MitoFit Preprints 2022.25.v2.
https://doi.org/10.26124/mitofit:2022-0025.v2
Open Access

Conceptual Communication

Cite
Donnelly C, Schmitt S, Cecatto C, Cardoso L, Komlódi T, Place N, Kayser B, Gnaiger E (2022) The ABC of hypoxia – what is the norm? MitoFit Preprints 2022.25.v2. https://doi.org/10.26124/mitofit:2022-0025.v2

Author contributions
All authors discussed the ABC of hypoxia in a workshop and contributed to completing the manuscript.

Conflicts of interest
The authors declare they have no conflict of interest. EG is founder and CEO of Oroboros Instruments, Innsbruck, Austria.

Online v1 2022-06-28
Received v2 2022-07-15
Online v2 2022-07-15

Keywords
ambient;
anoxia;
critical O_2 pressure p_c;
functional hypoxia;
hyperoxia;
hypoxia;
limiting O_2 pressure p_l;
normoxia;
oxygen O_2;
O_2 concentration c_{O2} [µM];
partial O_2 pressure p_{O2} [kPa]

The ABC of hypoxia – what is the norm

Chris Donnelly[1,2], Sabine Schmitt[1], Cristiane Cecatto[1], Luiza Cardoso[1], Timea Komlódi[1,3], Nicolas Place[2], Bengt Kayser[2], Erich Gnaiger[1*]

[1] Oroboros Instruments, Innsbruck, Austria
[2] Institute of Sport Sciences, Univ. Lausanne, Switzerland
[3] Dept. Med. Bioch., Semmelweis Univ., Budapest, HU

* Coresponding author: erich.gnaiger@oroboros.at

Abstract

Hypoxia is a condition of oxygen levels below normoxia and opposite to hyperoxia. We here define the normoxic reference state by three complementary precepts: (A) ambient normoxia at sea level in the contemporary atmosphere and corresponding dissolved O_2 concentration at air saturation of aqueous environments; (B) biological compartmental O_2 levels at ambient normoxia under physiological activity of healthy organisms in the absence of environmental stress (e.g. in a diving human, a stranded whale, a thermally stressed animal); and (C) O_2 levels above the control region, i.e., where the capacity for O_2 consumption is not compromised by partial O_2 pressure as evaluated by its kinetics. Conversely, the abc of hypoxia is concerned with deviations from these reference points caused by different mechanisms: (a) ambient alterations of oxygen levels; (b) biological O_2 demand exceeding O_2 supply under pathological or experimental limitations of convective O_2 transport or O_2 diffusion; and (c) critical oxygen pressure in oxygen kinetics shifted by pathological and toxicological effects or environmental stress. The ABC of hypoxia may be of help in the design and interpretation of *in vitro* and *in vivo* experimental studies.

Definitions always leak at the margins, where experts delight in posing counterexamples for their peers to ponder. Fortunately, the typical cases are clear enough that a little fuzziness around the edges does not interfere with the larger picture (Miller 1991).

1. Normoxia: ambient, biological compartments, and respiratory control

The terminology on 'oxia' — from normoxia to hypoxia and anoxia in contrast to hyperoxia — has a long history (Richalet 2021). Yet ambiguities persist. This is not surprising when anthropocentric and clinical perspectives on hypoxia (Burtscher et al 2022) clash with an evolutionary view of life in environments of different oxygen regimes (Mills et al 2022). The vagueness of the term hypoxia increases when penetrating further into mitochondrial physiology under conditions prevailing in the intracellular microenvironment in tissues under ambient normoxia: Microenvironmental oxygenation is in stark contrast to the ambient oxygen level in our macroscopic environment, which we often apply uncritically in studies with isolated mitochondria or cultured cells, when ambient normoxia implies effectively hyperoxic experimental conditions (Gnaiger et al 2000; Wenger et al 2015; Keeley, Mann 2019).

