

RESEARCH ARTICLES

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Hyperoxemia and hypoxemia impair cellular oxygenation: a study in healthy volunteers

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Abstract

Introduction Administration of oxygen therapy is common, yet there is a lack of knowledge on its ability to prevent cellular hypoxia as well as on its potential toxicity. Consequently, the optimal oxygenation targets in clinical practice remain unresolved. The novel PpIX technique measures the mitochondrial oxygen tension in the skin (mitoPO₂) which allows for non-invasive investigation on the effect of hypoxemia and hyperoxemia on cellular oxygen availability.

Results During hypoxemia, SpO₂ was 80 (77–83)% and PaO₂ 45(38–50) mmHg for 15 min. MitoPO₂ decreased from 42(35–51) at baseline to 6(4.3–9)mmHg ($p < 0.001$), despite 16(12–16)% increase in cardiac output which maintained global oxygen delivery (DO₂). During hyperoxic breathing, an FiO₂ of 40% decreased mitoPO₂ to 20 (9–27) mmHg. Cardiac output was unaltered during hyperoxia, but perfused De Backer density was reduced by one-third ($p < 0.01$). A PaO₂ < 100 mmHg and > 200 mmHg were both associated with a reduction in mitoPO₂.

Conclusions Hypoxemia decreases mitoPO₂ profoundly, despite complete compensation of global oxygen delivery. In addition, hyperoxemia also decreases mitoPO₂, accompanied by a reduction in microcirculatory perfusion. These results suggest that mitoPO₂ can be used to titrate oxygen support.

Keywords MitoPO₂, Hypoxia, Oxygen therapy, Hypoxemia, Hyperoxia, Hyperoxemia, Mitochondria, Cellular oxygenation

Background

Hypoxemia increases the risk of death in ICU patients by 50% [1, 2]. However, in 10% of all ICU patients, therapy with supplemental oxygen leads to supraphysiological arterial oxygen tensions [3]. As hyperoxemia is also associated with increased mortality [1–4], careful titration of oxygen therapy is imperative. Clinical trials have attempted to establish optimal oxygen targets but results are contradictory, partly due to the absence of a direct

biomarker of oxygen toxicity and oxygen debt. Most likely, determining oxygen dose is complicated by the lack of knowledge of the effects on a cellular level [5–8].

The rationale for supplemental oxygen therapy is to prevent cellular hypoxia [6]. The effects on a cellular level are unclear due to the variable effects of oxygen on organ perfusion. Hypoxemia increases cardiac output, recruits previously closed capillaries and decreases mitochondrial oxygen consumption, which improve global oxygen delivery and lower oxygen demand [9–12]. On the other end, hyperoxemia reduces cardiac output and increases ROS production, leading to impairments in microcirculatory perfusion [13, 14]. This could potentially offset the increase in arterial oxygen content and limit oxygen extraction by tissues [1, 2]. Potentially supporting this, multiple analyses have shown an association between hyperoxemia and mortality in critically ill patients [1–3]. However, this relationship is not always present in severity-adjusted models [4]. As such, the causality of

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hyperoxemia-associated harm remains doubtful, also partly due to an incomplete understanding of the cellular and physiological effects of supplemental oxygen [6, 15].

The development of the protoporphyrin IX delayed lifetime technique enables the measurement of mitochondrial oxygen tension non-invasively in the skin (mitoPO₂). Mitochondria are the utilizers of oxygen and therefore mitoPO₂ reflects the balance between oxygen supply and demand at the most downstream level [16, 17]. Cutaneous mitoPO₂ correlates well with organ mitoPO₂ and responds accurately to changes in FiO₂ or in tissue perfusion [16, 18–21]. Moreover, it also allows measurement of mitochondrial respiration (mitoVO₂) non-invasively [22, 23].

The aim of this study was to investigate the effect of hypoxemia and hyperoxemia on mitoPO₂ as marker of cellular oxygen availability in healthy human volunteers.

Methods

Study design and participants

This physiological cross-over intervention study in healthy human volunteers was conducted in the ICU of a teaching hospital (OLVG hospital, Amsterdam, The Netherlands). The study was approved by the institutional review board (MEC-u). Healthy human volunteers > 18 years and with BMI < 25 kg/m², were screened for eligibility. Participants were excluded if they had an allergy for plaster adhesives, mitochondrial disease, skin lesions, anemia or had a history of smoking or altitude exposure (> 1000 m) in the 3 months previous to inclusion.

Study procedures

Participants had an ALAcare patch placed on the sternum 4 h before start of the experiment and were monitored using ECG and pulse oximetry. An arterial catheter was placed for blood pressure monitoring and blood sampling. In the cross-over design, predetermined hypoxic and hyperoxic gas mixtures were delivered in a fixed order in all participants using high flow nasal cannula. After 30 min of accustoming to the setup, hypoxemia was titrated to an SpO₂ of 75–85% for 15 min. The target SpO₂ was achieved by titrating the FiO₂ of the high-flow nasal cannula between an FiO₂ of 9% and 12%. The flow-rate remained equal for all participants at 40L/h to ensure no rebreathing. When participants remained between an SpO₂ of 75% and 85% during a complete minute, the FiO₂ was set for the remainder of the hypoxic phase. After completion of 15 min, participants had a wash-out period of 45 min of breathing atmospheric air before commencement of hyperoxic gas breathing. Hyperoxic gas mixture was delivered for periods of 15 min with incremental FiO₂ of 40%, 60%, 80%

and 100%. After each step, clinical data was collected and measurements were done. MitoPO₂ was measured using the COMET (Photonics Healthcare, Utrecht, The Netherlands). The non-invasive cardiac output was recorded continuously using pulse wave contour analysis with the volume clamp method. The sublingual microcirculation was imaged using sidestream darkfield imaging (SDF) at baseline, after hypoxemia and after hyperoxemia to prevent the mixing of hyperoxic gas mixture with atmospheric air during the hyperoxic phases [24–26].

Measurements

COMET

MitoPO₂ was computed from the mean mitoPO₂ during the first 30 s of a dynamic measurement (1 Hz). MitoVO₂ is defined as the rate of mitochondrial deoxygenation after local occlusion of circulation by applying pressure on the probe. It is calculated by automatic linear fitting of the slope using MATLAB (The Mathworks Inc). The method of performing a dynamic measurement and calculating mitoVO₂ is described in detail elsewhere [27].

Non-invasive cardiac output

Cardiac index and systemic vascular resistance index were measured continuously throughout the experiment using the volume clamp method in the Nexfin device (BMEYE, Amsterdam, The Netherlands) as invasive arterial wave-form estimation of cardiac-output was not available in our ICU. The change between two measurements of Nexfin-CO has been shown to have very good agreement with invasive cardiac output and has been used previously to monitor hyperoxemia induced changes in cardiac output [25, 28, 29, 30].

