Original Communication

5-Hydroxymethylfurfural and α-ketoglutaric acid supplementation increases oxygen saturation during prolonged exercise in normobaric hypoxia

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Abstract: This double-blinded, randomized and placebo-controlled, crossover study investigated whether α-ketoglutaric-acid (α-KG) and 5-hydroxymethylfurfural (5-HMF) supplementation improves exercise performance in hypoxia and affects physiological responses during the exercise task. Eight moderately trained male participants (age: 25.3 ± 2.0 y, VO_{2max}: 48.0 ± 8.3 ml/min/kg) performed an incremental exercise test to exhaustion in normoxia and two 2-hour cycle time trial (TT) tests in hypoxia (3,500 m) each separated by 1-week. Prior to the TT, participants supplemented with either α-KG and 5-HMF or placebo (random order). Supplementation did not improve TT performance at altitude and did not affect heart rate, effort perception and oxidative stress levels ($p > 0.05$). Oxygen saturation (SpO_2) was enhanced during the α-KG and 5-HMF supplementation trial (79.5 ± 3.3 vs. 78.2 ± 3.7%, $p = 0.026$). Even though TT performance was unaffected, the enhanced SpO_2 – possibly originated from changed O_2-affinity – deserves further consideration as the exercise performance decline at altitude is strongly linked to the SpO_2 decline. The inclusion of moderately fit participants, not specifically cycle trained, might have prevented any visible performance enhancement.

Keywords: Altitude, antioxidants, oxidative stress, exercise performance

Introduction

Exposure to hypoxia, i.e., real or simulated altitude decreases aerobic exercise performance [1–3]. This impairment is greatest upon acute exposure [2] and the magnitude of the decrement depends on various conditions, especially on the degree of hypoxia and the individual fitness level [4, 5]. Either way, the decline in maximal as well as submaximal exercise performance at altitude have been mainly linked to the reduced oxygen availability [2, 4, 6]. Though, also an increased oxidative stress level was reported to contribute to performance losses by impairing mitochondrial function and muscle contraction [3, 7, 8].

Several studies investigated the effects of different antioxidant supplementation strategies on exercise performance in hypoxia. Simon-Schnass et al. for instance found that vitamin E supplementation in mountaineers had beneficial physical performance effects at altitude [9]. Subudhi et al. showed that broad-based antioxidant supplementation acutely improved submaximal exercise performance at altitude even though after an acclimatization period this effect vanished [10]. Similar, long-term (i.e. 3-week) α-ketoglutaric acid (α-KG) and 5-hydroxymethylfurfural (5-HMF) supplementation reduced the acute hypoxia induced performance loss during a graded exercise test to exhaustion [11], whereas no such beneficial effects were detected after short-term (i.e. 3 days) supplementation [12]. It has to be mentioned that these studies adopted a graded exercise test to exhaustion in hypoxia lasting for 10 to 20 min to evaluate exercise performance. Even though such tests are used to determine cardiovascular fitness, they do not necessarily reflect long duration

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competition outcomes [13, 14]. Additionally, such protocols might have been too short to identify a benefit of the short-term supplementation.

Next to this, it has to be recognized that different supplemental antioxidants have been studied e.g., vitamin E vs. broad-based antioxidant vs. combined intake of α-KG and 5-HMF, that might affect exercise performance to a different degree and in a different way. An interesting aspect of the latter substances, namely α-KG and 5-HMF, is that the combined intake, next to preventing oxidative stress, which may affect mitochondrial function and muscle contraction [3, 7, 8], is thought of having further performance enhancing properties, especially in a setting of reduced oxygen availability e.g., hypoxia. α-KG is the precursor of glutamine and glutamate [15], an intermediate of the citric acid cycle and the natural ubiquitous collector of amino groups in body tissue [16]. As a precursor of arginine, which is required for the formation of nitric oxide [17], the substance may affect muscle perfusion and contractility and lung diffusion capacity [12, 18]. Moreover, α-KG was found to improve exercise tolerance and training outcomes in untrained young adults [19]. 5-HMF is a low-molecular-weight five-carbon-ring aromatic aldehyde [20] and a common product of the Maillard reaction [16]. 5-HMF was found to reduce the decline of the mitochondrial membrane potential in human endothelial cells exposed to hypoxia [21]. Additionally, in severe hypoxia 5-HMF increased hemoglobin affinity [20, 22], prevented hemodynamic disturbance and partially was able to maintain microvascular oxygenation [20]. Thus, it seems reasonable to speculate that the combined intake of α-KG and 5-HMF may enhance prolonged exercise performance at altitude by improving mitochondrial function and muscle contraction through reductions of the oxidative stress levels [3, 7, 8], by increasing the oxygen saturation via a shift in the oxygen dissociation curve and improvements in lung diffusion capacity [12, 20, 22] and by maintaining microvascular perfusion and oxygenation [20].

