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ORIGINAL ARTICLE

Increased erythropoietin concentration after repeated apneas in humans

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Abstract Hypoxia-induced increases in red blood cell production have been found in both altitude-adapted populations and acclimatized lowlanders. This process is mediated by erythropoietin (EPO) released mainly by the hypoxic kidney. We have previously observed high hemoglobin concentrations in elite breath-hold divers and our aim was to investigate whether apnea-induced hypoxia could increase EPO concentration. Ten healthy volunteers performed 15 maximal duration apneas, divided into three series of five apneas, each series separated by 10 min of rest. Apneas within series were separated by 2 min and preceded by 1 min of hyperventilation to increase apnea duration and arterial oxygen desaturation. When EPO concentration after serial apneas was compared to baseline values, an average maximum increase of 24% was found ($P < 0.01$). No changes in EPO concentration were observed during a control day without apnea, eliminating possible effects of a diurnal rhythm or blood loss. We therefore conclude that serial apneas increase circulating EPO concentration in humans.

Keywords Erythropoiesis · Breath holding · Desaturation · Diving response · Bradycardia

Introduction

Increase in the total amount of erythrocytes, reflected by increased hematocrit (Hct) and hemoglobin concentration (Hb), is mediated by the hormone erythropoietin (EPO; Erslev 1953), which is produced mainly by the hypoxic kidney (Jacobson et al. 1957). The magnitude of EPO production appears to depend on the level of hypoxia (Eckardt et al. 1989; Knaupp et al. 1992) and is controlled at the cellular level by hypoxia-inducible transcription factors (HIF; Wang and Semenza 1993).

Few studies have focused on the effects of apnea-induced hypoxia on circulating EPO. There is some evidence that EPO levels and erythropoiesis are increased in sleep apnea patients. Cahan and associates (1995) reported that EPO levels in sleep apnea patients were reduced by 38% after 3 days of treatment, which suggests that the hypoxia experienced by these patients during sleep apneas could be a stimulus for an increased EPO release. This is further supported by the high Hct, Hb and EPO levels found in patients with severe obstructive sleep apnea (OSA; Choi et al. 2006; Hoffstein et al. 1994; Imagawa et al. 2001). The increased Hct levels were still within the clinically accepted range but appeared directly correlated to the severity of OSA (Choi et al. 2006).

Besides sleep apnea, a different and voluntary situation during which people may endure apnea-induced hypoxia is breath-hold diving, and benefits of a high erythrocyte level could include both increased O_2 storage and CO_2-buffering capacity, resulting in prolonged apneic duration. Comparison of resting Hb in elite breath-hold divers and untrained subjects showed a 5% higher Hb in the divers (de Bruijn et al. 2004). Training protocols for these divers consist of up to 20 h per week of apnea-related training, including successive maximal duration apneas on land and in shallow water, which raises the question whether the repeated hypoxic exposures could enhance erythropoiesis. Our aim was to investigate if repeated maximal duration apneas could cause an increase in serum EPO concentration, which has not previously been studied.

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Materials and methods

Subjects

Ten healthy volunteers, five females and five males, participated in the experiment. Their average (SD) age was 30 (6) years, weight 72 (19) kg and height 177 (9) cm. They performed 7 (6) h per week of recreational physical exercise. None of the subjects had been training breath-hold diving during at least 2 months before the experiments, nor had any of the subjects resided at altitude in the months preceding the experiments. One subject smoked and one used snuff-tobacco.

Protocol

All volunteers signed a consent form after being fully informed of the experimental protocol, which consisted of a serial apnea day and a control day. Experiments were approved by the regional human research ethics board at Umeå University, Sweden and complied with the Declaration of Helsinki. Subjects were asked not to exercise during the testing days and to refrain from eating, drinking or using tobacco at least 2 h prior to experiments. The experimental protocol started at 08:00 a.m. with a measurement of vital capacity (VC; Compact II, Vitalograph, Buckingham, England) in the standing and the supine position, which was followed by a 20 min period of horizontal rest.

A catheter was placed in an antecubital vein of the subject's arm for blood sampling, and probes for measuring cardiovascular parameters were placed on the hand of the opposite arm. The apnea protocol consisted of 15 maximal duration apneas, divided into three series of five apneas. Apneas were spaced by 2 min and series were spaced by 10 min of rest. In order to reduce pre-apneic alveolar PCO_2 to prolong apneic duration and increase arterial oxygen desaturation, subjects hyperventilated for 1 min prior to each apnea by increasing tidal volume. At 2 min before the apneas subjects were notified of the time, at 1 min subjects were instructed to start hyperventilating, at 30 s a nose-clip was administered and at 15 s the subject received the spirometer mouthpiece and continued hyperventilating through it during a 10 s countdown. Apnea started after a full exhalation and a deep, but not maximal, inhalation through the spirometer mouthpiece. During apnea the mouthpiece was removed, but at apnea termination the subject made a full exhalation through the mouthpiece. Subjects were instructed to try to reach below 85% SaO_2 during each apnea and received continuous visual feedback on their SaO_2 levels. Subjects reaching SaO_2 levels below 60% during apneas were told to resume breathing, in order to minimize risk of hypoxic syncope.