A finger cuff was placed on the index or middle finger according to the manufacturer's instructions. The mean of the last 2 min of each step was used for analysis. The cardiac index was used to calculate global oxygen delivery (DO₂) was calculated using the following formula: $DO_2 = CO * (10 * Hb / dl * SaO_2 + PaO_2 * 0.03)$. Hb, SaO₂ and PaO₂ were obtained from arterial blood gas analysis.

Sublingual microcirculation

The sublingual microcirculation was recorded using a handheld video microscope with sidestream darkfield imaging (SDF) with the MicroScan (MicroVision Medical, The Netherlands, Amsterdam). Sublingual measurements were done by one researcher trained in microcirculatory image recording. Directly after removal of the high-flow nasal cannula, SDF measurements were performed. Three anatomical sites were recorded: the medial and both lateral parts of the sublingual area. The validated AVA 4.3C software (Microvision Medical, Amsterdam, The Netherlands) was used for quality

control and analysis of images [31]. AVA 4.3C automatically assesses the focus, contrast and stability of the images. Additionally, good quality captures required the presence of flow in large vessels, to exclude possible pressure artefacts. Images were only evaluated in case the quality was sufficient. The proportion of perfused vessels (PPV), proportion of perfused small vessels (PPV small), density, and the perfused DeBacker density were determined automatically by the software. The perfused DeBacker Density are all vessels with visible microcirculatory flow. The Percentage of Perfused Vessels (PPV) is calculated as the percentage of perfused vessels in relation to the total number of all vessels and for small vessels in particular (PPV small). Small vessels (capillaries) have a diameter less than 20 μm . The mean values of three recordings of each parameter were used for final analysis.

Statistical analysis

Sample size calculation is based on the expected drop in mitoPO₂ during hypoxemia as predicted by a mathematical model as there are no previous data available for the expected effect size [32]. Modified Krogh equations predict that a saturation decrease from 98% to 85% results in a mitoPO₂ decrease of 40 mmHg. Since we expected that homeostatic mechanisms (cardiac output increase and microcirculatory recruitment) would attenuate this mitoPO₂ decrease, we set the minimum detectable difference at 20 mmHg. The standard deviation is expected to be 15 mmHg, corresponding to the sample standard deviation in healthy human volunteers [20]. The calculated sample size for a paired *t* test with a power of 90%, a significance level of 0.05, an effect size of 1.33 (minimum difference of 20 mmHg divided by the standard deviation of 15 mmHg) is 9 subjects. We also expected to see an increase of 20 mmHg in mitoPO₂ in response to hyperoxia, based on previous studies in rats [33, 34].

Data is presented in mean \pm SD or median (IQR) if non-normally distributed. Within-group differences over time were analyzed using repeated measures one-way ANOVA. Hypoxemia was compared with baseline. In case of significance, post-hoc tests were done with Mann–Whitney *U* test and Bonferroni correction for hyperoxia to determine at which FiO₂ the changes in variables occurred. For hyperoxemia and hypoxemia, a separate linear regression model was performed for mitoPO₂ and mitoVO₂. Parameters which differed significantly ($p < 0.05$) during hyperoxic/hypoxic phases in the ANOVA analysis were entered into the model as independent variables. Statistical analysis was done using Rstudio (Posit, Vienna, Austria).

Results

We enrolled 9 healthy volunteers, of which 6 were female. The median age was 25 (22–25) years and BMI was 21.7 (21.3–23.2) kg/m². Inhalation of 10% FiO₂ resulted in a decrease in PaO₂ from 107 (99–113) mmHg to 45 (38–50) mmHg and SaO₂ from 98 (98–98)% to 80 (77–83)%, ($p < 0.0001$). Some participants noted feeling drowsy during hypoxic gas breathing, which resolved rapidly during the wash-out step. No other discomfort was noted. Hyperoxic gas breathing increased the PaO₂ stepwise, with a plateau from 80% to 100% FiO₂, as shown in Fig. 1A.

Macro-hemodynamic and respiratory response

Hypoxic gas mixture breathing resulted in an increase in heart rate from 70 (60–79) bpm to 84 (71–88) bpm (Fig. 2). This paralleled an increase in cardiac output of 16 (12–16)% from baseline and a decrease in systemic vascular resistance index of 21 (17–28)%. Whereas arterial oxygen content (CaO₂) decreased with 20 (15–25)% during hypoxemia, DO₂ was maintained when compared to baseline ($p = 0.62$). The wash-out period restored all hemodynamic indices to baseline. Hyperoxia did not induce significant changes in macro hemodynamic parameters compared to baseline. PaCO₂ decreased significantly at 100% FiO₂. Hypoxic gas breathing did not result in hypocapnia (Table 1). Other arterial blood gas parameters were not significantly altered during hyperoxic or hypoxic breathing.

MitoPO₂ and mitoVO₂

Hypoxic mixture breathing induced a profound decline in mitoPO₂ from 42 (35–51) mmHg to 6 (4.3–9) mmHg (Fig. 3). Concurrently, MitoVO₂ decreased from 3.7 (2.9–5.0) mmHg/s to 0.80 (0.50–1.0) mmHg/s ($p < 0.01$). Wash-out recovered mitoPO₂ to 39 (13–52) mmHg and mitoVO₂ to 3.2 (0.92–4.8) mmHg/s.

Hyperoxic gas breathing also resulted in a median decrease in mitoPO₂ when compared to washout ($p = 0.03$). In all participants, mitoPO₂ decreased to 20 (9.0–27) mmHg when breathing 40% FiO₂ ($p < 0.05$), which remained significantly lower compared to wash-out until 80% FiO₂ ($p = 0.038$) (Fig. 3). Hyperoxia did not result in a significant decrease in mitoVO₂ when compared to wash-out (RM ANOVA $p = 0.2$). However, mitoVO₂ was strongly correlated with mitoPO₂ ($r = 0.84$, $p < 0.001$) during all experimental phases (Fig. 4). In linear mixed model analysis, mitoPO₂ remained the only predictor of mitoVO₂ with coefficient of -0.10 (-0.8 to -0.12).

