Int J Physiol Pathophysiol Pharmacol 2015;7(2):98-104
www.ijppp.org /ISSN:1944-8171/IJPPP0011544

Original Article

Effects of short-term antioxidant supplementation on oxidative stress and exercise performance in the heat and the cold

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Received June 17, 2015; Accepted July 9, 2015; Epub August 15, 2015; Published August 30, 2015

Abstract: The effects of short-term as well as long-term antioxidant supplementation on exercise performance in the heat or the cold are unknown. Based on our recent studies we hypothesized that short-term supplementation with alpha-ketoglutaric acid (α-KG) and 5-hydroxymethylfurfural (5-HMF) would decrease oxidative stress but without significant impairment of maximal exercise performance in the heat or the cold. During a 5-week period young and well-trained participants performed 5 incremental treadmill tests to exhaustion under different temperature conditions (normal: 20°C, cold: +7°C, heat: +33°C) and with different nutritional supplements (placebo or α-KG and 5-HMF) prior to the tests applying a randomized cross over design. The first test was performed under normal temperature, the second and fourth under cold and the third and fifth test under heat conditions. Reactive oxygen metabolites and the biological antioxidant activity in serum were determined (Free Carpe Diem, Diacron International) before the first and after each exercise test. We demonstrated that reactive oxygen metabolites and maximal exercise performance remained unchanged in the cold as well in the heat with and without short-term antioxidant supplementation. Thus, short bouts of intense exercise in the heat or the cold seem not to produce significant oxidative stress in well-trained subjects and therefore pre-treatment with antioxidants may not have beneficial effects. However, future studies will focus on potentially favorable effects in sedentary or diseased subjects and/or on effects of more prolonged antioxidant supplementation when performing endurance exercise for a long duration under extreme temperature conditions.

Keywords: Antioxidants, oxidative stress, biomarkers, heat, cold, exercise performance

Introduction

High intensity exercise produces free radicals including reactive oxygen species (ROS) associated with high rates of mitochondrial oxygen consumption but also related to various other mechanisms [1]. In addition, reactive nitrogen species (RNS) are generated by the contracting skeletal muscle cell [2]. Moreover, exercising under extreme environmental conditions, i.e. hypoxia, cold or heat, may all enhance the generation of free radicals [3-6]. There are several lines of evidence that high levels of free radicals might impair exercise performance by favoring muscle fatigue [7, 8] and/or the reduction of force production [9], e.g. by oxidizing regulatory proteins of sarcoplasmic reticulum calcium release channels and/or changes of myofilament structure and function [10].

Based on these findings, it might be suspected that supplementation with antioxidants when exercising under extreme environmental conditions should scavenge free radicals and therefore help maintaining exercise performance. However, research findings are controversial. Whereas some studies did not find any beneficial effects of supplementation with antioxidants on muscle strength, fatigue, and performance [11] others for example, demonstrated delayed muscle fatigue after supplementation with N-acetylcysteine (NAC) [12, 13]. Controversies might partly be explained by the use of different antioxidants and/or differences regarding the time and duration of supplementation. Recently, we found that short-term supplementation with alpha-ketoglutaric acid (α-KG) and 5-hydroxymethylfurfural (5-HMF) did not prevent the hypoxia induced decrease of

exercise performance despite attenuation of oxidative stress [14]. However, subsequently we demonstrated that aerobic exercise performance was less impaired in acute normobaric hypoxia after 3 weeks with supplementation of α-KG and 5-HMF when compared with a broad-based antioxidants supplement or placebo [15]. Thus, long-term α-KG and 5-HMF supplementation may have effectively prevented oxidative stress formation and may have increased NO bioavailability which in turn preserved exercise performance in hypoxia [16, 17]. Also other studies showed that the combined oral intake of α-KG compounded with 5-HMF improved exercise capacity and reduced oxidative stress, e.g. after lung surgery [18]. In addition, 5-HMF has been shown to elicit protective properties against hypoxic injury [19] and on the vascular endothelium as demonstrated in human umbilical vein endothelial cells [17, 20]. The effects of short-term as well as long-term antioxidant supplementation on exercise performance in the heat or the cold are unknown. Based on our recent studies we hypothesized that short-term supplementation with α-KG and 5-HMF would decrease oxidative stress but without significant impairment of maximal exercise performance in the heat or the cold.