Attempting to achieve clarity and generality, our proposal for an ABC of hypoxia considers (*1*) hyperoxic, normoxic, hypoxic, and anoxic conditions in the atmosphere and the hydrosphere down to the intracellular microenvironment, (*2*) adaptations to oxygen availability in geological time and biological evolution (Lane 2002), and (*3*) adaption of physiological responses to hypoxia from comparative to exercise physiology in health and disease (Hochachka et al 1993). We pursue a strategy of harmonization instead of standardization of terminology in an attempt to bridge the gap between apparently incompatible points of view.

Wherever oxygen gradients exist, **ambient normoxia** is distinguished from **normoxia in biological compartments** partitioned into organs, tissues, cells, and intracellular microenvironments along the respiratory cascade (Weibel 1984; 2000). Normoxia is not a norm but a reference condition for **respiratory control**, particularly for aerobic (Gnaiger et al 2000) and anaerobic energy metabolism (Gnaiger 1993), the control of redox state (Harrison et al 2015), and for oxygen sensing and hypoxic signaling in different organisms and tissues (Semenza, Wang 1992; Maxwell et al 1999; Clanton et al 2013; Ratcliffe 2022). Long-term evolutionary adaptation and short-term physiological, biochemical, and molecular acclimation and acclimatization re-set the *functional* normoxic reference points (Hochachka, Somero 2002).

For delimiting normoxia, we distinguish oxygen conditions in the (**A**) **a**mbient environment and (**B**) **b**iological compartments, from (**C**) **c**ontrol of mitochondrial oxygen consumption and signaling affected by oxygen availability relative to ambient normoxia. This leads to three connected definitions of normoxia, which together provide a reference for deriving three corresponding causes for deviations from normoxic conditions and normoxic function.

Conditions defined as normoxic from one perspective (e.g., **A** ambient) are not necessarily classified the same from other perspectives: (**B**) O_2 levels are low under normoxia in several biological compartments; or (**C**) control of mitochondrial oxygen consumption by intracellular p_{O2} becomes effective below 1 kPa or 1 % O_2 (5 % air saturation) with half maximal O_2 flux at a p_{50} of 0.02 kPa in isolated mitochondria respiring at OXPHOS capacity (Harrison et al 2015). Hence functional hypoxia is restricted to very low intracellular p_{O2} described as the 'oxygen control region' (see Figure 5 in Chance 1965). As a consequence, (**A**) relative to ambient normoxia, moderately hypoxic cell culture conditions (Gstraunthaler et al 1999; Al-Ani et al 2018; Klein et al 2021;

DiProspero et al 2021) may actually be hyperoxic compared to (**B**) tissue conditions *in vivo*, but are normoxic (**C**) if the p_{O2} is above the control region and thus above the critical p_{O2}, p_c, of mitochondrial respiration. Respiration drops sharply below the p_c. If we consider as normoxic any intracellular p_c (**B**) that is obtained at any physiological activity level of a healthy organism at ambient normoxia (**A**), then this definition of normoxia contrasts with the notion of physiologically induced tissue hypoxia, if the low intracellular p_{O2} drops below the p_c of mitochondrial respiration (**C**). The **ABC** of hypoxia links the three letters to the meaning of three complementary perspectives on normoxia across scientific disciplines. Several articles under the umbrella of *ABC of oxygen* (Bateman 1998; Leach 1998; Peacock 1998; Williams 1998; Wilmshurst 1998) use the ABC symbolically and provide overviews on specific areas related to normoxia, hypoxia, and hyperoxia.

2. Systematic definitions of normoxia as a reference for hypoxia

2.1. Categories of normoxia

A. Ambient normoxia

Comparable to referring to sea level for expressions of altitude, ambient normoxia may be defined as a p_{O2} of ~20 kPa (150 mmHg) prevailing at sea level in the environment. Modern sea level is the Earth's dominant elevation (Rowley 2013). We use the SI definition of standard pressure of 100 kPa as the reference barometric pressure and water vapour saturated air (20-30 °C) as the reference for the normoxic p_{O2} (Figure 1).

