EPO Modulation in a 14-Days Undersea Scuba Dive

Abstract

Erythropoiesis is affected during deep saturation dives. The mechanism should be related to a downregulation of serum Erythropoietin (s-EPO) concentration or to a toxic effect of the hyperbaric hyperoxia. We evaluated s-EPO and other haematological parameters in 6 scuba divers before, during and after a 14-days guinness saturation dive (8–10m). Athletes were breathing air at 1.8–2 ATA, under the control of a team of physicians. Serum parameters were measured before diving (T0) and: 7 days (T1), 14 days (T2) after the beginning of the dive and 2 h (T3) and 24 h (T4) after resurfacing. Hgb, and many other haematological parameters did not change whereas Ht, s-EPO, the ratio between s-EPO and reticulocytes (absolute, percent) declined progressively from T0 to T3. At T4 a significant rise in s-EPO was observed. Hgb did not vary but erythropoiesis seemed to be affected as s-EPO and reticulocyte counts showed. All these changes were statistically significant. The experiment, conducted in realistic conditions of dive length, oxygen concentration and pressure, allows us to formulate some hypotheses about the role of prolonged hyperbaric hyperoxia on erythropoiesis. The s-EPO rise, 24h after resurfacing, is clearly documented and related to the “Normobaric Oxygen Paradox”. This evidence suggests interesting hypotheses for new clinical applications such as modulation of s-EPO production and Hgb content triggered by appropriate O2 administration in pre-surgical patients or in some anemic disease.

Introduction

The well-known relationship between O2 administration and s-EPO production has been extensively studied under many clinical and experimental conditions. We evaluated serum Erythropoietin (s-EPO), some haematological and blood chemistry parameters in 6 volunteer scuba divers, 3 men and 3 women, who lived for 14 days on a wall-less platform anchored to the seabed of Ponza, an Italian island, at a depth of 8–10 meters (22–28 ft.). Athletes were continuously under the control of a specialized team of physicians called “Abyss Project dive medical group”. This medical organization was on location firstly to assure the safety of divers, and then to collect biochemical and clinical data on the experiment. Some results have been already published (30).

In a previous experiment 2 divers lived underwater for 10 days (240h) in the same place and under the same experimental conditions. This experience gave the opportunity to perform neuroendocrine and psychological assessments [28,29]. Few data on the effect of long-term diving on blood and stress parameters, both in professional and recreational scuba divers, is available [15,16,27,31,36]. These records were obtained in various experimental conditions, both in terms of length of exposure and O2 partial pressure. The purpose of the present study was to investigate the effects of prolonged dives, breathing air in hyperbarism (hyperbaric air – HBA) at a pressure ranging between 1.8–2 ATA in a relatively mild hyperbaric hyperoxia, on s-EPO production. It is well known that oxygen concentration at renal tubular cells is the main regulator of s-EPO synthesis, with hypoxia as the most important trigger for s-EPO production. Its effect has been widely studied because it represents a natural exhaustive model for many pathological conditions. Hyperoxic exposure, either due to high oxygen concentration or to a hyperbaric environment, as in the experimental conditions...
created for this experience, is far less frequent in human life. The effect on s-EPO production has been studied mainly in experimental setting in humans [1, 13, 19, 33], animals [21, 23] or in subjects undergoing hyperbaric exposure in professional or recreational diving or for hyperbaric medical treatment. During exposure to hyperoxia, the role of some mediator representatives of the oxidative stress seems to be crucial [5], s-EPO is a highly specific growth factor for erythroid lineage and can be considered as the final product of a complex regulatory system involving transcriptional factors such as Hypoxia – Inducible Factor -1α (HIF-1α), redox mechanism with Glutathione playing a pivotal role with its oxidized and reduced forms (2GSH↔GS-SG) acting as a scavenger on oxygen free radicals (OFR), and the enzymatic system involved both in glutathione biosynthesis (glutathione synthetase) and in restoring balance between its oxidized and reduced forms (glutathione peroxidase). In normal conditions, EPO assures the homeostasis between destruction and production of Red Blood Cells [25], and acts to prevent the apoptosis of erythropoietic precursors as well. So, during long exposure to a hyperbaric environment, erythropoiesis could be parallel s-EPO production.