Material and methods

Participants

Seven male sport students (age: 22.7 ± 2.6 years, height: 178.1 ± 3.9 cm, weight: 71.4 ± 6.9 kg) were informed about the study aims and gave written informed consent for participation. The participants were healthy and regularly active with a mean training volume of 8.9 ± 7.4 training hours per week and a maximal oxygen uptake (VO_{2max}) of 56.5 ± 8.7 ml/min/kg. The study was carried out in conformity with the ethical standard laid down in the 1975 declaration of Helsinki and was approved by the institutional review board and by the ethical Committee of the University of Innsbruck, Austria.

Study procedure and exercise performance

The study protocol for each participant consisted of 5 incremental treadmill tests to exhaustion each separated by a washout period of one week. Tests were performed under different temperature conditions with and without antioxidative supplementation prior to the tests applying a randomized crossover design. During the 5-week period participants were encouraged not to change their eating or training habits. Additionally, 24 hours before each test session participants were asked not to consume alcohol or nicotine, nor to perform a heavy training session.

All exercise tests were performed in a climate chamber (pole of cold, Austria) under 3 different climate conditions adapted from the study of Quindry et al. [23]. The conditions were room temperature (20°C, 40% humidity), cold (+7°C, 40% humidity) and heat (+33°C, 40% humidity). The order of the climate condition was as follows: the room temperature setting was the first test to be completed. During the second and fourth week, tests were completed under cold conditions and during week 3 and 5 under heat conditions.

Treadmill spiroergometry (Oxycon mobile, Viasys Healthcare GmbH Jaeger, Germany) test to exhaustion was performed as described in detail elsewhere [23]. Shortly, the test started at 5 km/h walking speed with an incline of 5% for 2 min followed by 2 min at 5 km/h walking speed with an incline of 10% and 1 min at 6 km/h walking speed with an incline of 10%, then the incline was increased by 2% per minute up to 20%, then participants started running and running speed was increased by 1 km/h per min up to exhaustion. VO_{2peak} was defined as the VO_2 averaged during the last 30 s before exhaustion. Before and after each exercise test participants rated their comfort [24], skin wetness [25], motivation and perceived exertion [26]. Body temperature (measurement at the ear by Thermoscan IRT 6022: Braun, Germany) was measured before and after the tests. Capillary blood samples were collected from fingertip at baseline and after each exercise test for the determination of lactate (Biosen C-Line, EKF, Magdeburg, Germany), oxidative stress and total antioxidant level (detailed description of theses parameters are provided below). For the oxidative stress and defense parameters blood was centrifuged at 3000 g for 5 min, and values were determined immediately from serum (Free Carpe Diem, DIACRON international).

Supplementation

Participants started with the supplementation of the substances (either placebo or α-KG plus

Table 1. Peak exercise performance and exercise responses at various conditions

Condition	Room temperature (°C)	Core temperature (°C)	Exercise time (minutes)	VO_{2peak} (ml/min/kg)	HR_{peak} (bpm)	Lactate (mmol/L)
Room temperature	+20	37.03 ± 0.35	12.60 ± 0.68	56.47 ± 8.73	191.00 ± 6.66	14.47 ± 1.99
Cold with placebo	+7	37.23 ± 0.57	12.51 ± 0.97	54.71 ± 9.06	188.86 ± 7.90	16.03 ± 3.65
Cold with supplement	+7	37.21 ± 0.52	12.51 ± 0.77	54.26 ± 5.57	189.14 ± 7.62	15.98 ± 2.21
Heat with placebo	+33	37.34 ± 0.43	12.34 ± 1.17	56.54 ± 8.87	189.86 ± 8.97	14.33 ± 3.41
Heat with supplement	+33	37.51 ± 0.48	12.47 ± 0.82	59.83 ± 7.23	191.43 ± 8.10	14.35 ± 2.70

Values are expressed as mean ± SD. There are no significant differences between conditions.