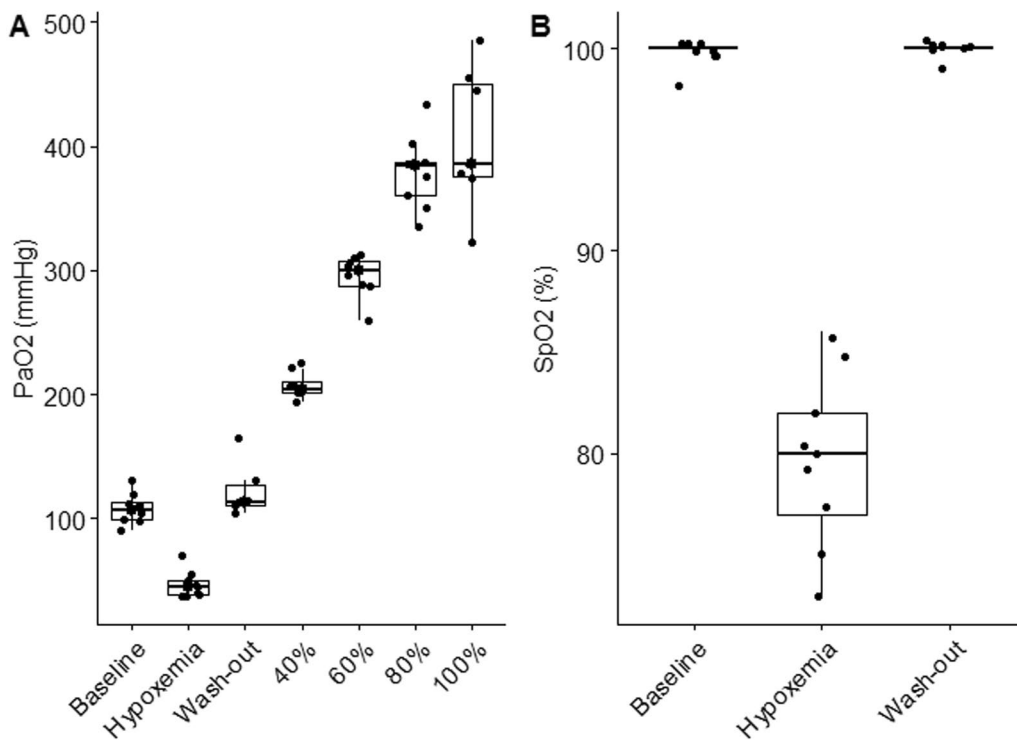


Fig. 1 **A** PaO₂ for individual experimental steps, individual datapoints and median and IQR presented. **B** boxplot of SpO₂ for baseline, hypoxemia and wash-out steps, with individual datapoints and median and IQR

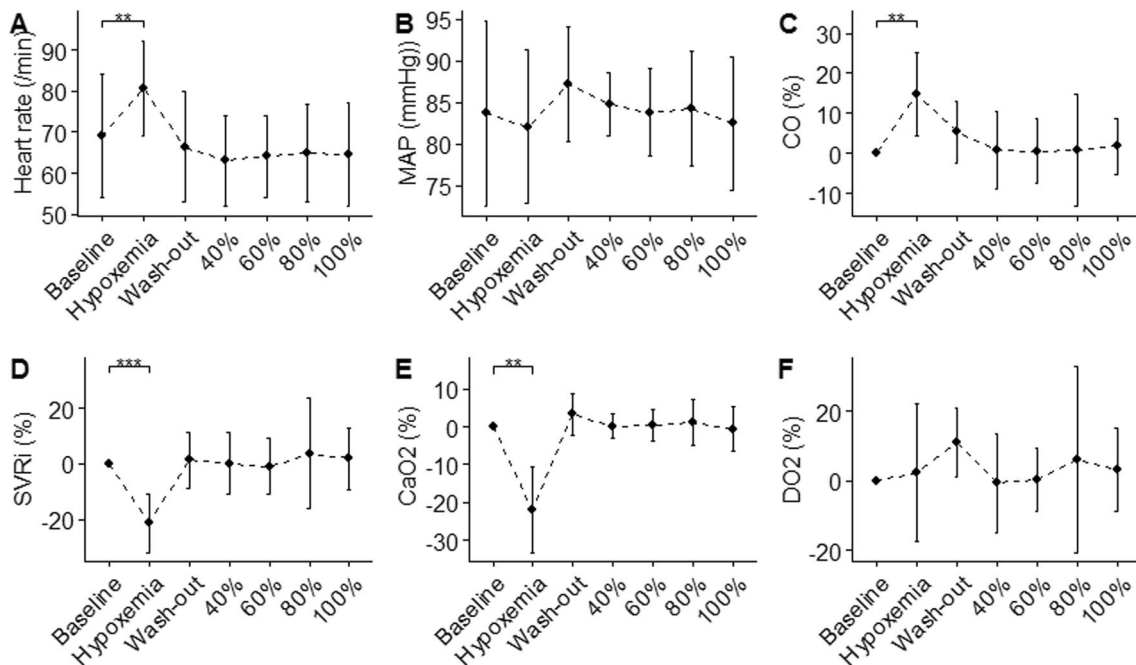


Fig. 2 Hemodynamic variables for all experimental steps. **A** Heart-rate, **B** Mean arterial pressure, **C** % change in cardiac output compared to baseline, **D** % change in systemic vascular resistance index compared to baseline, **E** % change in arterial oxygen content compared to baseline, **F** % change in global oxygen delivery compared to baseline. Pairwise paired Wilcoxon sign-rank test compared to baseline and wash-out: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; non significance

Table 1 Parameters of oxygenation and microcirculation

	Baseline	Hypoxia	Washout	40%	60%	80%	100%
SpO ₂ (%)	100 (100–100)	80 (77–82)#	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)
PaO ₂ (mmHg)	110 (99–110)	45 (38–50)	110 (110–130)	200 (200–210)*	300 (290–310)*	390 (360–390)*	390 (380–450)*
SaO ₂ (%)	99 (98–99)	83 (77–89)	99 (99–99)	100 (100–100)	100 (100–100)	100 (100–100)	100 (100–100)
Hb (g/dL)	12 (12–13)	13 (13–14)	13 (12–14)	13 (12–13)	13 (12–13)	13 (13–13)	13 (12–13)
Lactate (mmol/L)	0.60 (0.58–0.80)	0.80 (0.70–1.0)	0.55 (0.50–0.68)	0.60 (0.48–0.78)	0.50 (0.48–0.60)	0.50 (0.50–0.50)	0.50 (0.48–0.53)
pH	7.41 (7.38–7.59)	7.46 (7.41–7.59)	7.42 (7.39–7.47)	7.44 (7.41–7.52)	7.45 (7.42–7.57)	7.46 (7.42–7.52)	7.46 (7.42–7.47)
PaCO ₂ (mmHg)	36 (33–40)	33 (31–35)	38 (36–39)	35 (32–38)	33 (32–35)	34 (28–36)	34 (32–35)*
PPV (%)	96 (95–98)	95 (86–97)	–	–	–	–	83 (80–89)
Perfused DeBacker Density	3.6 (3.2–4.1)	3.8 (3.7–3.8)	–	–	–	–	2.4 (1.4–2.7)#
PPV small vessels (%)	100 (70–100)	100 (55–100)	–	–	–	–	75 (37–100)
Total vessel density	3.9 (3.3–4.3)	3.9 (3.7–4.2)	–	–	–	–	2.7 (1.7–3.3)
MitoPO ₂ (mmHg)	42 (35–51)	5.9 (4.3–9.0)#	39 (13–52)	20 (9.0–27)*	15 (13–32)*	18 (12–21)*	20 (15–25)
MitoVO ₂ (mmHg/s)	3.7 (2.9–5.0)	0.80 (0.50–1.0)#	3.2 (0.92–4.8)	2.0 (0.73–4.7)*	2.8 (1.7–4.4)	1.6 (0.77–3.2)*	2.4 (1.3–3.8)*

