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The oxygen cascade in hemodialysis patients and native high altitude dwellers – What can we learn from extreme physiology to benefit patients with end-stage renal disease (ESRD)

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Abstract

Haemodialysis patients repeatedly undergo intradialytic low arterial oxygen saturation (S_aO_2), reflecting hypoxemia, as well as low central venous oxygen saturation ($S_{cv}O_2$), reflecting an imbalance between upper body systemic oxygen supply and demand, are associated with increased mortality. Abnormalities along the entire oxygen cascade, with impaired diffusive and convective oxygen transport, may contribute to the reduced tissue oxygen supply. Dialysis treatment impairs pulmonary gas exchange and reduces ventilatory drive, whereas ultrafiltration can reduce tissue perfusion due to a decline in cardiac output. In addition to these factors, capillary rarefaction and reduced mitochondrial efficacy can further affect the balance between cellular oxygen supply and demand. Whereas it has been convincingly demonstrated that a reduced perfusion of heart and brain during dialysis contributes to organ damage, the significance of systemic hypoxia remains uncertain, although it may conceivably contribute to oxidative stress, systemic inflammation and accelerated ageing. These abnormalities along the oxygen cascade of dialysis patients appear to be diametrically opposite to the situation in Tibetan highlanders and Sherpas, whose physiology adapted to the inescapable hypobaric hypoxia of their living environment over many generations. Their adaptation includes pulmonary, vascular and metabolic alterations with enhanced capillary density, nitric oxide production and mitochondrial efficacy without oxidative stress. Why the repetitive intradialytic hypoxia experienced by dialysis patients does not afford protection via ischemic/hypoxic preconditioning remains unclear. Improving tissue oxygen supply in dialysis patients depends primarily on preventing hemodynamic instability by increasing dialysis time/frequency or prescribing cool dialysis. Whether dietary or pharmacological interventions, such as the administration of L-arginine, nitrate, Nrf2 agonists or prolyl hydroxylase 2 inhibitors may improve clinical outcome in dialysis patients warrants future research.

Introduction

Hemodialysis (HD) patients repeatedly undergo an intensive treatment that has profound effects on their internal physiological environment (1, 2) (3). Recent observations have suggested that the repeated intradialytic decline in central venous oxygen saturation ($S_{cv}O_2$) is related to mortality (4). $S_{cv}O_2$ is a marker of the balance between (upper body) systemic oxygen delivery (DO_2) and systemic oxygen demand (VO_2); thus, severely lowered $S_{cv}O_2$ levels may reflect global tissue hypoxia (5). In addition, prolonged intradialytic hypoxemia (PIH), defined as arterial oxygen saturation (S_aO_2) <90% for at least one third of the treatment time, is related to increased mortality (6). Thus, circumstantial evidence suggest that intradialytic hypoxemia, as well as tissue hypoxia, is related to adverse outcomes in dialysis patients.

On the other end of the spectrum, Sherpa, who are descendants of a Tibetan population and live in the Khumbu valley in Nepal, have resided at high altitude for >500 generations. Sherpa are known for their remarkable capacity for physical performance at high altitudes (7) (8). The largest population center in the Khumbu is Namche Bazaar (at 3440 m), whereas the highest seasonally populated settlement in the region is Gorak Shep at 5164m, just below the altitude of Everest Base Camp (EBC) at 5364 m. Atmospheric O_2 levels are approximately 67% at Namche Bazaar and 54% at EBC compared to sea level values; whereas in well acclimatized healthy trekkers mean S_aO_2 levels of 87% are typically observed in Namche and 73% at EBC (9), such levels are generally associated with severe pathology at sea level.

Sherpa and HD patients appear to be at the opposite ends of two extremes in human physiology with regards to adaptation to hypoxia. As argued for an intensive care setting, comparative physiology with respect to adaptation to hypobaric hypoxia at altitude may be of clinical relevance for patients experiencing hypoxemia at sea level (10). In this respect, it is also of interest that an inverse relationship between altitude and mortality has been observed in dialysis patients (11), which seems paradoxical in view of the deleterious effects of repetitive hypoxemia and consecutive tissue hypoxia in these patients (12).

The aim of this review is to look at the different mechanisms in the oxygen cascade of HD patients and compare those to findings obtained from altitude physiology.