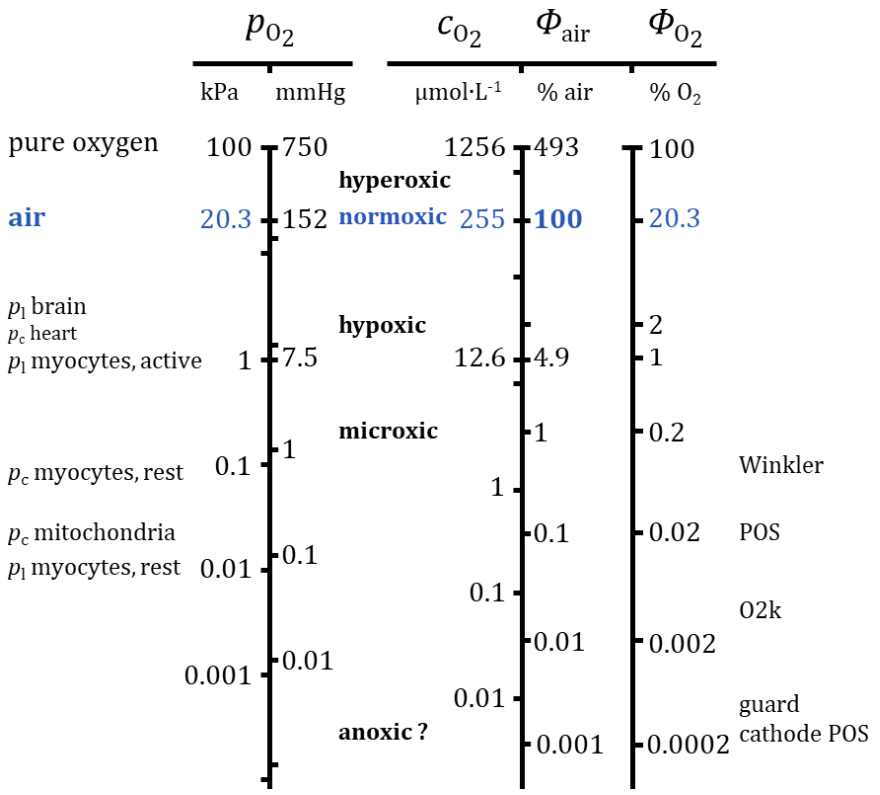

Figure 1. Deviations from ambient normoxia: logarithmic scale of partial pressure of oxygen p_{O2} (1 kPa = 0.133322 mmHg). The corresponding dissolved O_2 concentrations c_{O2} [µM] are given for pure water at 25 °C with O_2 solubility of 12.56 µM/kPa. The volume fractions of air Φ_{air} [% air] and volume fractions of O_2 Φ_{O2} [% O_2] are for the standard barometric pressure of 100 kPa and corrected for a 3.2 kPa water vapor saturation pressure at 25 °C. Left: some critical and limiting p_{O2}, p_c and p_l, for mammalian tissues and mitochondria. Right: limit of detection of selected methods (POS: polarographic oxygen sensor; O2k: Oroboros Oxygraph-2k). Modified after Gnaiger (1991).

B. Biological compartments of the respiratory cascade – compartmental normoxia

We propose to define biological compartmental normoxia as the p_{O2} in any compartment of a living organism (alveolar, arterial, capillary, interstitial, intracellular, venous, mixed-venous) observed under ambient normoxia. Biological compartmental p_{O2} is a function of aerobic metabolic activity and O_2 transport from the environment to the various compartments of an organism (Weibel 2000; Keeley, Mann 2019; Ortiz-Prado et al 2019; Poole et al 2020). Therefore, biological compartmental normoxia fluctuates within a range that is specific for the tissue. Compartmental p_{O2} may be regulated to remain within normoxic values up to critical degrees of ambient hypoxia (Grocott et al 2009). In some biological compartments p_{O2} is far lower compared to ambient oxygen levels (**A**) but may be effectively normoxic in terms of respiratory control (defined in **C**).

In isolated or cultured living cells and mitochondrial preparations – including isolated mitochondria, tissue homogenates, and permeabilized cells and tissues – ambient normoxia at air saturation of the incubation media (**A**) must be distinguished from biologically relevant normoxia as defined by the oxygen pressure prevailing in the cellular and mitochondrial microenvironment in the tissue of the intact organism (**B**).

