was approved by the local ethics committee at the Medical University of Graz, Austria.

Study Design

This study was double-blinded, randomized, and placebo controlled with a cross-over design. $\text{VO}_{2\text{max}}$ testing, anthropometric measurements, and a blood-chemistry

panel were carried out before start of investigation, followed by a 4-week wash-out. On Day 1, all baseline blood measurements were conducted and participants received placebo capsules or an EAC. After 12 days of treatment with placebo or EAC, the first 45% $\text{VO}_{2\text{max}}$ exercise test was carried out. On Day 14, the first 75% $\text{VO}_{2\text{max}}$ exercise test was conducted. After another 4-week wash-out the procedure was repeated using the opposite capsule treatment for each participant. A study timetable is provided in Table 1. Before all exercise tests participants were checked by a physician for health status as precondition to perform the tests.

Methods

All exercise tests were performed 3 hr after a standardized breakfast (Table 2). The week before the exercise tests participants received a 7-day menu plan with recipes to guarantee the same food, nutrient, and fluid intake the last 5–7 days before each exercise test. The menu plan provided carbohydrate-rich food such as pasta, rice, potatoes, and bread; at least five servings of fruit and vegetables daily; two servings of meat or fish per week; and plenty of water, fruit juice, and polyunsaturated fatty acids. The participants were instructed by a dietitian to meet all particular items of the menu plan. Food intake was analyzed using Opti Diet software (version 3.12, GOEmbH, Germany).

Before and after wash-out and treatment periods, and before all exercise tests, lean body mass and body-fat content and distribution were estimated by a computerized optical device, the Lipometer (Moeller Messtechnik, Austria).

EAC or placebo capsules were given for 2 weeks, always after a 4-week wash-out. Daily EAC treatment (two capsules of Oxytex Quatro-Quencher, Wörwag Pharma, Germany) contained 450 mg vitamin C (L-ascorbic acid), 107 IU vitamin E (RRR- α -tocopherol), 36 mg β -carotene (all-trans β -carotene), and 100 μg selenium (given as 100 mg selenium yeast). Placebo capsules had identical appearance and were filled with microcrystalline cellulose. Participants were instructed to consume one capsule with breakfast and the other with dinner. Compliance was assessed by the returned capsule count and by questionnaire.

The participants' training program was standardized during the whole study period, beginning 4 weeks before Day 1. Each week was documented in the training program: endurance training three or four times a week, mainly mountain biking, for at least 90 min with intensities at or between 45% and 75% $\text{VO}_{2\text{max}}$ and gymnastics two or three times a week for at least 60 min, with the exception of the week before the exercise tests.

All participants performed an incremental cycle-ergometer test (ERG 900S, Schiller, Switzerland) at 80 rpm to determine individual $\text{VO}_{2\text{max}}$. After a 3-min rest phase while they sat inactive on the ergometer, work rate started at 40 W for 3 min and was increased 20 W every minute until voluntary exhaustion. A standard electrocardiogram was recorded during the entire test, which was supervised by a physician.

Respiratory-gas-exchange variables were measured throughout all exercise tests using a breath-by-breath mode, with data being stored in 10-s intervals. During the tests, participants breathed through a facemask. Respiratory-gas-exchange data were continuously obtained by means of a portable open-air spirometry system (MetaMax I, Cortex Biophysik, Germany). Heart rate was