After the apneas, the subjects were allowed to drink at will and eat a light lunch, while they were instructed not to use tobacco until after the last blood sample.

To reveal any possible effects of diurnal variation during the experimental period (Cahan et al. 1992; Klausen et al. 1996), or influence of the blood sampling protocol on EPO production, the study included a control day with a mimic of blood sampling during the same time period, but without apneas. The control experiment was performed at least 72 h after the apnea protocol. The average (SD) air temperature was 23 (1) °C during both the apnea- and control day.

Measurements

The spirometer mouthpiece was connected to a CO_2/O_2 monitor (Normocap Oxy, Datex Ohmeda, Helsinki, Finland) for measurement of pre- and post-apneic end-tidal alveolar CO_2 ($F_{ET}CO_2$) and O_2 ($F_{ET}O_2$). Blood samples for analysis of EPO concentration were taken 2 min before the start of the first apnea, directly after the 15th apnea, and 1, 2, 3 and 5 h after the last apnea. Heart rate (HR) and arterial oxygen saturation (SaO_2) were measured via pulse oximetry (3900, Datex Ohmeda, Louisville, USA), skin blood flow (SkBF) via laser-Doppler flow meter (Periflux 5000, Perimed, Järfälla, Sweden) and mean arterial pressure (MAP) via photoplethysmometer (Finapres 2300, Ohmeda, Englewood, USA). Continuous data for HR, MAP, SkBF, and SaO_2 was recorded from 2 min before until 2 min after each apnea series using a data acquisition system (MP100A-CE, Biopac systems Inc., Santa Barbara, USA). Apneic time, from the end of pre-apneic inspiration to the start of post-apneic expiration, was marked with an analog switch.

Analysis

Blood samples were analyzed for EPO by an accredited lab at the Sundsvall hospital using the Immulite chemiluminescent immunoassay system (Immulite One, DPC, Los Angeles, USA). Post-apneic EPO values were compared to pre-apneic baseline values and to the corresponding control day values. Because the peak in EPO varied with time in different subjects, possibly due to the differences in apnea times, the individual maximum value was statistically compared to baseline. Average values for resting SaO_2 were obtained from the period of 120–60 s before the start of a series, after a period of at least 10 min of eupneic horizontal rest. To calculate the average values for the apnea-induced reduction in SaO_2, occurring in the finger with a circulatory delay of 20–30 s, the SaO_2-nadir after each apnea was identified. Control eupneic values for HR, MAP and SkBF were obtained from the period 90–60 s before each apnea and hyperventilation values from the initial 30 s of hyperventilation (60–30 s before apneas). Apnea values for cardiovascular parameters were obtained from the apneic period minus the first 30 s of the apnea, during which the diving response is established (Jung and Stolle 1981).

Subjects served as their own controls. For all cardiovascular comparisons the non-parametric Wilcoxon signed ranks test was used. In order to take individual variation in EPO levels into account, the EPO data from the apnea and the control day was analyzed via linear regression analysis followed by an ANOVA on the residuals from the regression analysis. Individual maximum EPO levels were compared to baseline using the non-parametric Wilcoxon signed ranks test. Significance was accepted at $P < 0.05$.

Results

Average (SD) $VC_{standing}$ was 5.0 (1) L and the VC_{supine} was 4.6 (1) L (92% of standing VC). The average pre-apneic inspiration was 3.2 (1) L (69% of supine VC). Hyperventilation reduced the pre-apneic $F_{ET}CO_2$ by 51%. The $F_{ET}CO_2$ increased from 2.4 (0.4)% before apneas to 5.5 (0.7)% after apneas, while $F_{ET}O_2$ decreased from 19.5 (0.2)% to 6.6 (1.1)% after apneas. Average apneic duration for the 15 apneas was 3 min 26 (51) s (range 1 min 34 s to 5 min 26 s). Apnea duration increased across each series, with slightly longer apneas in each successive series. Average total protocol duration was 109 (4) min. Subjects reached below 85% SaO_2 in 127 of 150 apneas. Average pre-series SaO_2 was 97.6 (1)% and average post-apneic nadir SaO_2 was 72.7 (11)%. Two subjects reached SaO_2 levels below 60% during apnea and resumed breathing on instruction. Subjects spent an average of 12 min 25 s (8) below 85% SaO_2. A registration of a typical series of five apneas performed by one subject is shown in Fig. 1.