At ambient normoxia, intracellular p_{O2} is a function of oxygen demand and oxygen supply, such that biological compartmental normoxia varies from aerobic resting or routine steady-state activity up to maximum aerobic activity $\dot{V}_{O2max}$ sustained for only a few minutes. Routine respiration (Chabot et al 2016; Nelson 2016) is higher than standard or basal respiration due to the oxygen consumption required to sustain various routine activities, not restricted to locomotory activity but including the effects of food intake. In cell cultures or isolated mitochondria, the concept of 'environmental normoxia' becomes ambiguous without a clear distinction between ambient normoxia from the perspective of the whole organism and experimental normoxia that mimics the corresponding extracellular or intracellular microenvironment *in vivo* (Figure 2). ROUTINE respiration of living cells is physiologically controlled by aerobic energy demand ranging from the minimum of LEAK respiration to the maximum of OXPHOS capacity (Gnaiger et al 2020). O_2 concentration in biological compartments of the living organism varies even under normoxia as a function of changing metabolic O_2 demand and supply. Correspondingly, normoxic oxygen conditions provided experimentally for cultured cells studied in ROUTINE, LEAK, and OXPHOS states need to be adjusted to the *in vivo* activity-dependent compartmental p_{O2} in the tissues of origin. The paradigm of ambient normoxia defines normoxic performance as the biological response that does not deviate from the physiological function measured under (**A**) ambient normoxia for the whole organism and (**B**) normoxia in biological compartments of the whole organism or mimetic biological normoxia in experiments with isolated cells and mitochondrial preparations.

C. Control of respiration – normoxia evaluated by function: functional normoxia

Control of respiration by O_2 pressure p_{O2} or O_2 concentration c_{O2} introduces a kinetic perspective with reference to kinetically saturating O_2 levels. Normoxic respiration can thus be defined as respiration at kinetic oxygen saturation, and hypoxic respiration is respiration below a critical oxygen pressure p_c, when the p_{O2} exerts control and respiration shows oxyconformance in the oxygen control region (Figure 2). 'Intracellular hypoxia is defined as (**B**) local oxygen pressure below normoxic reference states, or (**C**)

limitation of mitochondrial respiration by oxygen levels below kinetic saturation, resulting in oxyconformance. .. The high affinity of cytochrome *c* oxidase for oxygen implies independence of mitochondrial respiration of oxygen over a wide range of oxygen levels, which gives rise to the paradigm of "oxygen regulation", although "kinetic oxygen saturation" describes more accurately the underlying mechanism' (Gnaiger 2003).

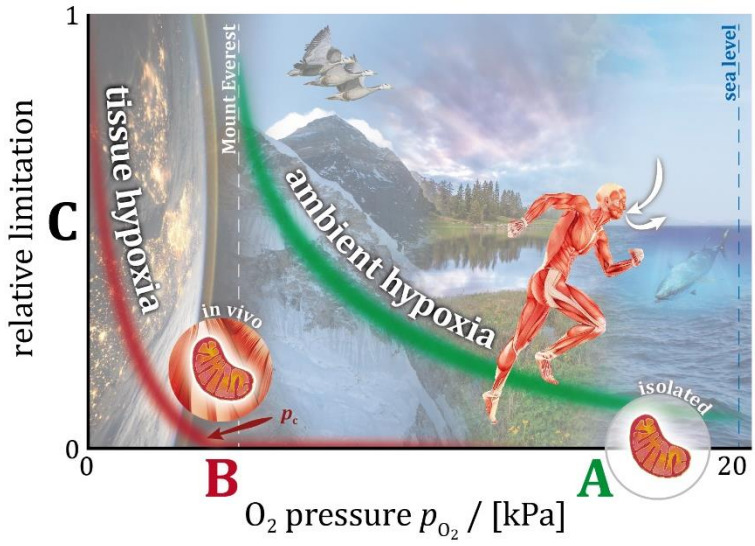

Figure 2. The ABC of hypoxia: Ambient p_{O2} (**A**, green line) and intracellular p_{O2} in tissues *in vivo* (**B**, red line) exert different limitations on critical physiological functions (**C**). At any ambient p_{O2}, intracellular p_{O2} varies between tissues and as a function of aerobic metabolic activity. Compared with in vivo conditions (**B**) isolated mitochondria are hyperoxic at ambient normoxia (**A**). Graphics by Paolo Cocco.