Table 1 Study Timetable

| Time point in study | Action |
|---------------------|--|
| -4 weeks | Informed consent Incremental exercise test, VO_{2max} testing Body-composition and physician check First blood-chemistry panel |
| -4 weeks to Day 1 | First wash-out from any supplements |
| -4 weeks to Day 56 | Physical activity records |
| Day 1 | First baseline blood measurements at rest: MDA, GPx, vitamins Second blood-chemistry panel Body-composition check First dispensal of placebo and EAC capsules |
| Days 1-14 | Placebo or EAC treatment |
| Day 7 | Handout and instructions for the 7-day menu plan with recipes |
| Days 8-14 | Food and fluid intake guided by menu plan No intense training (maximum heart rate 140 beats/min) |
| Day 12 | Body-composition and physician check First 45% VO_{2max} test with a duration of 90 min MDA, GPx, vitamin, and lactate measurements |
| Day 14 | Body-composition and physician check First 75% VO_{2max} exercise tests over 30 min MDA, GPx, vitamin, and lactate measurements Third blood-chemistry panel |
| Days 15-42 | Second wash-out from possible supplementation |
| Day 43 | Second baseline blood measurements at rest: MDA, GPx, vitamins Fourth blood-chemistry panel Body-composition check Second dispensal of placebo and EAC capsules |
| Days 43-56 | Placebo or EAC treatment |
| Days 50-56 | Food and fluid intake guided by the menu plan as provided on Day 7 No intense training (maximum heart rate 140 beats/min) |
| Day 54 | Body-composition and physician check Second 45% VO_{2max} test with a duration of 90 min MDA, GPx, vitamin, and lactate measurements |
| Day 56 | Body-composition and physician check Second 75% VO_{2max} exercise test over 30 min MDA, GPx, vitamin, and lactate measurements Fifth blood-chemistry panel |

Note. MDA = malondialdehyde; GPx = glutathione peroxidase; EAC = encapsulated antioxidant concentrate.

monitored throughout the tests using a commercially available heart-rate monitor (Polar Vantage NV, Polar Electro, Finland).

Two different exercise intensities were performed at 80 rpm: at 45% of individual VO_{2max} for 90 min and at 75% of individual VO_{2max} for 30 min. Participants performed each of the two 45% VO_{2max} tests after 12 days of placebo or EAC treatment and each of the two 75% VO_{2max} tests after 14 days of placebo or EAC

Table 2 Composition of the Standardized Breakfast Consumed 3 hr Before the Exercise Tests

| Food | kJ | Protein (g) | Fat (g) | Carbohydrates (g) |
|---|-------|-------------|---------|-------------------|
| Coffee with milk (low-fat) or tea with lemon and honey (10 g) | 180 | 0–2 | 0–2 | 4–10 |
| 3 slices wheat or rye bread | 1,390 | 8 | 1 | 75 |
| Butter 20 g | 652 | — | 16 | — |
| Marmalade/jam 30 g | 343 | — | — | 19 |
| One slice low-fat ham | 331 | 6 | 6 | — |
| One piece cheese | 490 | 16 | 5 | — |
| 250 ml fruit juice | 836 | 2 | — | 46 |
| 250 ml water | — | — | — | — |
| Total | 4,222 | 32–34 | 28–30 | 144–150 |
| Meal energy % | | 13% | 27% | 60% |

treatment. Similar to the $\text{VO}_{2\text{max}}$ test, participants started with the same procedure: rest phase, 40 W at the first step, thereafter work rate increased by 20 W every minute until the workload at 45% or 75% of $\text{VO}_{2\text{max}}$ was reached as calculated from the incremental $\text{VO}_{2\text{max}}$ test. Workload was adjusted every 5 min to maintain the target % $\text{VO}_{2\text{max}}$ intensity.

Before exercise (BE), immediately postexercise (IE), and 30 min (30M) and 60 min (60M) after each test, 12 ml of venous EDTA and heparinized blood were collected from a forearm vein with an indwelling cannula (17 G/1.4 mm: TriCath In). After centrifugation at 3,000 rpm for 10 min at 4 °C, EDTA and heparinized plasma were removed. EDTA samples were frozen at –70 °C until analysis of MDA and at –196 °C until analysis of vitamin E and β -carotene. The EDTA plasma for determination of vitamin C was mixed with 9% metaphosphoric acid and centrifuged again at 3,000 rpm for 4 min. The supernatant was placed into cryotubes and coated with nitrogen. Samples were frozen in liquid nitrogen at –196 °C until analysis. For lactate determination, 20 μl blood were drawn from the earlobe. Concentrations were measured at rest and every 5 min at 45% or 75% of $\text{VO}_{2\text{max}}$ via Eppendorf fully enzymatic analysis (EBIO Plus lactate analyzer, Eppendorf, Germany).