5-HMF; Cyl® Airnergy drink; both in the form of a liquid) approximately 48 h before exercise testing in the heat and the cold. Subjects ingested their supplements over 2 days prior to exercise testing, twice daily, once in the morning and once in the evening. The total antioxidant dose during these 2 days was 60 mg of 5-HMF and 4.8 g α-KG. The placebo was identical-looking and tasting. The order of supplementation under each condition was random.

Measurement of oxidative stress and total antioxidant capacity

Oxidative stress: Hydroperoxides were measured by the plasma levels of diacron reactive oxygen metabolites (d-ROMs) by using the Free Carpe Diem device (FREE® Carpe Diem; Diacron International, Italy). d-ROM levels are detected based on the ability of transition metals to catalyze, in the presence of peroxides, the formation of free radicals that are then trapped by an alchilamine. The alchilamine reacts to form a colored radical that can be detected by a spectrophotometer at 505 nm. The results are expressed in arbitrary units, namely Carratelli units (U.CARR). A single U. CARR corresponds to 0.08 ng/100 mL of H_2O_2 [27].

Total antioxidant capacity: The biological antioxidant activity of plasma (BAP) was determined using the Free Carpe Diem device (FREE® Carpe Diem; Diacron International, Italy). When trivalent $FeCl_3$ is dissolved in a colorless solution containing a chelation acid derivative, it turns red as a result of the action of the Fe^{+3} ions. However, it is decolorized by the reduction of Fe^{+3} to Fe^{+2} ions caused by the antioxidant activity of plasma added to the reaction solution. The antioxidant potential of plasma is evaluated spectrophotometrically by measuring the degree of decolorization. The normal value for BAP in healthy subjects is > 2200 μmol/L [27].

Statistical analysis

All statistical analyses were performed using the SPSS program version 16.0 (SPSS Inc., Chicago, IL). The paired t-test or Wilcoxon test was used to compare categorical variables or continuous variables between baseline and supplementation trials. ANOVA or Kruskal-Wallis test, with post hoc analysis (Bonferroni corrected), were used to compare differences among trials. Bivariate correlation analyses were performed by Pearson or Spearman as appropriate. Data are presented as means ± SD or median (interquartile range, IQR). A p-value < 0.05 was considered statistically significance.

Results

Exercise performance in the heat and cold with and without antioxidant supplement

Peak exercise time, VO_{2peak}, peak heart rates and blood lactate concentrations determined at the end of incremental exercise tests at room temperature, and with and without antioxidant supplement in the cold and the heat are shown in **Table 1**. None of the variables differed significantly between conditions. The ratings of perceived exertion (RPE) during submaximal and maximal exercise are depicted in **Figure 1**. Although not statistically different, RPE tended to increase at comparable exercise intensities from cold to warmer conditions.

Oxidative stress and antioxidant biomarkers at baseline and after maximal exercise in the heat and the cold with and without supplementation of α-KG and 5-HMF

Whereas the amount of blood oxidative stress (dROMs) after maximal exercise in various conditions did not differ significantly from baseline (**Figure 2**), the amount of antioxidant biomarkers (BAP) were elevated in both the cold with