Physiology of oxygen transport

The passage of oxygen from the atmosphere to the mitochondria is known as the oxygen cascade (13), which can conceptually be divided into five parts. The first part is characterized by *convective* mass transport through the bronchial tree into the alveoli. The second is the *diffusive* exchange of oxygen between air and blood at the interface of alveolar epithelial cells with the endothelial layer of the pulmonary capillaries, and

diffusion of oxygen via the blood plasma and through the cell membranes of red blood cells (RBC) to the oxygen binding hemoglobin molecules. The third step is the *convective* mass transport of oxygen from the lungs to the tissues. This is in larger blood vessels a function of the arterial oxygen content (mainly determined by hemoglobin level and S_aO_2) times the blood flow, according to the formula: $DO_2 = \text{cardiac output} \times (1.39 \times [Hb] \times S_aO_2 + 0.003 \times PaO_2)$. In the microcirculation, convective oxygen transport is based on red blood cell flow and oxygen content of the erythrocyte (14-16). In the fourth phase, *diffusive* exchange of oxygen takes place through the capillaries and tissue cells, with the functional capillary density as major determinant (16). The fifth and last part concerns the use of oxygen in cellular metabolism and its consumption in the mitochondria.

Tissue hypoxia can be due to *hypoxemia* (low arterial oxygen levels due to impaired gas exchange or reduced ambient oxygen levels) - e.g. to abnormalities in the 1st and 2nd part of the oxygen cascade, *impaired oxygen transport to the tissues* (corresponding to the 3rd and 4th part), and *impaired tissue oxygen extraction/utilization* (the 5th part). Whereas at high altitude, the potential for tissue hypoxia is principally due to the decline in atmospheric oxygen content due to the lower partial pressure of oxygen (i.e. the same volume of air containing less O_2 ; hypobaric hypoxia), the pathophysiology of tissue hypoxia in dialysis patients is far more complex (17). In the following paragraphs, the factors specific to dialysis patients are discussed, and will be juxtaposed to relevant findings derived from altitude medicine and those obtained in Sherpa and Tibetan highlanders (*Figure 1*).

Hypoxemia

The dialysis treatment itself plays a causal role in the pathogenesis of hypoxemia. Interest in dialysis-associated hypoxemia (DAH) has reemerged given the recently documented association with mortality (6). Meyring-Wosten et al (18) found that PIH, using the above definition, is present in 10% of HD patients. In the 1980s and 1990s, DAH - which occurred primarily within the first 30-60 minutes after the start of HD - was believed to be due to complement activation and leukocytopenia resulting from leukostasis in the pulmonary capillaries that in turn would lead to ventilation/perfusion abnormalities. However, later studies have suggested that a decline in arterial oxygen levels was predominantly due to a reduction in ventilatory drive. DAH was especially prevalent with the use of acetate dialysis, which was explained by the rapid efflux of CO_2 (19). With bicarbonate dialysis, influx of bicarbonate results in a rapid increase in pH and the resulting inhibitory effect on respiratory drive was supported by the fact that DAH was less prevalent during treatments with lower (29 mmol/l) vs. higher (36 mmol/l) dialysate bicarbonate levels (20). Whether the low amount of acetate currently present in dialysate has any effect on ventilator drive has not yet been investigated.

However, patient-related factors are also likely to play a major role in the pathogenesis of DAH. Based on anecdotal observations it has been shown that DAH also occurs during sleep (15), which is consistent with the high prevalence of sleep apnoea in HD patients and its association with adverse outcomes (21). Additionally, fluid overload could play a role in the relationship between DAH and outcome, given the inverse relationship between inter-dialytic hypertension and intra-dialytic S_aO_2 levels (18). This is in line with the finding in HD patients that both restrictive and obstructive lung function abnormalities can be observed (22), which appear to be related to the degree of fluid overload and can also be improved by ultrafiltration (23). Therefore, DAH may be either due to patient or treatment-related factors, resulting in either type I respiratory failure (e.g. due to fluid overload or immune system activation) or type II respiratory failure (sleep apnoea or reduced ventilatory drive due to rapid acid-base changes). The fact that the alveolar-arterial O_2 gradient ($\Delta A-a$) increased, especially during the first hour of HD, even with bicarbonate dialysis using polysulfone membranes, suggests that even in modern times a dialysis-related effect on pulmonary gas exchange or ventilation/perfusion mismatch cannot be excluded (24).

At altitude, a reduction in ambient oxygen content per volume of inspired air leads to an increase in respiratory drive, both in lowlanders and Sherpa, and is termed the hypoxic ventilatory response. However, in lowlanders the respiratory drive may be reduced due to the inhibition of central respiratory centers by the resultant decline in CO_2 levels, which may lead to periodic breathing and contribute to acute mountain sickness. One of the mechanisms by which acetazolamide is effective against mountain sickness is by inducing a mild metabolic acidosis (25). Interestingly, in Sherpa the inhibition of the respiratory response to CO_2 appears to be diminished or absent (7). In addition, the diffusive capacity of the lungs is higher in Sherpa, leading to an increase in the $\Delta A-a$ gradient and contributing to an optimal respiratory response under circumstances of low ambient oxygen levels (7).