To determine HPLC, 500 μl EDTA-plasma were used for determination by HPLC as previously described by Khoschsorur et al. (2000). Briefly, the MDA–thiobarbituric acid adduct was separated via HPLC, and detection followed fluorometrically at 550 nm. Concentrations were determined by calibration curve of a 1,1,3,3-tetramethoxypropane standard solution (0.1–0.2 μM).

Heparinized plasma was frozen at –70 °C until analysis. We used a sandwich ELISA assay (Biomedica, Vienna, Austria) for pl-GPx. The kit employs a microtiter plate precoated with affinity purified polyclonal antihuman pl-GPx. After washing, bound pl-GPx is detected spectrophotometrically (405 nm) using a biotinylated polyclonal antibody to pl-GPx, with amplification via streptavidin coupled to alkaline phosphatase.

α -Tocopherol, β -carotene, and vitamin C EDTA samples were used for determination of vitamin E and β -carotene via HPLC by UV detection as described by Jakob and Elmadfa (1995). Vitamin C was analyzed electrochemically as described by Maxwell, Jakeman, Thomason, Leguen, and Thorpe (1993).

Standard blood-chemistry panels were run before and after wash-out periods and treatments, using EDTA plasma from peripheral blood. Analysis was performed using routine clinical chemistry methods and a Eurolyser clinical chemistry analyzer (Dia Team, Diagnostica und Arzneimittel Großhandel GmbH, Linz, Austria). In addition, creatine kinase, uric acid, total protein, and C-reactive protein were measured before and after all exercise tests by Eurolyser. This goes for hemoglobin and iron concentrations, as well, which were assessed using an Advia clinical analyzer (Bayer, Leverkusen, Germany). To avoid circulatory hemodynamic artifacts caused by exercise dehydration, we corrected all blood values by total plasma protein and hemoglobin concentrations.

Statistical Analysis

MDA, pl-GPx, and vitamin data were analyzed using a 2 (treatment = grouping factor) \times 2 (type of exercise = grouping factor) \times 4 (time = within factor) repeated-measurements analysis of variance (ANOVA). If appropriate, for selected time points, additional analyses were conducted by *t* test. Participant characteristics, lactate, and standard blood variables were also compared by repeated-measures ANOVA. Statistical analysis was performed with SPSS software, version 12.0. All data are presented as $M \pm SD$. Statistical significance was set at $p < .05$.

Results

No significant changes were noted for weight, body fat, lean body mass, or watt performance over the study period ($p > .05$). In addition, no changes were noted for lactate and creatine kinase kinetics during the intensity-equal exercise tests ($p > .05$). Table 3 provides the characteristics of the 8 participants. Clinical blood-chemistry parameters showed no remarkable concentrations or changes during the study period (data not shown). Supplement compliance was $>95\%$, and no indigestibility or intolerance was reported. Before each exercise series, food intake was matched. Analysis of the 7-day menu plan is presented in Table 4.

There was no treatment effect on the observed oxygen uptakes for either intensity, 45% or 75% VO_{2max} ($p > .05$). In the 75% VO_{2max} tests, participants performed at $74.8\% \pm 2.4\%$ of individual VO_{2max} . In the 45% VO_{2max} tests, they performed at $45.1\% \pm 2.2\%$ of individual VO_{2max} .

BE concentrations of MDA increased from $0.78 \pm 0.07 \mu\text{mol/L}$ at baseline and after placebo treatment to $1.16 \pm 0.11 \mu\text{mol/L}$ ($p < .05$) after EAC treatment (Figure 1). After the 45% VO_{2max} tests (Figure 1[a]), MDA concentrations increased at IE, 30M, and 60M from $0.76 \pm 0.10 \mu\text{mol/L}$ after placebo treatment to $1.21 \pm 0.14 \mu\text{mol/L}$ after EAC supplementation ($p < .05$). There was an Exercise \times Time \times Treatment effect on the strenuous 75% VO_{2max} test ($p < .05$; Figure 1[b]): With EAC treatment, MDA concentrations decreased from BE to IE to