The purpose of this study was to investigate if short-term supplementation with α-KG and 5-HMF improves long duration (i.e. 2 hours) time trial performance in hypoxia and/or is able to alter submaximal exercise responses (oxygen saturation (SpO_2), heart rate, oxidative stress).

Materials and methods

Subjects

Ten male sport students from the University of Innsbruck volunteered to participate in the study. All participants were healthy and regularly active (they practice different sports for at least 6 hours per week), without cycling specific training background. During the study, two participants dropped out and had to be excluded from analysis. One participant stopped the hypoxia test due to dizziness. Another participant apparently used different pacing strategies during the trials even though participants were advised not to use such strategies. An overview of the characteristics of the remaining eight participants is shown in Table 1. The study was carried out in conformity with the ethical standards of the declaration of Helsinki and has been approved by the Institutional Review Board of the Department of Sport Science of the University of Innsbruck.

Table 1. Baseline characteristics and incremental exercise test outcome of the participants (n = 8)

	mean ± sd
age (years)	25.3 ± 2.0
height (m)	1.81 ± 0.07
body weight (kg)	78.9 ± 10.5
BMI (kg/m^2)	24.1 ± 2.7
VO_{2max} (mL/min/kg)	48.0 ± 8.3
power output (W/kg)	4.48 ± 0.56
HR_{max} (b/min)	191 ± 8
Borg RPE scale	19.4 ± 0.9

Body Mass Index, BMI; maximal oxygen uptake, VO_{2max}; maximum heart rate, HR_{max}; rating of perceived exertion, RPE

Design

The study was conducted as a double-blinded, randomized and placebo-controlled crossover study. An incremental test to exhaustion with gas analyses in normoxia was performed as a baseline-test. Thereafter, participants were randomly assigned, stratified by the maximal oxygen uptake, to a group that performed the time trial with the α-KG and 5-HMF supplementation first and a group that ingested the placebo supplement first. All sessions were separated by one week. Participants ingested the α-KG and 5-HMF or the placebo supplement in the evening before the test, 1 hour before the test and after 1 hour of exercise. During the study period, participants were advised to maintain nutritional habits and physical activities.

Baseline exercise test

After presenting to the laboratory, capillary blood samples were taken from the fingertip to measure oxidative stress levels (d-ROMs-test, FREE Carpe diem from Diacron (Grosseto, Italy)). Details on the oxidative stress level measurement is provided elsewhere [23]. Shortly, 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. Baseline incremental exercise tests were performed on a cycle ergometer (Cyclus 2, RBM, Germany) in normoxic conditions (Innsbruck, FiO_2 = 20.9%, 574 m). Oxygen uptake was measured continuously with a mobile gas analyser (Cortex Metalyzer 3B CORTEX Biophysics GmbH, Leipzig, Germany) which also recorded heart rate from a Polar chest belt (Polar H7 heart rate sensor, Polar Electro, Kempele, Finland). Before starting the incremental exercise test, a warm up period lasting for 10-minutes at a load of 1 watt per kilogram of body weight was performed. The test started at 75 watts and power output was increased each minute by 25 watts until exhaustion. Participants were advised to keep pedalling frequency rpm > 70. Rating of perceived exertion (RPE) was examined every second minute by using the Borg scale. Exhaustion criteria were: RQ > 1.1, a plateau of VO_2 despite increased power output, achievement of 90% of theoretical maximum heart rate (220 minus age), and RPE scale > 17 of 20. Exhaustion was accepted if 3 of the 4 criteria were reached [24].

Hypoxia test sessions

Participants presented to the laboratory 30 minutes before beginning the test. Immediately after arrival, capillary blood was collected from the fingertip to measure oxidative stress levels. After ingesting the supplement or the placebo participants entered the normobaric hypoxic chamber set at a FiO_2 of 13.5% corresponding to approximately 3500 m. After a 15 min resting period in the chamber the cycling session started with a five-minute warm-up period at an intensity corresponding to 1 watt per kilogram of body weight. Thereafter the two-hour hypoxia time trial test started. The pedal force was fixed so that at a pedalling frequency of 75 rpm the power output corresponded to an intensity of 80% of the adjusted maximal oxygen uptake of the pre-test. As aerobic performance decreases by approximately 10–15 % every 1000 m above 1500 m [2] a 20 % lower maximal oxygen uptake from the pre-test was anticipated (3500 m). Lowering pedalling frequencies resulted in a reduction of power output and vice versa. Participants were asked to pedal at a maximal frequency for two hours. Pedal force was reduced manually, if participants despite lowering the pedalling frequency were not able to proceed. As mentioned, after an hour of exercise a further α-KG and 5-HMF or placebo dose was ingested. The second hypoxia test followed the same protocol. Power output and heart rate were measured continuously, RPE and SpO_2 (Pulsox-3i, Konica Minolta, Tokyo, Japan) were documented every ten minutes.