Whereas normoxic respiration of isolated mitochondria can be measured as a constant rate in a wide range of O_2 concentrations (oxyregulators), H_2O_2 production is a continuous function of O_2 concentration (oxyconformance; Komlódi et al 2021). Unlike nearly constant mitochondrial respiration above the p_c, a corresponding p_c cannot be defined for H_2O_2 production (see Figure 7a in Komlódi et al 2021). It is for this reason that we use oxygen control of respiration to define functional normoxia.

2.2. Causes of deviations from normoxia

Based on definitions of the categories (**A ambient**) environmental normoxia, (**B biological**) compartmental normoxia, and (**C control**) functional normoxia, the causes for deviations from normoxia are distinguished by three categories: (**a**) ambient hypoxia and hyperoxia, (**b**) biological compartment hypoxia and hyperoxia, and (**c**) critical function-induced hypoxia and hyperoxia (Table 1).

Maximum aerobic activity may induce compartmental hypoxia, gauged from a comparison of intracellular p_{O2} — which declines in skeletal muscle at $\dot{V}_{O2max}$ relative to routine activity (Richardson et al 2006) — and oxygen kinetics of isolated mitochondria (Gnaiger 2001; Harrison et al 2015). Since OXPHOS capacity (Gnaiger et al 2020) of isolated mitochondria is already slightly limited at intracellular tissue p_{O2} observed at $\dot{V}_{O2max}$, a high workload can entail physiological hypoxia (Richardson et al 1999; Richardson 2000). At differing ambient p_{O2} the fractional limitations of $\dot{V}_{O2max}$ may be redistributed amongst compartments of the respiratory cascade (di Prampero, Ferretti 1995). $\dot{V}_{O2max}$ cannot be maintained over prolonged periods of time, such that upon functionally induced hypoxia the organism returns to a normoxic steady state.

Categories **A** and **a** appear similar, distinguished only as (**A**) a description of a state in terms of a given p_{O2} (static), in contrast to (**a**) including the causes for deviations of the p_{O2} from normoxia (dynamic). Therefore, **A** and **a** are tightly linked (Table 1).

Table 1. Categories (static) and causes of deviations (dynamic) from normoxia

Oxia category	Deviation from normoxia	Examples for deviations from normoxia
A ambient	**A** ambient hypoxia or hyperoxia	• hypobaric conditions: high altitude or low-pressure chamber • hyperbaric conditions: high-pressure chamber, diving • normobaric conditions: O_2 deprivation in the environment (environmental normobaric hypoxia), O_2 supplementation (environmental normobaric hyperoxia)
B biological compartment	**B** biological compartment hypoxia or hyperoxia	• ambient-induced hypoxia or hyperoxia on the compartmental level (living organism) • pathologically and toxicologically induced hypoxia or hyperoxia on the compartmental level (living organism) • experimental for isolated organs, tissues, cells, and organelles: deviations of incubation p_{O2} of experimental preparations from (**B**) compartmental normoxia in the intact organism
C control of respiration	**C** critical function-induced hypoxia or hyperoxia	• environmental: respiratory O_2 depletion or photosynthetic O_2 accumulation in eutrophic aqueous environments (Gnaiger 1983) • physiologically induced on the compartmental level. Hypoxia: tissue-work related; living organism at high workload of a tissue; (mal)adaptive responses of the respiratory cascade to (de)training and lifestyle. Hyperoxia: endosymbiotic algae at high light intensities (e.g. corals) • pathological-pharmacological-toxicological O_2-transport related hypoxia (ischemia and stroke, anaemia, chronic heart disease, chronic obstructive pulmonary disease, disordered regional distribution of blood flow, obstructive sleep apnea, CO poisoning), inhibition or acceleration of O_2-linked pathways (cyanide, rotenone, NO, ..; doping, ..) • genetic: inhibition or acceleration of O_2-linked pathways (mutations, inherited diseases, knockout, knock-in)