Material and Methods

Selection of scuba divers
21 volunteers, all expert divers, (mean age 32; range 18–54 years) underwent preliminary tests including: evaluation of medical history, physical examination, principal haematological and blood chemistry parameters assays, complete cardio-pulmonary study (chest X-ray, resting and exercise ECG testing, 2D echocardiogram, spirometry, blood gas analysis, 24h Holter ECG monitoring), and psychological assessment (State and Trait Anxiety Inventory and the Zung self-rating depression scale). According to the normality of these tests, 6 subjects were selected to participate to the experience: 3 males M1, M2, M3 aged respectively 40, 34 and 28 years; 3 females: F1, F2, F3 aged respectively 33, 31 and 26 years. All of them gave their written informed consent to the experiment and no one received any compensation for the performance.

Conditions of the experiment
This study, called "Abyss Project", took place in Cala Feola, to the north west of Italy's island of Ponza (40°53 84N 12°57 80E), in the central Tirrenian (Mediterranean) sea. The scuba divers spent a total of 360h (14 days) at a depth of 8–10m (22–28ft). The temperature of the sea water varied (24 and 26 °C), being spent a total of 360 h (14 days) at a depth of 8–10 m (22–28 ft). The 6 subjects lived on a wall-less platform anchored to the lower during the night and depending on the prevalent winds. The temperature of the sea water varied (24 and 26 °C), being spent a total of 360 h (14 days) at a depth of 8–10 m (22–28 ft). The scuba divers underwent preliminary tests including: evaluation of medical history, physical examination, principal haematological and blood chemistry parameters assays, complete cardio-pulmonary study (chest X-ray, resting and exercise ECG testing, 2D echocardiogram, spirometry, blood gas analysis, 24h Holter ECG monitoring), and psychological assessment (State and Trait Anxiety Inventory and the Zung self-rating depression scale). According to the normality of these tests, 6 subjects were selected to participate to the experience: 3 males M1, M2, M3 aged respectively 40, 34 and 28 years; 3 females: F1, F2, F3 aged respectively 33, 31 and 26 years. All of them gave their written informed consent to the experiment and no one received any compensation for the performance.

Results

Table 1 shows the results of the hematological parameters and of O/P ratio observed before immersion (T0) and 7 days (T1), 14 days (T2) after beginning the dive, 2h (T3) and 24h (T4) after resurfacing. Hgb did not change while Ht, s-EPO, O/P ratio, absolute and percentual reticuloocyte counts declined progressively from T0 until T3. At T4 (24h after resurfacing) a rise of s-EPO production was observed. This phenomenon, according to the results of other authors can be explained by the so-called “Normobaric Oxygen Paradox” (NOP) [1–3, 10, 11]. All these changes are statistically significant according to the repeated measure ANOVA test. After applying the post-hoc Scheffe test a significant difference was present between T0 observation and T1, T2, T3 (p<0.01), while between T0 and T1 there was only a trend towards significance (p=0.1). No difference is observed between T0 and T4, one day after resurfacing. A significant difference was present between T4 and T1, T2 and T3, respectively (p<0.001).
Fig. 1 shows s-EPO for each subject. In Fig. 2 the s-EPO mean values obtained during the dive and after the resurface are expressed as percentage of pre-dive s-EPO mean value.

No statistically significant variations were observed in other main blood chemistry parameters. Particularly renal function, which might negatively influence s-EPO production, remained constant without variations.

Discussion

Evaluation of s-EPO production in vivo, during and after a continuous exposure to HBA (1.8-2 ATA) had never reported before. Similar experiments have been conducted by other authors [1, 19, 32] although with wide variations, both in terms of length of exposure and of \( O_2 \) partial pressure. In 2005, 16 healthy subjects were exposed to normobaric hyperoxia (100% \( O_2 \), for 2 h, via a “nonrebreather” mask) for the first stage of the experiment, and to hyperbaric hyperoxia (100% \( O_2 \), 2.5 ATA, for 1.5 h, in a hyperbaric chamber) for the second stage, 7 days later [1]. A clear impairment of s-EPO production without any variation of Hgb and Ht was found after the hyperbaric exposure. Notably this impairment was still present 24 h after the end of the experiment in hyperbarism, whereas 24 and 36 h after the experiment in normobarism, a stable increase in s-EPO production was observed. No further data is available about s-EPO determination beyond 24 h of hyperbarism, except in Hofso et al. [19] but under different experimental conditions. Nevertheless a similar decrease the day after the resurfacing was reported. Data from our study essentially correspond to the trend of experimental hyperbaric hyperoxia reported by Balestra et al. [1], remarkably in the progressive decline of s-EPO production from T0 until T3.