Table 3 Participant Characteristics, $N = 8$, $M \pm SD$

| Variable | |
|--|------------------|
| Age, years | 28.0 \pm 2.2 |
| Height, cm | 178.2 \pm 5.4 |
| Weight, kg | 72.6 \pm 4.1 |
| Total body fat, % | 11.3 \pm 2.3 |
| Lean body mass, kg | 64.7 \pm 3.6 |
| VO _{2max} , ml · kg ⁻¹ · min ⁻¹ | 60.4 \pm 4.5 |
| Maximum workload, W | 360 \pm 20 |
| Maximum lactate, mmol/L | 11.9 \pm 2.7 |
| Maximum heart rate, beats/min | 189 \pm 8 |
| Ventilatory threshold, % of VO _{2max} | 78.6 \pm 3.1 |
| Lactate turn point 2, % of VO _{2max} | 77.8 \pm 2.8 |
| Lactate during exercise, mM/L | |
| at 45% of VO _{2max} | 1.72 \pm 0.41 |
| at 75% of VO _{2max} | 3.63 \pm 2.34* |
| Creatine kinase, U/L | |
| at 45% of VO _{2max} | 127 \pm 24 |
| at 75% of VO _{2max} | 251 \pm 58* |

Note. Comparison of repeated-measures data resulted in $p > .1$ (repeated-measures ANOVA). Lactate and creatine kinase concentrations showed differences between the 2 exercise intensities.

* $p < .05$, repeated-measures ANOVA.

levels similar to those after placebo treatment. These IE concentrations remained stable at 30M and 60M with both treatments ($0.83 \pm 0.13 \mu\text{mol/L}$).

PI-GPx concentrations decreased at all time points after EAC supplementation: from $1.16 \pm 0.18 \text{ mg/L}$ at baseline and after placebo treatment to $0.46 \pm 0.16 \text{ mg/L}$ after EAC supplementation ($p < .05$). The type of exercise had no influence on pl-GPx at any time point (Figure 2).

There were no effects of EAC or placebo treatment observed on vitamin C concentrations on the 45% VO_{2max} tests (data not shown). There was a time and an Exercise \times Time effect at the strenuous 75% VO_{2max} test with EAC treatment ($p < .05$; Figure 3): We observed increased values from BE to IE (from $9.1 \pm 1.4 \text{ mg/L}$ to $13.9 \pm 1.9 \text{ mg/L}$), returning again to baseline concentrations at 30M.

There was a significant effect of EAC supplementation in both vitamins, α -tocopherol, and β -carotene, showing increased concentrations at all measured time points. α -Tocopherol concentrations increased from $7.9 \pm 2.3 \text{ mg/L}$ to $13.6 \pm 3.0 \text{ mg/dl}$ ($p < .05$, data not shown). β -Carotene concentrations increased from $31 \pm 22 \mu\text{g/L}$ to $89 \pm 18 \mu\text{g/L}$ ($p < .05$; data not shown). The types of exercise had no influence on these vitamins.

Table 4 Analysis of the 7-Day Menu Plan Compared With the Recommended Daily Allowance (RDA) of the German, Austrian, and Swiss Nutrition Societies (2000)