PPV, proportion of perfused vessels

**p* < 0.05 compared to wash-out

p < 0.05 compared to baseline

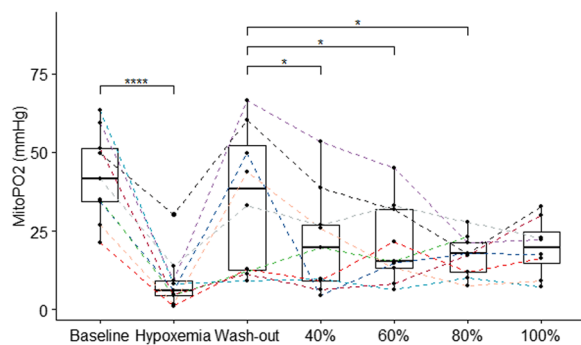


Fig. 3 Paired mitoPO₂ data for all participants. Kruskal–Wallis *p* < 0.001. Pairwise paired Wilcoxon sign-rank test compared to baseline and wash-out: **p* < 0.05, ***p* < 0.01, ****p* < 0.001, ns: non significance

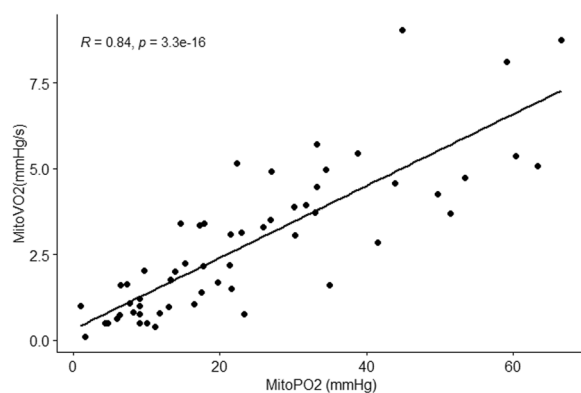


Fig. 4 Correlation of mitoPO₂ with mitoVO₂ for all experimental steps. Pearson's Rho and significance level displayed

Microcirculation

During hypoxemia, no significant recruitment of the microcirculation was observed (Fig. 5 and Table 1). During hyperoxic gas breathing, parameters of sublingual microcirculation worsened (Fig. 5). Proportion of perfused vessels (PPV) decreased from 96 (95–98)% to 80 (80–89)% at the end of the hyperoxia period. The absolute number of perfused vessels decreased in parallel, from 3.6 (3.2–4.1) to 2.4 (1.4–2.7).

Determinants of mitoPO₂

A linear regression model was performed to determine the influencing factors on mitoPO₂. PaO₂ and Perfused DeBacker density were identified as significant predictors of mitoPO₂ during hyperoxia and baseline/wash-out (*p* < 0.05). During the hyperoxic phase, mitoPO₂ was linearly correlated with PaO₂ (*r* = − 0.44, *p* < 0.01) and the perfused DeBacker Density (*r* = 0.6, *p* = 0.023). Figure 6 shows the fitted general model with PaO₂ and perfused DeBacker Density as explanatory variables. Inclusion of the hypoxic phase results in a non-significant correlation between perfused DeBacker density and mitoPO₂, due to an inability of perfused DeBacker density recruitment to restore mitoPO₂ to baseline values (Fig. 5C).

Discussion

We assessed the effect of acute hypoxemia and hyperoxemia on mitoPO₂ as a marker of oxygen debt and toxicity. Despite complete compensation of global oxygen delivery during hypoxemia, mitoPO₂ decreased in all participants. Also, we demonstrated that hyperoxemia is detrimental to mitoPO₂, through reduced microcirculatory perfusion.

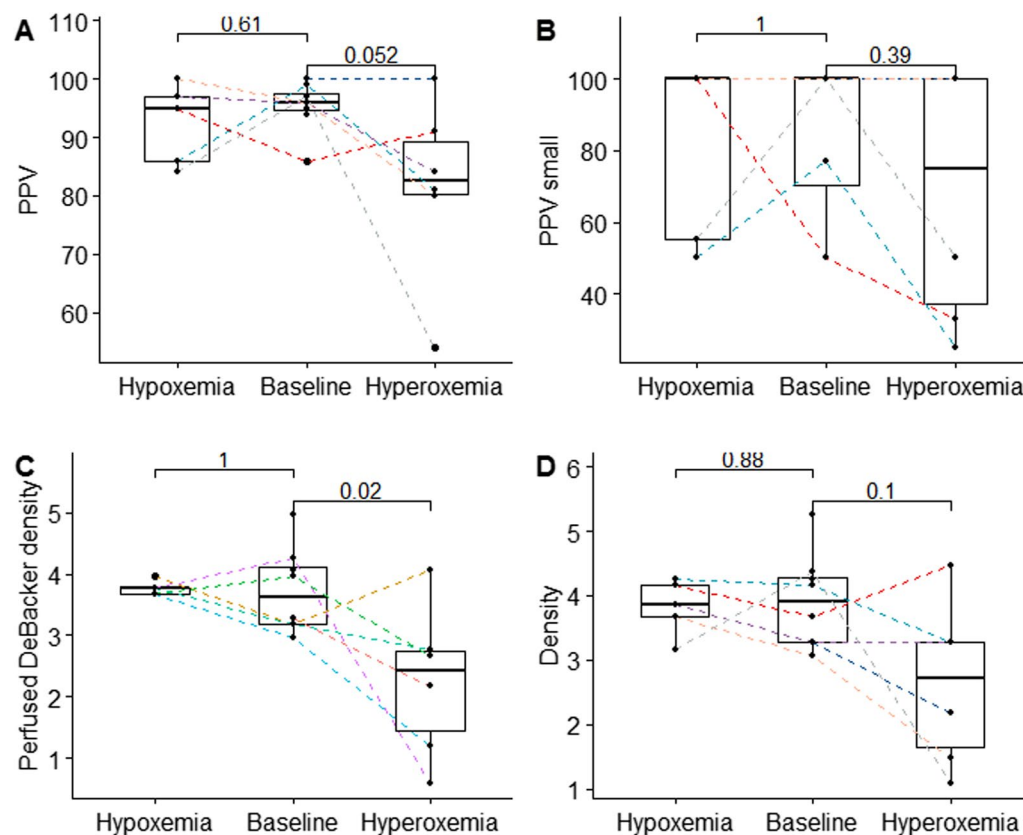


Fig. 5 Boxplots of microcirculatory parameters at baseline, hypoxemia and hyperoxia (100% FiO_2) with individual data points **A** Proportion of perfused vessels, **B** Proportion of small perfused vessels, **C** Perfused vessel (DeBacker) density, **D** Total vessel density. *P* values of paired-Wilcoxon sign rank test displayed