20 minutes after the time trial, oxidative stress levels were measured again from capillary blood taken from the fingertip.

Supplementation

The α-KG and 5-HMF supplement and placebo were obtained from C.Y.L. Pharmazeutika GmbH. A single dosage of the α-KG and 5-HMF supplement included 30 ml of fluid (in two sachets) containing water, sugar (12.3 g), α-KG (2.4 g), magnesium chloride, 5-HMF (0.24 g), potassium hydroxide and sodium hydroxide. The content of the sachets was solved in 300 ml of water. At each TT session, participants ingested three times a single dosage (i.e. in the evening before the test, 1 hour before the test and after 1 hour of exercise). Placebo did not differ in appearance and taste from the supplement and contained the same amount of carbohydrates as the α-KG and 5-HMF supplement. The placebo did not contain any other substance.

Statistical analyses

An a priori power analysis was performed to determine the number of participants needed in this study. Assuming greater performance effects of the substances during long duration than short duration exercise, we based the power calculation on the results of Mariacher et al. [11]. According to the results of this study a total sample size of 9 subjects was required to detect significant differences in submaximal performance between trials (Cohens d = 1.12; power of 0.80). Data were analyzed with IBM SPSS Statistics 20.0. Shapiro-Wilk test was used to estimate normal distribution. Changes between the placebo and the α-KG and 5-HMF were assessed by using paired student t-tests (mean values over the entire 2-hour time trial for power output, heart rate, SpO_2 and RPE). An analysis of variance with repeated measurement design was used to identify changes in oxidative stress values. Effect sizes (ES, Cohens d or η^2) were calculated and interpretations of results are based on scale of magnitudes. Data are presented as means ± SD. The level of significance was set at $p \leq 0.05$.

Results

Time trial outcomes are shown in Table 2. α-KG and 5-HMF supplementation neither improved performance in hypoxia (p = 0.634, ES: 0.16, qualitative inference: very likely trivial) nor did it affect HR responses (p = 0.815, ES: 0.13, qualitative inference: unclear) and perceived exertion (p = 0.910, ES: 0.10, qualitative inference: unclear). SpO_2 on

Table 2. Mean values for power output and exercise response parameters during the α-KG and 5-HMF and placebo trial

	α-KG and 5-HMF	Placebo	P value	ES	Qualitative inference
Mean power output (W)	155.1 ± 24.8	154.3 ± 24.0	0.634	0.16	Very likely trivial
Mean HR (b/min)	158 ± 15	157 ± 17	0.815	0.13	unclear
Mean RPE	16.3 ± 1.5	16.2 ± 1.4	0.910	0.10	unclear
Mean SpO_2 (%)	79.5 ± 3.3	78.2 ± 3.7	0.026	1.02	likely positive

Effect size (Cohens d), ES; heart rate, HR; rating of perceived exertion, RPE; Peripheral oxygen saturation, SpO_2
Power output and HR were measured continuously, while SpO_2 and RPE were measured every 10 minutes.

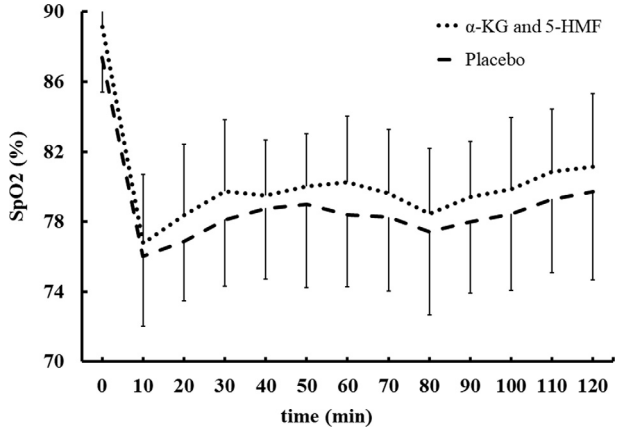

Figure 1. Oxygen saturation in the course of the α-KG and 5-HMF and placebo test sessions.

the other hand was increased during the α-KG and 5-HMF trial (79.5 ± 3.3 vs. 78.2 ± 3.7% for the supplementation and the placebo trial, respectively, p = 0.026, ES: 1.02, qualitative inference: likely positive, Figure 1). The oxidative stress levels were not affected by the supplementation (α-KG and 5-HMF trial: 222.0 ± 50.0 to 214.5 ± 32.3 Ucarr; placebo trial: 199.8 ± 40.0 to 214.6 ± 40.5 Ucarr; p = 0.426; $\eta^2 = 0.093$).

