Original articles

The 'normobaric oxygen paradox': does it increase haemoglobin?

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Abstract

(De Bels D, Theunissen S, Devriendt J, Germonpré P, Lafere P, Valsamis J, Snoeck T, Meeus P, Balestra C. The 'normobaric oxygen paradox': does it increase haemoglobin? *Diving and Hyperbaric Medicine*. 2012;42(2):67-71.)

Background: A novel approach to increasing erythropoietin (EPO) using oxygen (O_2) (the 'normobaric oxygen paradox') has been reported in healthy volunteers. We investigated whether the EPO increase is sufficient to induce erythropoiesis by comparing two protocols of O_2 administration.

Methods: We compared the effect of daily versus alternate days 100% O_2, breathed for 30 minutes, on haemoglobin concentrations during a 12-day period. Nine subjects underwent the two protocols six weeks apart.

Results: We observed a significant increase in haemoglobin (as a percentage of baseline) in the alternate-days group compared to the daily group and to baseline after four days (105.5 ± 5.7 % vs. 99.6 ± 3.3 % difference from baseline; $P < 0.01$). At the end of the experimental period, haemoglobin values increased significantly compared to baseline in both groups. There was a significant percentage rise in reticulocyte count in the alternate-days group compared to the daily group (182 ± 94 % vs. 93 ± 34 %; $P < 0.001$).

Conclusion: The normobaric oxygen paradox seems effective in increasing haemoglobin in non-anaemic, healthy volunteers, providing sufficient time is allowed between O_2 applications. The exact time interval is not clearly defined by this study but should probably be at least or greater than two days. Further studies are needed to define more precisely clinical applications in the use of O_2 as a pharmaceutical agent.

Key words

Oxygen, haematology, reactive oxygen species (ROS), physiology

Introduction

There has been increasing concern during the last decade about transfusion hazards.[1,2] Furthermore, no clear evidence has arisen about the benefits of transfusion and even questions about increasing mortality have even been raised.[3,4] The use of a red blood cell progenitor enhancer such as exogenous erythropoietin (EPO) is extensively recognised, and a relatively low rate of adverse effects has been reported in patients adequately followed in medical institutions.[5] However, the price of such medications is very high and its availability is limited in some countries.

A recently described phenomenon called the 'normobaric oxygen paradox' (NOP), may show possible clinical applications.[6] The technique consists of the simple application of high-concentration oxygen (O_2) breathing (monitored by transcutaneous oxygen tension) to spontaneously breathing subjects. This has been shown to provoke a significant increase in endogenous erythropoietin production.[7] The purpose of this paper is to report a potential clinical application of NOP to increase haemoglobin (Hb) concentration in healthy humans.

THE NORMOBARIC OXYGEN PARADOX

The mechanism proposed to explain this phenomenon lies deep within the fundamental cellular mechanisms of adaptation to hypoxia. This depends on the availability of oxygen-free radicals (reactive oxygen species, ROS). In fact, in the presence of ROS, the hypoxia-inducible factor alpha, (HIF-1 α), is linked constantly to the tumor-suppressing Von Hippel Lindau protein (VHLp). This formed complex is subsequently bound to ubiquitin ligase and finally recycled in the proteasome (Figure 1).[8] In case of limited availability or absence of ROS, the total amount of HIF-1 α available will not link with VHLp and thus can be dimerised with HIF-1ß. This HIF complex can thus start the cascade of erythropoietin (EPO) gene expression through binding to promoters such as hypoxia-responsive elements, and subsequently lead to EPO de novo synthesis.[9]

Increasing the patient's inspired oxygen (and thus the intracellular availability of ROS) will increase the production of protective agents against ROS, i.e., up-regulation of glutathione synthetase enzyme activity (gamma glutamyl cysteine synthetase). This enhanced activity will increase the glutathione production and subsequently the scavenging of ROS. During the hyperoxic period, the amount of reduced glutathione (GSH) will therefore rapidly increase to overcome the increased oxidative agents. When stopping hyperoxia, this increased amount of GSH together with an ongoing (slow) conversion of oxidised glutathione to GSH will produce an excess of ROS scavenging. The importance