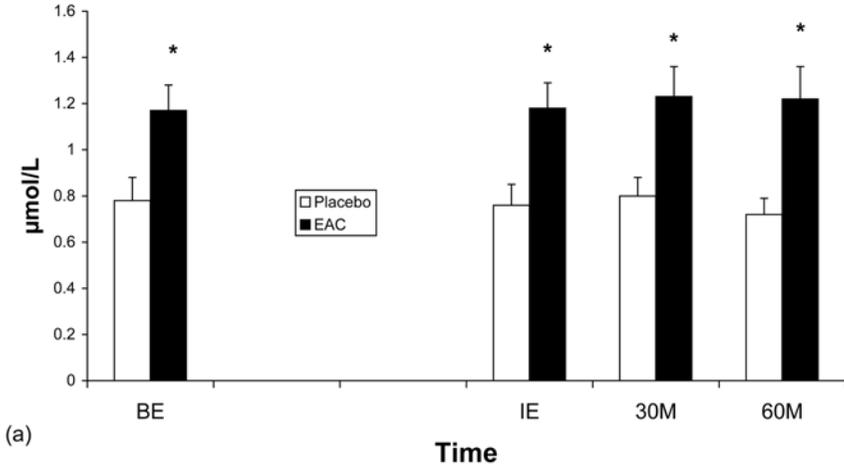
| Nutrient | RDA | Menu plan | % RDA |
|-----------------------------------|--------|-----------|-------|
| kJ | 13,011 | 12,935 | 99 |
| Protein, g/kg body weight | 0.8 | 1.37 | 17.1 |
| Carbohydrate, % of kJ | >50 | 61 | — |
| Fat, % of kJ | 30 | 25.5 | 85 |
| Cholesterol, mg | 300 | 277 | 92 |
| Water, L | 1.41 | 3.8 | 270 |
| Vitamin C, mg | 100 | 99 | 99 |
| Vitamin E, mg | 14 | 13.3 | 95 |
| β -Carotene, mg | 6 | 5.7 | 95 |
| Folate, μ g | 400 | 409 | 102 |
| Vitamin B ₁₂ , μ g | 3 | 4 | 133 |
| Sodium, mg | 550 | 1050 | 192 |
| Calcium, mg | 1,000 | 1,101 | 110 |
| Magnesium, mg | 350 | 423 | 121 |
| Iron, mg | 10 | 14.1 | 141 |
| Copper, mg | 1–1.5 | 1.6 | 107 |
| Zinc, mg | 10 | 12.8 | 128 |
| Manganese, mg | 2–5 | 2.9 | ~100 |
| Selenium, μ g | 30–70 | 67 | ~100 |

Discussion

Data from this study show that (a) EAC supplementation led to an increase of MDA concentrations at rest BE, indicating an increase of LIPOX in trained men; (b) with EAC treatment, strenuous aerobic exercise for 30 min at 75% of individual VO_{2max} led to a decrease of postexercise MDA concentrations; (c) with EAC treatment and at 75% VO_{2max} exercise intensity for 30 min, increase in plasma vitamin C concentrations was observed IE, returning to BE values at 30M; and (d) EAC supplementation attenuated pl-GPx concentrations in plasma at all measured time points.

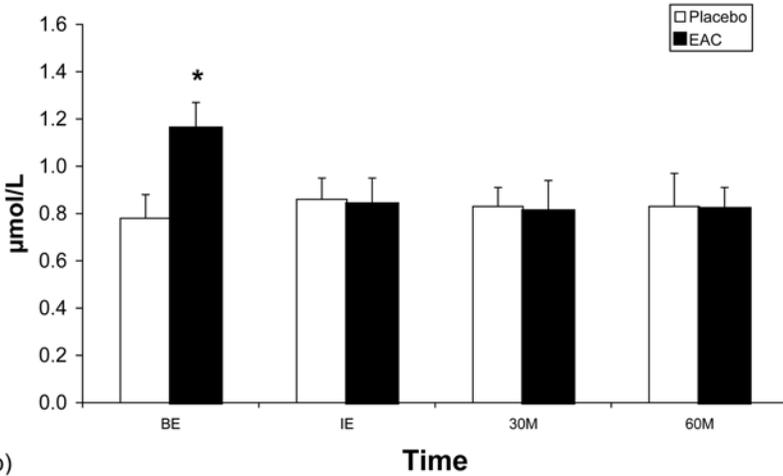
MDA is a widely used marker to estimate LIPOX (Deepa, Jayakumari, & Thomas, 2008; Ishii et al., 2008; Khoschsorur et al., 2000; Toroser, Orr, & Sohal, 2007). The main finding in our study was the significantly higher MDA concentrations after 2 weeks of EAC treatment at rest BE and after 45% of individual VO_{2max} (Figure 1). These results contrast with those of other exercise studies investigating LIPOX and MDA, in which trained men were supplemented with vitamins E and C (Bloomer et al., 2006; Bryant et al., 2003). Bloomer et al. found no effect on MDA after 2 weeks of supplementation with vitamins C (1,000 mg/day) and E (400 IU/day). Bryant et al. found a nonsignificant increase in preexer-

MDA at 45% VO_{2max}



(a)

