# Erythropoietin production can be enhanced by normobaric oxygen breathing in healthy humans.

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## INTRODUCTION

Erythropoietin (EPO) induces red blood cell production by activating red bone marrow progenitor cells, and is used therapeutically in chronic anemia. It is also used as an unauthorized adjunct to increase the oxygen transport capacity in the blood of athletes. Renal tissue hypoxia is the only widely accepted trigger for EPO production (1-3), even if new oxygen-sensitive sites have been recently proposed (4-6). This is well established in models of reduced-oxygen delivery during anemia (7), reduced renal perfusion (8), or hypobaric or normobaric hypoxia (9, 10) or hypoxemia. In one report, hemoconcentration, following sporting activities (11), has been reported to increase EPO secretion. There does not seem to be agreement on the existence of a circadian variation in EPO secretion (12, 13), although the exact timing and magnitude of the nadir and zenith are not unequivocally established, nor is the EPO plasma concentration.

Previous experiments in breath-hold divers have led us to hypothesize that another triggering mechanism might exist, independent from renal tissue hypoxia. After a series of deep breath-hold dives, two out of five divers showed a marked augmentation of serum EPO levels. During descent to depth, intra-alveolar oxygen tensions increase. During ascent from depth, oxygen tension falls to atmospheric values. During these (unpublished) experiments, no severe alveolar hypoxia was observed after surfacing, although EPO production seemed to increase. Recently, a Spanish study reported that short exposure to intermittent hypobaric hypoxia increased EPO production (14). We hypothesize that a sudden decrease in tissue-oxygen tension from hyperoxia back to normoxia might act as a trigger for EPO release.

## MATERIALS AND METHODS

32 healthy subjects (23 males and 9 females), ranging in age from 22 to 47 years, participated in this study after written informed consent. They did not smoke for 24 hours before the test, nor did they take any medication or perform strenuous physical exercise. Sixteen of the participants were subjected to normobaric oxygen breathing (15 lpm) for two hours. In order to obtain more thorough tissue denitrogenation, they were asked to perform moderate physical exercise (twenty knee bends) every ten minutes (15, 16). Continuously monitoring the following clinical signs controlled oxygen breathing: mask fit, movement of the three one-way valves, movement of the reservoir bag, and moisture formation on the transparent mask during

expiration. Furthermore, transcutaneous oxygen tension ($PTcO_2$) was measured sequentially in all subjects using a Radiometer TCM3 (Radiometer, Copenhagen).

Blood samples were taken before the start and at the end of oxygen breathing, at time 0, then, at times 2, 4, 5, 7, 10, 24 and 36 hours after oxygen breathing. Total blood volume taken was less than 30 milliliters; this had no influence on hematocrit. The blood was immediately centrifuged (10 minutes at 3000 rpm), and the separated serum was frozen immediately to –80°C for a maximum of 24 hours before analysis. Serum EPO concentration was measured using a radioimmunoassay (EPO-Trac $^{125}$I RIA, INCSTAR, Stillwater, USA). Hematocrit and hemoglobin concentrations were measured before and after oxygen breathing, as hemoconcentration is known to influence EPO production (11). Body impedance was measured before and after oxygen breathing. For this, a Tanita TBF-310-GS scale was used to test the eventual difference in body fat mass and the EPO response, as tissue denitrogenation rate can differ because of fat mass (17).

Without oxygen breathing, 16 healthy subjects were randomly submitted to the same blood sampling in order to establish a circadian EPO production rhythm, as reported in the literature (12).

Standard statistical analysis was performed, including mean, standard deviation, normality, median and the analysis of variance (ANOVA) as repeated measures to test the effects between and within subjects after Kolmogorov-Smirnov's test for normality. The post-test performed was Bonferroni or Dunnet comparison versus control values.

## RESULTS

All subjects had baseline hematocrit, serum hemoglobin and impedance values within the normal range. These were not significantly altered after the oxygen-breathing period (**Figures 1, 2**).

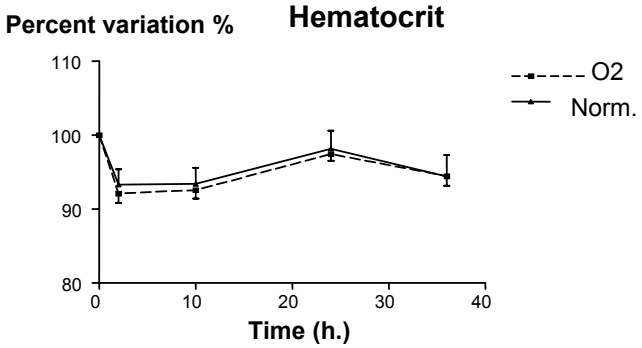



**Figure 1.** Percent variation of hematocrit over 36 hours after two hours of normobaric oxygen breathing and the control group (Norm.).

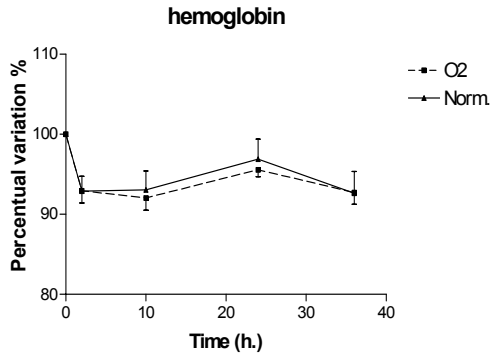



**Figure 2.** Percent variation of hemoglobin over 36 hours after two hours of normobaric oxygen breathing and the control group .