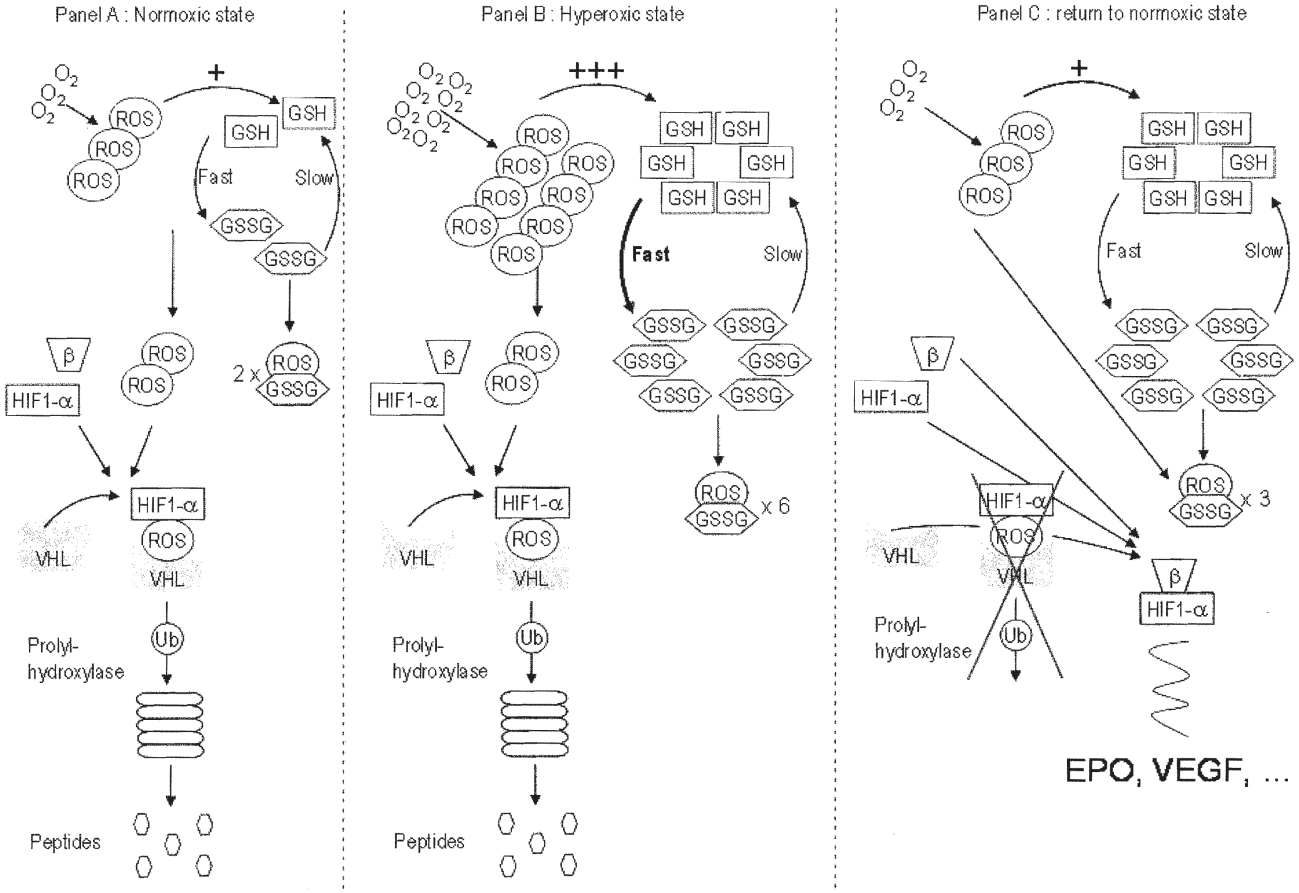
Figure 1

The normobaric oxygen paradox (reproduced with permission)[8]

Panel A: Normoxia – normal intracellular function

Panel B: Hyperoxia – during normobaric hyperoxia, reactive oxygen species (ROS) stimulate glutathione (GSH) production; hypoxia-inducible factor alpha (HIF-1 α) is continuously produced, but continuously inactivated by its binding to another protein, Von Hippel Lindau tumor-suppressor protein (VHL), and by subsequent ubiquitous metabolisation by hydroxylation of proline residues

Panel C: Return to normoxic conditions. All ROS are neutralised by the increased intracellular GSH. This induces exogenous erythropoietin (EPO) gene expression similarly to hypoxia, and this situation could be called the normobaric oxygen paradox; GSSG – glutathione disulphide; VEGF – vascular epithelial growth factor; Ub – ubiquinone (reproduced with permission)



of optimal glutathione availability to increase EPO synthesis in this setting has recently been emphasised by n-acetylcysteine administration (NAC).[10] This phenomenon will last long enough after the O_2 concentration reduction to mimic a 'hypoxic' situation, where the availability of ROS is reduced.[11,12] This complex situation will allow the binding of HIF dimmers, triggering EPO gene expression.