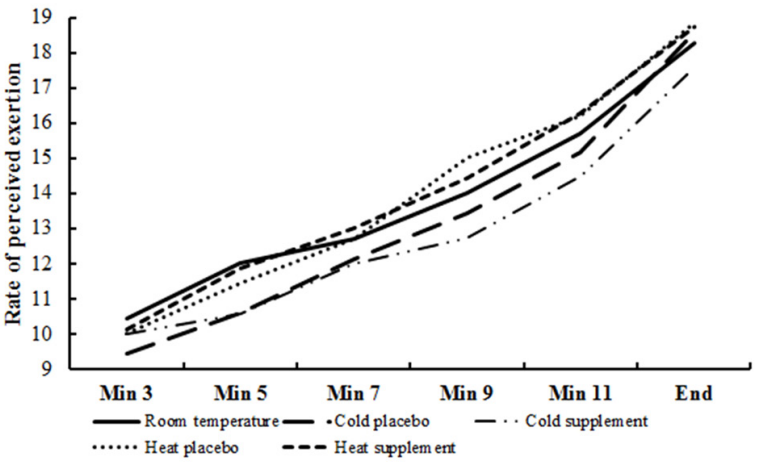

Figure 1. Ratings of perceived exertion during incremental exercise testing under various conditions.

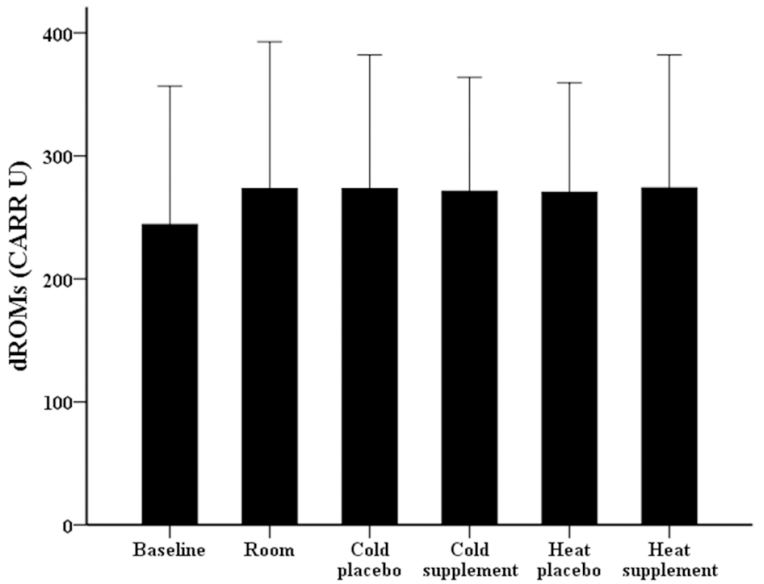

Figure 2. The amount of oxidative stress (dROMs) at baseline (rest) and after maximal exercise under various conditions. There are no significant differences between conditions.

placebo (P = 0.040) and the cold with supplementation of α-KG and 5-HMF (P = 0.008) compared to baseline (**Figure 3**).

Discussion

The main findings of the present study are that short-term supplementation with α-KG and 5-HMF did not change peak exercise performance and related physiological responses and did not affect oxidative stress during exercise neither in the cold nor the heat. However, the amounts of antioxidant biomarkers (BAP) were elevated after maximal exercise in the cold with placebo and with supplementation of α-KG and 5-HMF. RPE during submaximal exercise tended to increase with increasing temperature independently of antioxidant supplementation.

Unexpectedly, there were no changes in maximal performance and related physiological responses neither in the heat nor the cold even without antioxidant supplementation compared to normal ambient temperature conditions. Thus, no performance reductions occurred that could have been compensated for by short-term antioxidant supplementation. Some studies reported a performance decline at extreme temperature conditions [22, 28, 29] but others did not [22, 30, 31]. With respect to cold environment, studies indicate that only under extreme conditions (i.e. approximately below -10°C) performance impairment would occur [28, 29]. The very cold exposure is thought to induce bronchus constriction due to ventilating heavily cold air, to reduce blood flow in superficial parts of the muscle, to reduce muscle contraction velocity, to alter motor unit recruitment and to induce oxidative stress, and thus reducing exercise performance [3, 28, 29, 32]. The degree of cold exposure in the present investigation was probably too low to induce such detrimental effects. With respect to hot exposure, time seems to be critical. Pirnay et al. showed that performance of an incremental exercise test was only affected after thermal exposure leading to a significantly increased body temperature (body temperature was already increased to 38.6°C when the test started and was 39.2°C after exhaustion), but this was not the case when starting the exercise immedi-