Mito PO_2 at baseline was 40 mmHg, corresponding to previous studies in healthy volunteers [19, 27]. Mito PO_2 decreased sharply in acute hypoxemia. This corroborates predictions from mathematical experimental models which showed significant reductions in mito PO_2 in response to hypoxemia [32, 35]. Although intuitive, this finding is in contrast with some studies in healthy volunteers which show that indirect markers of tissue oxygenation are largely unchanged during acute hypoxemia [11, 15, 36–38]. Accordingly, it was proposed that adaptive mechanisms, such as an increase in CO and recruitment of the microcirculation could maintain oxygen delivery to cells and has led to the speculation that lower SpO $_2$ targets could be beneficial in critically ill patients [15]. Indeed, we found an increase in CO through an increase in heart rate, causing a maintained global DO $_2$ during hypoxemia. Nevertheless, the sharp decrease in mito PO_2 shows that hypoxemia decreases oxygen delivery into the parenchyma. The probable explanation is that in the microcirculation, the augmented blood flow above physiological levels is not beneficial during acute hypoxemia because the time for red blood cells to unload

their (limited) oxygen content decreases, thus causing hypoxic tissue hypoxia [12, 32, 39]. Although systemic DO $_2$ reflects the total oxygen content carried per unit time, it does not reflect the ability of the microcirculation to unload oxygen into the parenchyma. Mathematical models and experimental studies corroborate this disconnection showing a decrease in ScvO $_2$ and increase in oxygen extraction ratio during hypoxemia [32, 40]. An alternative explanation is that despite an increase in CO, the observed decrease in mito PO_2 is mediated by redistribution of blood flow away from the skin, kidneys, GI and liver to the heart and brain during hypoxemia [41–44]. In shock and critical illness, skin blood flow closely resembles visceral organ blood flow. However, it remains unknown whether this close relation remains during a combination of hypoxemia and shock. The detrimental effects of hypoxemia on internal organs is further supported by significant cognitive decline in healthy human volunteers during acute hypoxemia, likely representing slight cerebral oxygen debt [32, 45]. Commonly used markers of tissue oxygenation/perfusion such as NIRS and lactate, may not be suitable to ensure adequate

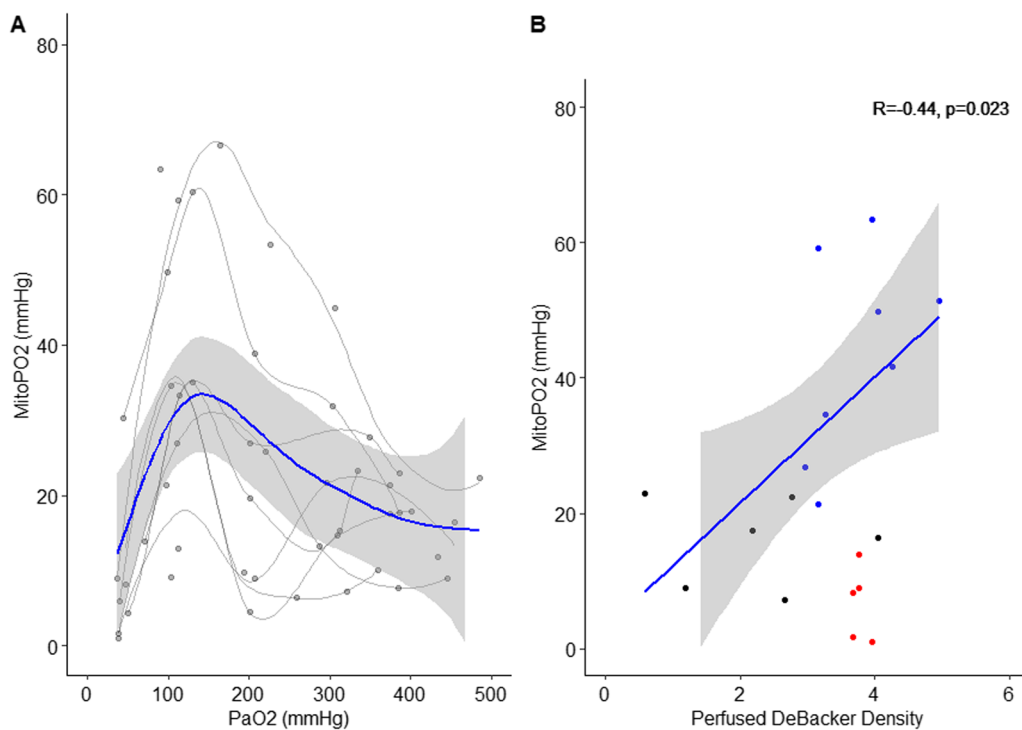


Fig. 6 **A** Correlation of mitoPO₂ and PaO₂ for all experimental steps, regression line (blue) with confidence interval fitted by generalized additive model. **B** Correlation of mitoPO₂ and perfused vessel density for all experimental steps, in red data points during hypoxemia, in blue during baseline. Linear regression fit and its Pearson's Rho and significance level displayed for hyperoxia and baseline steps only

cellular oxygenation in the context of acute hypoxemia [15].

Hyperoxemia also causes a profound decrease in mitoPO₂, occurring at a PaO₂ level of 200 mmHg. This occurred in parallel with a decrease in sublingual perfused vessel density. Although previous studies have shown a decrease in microcirculatory perfusion [30, 40, 46, 47], it was also shown that oxygen delivery increased with increased PaO₂ [48–50]. This combination has led to the accepted hypothesis that an increased tissue oxygen tension induces radical oxygen species mediated damage. However, tissue pO₂ measurements were performed using devices that disturb the integrity of the tissue. Also, the SDF technique has been unable to elucidate the downstream effect of hyperoxemia on oxygen toxicity and debt as it is not able to evaluate the oxygen content of capillaries [46, 49, 51, 52]. This study is the first to directly measure cellular oxygen availability in response to hyperoxemia and indeed demonstrates that a PaO₂ above 200 mmHg has a detrimental effect on cellular oxygenation, at least in healthy volunteers. Of note, this cutoff value (200 mgHg) corresponds well with PaO₂-associated mortality in critically ill patients [1, 2]. Our results show no dose-dependency of decreasing mitoPO₂ while increasing PaO₂, with the effect plateauing at an FiO₂ of

40%. This corresponds to meta-analyses showing that PaO₂ has no dose-dependent effect on mortality [1, 5]. Taken together, our findings suggest that a reduction in tissue oxygenation, through reduction in microcirculatory perfusion might account for the observed harm of hyperoxemia in hospitalized patients.

Our study has potential implications for future clinical investigations into hyperoxemia and hypoxemia. Intensivists frequently assess markers of tissue oxygenation (lactate, microcirculation) when hypoxemia is refractory. However, whereas no evidence exists that lactate reflects tissue hypoxia during hypoxemia, we demonstrate that mitoPO₂ may be an alternative [11, 15, 37, 53]. Increasing PaO₂ during normoxemia to attempt to increase oxygen delivery is done frequently [54]. However, this has never been empirically demonstrated to be effective and guidelines provide contradictory recommendations for supplemental oxygen therapy during normoxemia [55–60]. We show that from a PaO₂ of 200 mmHg and above, median mitoPO₂ is lower than the 25 mmHg threshold associated with organ failure in critically ill patients. As such, our results suggest that O₂-supplementation should probably not exceed an upper PaO₂ limit of 200 mmHg, as it is associated with a decrease in tissue oxygenation. However, it remains to be investigated whether a low

mitoPO₂ reflects adverse effects on cellular integrity and organ function in patients [61]. In addition, the heterogeneous effect of PaO₂ on hemodynamics warrants further investigation of mitoPO₂ as a biomarker of oxygen toxicity and debt for personalized titration of PaO₂ in critically ill patients.

