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PLASMA ANTIOXIDANT CAPACITY IN HIGHLAND SUBJECTS EXPOSED AT 5200 METERS OF ALTITUDE

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ABSTRACT

Human exposure to high altitude conditions (reduced barometric pressure) leads to the formation of free radicals, which could be a major cause of altitude sickness. In the present study the total antioxidant capacity (TAC) was measured by two methods, FRAP (ferric reducing antioxidant power) and ABTS (2,2 '-azino-bis-3-6-sulfonic acid ethylbenzotiazolin acid) in the blood plasma samples of 15 non-smoking Bolivians (10 men and 5 women), who were exposed to large changes in altitude from 3,600 to 5,200 meters. The average antioxidant activity for ABTS at 3600 m was 560 µmol Trolox Equivalent./l of plasma and for FRAP 569 µmol Trolox Equivalent./l of plasma.. .After exposure to 5200 m, the ABTS and FRAP showed an increment in average of 602 µmol Trolox Equivalent./l of plasma and 642 µmol Trolox Equivalent./l of plasma. Both methods showed a high linear correlation for all samples. After exposure of subjects to extreme altitude, the results showed a significant increase in the level of antioxidants in blood plasma samples especially for the FRAP method.

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INTRODUCTION

Altitude sickness is a common problem to people exposed to high altitude. This disease starts as primary disorder of the central nervous system and for most of non acclimatized people, headache is the main symptom observed after 6-12 hrs of arrival [1]. This disorder affects millions of visitors in high altitude destinations (above 2500 m) such as travellers, tourists, climbers, athletes, or football players among others every year. Although the cause of altitude sickness is not fully understood yet [2], there is some scientific evidence that hypoxia can be one of the main factors for altitude sickness due to the formation of reactive oxygen species (ROS) at the mitochondrial level [3].
ROS released during hypoxia can lead to a number of consequences after headache such as lack of appetite, nausea, vomiting and, in the worst of cases, pulmonary edema and high altitude cerebral edema [4]. As a protective response to the oxidative stimuli from the formed ROS, the human organism may generate endogenous antioxidants [4,5,6] . For example, Baille et al., 2007 [6] reports that urate levels increased in human blood when people from lowlands were exposed about nine days to a high altitude, showing a possible relationship between endogenous antioxidants and ROS formed during hypoxia.

As an important destination in South America, La Paz (Bolivia) is an urban place example for exposing to high altitude since it is situated at 3632 m.a.s.l [7]. The city of La Paz, is one the mains destinations in Bolivia and it has about 2 million inhabitants, living in the urban sprawl, adapted to an average altitude from 3300 to 4000 m.a.s.l . The aim of the present study was to observe the variation in blood plasma antioxidant levels in healthy, native individuals living in La Paz who were moved and exposed to 5200 m.a.s.l. during about 2 hours in the surrounding mountains. The results showed a significant increase in the endogenous antioxidant level, as it was observed before in people native from the low lands. The main conclusion from the present study is that there is a significant increment in endogenous plasma TAC in highlander population after the exposure to a higher altitude.

EXPERIMENTAL

Chemicals

ABTS [2,2'-azino-bis(3-ethylbenzotiazoline-6-sulphonic acid)], potassium persulfate, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, 97%), TPTZ (2,4,6-tripyridyl-s-triazine), were purchased from Sigma-Aldrich (St. Louis, USA), acetic acid (glacial p.a.), and sodium acetate from BDH Chemicals Ltd. (Poole, UK).Ferric chloride from ICN Biomedicals (Costa Mesa, CA, USA).

Samples

Samples of human blood were obtained from fifteen healthy, non-smokers highland natives volunteers of both sexes (10 males and 5 females) aged 22 ± 1.1 years old. They provided written informed consent following the local research committee's exigencies in accordance with procedures outlined by the *Declaration of Helsinki*. From each individual a first blood sample (5 ml) was taken at 3600 m.a.s.l (La Paz city) at 8:00 in the morning and a second sample was taken in the Huayna Potosí Mountain at 5200 m.a.s.l of altitude after 2 hours (11:00 am). Subjects were fasting during the first sampling period and they ate a sandwich consisting in jam, lettuce and bread before the second sampling period. Samples were immediately transported in dry ice to the laboratory in La Paz and were centrifuged at 3000 rpm for ten minutes to obtain 2.5 mL of plasma for each sample. The blood plasma samples were stored at -80ºC until analysis. Samples were analysed by triplicates during two different days.

Measurement of TAC

The total antioxidant capacity (TAC) was measured using the ABTS and FRAP methods as described by [8]. The results are expressed as µmol Trolox™ equivalents per litre of plasma (µmol TE/l).

Statistical Analysis

The results were expressed as mean values ± standard error mean (SEM) of six replicates measured over two days. Linear correlation coefficient was calculated according to the Pearson method. The significance of differences between groups was assessed by the paired t-test (SPSS, version 16, Chicago Ill, USA).