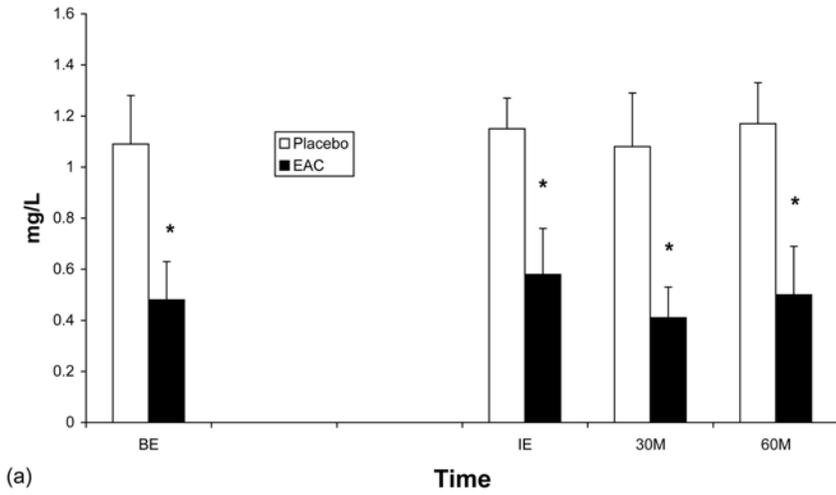
MDA at 75% VO_{2max}



(b)

Figure 1 — Malondialdehyde (MDA) concentrations in $\mu\text{mol/L}$ plasma, 3 hr after a standardized breakfast, at 45% (duration 90 min) and 75% (duration 30 min) of individual VO_{2max} exercise intensity. Supplementation with either encapsulated antioxidant concentrate (EAC) or placebo ($N = 8$). Data are presented as $M \pm SD$. Statistical analyses via repeated-measures ANOVA. Four time points of blood collection: BE, before exercise; IE, immediately after the exercise test; 30M, 30 min after the exercise test; and 60M, 60 min after the exercise test. *Statistically significant. (a) Effect of EAC supplementation (dark columns) to increased MDA concentrations pre- and postexercise ($p < .05$). (b) Effect of Exercise \times Time \times Treatment on the strenuous 75% VO_{2max} test after EAC treatment ($p < .05$). At BE, MDA was increased after 2 weeks EAC supplementation followed by decreased postexercise concentrations, similar to placebo values.

pl-GPx at 45% VO_{2max}



pl-GPx at 75% VO_{2max}

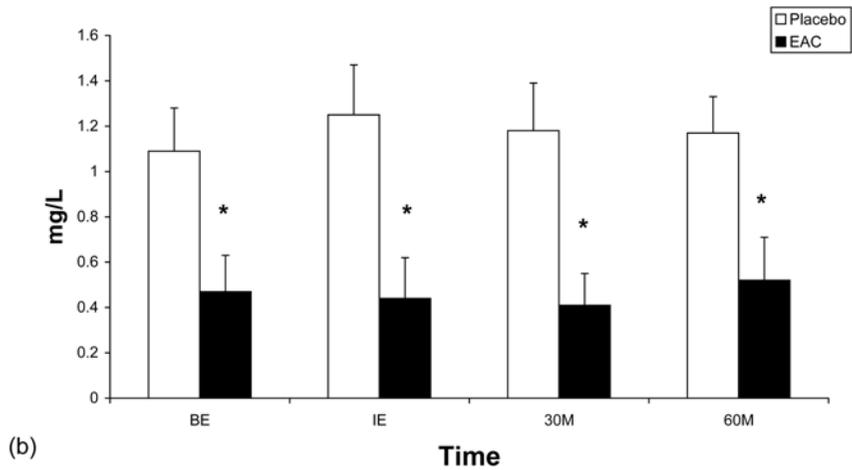


Figure 2 — Plasma glutathione peroxidase (pl-GPx) concentrations in mg/L plasma. See notes for Figure 1. Significant effect of EAC supplementation (dark columns) led to decreased enzyme concentrations at all measured time points ($p < .05$).