In our study, after 24 h, a sharp increase in s-EPO production was detected in all subjects of the study, this is in line with the mild hyperoxia proposed in Balestra’s study showing the same increase after 100% oxygen breathing, whereas in the hyperbaric phase of the experiment, with an exposure rising up to 2.5 ATA of pure oxygen, the decline of s-EPO concentration was still present at 24 h.

Nevertheless the particular conditions of this dive were quite different to those of other experiments reported. First of all our divers continuously breathed air throughout the entire experiment, without any \( O_2 \) supplement, but at a pressure ranging between 1.8 and 2 ATA which is lower compared both to the mixture used by Balestra et al. [1] (in hyperbaric phase) and to that used by Hofso et al. [19]. The duration of this experiment was also different to those aforementioned: more prolonged than [1] and shorter than [19]. In agreement with Balestra et al. [1], our results showed that s-EPO production decreased not

![](image)

Table 1  Mean values and SD of Hgb, Ht, reticulocyte count (perceptual and absolute), s-EPO and O/P ratio before (T0), during (T1; T2) and after the dive (T3; T4). p is calculated using repeated measure ANOVA Test.

<table>
<thead>
<tr>
<th>T</th>
<th>Hgb g/dl</th>
<th>Ht</th>
<th>Ret %</th>
<th>Ret 10^9/l</th>
<th>s-EPO mU/ml</th>
<th>O/P ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>14.03 ± 1.25</td>
<td>41.32 ± 2.81</td>
<td>1.19 ± 0.35</td>
<td>56.40 ± 22.09</td>
<td>11.58 ± 3.09</td>
<td>0.89 ± 0.10</td>
</tr>
<tr>
<td>1</td>
<td>13.72 ± 1.39</td>
<td>40.03 ± 3.37</td>
<td>1.08 ± 0.50</td>
<td>50.72 ± 26.16</td>
<td>6.28 ± 3.20</td>
<td>0.60 ± 0.19</td>
</tr>
<tr>
<td>2</td>
<td>13.33 ± 1.80</td>
<td>38.83 ± 4.35</td>
<td>0.72 ± 0.23</td>
<td>32.58 ± 12.82</td>
<td>4.23 ± 1.59</td>
<td>0.46 ± 0.10</td>
</tr>
<tr>
<td>3</td>
<td>13.40 ± 1.43</td>
<td>39.77 ± 3.38</td>
<td>0.67 ± 0.17</td>
<td>30.18 ± 8.90</td>
<td>4.50 ± 1.73</td>
<td>0.49 ± 0.17</td>
</tr>
<tr>
<td>4</td>
<td>13.67 ± 1.35</td>
<td>39.82 ± 3.14</td>
<td>0.71 ± 0.16</td>
<td>32.96 ± 9.59</td>
<td>14.02 ± 5.05</td>
<td>0.90 ± 0.12</td>
</tr>
<tr>
<td>P n.s.</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
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</tr>
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</table>

**Fig. 1** s-EPO results at the different times. Each line refers to a single diver. In all athletes s-EPO 24 h after resurfacing is higher than in T0 while Hgb levels are unchanged. In one scuba diver s-EPO post-dive exceeds 20 mU/ml.

**Fig. 2** Mean values of s-EPO at 7, 14 days during the dive and 2 h and 24 h after the resurface, expressed as percentage of mean pre dive s-EPO.
only in absolute values but also in relation to Ht, as seen in the O/P ratios. This is also evident in the expression of s-EPO mean values as a percentage of pre-dive s-EPO (Table 1). This data does not seem to be related to renal function impairment, because serum creatinine values remained unchanged throughout the whole period of observation. This reduction is then followed by an increase after the exposure, if the PO2 is not too high (Balestra’s normobaric phase) [1]. Although the Hgb did not change, some signs of impairment of erythropoiesis are already present: Ht and absolute and percentage reticulocytes count declined from T0 to T4 [14]. The different behaviour between Hgb and Ht can be influenced by hemodilution.