Discussion

The present investigation shows that short-term supplementation with α-KG and 5-HMF was neither able to improve two-hour time trial performance in hypoxia nor did it affect heart rate, perceived exertion and oxidative stress levels in moderately trained individuals without cycling specific training background. SpO_2, on the other hand, was increased during the α-KG and 5-HMF supplementation trial.

Several studies investigated the effects of dietary supplements on the exercise performance at altitude. Depending on the duration of the supplementation, either beneficial [9–11], or no effects on exercise performance were found [12]. The result of the present investigation is in agreement with a study using a similar supplementation strategy, though investigating outcomes of short incremental exercise tests [12]. Yet, the present study and the study performed by Gatterer et al. (i.e., muscle oxygenation differences) [12] found physiological responses that would have been expected of having some performance influencing effects. In the present study, for instance, an increased SpO_2 during the α-KG and 5-HMF trial (Figure 1) was found. 5-HMF reduces the p50 via allosteric modification of the haemoglobin [20, 22]. This shift in the oxygen-haemoglobin dissociation curve was found to improve arterial O_2 saturation and O_2 delivery under hypoxia conditions in a swine model [22]. As decrements in cycling performance from sea-level to altitude were found to be closely related to the decline in SpO_2 [6] it may be argued that increasing SpO_2 should also improve performance. One reasons for the unchanged exercise performance despite increased SpO_2 might be that the participants in the present study were not specifically trained in cycling and thus have not been able to profit from the higher SpO_2 after α-KG and 5-HMF ingestion. This leads to the assumption that other factors than oxygen availability might have determined time trial performance in our moderately trained participants. These factors might include muscular fatigue [25] and/or the effects of oxidative stress on muscle contraction [26]. With respect to the latter, the unchanged oxidative stress level found in the present investigation argue against such a contribution. However, the oxidative stress evaluation in the present study was based on a single parameter and measured in blood plasma, which might not necessarily reflect the conditions within the working muscles [10]. In addition, participants of the present study, even though advised to select the highest possible pedalling frequency in order to increase power output, mostly chose to use a frequency that was the most comfortable for them. The fixed pedal force in conjunction with the unchanged pedalling frequency might thus have determined the unchanged power output as well. Alternatively, it could be assumed that short-term supplementation is not effective in enhancing performance and that a longer supplementation period would have been necessary as was suggested earlier [11].

Some limitations of the present study have to be acknowledged. As mentioned the participants were not specifically

trained for cycling which might have influenced the outcomes of the present investigation as outlined before. Nonetheless, the finding of an increased SpO_2 during the α-KG and 5-HMF trial should not have been influenced by the inexperience of the participants especially when considering the unaltered mean power output. Furthermore, we only measured a single approximate for the oxidative stress level, namely d-ROM, once 20 minute after exercising. This procedure might have limited the probability to detect an effect on the oxidative stress level. The double-blinded cross-over design of this study can certainly be considered a strength of the investigation.

Conclusion

In conclusion, α-KG and 5-HMF was not able to enhance TT performance at altitude in moderately fit participants, not specifically cycle trained. Yet, due to the enhanced SpO_2, performance enhancing capabilities of the supplementation in hypoxia cannot be ruled out. This applies in particular when considering that the exercise performance decline at altitude is strongly linked to the SpO_2 decline [6] and that high performance athletes suffer the largest performance losses at altitude [4]. The specific study design and the inclusion of moderately fit participants, not specifically cycle trained, might have prevented any visible performance enhancement, leading to the speculation that elite athletes competing at altitude might better profit from such a supplement due to maintenance of a higher SpO_2 for a given workload.

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Authorship
The study was designed by HG, MB, FK, and LM; data were collected and analyzed by LM, FK and HG; data interpretation and manuscript preparation were undertaken by HG, MB, FK, and LM. All authors approved the final version of the paper.

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Conflict of interest
The authors declare that there are no conflicts of interest.

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