In the control group of 16 divers who were not breathing oxygen, a circadian variation of serum EPO concentration was found. The nadir occurred at around 14.00 Hrs (8.96 mU/ml ± 2.1) and the zenith at about 22.00 Hrs (12.1 mU/ml ± 3.5) (**Figure 3**).

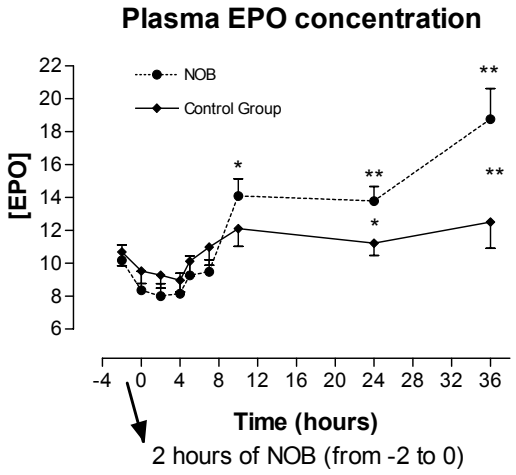
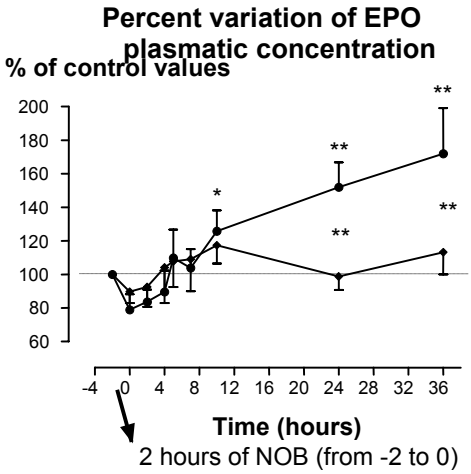



**Figure 3.** Evolution of mean (± SEM) plasma EPO concentration (mU/ml) before and after two hours of 100% oxygen breathing over 36 hours (N=16); Time 0 = from end of two hours of oxygen breathing. (***: P<0.01). Circadian variation of serum EPO in control group (n=16) (not breathing oxygen) is also shown.

In the oxygen-breathing group, an initial decrease in serum EPO concentration after the oxygen breathing was followed by a significant increase at 24 and 36 hours (**Figure 3**). This increase was statistically significant, both with regard to the initial (pre-oxygen-breathing) value and to the mean variation of the control group (circadian rhythm group) (**Figure 4**). This increase seemed to start after a lag of about eight hours after cessation of oxygen breathing. This is consistent with the time lapse needed for message transcription and EPO protein synthesis by the renal peritubular cells (18).

**Figure 4.** Percent variation of plasma EPO concentrations of the oxygen-breathing group (●) vs. the control group control group (♦)




## DISCUSSION

A significant increase in serum EPO levels was measured after two hours of 100% oxygen-breathing. This increase was not related to a circadian EPO variation, nor could it be explained by changes in hematocrit level or whole body dehydration. By breathing 100% oxygen for two hours, a significant nitrogen washout was obtained, thereby increasing the relative oxygen content in all body tissues, including the kidneys. The cessation of oxygen breathing results in a rapid decline in oxygen partial pressure in the blood and tissues, followed by a gradual increase of the nitrogen content of these tissues. It has been reported that hypoxic periods of 45 minutes, but not of 30 minutes, trigger EPO production (13). A change in oxygen content induced by stopping the oxygen breathing, which could be called a "relative hypoxia," persists over a prolonged period . Since it is actually a return to the normal oxygenation state, it could constitute a sufficient trigger for increasing EPO transcription and secretion. It is not likely that an oxygen-induced arteriolar

vasoconstriction, via a decreased perfusion of the renal parenchyma, could be responsible for this increased EPO production. On one hand, hyperoxic vasoconstriction only attains significant levels in hyperbaric hyperoxia. On the other hand, renal oxygen supply would be balanced by the increase in arteriolar blood oxygen content.

## CONCLUSION

These data demonstrate a previously unreported triggering mechanism, unrelated to an absolute decrease in oxygen delivery to the renal peritubular cells, for EPO production in healthy humans. Potentially, this mechanism could have clinical applications even if our results do not demonstrate significant hematocrit or reticulocyte increases (because of an insufficiently long follow-up). It may be that this mechanism induces an increase in red blood cell production. Moreover, recent studies demonstrate an important neuroprotective effect of EPO (19-23). This is a promising, multipurpose medical benefit for any pathology or sport susceptible to promoting adverse neurological effects as a result of environmental factors. Further studies are necessary to determine the duration and final magnitude of the increased EPO production, and the possibility of repeating the triggering mechanism over several consecutive days in order to get an additive effect on serum EPO levels. The administration of hyperbaric oxygen, instead of normobaric oxygen, should also be investigated (24, 25).

## ACKNOWLEDGEMENTS

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