Limitations

Our study has several limitations. As this was a study in healthy volunteers, results might not apply to the critically ill patient. Patients in the ICU often have disturbed Hb-O₂ dissociation curves and impaired vascular reactivity, meaning high/low PaO₂ and ROS could have a different effect on the visceral and skin microcirculation in patients with systemic inflammation compared to healthy volunteers. Furthermore, the coupling between skin and visceral mitoPO₂, blood flow and microcirculation that is seen in experimental hypoxemia could potentially be absent in critically ill patients with shock.

Also, we did not control for normocapnic hypoxemia. Alkalosis was observed in most participants throughout the experiment. However, we found that decreases in mitoPO₂ were not explained by hypocapnia as continuous variable in the mixed model but this may have been due to the limited sample size. Furthermore, gas mixture breathing was not randomized as we used a cross over study setting. It is known that both hyperoxemia and hypoxemia have long lasting effects on the microvasculature and arteriolar tone due to increased sympathetic activity, even after cessation of hypoxic and hyperoxic stimuli [62–64]. As such, we cannot exclude the possibility that exposure to hypoxia may have altered the response during hyperoxemia. However, SVR was restored during wash-out and no significant microcirculatory hypoperfusion was noted during hypoxemia. Finally, it is unknown whether a low mitoPO₂ during hypoxemia and hyperoxemia reflects cellular damage and organ function. We call for clinical investigations for mitoPO₂ as a marker of organ function during resuscitation.

Conclusion

Acute hypoxemia decreases skin mitoPO₂ profoundly, despite complete compensation of global oxygen delivery. Hyperoxemia decreases skin mitoPO₂ dose-dependently through decreased microcirculatory perfusion. We identified a maximum PaO₂ of 200 mmHg for optimal tissue oxygenation in healthy volunteers. These results suggest that mitoPO₂ could be used as a marker of oxygen debt during oxygen therapy.

Abbreviations

CaO ₂	Arterial oxygen content
CO	Cardiac output

DO ₂	Global oxygen delivery
FIO ₂	Fraction of inspired oxygen
MitoPO ₂	Mitochondrial oxygen tension
MitoVO ₂	Mitochondrial oxygen consumption
PaCO ₂	Partial pressure of carbon dioxide in arterial blood gas
PaO ₂	Partial pressure of oxygen in arterial blood gas
PPV	Proportion of perfused vessels
ROS	Radical oxygen species
SaO ₂	Arterial haemoglobin oxygen saturation
SDF	Sidestream darkfield imaging
SpO ₂	Saturation of oxygen measured using plethysmography
SVR _i	Systemic vascular resistance index

Acknowledgements

We would like to thank all volunteers for their participation. We would like to thank Nico Hilderink and Richard Petersen for their excellent technical support of construction of the gas-delivery system.

Author contributions

BH, JP and NJ conceived and designed the study. BH performed study interventions and measurements. BH and NJ drafted the first version of the manuscript. All authors critically read and reviewed the manuscript.

Funding

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The MEC-u approved this study protocol with title “The effect of arterial oxygen content on mitoPO₂ in healthy human volunteers” and number NL79079.100.21. Informed consent was obtained from all participants prior to enrollment and study procedures were performed in concordance with the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

NJ is editor-in-chief of *Intensive Care Medicine Experimental*.

Received: 6 September 2023 Accepted: 28 March 2024

Published online: 15 April 2024

References

- de Jonge E, Peelen L, Keijzers PJ et al (2008) Association between administered oxygen, arterial partial oxygen pressure and mortality in mechanically ventilated intensive care unit patients. *Crit Care* 12(6):1–8. <https://doi.org/10.1186/cc7150>
- Eastwood G, Bellomo R, Bailey M et al (2012) Arterial oxygen tension and mortality in mechanically ventilated patients. *Intensive Care Med* 38(1):91–98. <https://doi.org/10.1007/s00134-011-2419-6>
- Schjørring OL, Jensen AKG, Nielsen CG et al (2020) Arterial oxygen tensions in mechanically ventilated ICU patients and mortality: a retrospective, multicentre, observational cohort study. *Br J Anaesth* 124(4):420–429. <https://doi.org/10.1016/j.bja.2019.12.039>
- Hochberg CH, Semler MW, Brower RG (2021) Oxygen toxicity in critically ill adults. *Am J Respir Crit Care Med* 204(6):632–641. <https://doi.org/10.1164/rccm.202102-0417CI>
- Palmer E, Post B, Klapaukh R et al (2019) The association between supraphysiologic arterial oxygen levels and mortality in critically ill patients