2.3. Extents of hypoxia: approaching anoxia

There is a continuous transition of hypoxia to anoxia, which is best represented on a logarithmic scale of p_{O2} (Figure 1). Respiration declines below the critical p_{O2}, p_c, and anaerobic metabolism is stimulated below the limiting p_{O2}, p_l (Gnaiger 1991). If the transition to anoxia is of interest, then further differentiation of deep hypoxia (microxia) and anoxia is made, taking into account the limit of detection of methods applied for determining p_{O2} and different methods to detect functional responses to the presence (deep hypoxia) or absence (anoxia) of trace amounts of oxygen (Gnaiger 1993; Harrison et al 2015). Oxic versus anoxic conditions (in the presence or absence of molecular oxygen) must be distinguished from aerobic and anaerobic metabolism. Aerobic metabolism requires oxic conditions, whereas anaerobic metabolism may proceed under oxic conditions (aerobic glycolysis; glucose → lactate) or under anoxia (anaerobic glycolysis; Poole et al 2021; Brooks et al 2022).

2.4. Extents of hyperoxia: experimental conditions for studies of cultured cells and isolated mitochondria

There is wide concern that the routine application of hyperoxic conditions for the study of biology is hindering the translation of *ex vivo* experimental findings to *in vivo* contexts (Keeley, Mann 2019). Striking examples are the use of ambient normoxia in cell cultures (Keeley, Mann 2019) or in studies of isolated mitochondria (Gnaiger et al 1998). Whereas effectively hyperoxic conditions may have negligible short-term consequences on mitochondrial oxygen consumption, the immediate effects of oxygen levels on mitochondrial efficiency (Gnaiger et al 2000), redox states (Harrison et al 2015) and ROS production (Komlódi et al 2021) underline the importance of choosing appropriate experimental oxygen conditions depending on the research question addressed. Further evidence of oxygen control of molecular signaling (Jiang et al 1996) and numerous cellular

processes (Keeley et al 2018) suggest that *ex vivo* experimental conditions for tissues, cells and mitochondria ought to be in the p_{O2} ranges of biological compartments (**B**).

2.5. Partial pressure and concentration of oxygen

The concept of biological compartmental normoxia — and consideration of terrestrial and aqueous organisms — raises the issue of the preference of expressing O_2 'levels' in terms of amount, *i.e.* concentration c_{O2} in units [mol·dm^{-3} $\stackrel{\text{def}}{=}$ M] or partial pressure p_{O2} in SI units [J·m^{-3} $\stackrel{\text{def}}{=}$ Pa]. At ambient normoxia, the concentration of O_2 in dry air at 25 °C equals $\Phi_{O2}\cdot(RT)^{-1}$ = 0.20946·(100-3.17) kPa·(2.479 kJ·mol^{-1})$^{-1}$ = 8.18 mM. In contrast, the oxygen concentration in air-saturated pure water is 0.255 mM or 255 µM (Figure 1). The oxygen concentration in air thus is 32-fold higher than in air-saturated water (https://wiki.bioblast.at/index.php/Oxygen_solubility) while the oxygen partial pressure is identical at 20.3 kPa in both media. At room temperature or 37 °C, the concentration of oxygen is 30- to 40-fold higher in the gas phase compared to the aqueous phase in equilibrium with the gas phase. The oxygen solubility S_{O2} in serum is 9.40 µM/kPa or 0.89 relative to pure water at 37 °C (Baumgärtl, Lübbers 1983). Taken together, these are the physicochemical reasons why tracheal oxygen supply through the gas phase is very effective in supporting high oxygen demand of flying insects, and why we need red blood cells with hemoglobin to boost the total amount of oxygen carried per volume of blood (Weibel 1984).

Continuous oxygen concentration gradients dc_{O2}/dz, considering diffusion in a single direction with distance z, are distinguished from discontinuous differences of oxygen concentration Δc_{O2} between compartments. Multiplied by RT, $\Delta c_{O2}\cdot RT$ equals a pressure difference $\Delta\Pi_{O2}$ (Gnaiger 2020). Discontinuous differences Δc_{O2} are caused by diffusion limitation across compartmental barriers, and are additionally a function of differing oxygen solubilities between compartments, such as the gaseous-aqueous compartments in the lung and the aqueous-membraneous compartments in the cell (Weibel 1984).