The reported data may be explained by a complex mechanism controlling s-EPO gene transcription. The pivotal role in this mechanism is played by Hypoxia Inducible Factor -1α (HIF-1α), a transcriptional factor, which in turn, undergoes a strict regulation involving intracellular oxygen free radicals (OFR) and redox mechanism, a balance of oxidized and reduced form of Glutathione (2GSH ↔ GS-SG) [17].

In our experiment divers underwent a chronic hyperoxic stimulation due to hyperbarism itself, throughout the dive, creating the conditions for the reported decline in EPO production [1,11]. The key role in this phenomenon is played by the increase of oxygen partial pressure and of its by-products OFR that, despite the stimulus for the ex-novo synthesis of GSH synthase and, in turn, of GSH, leads to ubiquitination of the complex HIF-1α-Von Hippel Lindau protein and to a consequent proteosomal inactivation [17]. Nevertheless, the hyperoxic stimulation which our divers underwent, may be considered mild when compared to those reported in the literature – not so intense as it would be if an O2 supplement was administered, but more prolonged. In our opinion, 24h after the end of the dive, in the cytoplasmatic milieu, the high level of GSH and of glutathione peroxidase induced by the chronic hyperoxic stimulation, lasting 14 days, coupled with the sudden decrease of OFR at the end of hyperbaric stress seems similar to the conditions called NOP [1–3]. According to this hypothesis, the registered rise of EPO at time 4 may be the final result of a total and complete scavenging of OFR, by GSH oxidated to GS-SG and experienced by the cells as a hypoxic state.

Although Hgb did not change, some signs of impairment of erythropoiesis were already present: Ht and absolute and percentage reticulocytes count declined from T0 to T4. The different behaviour between Hgb and Ht can be influenced by hemodilution.

Taking into account the timing of erythropoiesis [22], the appearance of anemia would have been expected, had this experiment been continued. Reduction of Hgb after 28 days of a deep saturation dive in a hyperbaric chamber has already been reported [32]. In our experiment, only one subject (F2), the same who had the lowest Hgb value at the beginning of the study, showed mild anemia at the end (12.9 g/dl at T0; 11.2 g/dl 24h after resurfacing). Nevertheless, it could be argued that s-EPO response to anemia is enhanced by a lower Hgb. It begins to be relevant for a Hgb level below 10.5 g/dl [9] that under stress conditions may become clinically effective even in a young and healthy subject. In this case, s-EPO production would receive two opposite stimuli: hypoxia (which is Hgb related) and an increase in oxygen dissolved in plasma. It would be interesting to verify the final effect.

HBO has a well-documented effect on apoptosis in vitro [9,20,34] and could affect erythropoiesis in vivo either with a direct effect of hyperbarism and with the reduction of s-EPO production (the main action of which is just the prevention of erythroid precursor’s apoptosis) [25].

Finally, we clearly documented the NOP in all subjects, 24h after their return to the atmospheric pressure. The results do not differ from those reported by Balestra et al. [1], even though there was a difference in oxygen concentration and partial pressure used during the dive. Even if the decrease of oxygen partial pressure is of a lesser amplitude, the length of the stimulus alone could lead to an increase in the glutathione activity, preparing the system to react. This has already been shown in healthy volunteers even without an oxygen drop (from hyperoxia – 100% of oxygen to normoxia – 20% of oxygen) but only increasing the glutathione supplementing N-Acetyl-Cysteine (NAC).

As pointed out in recent reports, the optimal stimulus to be given in order to reach the best response is still a matter of controversy, especially considering the dose [2,3,8,10–12,24] and sequence [7,26] of oxygen supplement, with or without NAC [35]. In conclusion, this data suggests that a prolonged exposure to hyperbarism may affect erythropoiesis despite the persistence of the same Hgb levels. The clear reduction in serum s-EPO leads us to suppose that anemia would become a clinical problem, should exposure continue.

This data represents a basis to verify if treatments such as prolonged oxygen-therapy with or without positive pressure in respiratory failure or the hyperbaric chamber, affect erythropoiesis. In the near future, this issue will be thoroughly investigated. If anemia is a possible consequence of hyperoxia, the demonstration of NOP could also offer new interesting ways to stimulate erythropoiesis [6].

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