- a multicenter observational cohort study. *Am J Respir Crit Care Med* 200(11):1373–1380. <https://doi.org/10.1164/rccm.201904-0849OC>
6. Singer M, Young PJ, Laffey JG et al (2021) Dangers of hyperoxia. *Crit Care* 25(1):1–15. <https://doi.org/10.1186/s13054-021-03815-y>
 7. Schumacker PT (2010) Is enough oxygen too much? *Crit Care*. <https://doi.org/10.1186/cc9201>
 8. Jones GAL, Peters MJ (2022) Towards causality with liberal oxygen use?*. *Pediatr Crit Care Med* 23(2):135–137. <https://doi.org/10.1097/PCC.0000000000002876>
 9. Cain SM (1965) Appearance of excess lactate in anesthetized dogs during anemic and hypoxic hypoxia. *Am J Physiol* 209(3):604–610. <https://doi.org/10.1152/ajplegacy.1965.209.3.604>
 10. Bickler PE, Feiner JR, Lipnick MS, Batchelder P, Macleod DB, Severinghaus JW (2017) Effects of acute, profound hypoxia on healthy humans: implications for safety of tests evaluating pulse oximetry or tissue oximetry performance. *Anesth Analg*. <https://doi.org/10.1213/ANE.00000000000001421>
 11. Hobler KE, Carey LC (1973) Effect of acute progressive hypoxemia on cardiac output and plasma excess lactate. *Ann Surg*. <https://doi.org/10.1097/0000658-197302000-00013>
 12. Schumacker PT, Samsel RW (1989) Analysis of oxygen delivery and uptake relationships in the Krogh tissue model. *J Appl Physiol* 67(3):1234–1244. <https://doi.org/10.1152/jappl.1989.67.3.1234>
 13. Smit B, Smulders YM, van der Wouden JC, Oudemans-van Straaten HM, Spoelstra-de Man AME (2018) Hemodynamic effects of acute hyperoxia: systematic review and meta-analysis. *Crit Care* 22(1):1–10. <https://doi.org/10.1186/s13054-018-1968-2>
 14. Spoelstra-De Man AME, Smit B, Oudemans-Van Straaten HM, Smulders YM (2015) Cardiovascular effects of hyperoxia during and after cardiac surgery. *Anaesthesia* 70(11):1307–1319. <https://doi.org/10.1111/anae.13218>
 15. Kiers HD, Pickkers P, Kox M (2022) Hypoxemia in the presence or absence of systemic inflammation does not increase blood lactate levels in healthy volunteers. *J Crit Care* 71:154116. <https://doi.org/10.1016/j.jcrc.2022.154116>
 16. Römers LHL, Bakker C, Dollée N et al (2016) Cutaneous mitochondrial Po₂, but not tissue oxygen saturation, is an early indicator of the physiologic limit of hemodilution in the pig. *Anesthesiology*. <https://doi.org/10.1097/ALN.0000000000001156>
 17. Ince C, Mik EG (2016) Microcirculatory and mitochondrial hypoxia in sepsis, shock, and resuscitation. *J Appl Physiol*. <https://doi.org/10.1152/japplphysiol.00298.2015>
 18. Neu C, Baumbach P, Plooij AK et al (2020) Non-invasive assessment of mitochondrial oxygen metabolism in the critically ill patient using the protoporphyrin IX-triplet state lifetime technique—a feasibility study. *Front Immunol* 11(May):1–9. <https://doi.org/10.3389/fimmu.2020.00757>
 19. Baumbach P, Neu C, Derlien S et al (2019) A pilot study of exercise-induced changes in mitochondrial oxygen metabolism measured by a cellular oxygen metabolism monitor (PICOMET). *Biochim Biophys Acta Mol Basis Dis* 1865(4):749–758. <https://doi.org/10.1016/j.bbadis.2018.12.003>
 20. Baumbach P, Schmidt-Winter C, Hoefler J et al (2020) A pilot study on the association of mitochondrial oxygen metabolism and gas exchange during cardiopulmonary exercise testing: is there a mitochondrial threshold? *Front Med*. <https://doi.org/10.3389/fmed.2020.585462>
 21. Harms FA, Ubbink R, de Wijs CJ, Ligtenberg MP, ter Horst M, Mik EG (2022) Mitochondrial oxygenation during cardiopulmonary bypass: a pilot study. *Front Med* 9:1–13. <https://doi.org/10.3389/fmed.2022.785734>
 22. Wefers Bettink MA, Harms FA, Dollee N et al (2020) Non-invasive versus ex vivo measurement of mitochondrial function in an endotoxemia model in rat: toward monitoring of mitochondrial therapy. *Mitochondrion*. <https://doi.org/10.1016/j.mito.2019.11.003>
 23. Harms FA, Voorbeijtel WJ, Bodmer SIA, Raat NJH, Mik EG (2013) Cutaneous respirometry by dynamic measurement of mitochondrial oxygen tension for monitoring mitochondrial function in vivo. *Mitochondrion* 13(5):507–514. <https://doi.org/10.1016/j.mito.2012.10.005>
 24. Ubbink R, Bettink MAW, Janse R et al (2017) A monitor for cellular oxygen METabolism (COMET): monitoring tissue oxygenation at the mitochondrial level. *J Clin Monit Comput* 31(6):1143–1150. <https://doi.org/10.1007/s10877-016-9966-x>
 25. Broch O, Renner J, Gruenewald M et al (2012) A comparison of the Nexfin[®] and transcatheter pulmonary thermodilution to estimate cardiac output during coronary artery surgery. *Anaesthesia* 67(4):377–383. <https://doi.org/10.1111/j.1365-2044.2011.07018.x>
 26. Coppel J, Bountziouka V, Martin D, Gilbert-Kawai E (2021) A comparison of the quality of image acquisition between two different sidestream dark field video-microscopes. *J Clin Monit Comput* 35(3):577–583. <https://doi.org/10.1007/s10877-020-00514-x>
 27. Harms F, Stolker RJ, Mik E (2016) Cutaneous respirometry as novel technique to monitor mitochondrial function: a feasibility study in healthy volunteers. *PLoS ONE* 11(7):1–11. <https://doi.org/10.1371/journal.pone.0159544>
 28. Ameloot K, Van De Vijver K, Broch O et al (2013) Nexfin noninvasive continuous hemodynamic monitoring: Validation against continuous pulse contour and intermittent transpulmonary thermodilution derived cardiac output in critically ill patients. *Sci World J* 2013:1. <https://doi.org/10.1155/2013/519080>
 29. Stolmeijer R, Van Ieperen E, Lameijer H, Van Beest P, Ter Maaten JC, Ter Avest E (2022) Haemodynamic effects of a 10-min treatment with a high inspired oxygen concentration in the emergency department: a prospective observational study. *BMJ Open* 12(9):1–8. <https://doi.org/10.1136/bmjopen-2021-059848>
 30. Smit B, Smulders YM, Eringa EC et al (2018) Hyperoxia does not affect oxygen delivery in healthy volunteers while causing a decrease in sublingual perfusion. *Microcirculation* 25(2):1–8. <https://doi.org/10.1111/micc.12433>
 31. Dobbe JGG, Streekstra GJ, Atasever B, van Zijderveld R, Ince C (2008) Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. *Med Biol Eng Comput* 46(7):659–670. <https://doi.org/10.1007/s11517-008-0349-4>
 32. Hilderink BN, Crane RF, Baysan M et al (2023) A simulation of skin mitochondrial PO₂ in circulatory shock. *J Appl Physiol* 134:1165–1176. <https://doi.org/10.1152/japplphysiol.00621.2022>
 33. Mik EG, Van Leeuwen TG, Raat NJ, Ince C (2004) Quantitative determination of localized tissue oxygen concentration in vivo by two-photon excitation phosphorescence lifetime measurements. *J Appl Physiol*. <https://doi.org/10.1152/japplphysiol.01399.2003>
 34. Mik EG, Johannes T, Zuurbier CJ et al (2008) In vivo mitochondrial oxygen tension measured by a delayed fluorescence lifetime technique. *Biophys J*. <https://doi.org/10.1529/biophysj.107.126094>
 35. Sharan M, Gupta S, Popel AS (1998) Parametric analysis of the relationship between end-capillary and mean tissue PO₂s predicted by a mathematical model. *J Theor Biol* 195(4):439–449. <https://doi.org/10.1006/jtbi.1998.0805>
 36. Bickler PE, Feiner JR, Lipnick MS, Batchelder P, MacLeod DB, Severinghaus JW (2017) Effects of acute, profound hypoxia on healthy humans. *Anesth Analg*. <https://doi.org/10.1213/ane.00000000000001421>
 37. Lundsgaard-Hansen P, Augustin E, Dawert I, Schülgen C (1966) Regional differences of the lactate/pyruvate response to progressive arterial hypoxemia. *Pflügers Arch Gesamte Physiol Menschen Tiere* 292(1):60–75. <https://doi.org/10.1007/BF00413125>
 38. Wolff CB, Richardson N, Kemp O et al (2008) Near infra-red spectroscopy and arterial oxygen extraction at altitude. *Adv Exp Med Biol*. https://doi.org/10.1007/978-0-387-71764-7_24
 39. Schumacker PT, Cain SM (1987) The concept of a critical oxygen delivery. *Intensive Care Med* 13(4):223–229. <https://doi.org/10.1007/BF00265110>
 40. Damiani E, Casarotta E, Orlando F et al (2021) Effects of normoxia, hyperoxia, and mild hypoxia on macro-hemodynamics and the skeletal muscle microcirculation in anesthetised rats. *Front Med* 8:1–10. <https://doi.org/10.3389/fmed.2021.672257>
 41. Bernstein D, Teitel D, Sidi D, Heymann MA, Rudolph AM (1987) Redistribution of regional blood flow and oxygen delivery in experimental cyanotic heart disease in newborn lambs. *Pediatr Res* 22(4):389–393. <https://doi.org/10.1203/00006450-198710000-00004>
 42. Smit B, Smulders YM, Eringa EC et al (2018) Effects of hyperoxia on vascular tone in animal models: systematic review and meta-analysis. *Crit Care* 22(1):1–16. <https://doi.org/10.1186/s13054-018-2123-9>
 43. Dyess DL, Christenberry DP, Peebles GL et al (1998) Organ blood flow redistribution in response to hypoxemia in neonatal piglets. *J Investig Surg* 11(6):381–392. <https://doi.org/10.3109/08941939809032215>