For convective O_2 transport the total O_2 concentration in the medium that is moved from the source to the sink matters. A larger amount of molecular O_2 is transported per volume of gas compared to a volume of aqueous solution. Given the low S_{O2} in serum, high affinity O_2 carriers such as hemoglobin greatly enhance the convectional efficacy by increasing the total amount of O_2 transported by a volume of blood. Just having a carrier is not sufficient. The regulation of loading of the carrier with O_2 at the source and unloading at the sink are essential. The interaction between the sigmoidal shape of the hemoglobin O_2 dissociation curve and the Bohr effect is the obvious mammalian example. Molecular O_2 diffusion along a gradient of O_2 pressure is driven by dp_{O2}/dz, where Fick's law of diffusion represents a special case of the linear flux-pressure relationships which can be extended to discontinuous descriptions based on pressure differences Δp_{O2} (Gnaiger 2020). In diffusion, O_2 is transferred across the medium, which may be facilitated by O_2 carriers such as myoglobin, again dependent on the loading/unloading kinetics. The O_2 solubility is a decisive component of O_2 transfer by diffusion (Hitchman, Gnaiger 1983), implicit in the diffusion coefficient or mobility (Gnaiger 2020).

3. Conclusions

'*Full standardisation of definitions and analytical procedures could be feasible for new research efforts. .. For existing datasets and studies, harmonisation attempts to achieve some, but not necessarily perfect, homogeneity of definitions might need substantial effort and coordination* (Ioannidis et al 2014). The concept of harmonization instead of standardization of terminology pursues a strategy that may be commonly acceptable across apparently incompatible points of view: Instead of proposing a guideline on terminology, the **ABC** of hypoxia and corresponding norms is intended to (*1*) bridge the gap between different points of view (*static*: **A** versus **B** versus **C**) and (*2*) clarify the causes and processes of altered oxygen availability and supply (*dynamic*: **a** versus **b** versus **c**), and (*3*) provide a simple framework for the labelling and communication of O_2 concentrations and pressures at various levels and across disciplines. Each investigator may consider if the important field of oxygen-regulated biological function will gain (*4*) from a consensus on general definitions provided by the **ABC** of hypoxia. Clarification of concepts aims at resolving current controversies to facilitate future research.

Acknowledgements

We thank Martin Burtscher for making us aware of the *ABC of oxygen*, Adam Chicco for critical comments on absolute versus evolutionary definitions of normoxia, and Malcolm J Shick and Adalberto L Val for discussions. Chris Donnelly was supported by the Swiss National Science Foundation under grant agreement nº 194964.

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Supplement

Harmonization with terms in the literature

S1. Definitions of hypoxia

Several definitions of hypoxia are restricted to a single category or specific combination of categories and lack, therefore, generality.

C.c: Hypoxia is defined as functional hypoxia by the European Environmental Agency as "a state of low oxygen concentration in water and sediments, relative to the needs of most aerobic species" (https://www.eea.europa.eu/help/glossary/chm-biodiversity/hypoxia; retrieved 2022-05-21).

A or **B.c**: Hypoxia - 'a condition in which there is not enough oxygen available to the blood and body tissues'
(https://dictionary.cambridge.org/dictionary/english/hypoxia , retrieved 2022-05-21).

A or **B.c**: Hypoxia – 'deficiency in the amount of oxygen delivered to the body tissues' (https://www.collinsdictionary.com/dictionary/english/hypoxia, retrieved 2022-05-21)

A or **B.c**: Hypoxia – 'a deficiency of oxygen reaching the tissues of the body' (https://www.merriam-webster.com/dictionary/hypoxia, retrieved 2022-05-21)