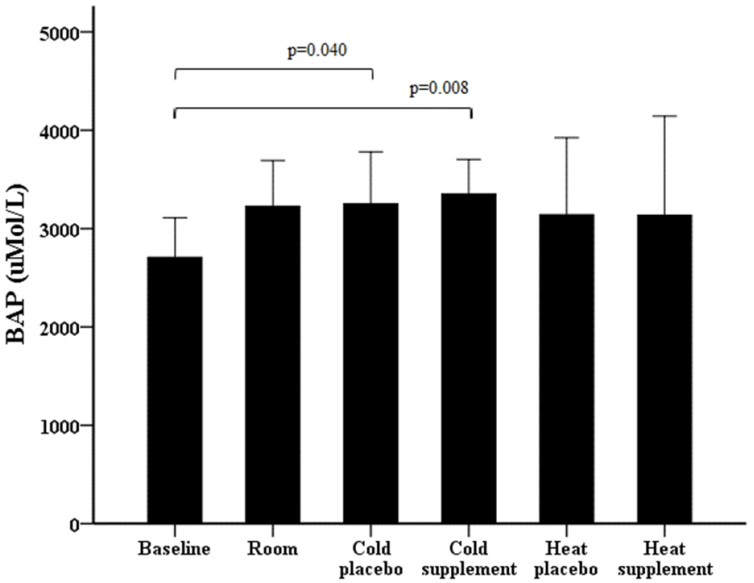

Figure 3. The amount of antioxidants (BAP) at baseline (rest) and after maximal exercise under various conditions.

ately at the beginning of heat exposure (body temperature was 36.9°C when the test started and was 37.4°C after exhaustion) [22]. Heat stress is thought to induce oxidative stress and redistribute blood flow away from the working muscles to the skin, thereby reducing performance [6, 22]. In the present study, exercise tests started immediately after entering the climate chamber and body temperature did not significantly increase. Thus detrimental effects of a raised body temperature should not have developed. Additionally, in the present study participants were really well trained (VO_{2max}: 56.5 ± 8.7 ml/min/kg). Farney et al. showed that in trained men strenuous exercise did not increase blood oxidative stress [33]. This may explain why we did not see any significant increases in d-ROMs and related performance changes.

We found that the amount of antioxidant biomarkers (BAP) were elevated in cold with placebo and with supplement but this was not the case in the heat. Our test series was conducted in October when outside temperatures reached already low values (approximately 0°C) during some days. Therefore it could be speculated that some adaptation to cold may have occurred, which was shown to enhance antioxidant defense after exercise [34].

Our data indicate that RPE during submaximal exercise slightly increases with temperature elevation independent of antioxidant supplementation. These observations are in accordance with Maw et al. who demonstrated that RPE was significantly lower in the cold compared to exercise performed in the heat [35]. Supplementation did not show any effect on RPE as was also reported by Keong et al. [36].

Some limitations of this study should be mentioned. Firstly the small sample size could have led to a type II error which however, is limited by the use of a cross over design. Secondly, the timing of blood sampling may have influenced present outcomes. Accordingly, Michailidis et al. noted that sampling time is crucial when investigating aerobic exercise-induced oxidative stress [37].

Conclusion

The present findings demonstrate that reactive oxygen metabolites and maximal exercise performance remained unchanged in the cold as well in the heat with and without short-term antioxidant supplementation. Interestingly, the amounts of antioxidant biomarkers were elevated after maximal exercise in the cold with placebo and with supplementation of α-KG and 5-HMF. Since short bouts of intense exercise in the heat or the cold seem not to produce significant oxidative stress in well-trained subjects pre-treatment with antioxidants may not have beneficial effects. However, future studies will focus on potentially favorable effects in sedentary or diseased subjects and/or on effects of more prolonged antioxidant supplementation when performing endurance exercise for a long duration under extreme temperature conditions.

Acknowledgements

We are especially grateful to the University of Innsbruck for continuous support of our research work.

Disclosure of conflict of interest

None.

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