Material and methods

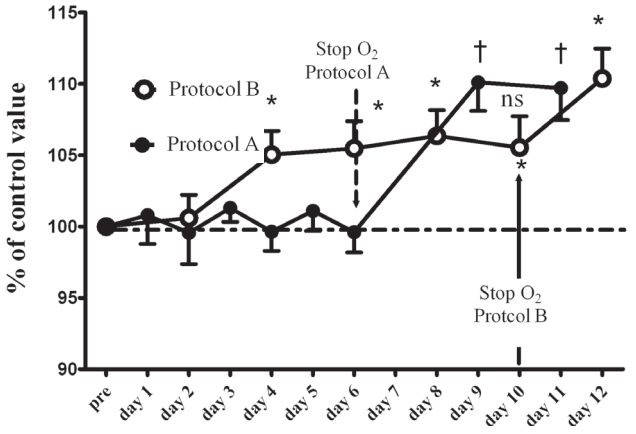
SUBJECTS

Nine healthy volunteers (five men and four women; aged 18 to 41 yrs, mean 30 yrs) participated in this study after Academic Ethics Committee, ISEK, Brussels, Belgium approval and written, informed consent was obtained. The study was performed in accordance with the Declaration of Helsinki Subjects were asked not to smoke or to take any medication (NAC or iron) or perform strenuous physical exercise 24 hours before and during each study period or stay at altitude within two weeks before the experiments.

EXPERIMENTAL PROTOCOL

Subjects underwent two protocols of O_2 breathing. Each one subsequently underwent the two protocols. Protocol A involved 100% O_2 daily and protocol B 100% O_2 on alternate days. The two protocols took place six weeks apart, so that values for the measured parameters had returned to baseline in between. The sequence was not randomised since all subjects underwent both protocols. The subjects breathed 100% normobaric O_2 (15 L min^{-1} via a non-rebreathing facemask) for 30 minutes. Constant monitoring of the following parameters controlled O_2 breathing: mask fit, movement of the three one-way valves on the mask, movement of the reservoir bag and moisture formation on

Figure 2
Comparison baseline haemoglobin after 30 minutes of 100% O_2 breathing daily (protocol A; † $P < 0.01$) or on alternate days (protocol B; * $P < 0.01$); ns – not significant



the transparent mask during expiration. Only 7 ml of blood were withdrawn for analysis each day so that plasma volume contraction did not occur, since this can interfere with EPO production.[13]

ANALYSES

Samples were drawn in EDTA tubes by repeated needle sticks in the antecubital fossa. They were centrifuged within 4 hours of their sampling. Haemoglobin (Hb) and haematocrit (Hct) levels were measured in a routine automated manner using red laser light to measure both volume and haemoglobin concentrations on a cell-by-cell basis (ADVIA 2120, Siemens AG Healthcare Sector, Erlangen, Germany); reticulocyte count was done by colorimetry on the same apparatus.

STATISTICS

Standard statistical analyses were performed, including mean, standard deviation, and a one-sample Student's paired t-tests for between- and within-subject effect after Kolmogorov Smirnov tests for normality of distribution of the data. Taking the initial value as 100%, percentage changes in Hb were calculated, thereby allowing an appreciation of the magnitude of change rather than the absolute values. The raw data Hb are available on Excel™ file from the journal office.

Results

HAEMATOCRIT AND HAEMOGLOBIN

All subjects had baseline haematocrit (41.3 ± 2.8 %) and haemoglobin values (14.8 ± 1.1 g L^{-1}) within the normal population range. No significant increase of haematocrit was seen (43.9 ± 1.2 % and 44.0 ± 1.7 %) after either of the two protocols ($P > 0.05$). This reassured us as to the hydration status of our subjects. The Hb level started to rise after day 4 of protocol B (alternate days; $P < 0.01$) and this rise was maintained throughout that study period. For protocol A (daily O_2) the Hb level did not change from baseline until O_2 administration ceased ($P < 0.01$). Figure 2 shows the percentage changes of Hb during the two study periods. Some Hb values fell transiently before rising, and the rate of rise varied between individuals.