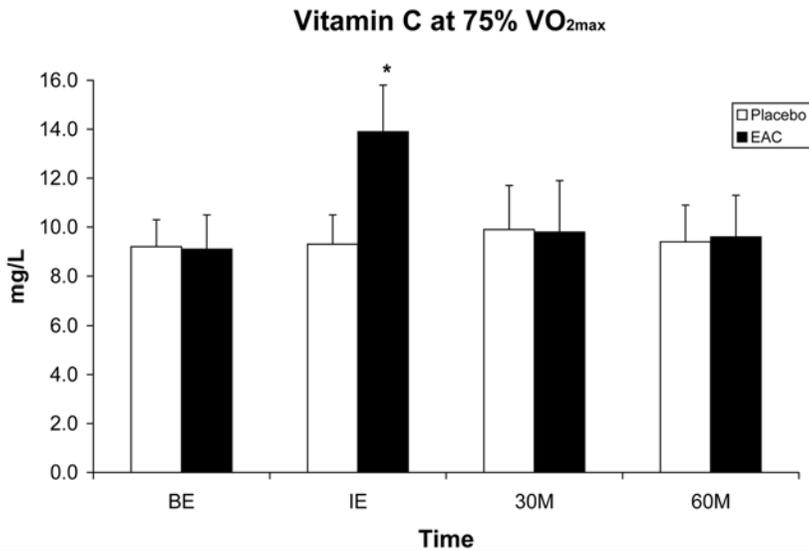


Figure 3 — Vitamin C concentrations in mg/L plasma. See note for Figure 1. There was a time and an Exercise \times Time effect on the strenuous 75% VO_{2max} test at the end of the encapsulated antioxidant concentrate treatment period (dark columns; $p < .05$). Values increased from BE to IE, reaching BE and placebo values again at 30M.

cise MDA concentrations after 3 weeks ingestion of 1,000 mg/day vitamin C + 200 IU/day vitamin E. Neither study used β -carotene. Therefore, we tended to assume that the lack of a significant MDA increase might be attributable to the additional β -carotene content of 36 mg/day provided by our EAC.

This hypothesis is attenuated by the findings of Kanter et al. (1993), who observed significantly decreased MDA concentrations after supplementation with a vitamin mixture consisting of 1,000 mg/day vitamin C, ~880 IU/day vitamin E, and 30 mg/day β -carotene. Consequently, the increase of MDA in our study cannot be ascribed to β -carotene alone.

The treatments of the compared studies were all higher in daily concentrations of vitamin C (1,000 mg) and vitamin E (200–880 IU) than ours (vitamin C 450 mg, vitamin E 107 IU). Kanter et al.'s (1993) study was equivalent to Bloomer et al.'s (2006) and Bryant et al.'s (2003) studies in vitamin C (1,000 mg daily) but higher in vitamin E (880 IU/day vs. 200–400 IU/day) and used additional β -carotene (30 mg/day); the latter was similar to our study (32 mg/day β -carotene). Furthermore, Kanter et al.'s treatment lasted 6 weeks, Bloomer et al.'s 2 weeks, Bryant et al.'s 3 weeks, and ours 2 weeks. Therefore, the influence of a vitamin C and E and β -carotene mixture on LIPOX must depend on the applied proportions of concentrations of these vitamins and on duration of treatment, as well.

In our study, the plasma concentrations of vitamin C—a possible scavenger of generated vitamin E and carotene radicals, and those may contribute to LIPOX (Buettner, 1993)—showed no increase after EAC supplementation. On the other

hand, plasma vitamin E and β -carotene concentrations increased significantly, β -carotene even above normal values (Stacewicz-Sapuntzakis, Bowen, Kikendall, & Burgess, 1987): from 31 $\mu\text{g/L}$ in mean up to 90 $\mu\text{g/L}$.

We conclude that a lack of vitamin C availability in plasma at rest and after 45% $\text{VO}_{2\text{max}}$ intensity exercise, combined with the significantly increased β -carotene concentrations, was responsible for insufficient regeneration of vitamin E and particularly of β -carotene. Hence, the disproportion of these three vitamins in plasma led to enhanced LIPOX as indicated by increased MDA concentrations.