RESULTS

Total Antioxidant Capacity (TAC)

In most of the samples an increase was observed in TAC values by both methods after the exposure at 5200 m.a.s.l. For instance, the mean obtained at 3600 meters by the FRAP method was 544 µmol of Trolox /l of blood plasma and increased to 629 µmol/l after 2h-of exposition at 5200 m.a.s.l. Similar behaviour was observed by the ABTS method where the mean increased from 518 to 561 µmol of Trolox /l after the high altitude exposure.

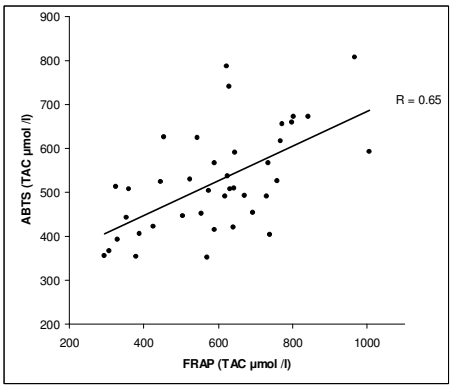

Figure 1*: Linear correlation between ABTS and FRAP methods for all plasma samples, each value being the mean of triplicate measurements.*

Statistic determinations

TAC values assessed by both methods showed a significant linear correlation ($r = 0.65$; $p < 0.01$, Figure 1) for all determinations according to the Pearson method. The correlation of both methods was also observed in other studies [8,9,10]. In the present work significant differences were found in total antioxidant capacity (TAC) by the FRAP method after the high altitude exposure of blood plasma sample using the T paired test ($p< 0.05$) .However the increases showed by the ABTS method were not significantly different. (Figure 2).

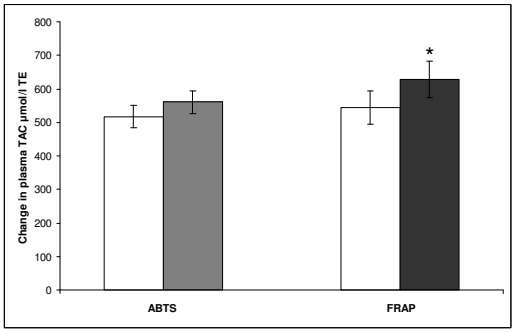

Figure 2: *FRAP and ABTS values from human plasma antioxidant levels before and after exposure to 5200 m.a.s.l. Each column represents the mean ± S.E.M. of 15 values corresponding to 15 highlander volunteers. *T paired test ($p< 0.05$).*

DISCUSSION

Total antioxidant capacity TAC

The data obtained in the present investigation assessed by the ABTS method at 3600 m were in a similar range to those obtained in human blood plasma in another study assessed by the same methodology collected in subjects from Poland living at the sea level [11]. However, our data assessed by the FRAP method were 50% higher in comparison with Janaszewska et al. 2002[11] and also they were somewhat higher than the values obtained in blood plasma samples before and after wine consumption collected in Croatia [12]. A possible difference can be that the FRAP method would be more sensitive to the changes of the different types of antioxidants at high altitude present in blood plasma in comparison with ABTS. For instance, the contribution of the total antioxidant capacity (TAC) in plasma is a combination of urate which is responsible of 65% of the TAC, and phenolic compounds, vitamin C and E representing the remaining 35% [12]. On the other hand, as it is shown above, the FRAP method also evidenced a statistical significant increase in TAC after the high altitude changes in comparison with those obtained by the ABTS method.

The present data were also in accordance with data measured in plasma obtained from healthy lowlanders by the ABTS method exposed to an altitudinal gradient from sea level to 5200 meters when travelling from London to La Paz and being after exposed during 13 days to such gradient. In comparison, our data were higher than those obtained at sea level (London) and up to 5200 m after 6 days of acclimatization. In that experiment during the first period (London – La Paz), the TAC values also were lower in comparison with those obtained by Janaszewska et al., 2002[11] assessed in polish population. However, when individuals were exposed to 5200 m from the 6^{th} to the13^{th} day of acclimatization, data were in the same range of the present research.[13]

A possible explanation could be that the endogenous urate concentration, which is the main antioxidant found in plasma [6], remains higher in native populations adapted at high altitude in comparison with people leaving at sea level, as a normal process of long adaptive mechanism to offset hypoxia. [13]

In the present work it has been found a significant correlation between values obtained in blood plasma by FRAP and ABTS methods contrasting with the study by Cao et al., [14] where no correlation was observed in human blood plasma measured by both methods. This could be due to increments of urate concentration by the altitudinal changes which make more comparable both methods. However, in other studies, FRAP and ABTS methods showed high linear correlation in samples from living organism for example in differed animal tissue as heart, kidney liver and brain [15].

The statistical analysis reveals that the FRAP method shows a significant increment in comparison with the ABTS method and indicates that FRAP should be recommended as an appropriate method in similar studies when exposing humans to high altitudes.

These data suggest that the TAC levels also increases in populations born and inhabiting at high altitudes when they are exposed to an extra altitudinal change. It will be very important in the future to study the several factors that could influence in some degree these levels, for instance dietary factors or chemicals (antihypoxia new drugs).

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