RETICULOCYTE COUNT

Reticulocyte counts were measured at baseline and at day 7, any changes from baseline being expressed as percentage change in count. There was a significant rise in reticulocyte count in the alternate-day group as compared to the daily group (182 ± 94 % vs. 93 ± 34 %, $P < 0.001$), demonstrating enhanced erythrocyte production.

Discussion

At cessation of O_2 breathing, the arterial O_2 partial pressure falls within minutes to a normal baseline level. After this situation, transcription of EPO starts within 4–8 hours.[14] In protocol B, a significant increase in Hb was seen after four days and remained elevated throughout that study period. This is consistent with the NOP hypothesis that permits an increase in EPO synthesis, thus increasing Hb.[7,15] The literature shows a rapid increase in reticulocytes and Hb with high rh-EPO doses in patients with normal bone marrow function.[16] If, during this time lapse, and according to the NOP physiology, one re-introduces O_2, the effect could potentially be cancelled. This could explain the absence of Hb increase until after O_2 cessation in protocol A. The interval between two periods of O_2, on a daily basis is probably too short to permit a sufficient rise in EPO to stimulate an increase in Hb.

Both protocols resulted in a significant increase of Hb after stopping O_2 administration when compared to baseline values. It is important to bear in mind that these people were healthy volunteers. The optimal time between two periods of O_2 is not clearly determined by our study, but we do see an increase in Hb in the alternate-day protocol earlier compared to the daily protocol, proof that every day is too frequent but that when O_2 administration ceases, Hb rises according to the NOP hypothesis. It is interesting to see that Hb remains high for several days, perhaps allowing for a twice-a-week protocol.

A drop from 100% to 21% of O_2 in the breathing gas induces an NOP effect in healthy volunteers.[6] The NOP appears to be an efficient way to increase EPO. This could have clinical potential, as EPO has been shown to be active in both cardio- and neuroprotection and it could reduce costs.[16–18] Repetition of this experimental protocol in anaemic patients could increase circulating EPO levels and

thereby increase erythropoiesis and thus Hb. The important point appears to be to leave a sufficient time interval between two O_2 administrations for EPO synthesis in order to avoid competing against this mechanism reapplication of O_2 too soon.

Even though the exact amount of O_2 required to produce a NOP effect is not known, the minimal concentration of inspired O_2 seems to be around 40–50% providing that glutathione availability is optimal.[19] Increasing the variation of pO_2 shows less consistent results especially when associated with hypoxia, as shown by Debevec.[20] Further investigations to determine the optimal 'dose' are welcomed, and recent publications suggest that 100% O_2 may not be optimal.[21] NOP is a recent protocol and the time frame as well as the precise dose of O_2 needed are crucial to establishing clear recommendations for clinical use.

Whilst the Haldane effect may be present in bed-rest patients with hypoventilation, this seems unlikely in young, healthy adults without pulmonary disease. If this were the case in our study, it would be present in both groups. We also know that NAC by itself has been shown to raise EPO levels and this phenomenon is increased by addition of oxygen.[10,22] Therefore, we asked our volunteers not to undergo strenuous activities or to take any antioxidants such as NAC.

The main limitation of this study is the small sample size. Further clinical investigations are needed to achieve the optimal use of the NOP and to better understand which patients would benefit from the induction of this physiological pathway. Potential clinical applications of the NOP could be twofold. Firstly, a small number of O_2 breathing sessions of limited duration (e.g., 30 minutes) appear to be sufficient to increase circulating endogenous EPO levels, thus leading to a cytoprotective effect on brain and cardiac cells. Secondly, increasing endogenous EPO seems to increase Hb levels of volunteers if sufficient time is allowed between two O_2 sessions.

Conclusion

Alternate-day O_2 breathing for 30 minutes stimulated an early increase in Hb, whereas a rise did not occur with daily O_2 until administration ceased. Likewise, the reticulocyte count was elevated more by the alternate-day protocol than the daily protocol. Daily O_2 is too frequent an exposure in a non-anaemic population, but the exact best time course for the use of the normobaric oxygen paradox to stimulate erythropoiesis is not clearly established in this study.

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Submitted: 15 September 2011
Accepted: 09 April 2012

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Editor's note:
All nine authors contributed to several components of the planning, conduct, analysis and reporting of this study..