The question remains: Why was there a lack of vitamin C availability in plasma under resting and mild exercise conditions? We suggest that the concentration of vitamin C in the EAC and/or its bioavailability was not adequate to increase plasma concentrations and/or that a surplus in plasma was stored up into the adrenal gland (see next paragraph).

Another finding was the significant decrease of MDA values from BE to IE after EAC treatment with the 75% $\text{VO}_{2\text{max}}$ test and the increase of vitamin C concentrations at the same time. In contrast, no effect of exercise on either substance was found in the other three tests. We provide a hypothesis discussing a connection between MDA and vitamin C to explain the phenomenon that vitamin C increased from BE to IE after EAC treatment at the 75% $\text{VO}_{2\text{max}}$ test (Figure 3). Although this change was still within the normal range of 8–14 mg/L (Baessler, Golly, Loew, & Pietrzik 2002), this significant effect indicates a mobilization of vitamin C during strenuous aerobic exercise. The adrenal gland is the major source of vitamin C efflux into the circulation during exercise, and the rapid rise in plasma vitamin C is associated with the release of cortisol during exercise (Pedersen, Rohde, & Ostrowski, 1998). We assume that vitamin C has mainly been delivered by the adrenal gland, which can save vitamin C in concentrations more than 150 times higher than plasma (Gleeson, Robertson, & Maughan, 1987). The EAC treatment provided a surplus of vitamin C in plasma, and this was stored up into the gland. The vitamin C supply to plasma at 75% $\text{VO}_{2\text{max}}$ intensity braked LIPOX processes and decreased postexercise MDA concentrations. At 30M, vitamin C concentrations returned to BE values because the vitamin was primarily consumed for vitamin E and β -carotene regeneration, and furthermore, in recovery, no more vitamin C affecting cortisol efflux was needed.

GPx is a selenocysteine-containing antioxidant enzyme that scavenges hydrogen peroxide and organic hydroperoxides (Maddipati & Marnett, 1987). Of the five known GPx isoforms, only one is found in extracellular space, and it is primarily synthesized in the kidneys' proximal tubules and hepatic cells (Avisar et al., 1994, 1989; Chu, Esworthy, Doroshov, Doan, & Liu, 1992). Decreased pl-GPx concentrations and activities are found in cerebrovascular and hemodialysis patients and are associated with decreased bioavailability of vascular nitric oxide, enhanced platelet activation, and extracellular oxidative stress (Voetsch et al. 2007).

In this investigation we found decreased pl-GPx concentrations after 2 weeks of EAC treatment (Figure 2), although 100 μg selenium was supplemented daily. Compared with reported pl-GPx concentrations from Australia and Canada (Jacobson, Narkowicz, Tong, & Peterson, 2006, Rush & Sandiford, 2003), baseline values of our participants were lower: ~ 1 mg/L compared with 3–30 mg/L.

Our detected concentrations were closer to those reported from Latvian people (Hagmar, Persson-Moschos, Akesson, & Schuetz, 1998). Seven-day food selenium intake of our participants was adequate (Table 4), but unfortunately we did not measure plasma selenium at baseline to detect possible deficiency before the experimental procedure.

In untrained men and women, Rush and Sandiford (2003) detected higher pl-GPx concentrations than we did in our trained participants. These researchers reasoned that the level of fitness might have an influence on pl-GPx concentrations. To the best of our knowledge, no study has compared different levels of fitness with pl-GPx concentrations. Therefore, the hypothesis is still critical that level of fitness might influence pl-GPx concentrations until further research provides adequate data. However, because of the crossover design, the 7-day menu plan before the exercise tests, and the 3-day recovery time before each test, nutritional influences (between baseline and exercise tests) or effects of heavy exercise (before the testing date) on pl-GPx can be excluded. Consequently, evidence remains that the EAC treatment led to a kind of down-regulation of expression for this enzyme and/or to decreased secretion from hepatic or renal cells.

Although the number of participants (8) in this study was small, our results are substantive; we standardized strictly nutritional intake, training regimen, the exercise protocol, and level of fitness. We conclude that the antioxidant supplementation used induced an increase of LIPOX accompanied with effects on pl-GPx to lower concentrations. Therefore, we did not recommend this supplement to our participants for daily use.

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