44. Pinsky MR, Schlichtig R (1990) Regional oxygen delivery in oxygen supply-dependent states. *Intensive Care Med* 16(2):169–171. <https://doi.org/10.1007/BF01785248>
45. Wang L, Sang L, Cui Y et al (2022) Effects of acute high-altitude exposure on working memory: a functional near-infrared spectroscopy study. *Brain Behav*. <https://doi.org/10.1002/brb3.2776>
46. Orbegozo D, Pu F, Donadello K et al (2015) Normobaric hyperoxia alters the microcirculation in healthy volunteers. *Microvasc Res* 98:23–28. <https://doi.org/10.1016/j.mvr.2014.11.006>
47. Fink S, Ray CW, McCartney S, Ehrlich H, Shoemaker WC (1984) Oxygen transport and utilization in hyperoxia and hypoxia: relation of conjunctival and transcutaneous oxygen tensions to hemodynamic and oxygen transport variables. *Crit Care Med*. <https://doi.org/10.1097/00003246-198411000-00004>
48. Hafner S, Beloncle F, Koch A, Radermacher P, Asfar P (2015) Hyperoxia in intensive care, emergency, and peri-operative medicine: Dr. Jekyll or Mr. Hyde? A 2015 update. *Ann Intensive Care* 5(1):1–14. <https://doi.org/10.1186/s13613-015-0084-6>
49. Bredle DL, Bradley WE, Chapler CK, Cain SM (1988) Muscle perfusion and oxygenation during local hyperoxia. *J Appl Physiol* 65(5):2057–2062. <https://doi.org/10.1152/jap.1988.65.5.2057>
50. Shykoff BE, Lee LR, Gallo M, Griswold CA (2021) Transcutaneous and end-tidal CO₂ measurements in hypoxia and hyperoxia. *Aerosp Med Hum Perform*. <https://doi.org/10.3357/AMHP.5856.2021>
51. Bredle DL, Petrini MF, Norman JR, Schuller D, Tuchs Schmidt JA (1993) Elevation of cardiac output and oxygen delivery improves outcome in septic shock. *Chest*. <https://doi.org/10.1378/chest.103.4.1311c>
52. Popel AS, Gross JF (1979) Analysis of oxygen diffusion from arteriolar networks. *Am J Physiol Heart Circ Physiol*. <https://doi.org/10.1152/ajpheart.1979.237.6.h681>
53. Huckabee WE (1961) Abnormal resting blood lactate. I. The significance of hyperlactatemia in hospitalized patients. *Am J Med*. [https://doi.org/10.1016/0002-9343\(61\)90171-1](https://doi.org/10.1016/0002-9343(61)90171-1)
54. Helmerhorst HJF, Schultz MJ, van der Voort PHJ et al (2014) Self-reported attitudes versus actual practice of oxygen therapy by ICU physicians and nurses. *Ann Intensive Care* 4(1):1–9. <https://doi.org/10.1186/s13613-014-0023-y>
55. Beasley R, Chien J, Douglas J et al (2015) Thoracic Society of Australia and New Zealand oxygen guidelines for acute oxygen use in adults: swimming between the flags. *Respirology* 20(8):1182–1191. <https://doi.org/10.1111/resp.12620>
56. Kallstrom TJ (2002) AARC Clinical Practice Guideline: oxygen therapy for adults in the acute care facility—2002 revision and update. *Respir Care* 47:717
57. Mugo C, Chojecki D (2016) Oxygen therapy in acute care settings. *Institute Health Economics, Alberta*, pp 1–10
58. Casaubon LK, Boulanger JM, Blacquiere D et al (2015) Canadian stroke best practice recommendations: hyperacute stroke care guidelines, update 2015. *Int J Stroke* 10(6):924–940. <https://doi.org/10.1111/ij.12551>
59. Nikolaou NI, Welsford M, Beygui F et al (2015) Part 5: Acute coronary syndromes. 2015 International consensus on cardiopulmonary resuscitation and emergency cardiovascular care science with treatment recommendations. *Resuscitation* 95:e121–e146. <https://doi.org/10.1016/j.resuscitation.2015.07.043>
60. Soar J, Nolan JP, Böttiger BW, et al. (2015) European resuscitation council guidelines for resuscitation 2015. Section 3. Adult advanced life support. *Resuscitation* 95:100–147. <https://doi.org/10.1016/j.resuscitation.2015.07.016>
61. Favaron E, Ince C, Hilty MP et al (2021) Capillary leukocytes, microaggregates, and the response to hypoxemia in the microcirculation of Coronavirus disease 2019 patients. *Crit Care Med*. <https://doi.org/10.1097/CCM.0000000000004862>
62. Bertuglia S, Colantuoni A, Coppini G, Intaglietta M (1991) Hypoxia- or hyperoxia-induced changes in arteriolar vasomotion in skeletal muscle microcirculation. *Am J Physiol Heart Circ Physiol* 260(2):29–32. <https://doi.org/10.1152/ajpheart.1991.260.2.h362>
63. Davies T, Gilbert-Kawai E, Wythe S et al (2018) Sustained vasomotor control of skin microcirculation in Sherpas versus altitude-naïve lowlanders: experimental evidence from Xtreme Everest 2. *Exp Physiol* 103(11):1494–1504. <https://doi.org/10.1113/EP087236>
64. Milstein DMJ, Helmers R, Hackmann S, Belterman CNW, van Hulst RA, de Lange J (2016) Sublingual microvascular perfusion is altered during normobaric and hyperbaric hyperoxia. *Microvasc Res* 105:93–102. <https://doi.org/10.1016/j.mvr.2016.02.001>

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