After apneas, the average serum EPO concentration had increased by 15% from baseline at 1 h, by 12% at 2 h and by 16% at 3 h. At 5 h after the apnea series circulating EPO had returned to baseline. While baseline EPO values for the apnea day and the control day were the same, no changes in EPO concentration were observed on the control day. Resting EPO levels were shown to vary significantly between individuals (Fig. 2). In order to weigh for individual variation the residuals from the linear regression were used to further analyze the effects of apnea on EPO concentration. Statistical analysis of the residuals showed significant effects of apnea on EPO with time after apnea (Fig. 3). Because the peak in EPO varied with time among subjects, the individual maximum value was also compared to baseline. Serum EPO increased from baseline (8.6 U/L) by an average individual maximum of 24% after apneas ($P < 0.01$).

A 12% increase in heart rate occurred during hyperventilation compared to the preceding eupneic value ($P < 0.01$), followed by a 9% reduction ($P < 0.05$) back to the pre-hyperventilation eupneic level during apnea. Blood pressure decreased by 4% during hyperventilation compared to the preceding eupneic value ($P < 0.05$), followed by an

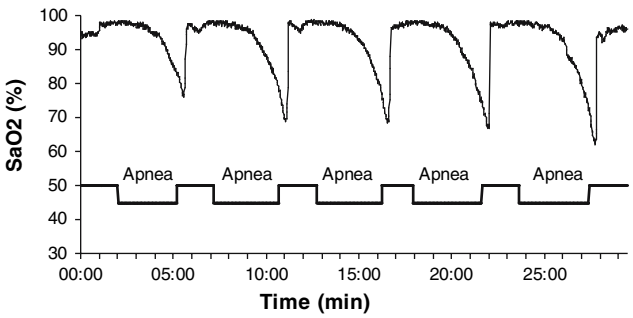

Fig. 1 Original recording of arterial oxygen saturation (SaO_2) in a single subject during a series of five apneas. The nadir corresponds to the end of the apnea, but is slightly shifted due to a circulatory delay

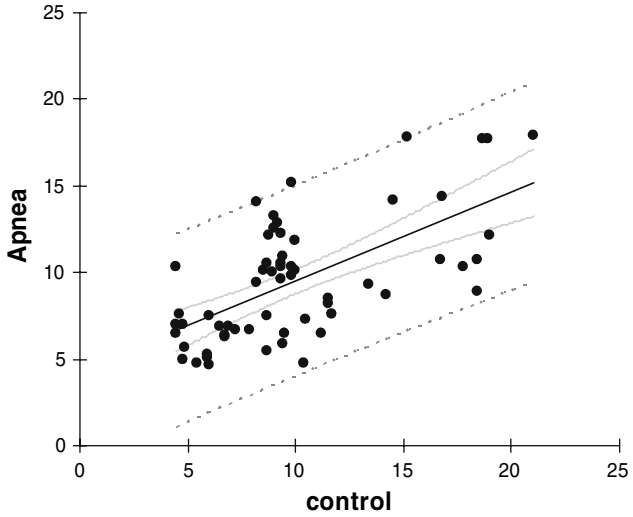

Fig. 2 Relationship between individual EPO values on the apnea and the control day. The equation of the model writes: Apnea = 4.303 + 0.517 × control, $F = 39.59$, $P < 0.0001$

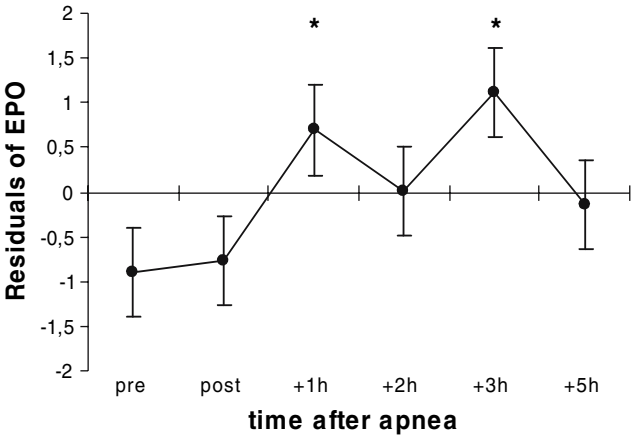

Fig. 3 Residual variation in EPO after taking individual variation into account. * Significant at $P < 0.05$

increase of 20% during apnea compared to during hyperventilation ($P < 0.01$). Skin blood flow decreased by 20% during hyperventilation ($P < 0.01$) and was reduced by another 22% during apnea compared to hyperventilation ($P < 0.01$).