S2. Various 'oxia' terms

Various 'oxia' terms are used in the literature to point out a particular ABC category. Our work aims at simplifying the nomenclature without loss of conceptual detail. For harmonization, the following 'oxia' terms are linked to the **ABC** categories. The history of the terms related to hypoxia has been reviewed from a clinical and high-altitude medicine perspective by Richalet (2021). He refers to Opitz (1941): '*In 1941, Opitz says, in the introduction of his review paper "Über akute Hypoxie" (About acute hypoxia): "Die Bezeichnung 'Hypoxie' soll immer dann verwendet werden, wenn die Sauerstoffversorgung der Gewebe gegenüber der Norm erschwert ist." (The term "hypoxia" should always be used when the oxygen supply to the tissues is more difficult than the norm).*' This definition even includes 'silent hypoxia' ascribed to SARS-CoV-2 infected patients without symptoms of dyspnoea yet low O_2 saturation in the blood (Rahman et al 2021).

The early book 'Anoxia' (van Liere 1942) was published later under the title 'Hypoxia' (van Liere EJ, Stickney JC 1963) with an identical table of contents and largely identical text, mainly replacing the term anoxia by hypoxia.

B: "Physoxia: physiological oxygen level in peripheral tissues with an average of approximately 6 % (ranging from approximately 7.5 % to 4 % depending on the tissue; lower limit approximately 1 %). For experimental studies, 5 % is the proposed compromise since this is often used" (McKeown 2014). — The term 'physoxia' or 'physioxia' (Carreau et al 2011) suggests physiological control in contrast to responses to ambient hypoxia. Without further specification, physoxia may be interpreted as (**B.a**) compartmental oxygen levels under any environmental conditions, (**B.c**) for any level of physiological activity, and (**Ba.c**) their combination (e.g. muscle p_{O2} at $\dot{V}_{O2max}$ at high altitude). In addition, physoxia does not separate the categories **B** and **C** of normoxia, and it may include any pathological cause of deviation from normoxia.

C.b: "Pathological hypoxia: shows persistence of poor oxygenation suggesting disruption to normal homeostasis. Below this level pathological hypoxia applies" (McKeown 2014). Besides regulation of hypoxia response genes, the critical physiological function should be specified. — High altitude exposure may result in prolonged poor oxygenation of tissues. But is this pathological hypoxia?

A,B: Under the term 'anoxic anoxia' 48 results were retrieved in a PubMed search, one from 2000 and all others from the 1990's and older (retrieved 2022-05-20). Anoxic anoxia, 'true anoxia' (Krumschnabel et al 1997; Ossum et al 2006), or acute anoxic anoxia refer to the use of N_2 to decrease the O_2 concentration. Ludvigsen and Folkow (2009) refer to true and chemical anoxia for the combination of cyanide & N_2. Physical hypoxia is used in the context of cell culture, when O_2 concentrations were kept low (Zhao et al 2019; Wu et al 2020). Based on the electrolysis of H_2O, gaseous H_2 can be used instead of N_2 to lower experimental O_2 concentrations (Schmitt et al 2022).

S3. Chemical anoxia and hypoxia

The concept of 'chemical anoxia' is based on inhibitors of the electron transfer system without concern of O_2 concentrations. As such, chemical anoxia fits into category **c** (inhibition of O_2-linked pathways; Table 1). A PubMed search for the term 'chemical

anoxia' retrieved 73 results (2022-05-20). Several inhibitors of the electron transfer system are used — such as as cyanide, azide, rotenone, antimycin A, deoxyglucose, iodoacetate, 3-nitropropionic acid, alone or in combination — preventing mitochondrial electron transfer to O_2 and hence inhibiting respiration. This is the state of residual oxygen consumption ROX in mitochondrial physiology (Gnaiger et al 2020).

452 results were retrieved for 'chemical hypoxia' (PubMed, 2022-05-20), where the majority uses cobalt as a hypoxia mimetic. Cobalt stabilizes hypoxia-inducible factors 1α and 2α under normoxic conditions (Muñoz-Sánchez, Chánez-Cárdenas 2019). Yet some publications on chemical hypoxia use the same inhibitors mentioned above for chemical anoxia and additionally deferoxamine, dimethyloxaloylglycine, 2,4-dinitrophenol, and isoflurane (Nowak-Stępniowska et al 2022). In one case 'chemical hypoxia' and 'chemical ischemia' are used synonymously (Iwai et al 2018).

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