Discussion

This study suggests that repeated voluntary apneas may induce increased serum EPO concentration. No changes in EPO were found on the control day, which suggests that the increase found after the apnea series was likely caused by the apnea-induced hypoxia and not by e.g. a circadian rhythm of EPO production (Cahan et al. 1992; Klausen et al. 1996) or by blood loss from the sampling protocol. It is also unlikely that the changes in EPO concentration were caused by changes in plasma volume, since no differences were found between the samples from directly before and after the apneas. The results show that there is a large individual variation in EPO concentration, with both individually high and low EPO resting levels found in the current group of subjects. Further analysis of the residuals of the correlation analysis showed that serum EPO levels after apnea are not correlated to the individual variation and are thus most likely an effect of the apneas.

EPO production has previously been shown to peak around 3 h after the end of high altitude exposure, with a half-life of around 5 h (Eckardt et al. 1989). The change in EPO found in this study peaked at approximately 3 h after the last apnea—or 4 h 48 min after the start of the first hypoxic exposure—and returned to baseline 5 h after the last apnea. Despite the relatively short, intermittent hypoxia used in the current study, our 24% increase in EPO is comparable to an increase of 24% found by Ge and associates (2002) after 6 h at an altitude of 1,780 m. The average SaO_2 levels found in the current study compared to an altitude of around 2,000 m in that same study (Ge et al. 2002), while much lower SaO_2 levels were reached for short periods during our protocol.

There have been conflicting results regarding the effects of normobaric intermittent hypoxia on EPO changes. One obvious source of the discrepancies is the variation in hypoxic stimulus. By alternating 2.5 min of breathing a mixture of 10% O_2 and 89.5% N_2, by 1.5 min of breathing room air, EPO levels were increased by 52% after 240 min of exposure (Knaupp et al. 1992). A shorter daily exposure of 70 min over a course of 4 weeks, with a graded decline in fraction of inspired O_2 from 0.12 to 0.10, did not induce any changes in EPO levels (Julian et al. 2004). This may suggest that the rate of change in oxygenation is the stimulus. The rapid reduction in tissue-oxygen levels that occur during breath-hold diving, especially after hyperoxic exposure during deep dives, has been suggested as a possible stimulus for an enhanced EPO production (Balestra et al. 2006). The periodic decreases in oxygen saturation during repeated maximal apneas in our study may thus be a sufficient stimulus, despite the relatively short total duration of hypoxia.

The possibility of apnea as a stimulus for an increased production in EPO is supported by observations that OSA-patients, known to endure repeated nocturnal hypoxic periods, have increased levels of Hb, Hct and EPO when compared to control subjects (Imagawa et al. 2001). Furthermore, treatment of OSA, and thus cessation of the nocturnal apneas, leads to a reduction in EPO levels (Cahan et al. 1995).

In this study, we have shown that repeated short-term hypoxia induced by voluntary apnea can cause increases in circulating EPO, supporting the idea that apnea could be a stimulus for erythropoietin generation. However, it remains to be investigated in a longitudinal study whether this is sufficient to cause increased erythropoiesis leading to higher Hb. Nevertheless, the high levels of Hb observed in trained breath-hold divers (de Bruijn et al. 2004) make it interesting to further study such a possibility. In this study a single sequence of 15 apneas was performed by subjects untrained in breath-hold diving, while the apnea specific training of competitive apneic divers is much more extensive and would likely exert a more powerful stimulus.

A diving response, consisting of bradycardia, reduced skin blood flow and hypertension, was found, which complies with earlier studies (Andersson et al. 2004; Gooden 1994; Schagatay et al. 1999). However, hyperventilation seems to have a negative effect on the cardiovascular diving response, when values are compared to pre-hyperventilation eupneic baselines, which deserves further investigation. A reduction in blood flow to the kidney caused by the apnea-induced vasoconstriction, as found in Weddell seals (Zapol et al. 1979), could possibly result in a local ischemic hypoxia in the EPO producing kidney. This could provide an additional explanation for the increase in EPO concentration found, despite the apparently small stimulus used.

In this study, we successfully used hyperventilation to make it possible for untrained subjects to reach SaO_2 levels of below 85%. One minute of hyperventilation reduced alveolar PCO_2 levels by half and prolonged apneic duration to an average of 3 min 26 s, which by far exceeds the durations of approximately 40–100 s previously reported for non-divers without hyperventilation (Schagatay and Andersson 1998; Schagatay et al. 1999, 2000). Hypoxic syncope was avoided by asking subjects to start breathing when SaO_2 levels reached 60% and it should be emphasized that hyperventilation should never be combined with under water activity, as the stimulus to resume breathing may be delayed until hypoxic syncope occurs and the diver

may easily drown. Using only non-immersion apneas prevents this and with careful monitoring of SaO_2 levels, or by avoiding hyperventilation, the risk of syncope could be minimized in a training program.

In conclusion, intermittent hypoxia, induced by repeated voluntary maximal duration apneas, increased circulating serum EPO concentration. We suggest that this may connect the high Hb-levels observed in elite breath-hold divers to their intense apnea training.

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