Oxygen transport to the tissues

Haemoglobin and oxygen affinity

Hypoxic stress leads to an increased transcription of hypoxia inducible factors (HIFs), as well as to a reduced breakdown by prolyl hydroxylase domain-containing enzymes (PHDs) (26). HIFs are evolutionarily highly conserved factors that are involved in oxygen

homeostasis in organisms ranging from invertebrates to humans (27), with HIF1 α and HIF2 α mediating the short and sustained response to hypoxia, respectively (28). Among the ubiquitous effects of HIF is an increase in erythropoietin production, which increase arterial oxygen content and convective transport. Whereas this response is inherently limited in dialysis patients, an increase in haemoglobin has been observed in the blood of lowlanders sojourning at altitude and indigenous populations who reside at high altitude, such as Andeans. This response, especially when resulting in polyglobulia, is associated with severe pulmonary hypertension and heart failure in Andeans (29). Interestingly, in Sherpa and Tibetans the hypobaric hypoxia of their living environment does not elicit an increase in the erythropoietic response.(30) This is likely due to a polymorphism in the EPAS1 gene, which results in a down-regulation of HIF2 α , or the *EGLN1* encoding prolyl hydroxylase 2 (PHD2) polymorphism, a negative regulator of HIF (7, 30-32). The EPAS1 gene polymorphism appears to prevent the pulmonary vasoconstriction associated with hypoxia at altitude (8). Remarkably, the rare EPAS1 polymorphism has also been encountered in the DNA of the Denisovans, an extinct human population living around 20.000 years ago, of whom only small fossil remnants were found in a Siberian cave (33). As this polymorphism, which appears to be beneficial in chronic mountain disease, is present in Sherpa it can be suggested that there is an optimal balance between the effect of increasing oxygen delivery by higher haemoglobin levels and the increase in blood viscosity by higher red blood cell (RBC) densities. Alternatively, it may indicate there are multiple evolutionary solutions to overcoming the threat of hypoxia (30). The oxygen affinity of the RBC, as measured by the ratio of 2,3 diphosphoglycerate (2,3 DPG) concentration over that of haemoglobin, is usually decreased with ascent to altitude or in highlanders, which may increase oxygen delivery to the tissues. However, this may be partly offset by the Bohr effect due to a reduction in CO₂, which in turn increases oxygen affinity of the RBC (34). Of note, the oxygen affinity of RBCs in Sherpa is not much different from that of acclimatized lowlanders (35). Continuous renal replacement therapy (CRRT) resulted in a reduction of 2,3 DPG levels possibly due to phosphate depletion (36) and start of dialysis (37). To which extent this may play a clinically important effect by impairing oxygen delivery to the tissues remains as yet uncertain, although in patients treated with CRRT, reductions in 2,3 DPG were associated with an increased risk of death (36).

Another consideration is that there is subtle cell damage during the first 10 minutes of dialysis due to mechanical stress. This is consistent with observations detailing a spike in production of breath ethane in dialysis patients during this period, as a result of cell membrane rupture and cell loss, which would be expected to have an impact on oxygen carrying capacity (38, 39). Importantly, hemolysis also increases cell-free hemoglobin levels in blood, which creates an effective sink for nitric oxide (NO), triggers oxidative

stress, gives rise to endothelial dysfunction and has been linked to acute renal failure (40-42).

Macro and microcirculation

Convective oxygen transport in the arterial system is dependent on the product of arterial oxygen content times blood flow. In healthy people at altitude, one of the *consequences* of hypobaric hypoxia is a compensatory increase in cardiac output. In contrast, the decline in cardiac output during HD leads to a decline in tissue perfusion and is therefore likely one of the major *causes* for tissue hypoxia, which may be aggravated by a flow reduction due to stenosis in the arterial system and an impairment in cerebral autoregulation (43). Various studies have shown that ultrafiltration has a major untoward effect on cardiac and cerebral perfusion (2, 3), although myocardial stunning during dialysis was even observed in the absence of ultrafiltration (44). Ultrafiltration volume was also related to the decline in $S_{cv}O_2$ levels in HD patients (45). Furthermore, abnormalities in the microcirculation can play a major role in the impaired tissue oxygen supply in HD patients, both by a decline in the number of perfused capillaries as via a loss of hemodynamic coherence between the macro- and microcirculation (16). Indeed, whereas tissue edema and haemodilution can impair diffusive oxygen transfer (16), abnormalities in the number of capillaries *per se* may also play an important role in tissue hypoxia. Yeh et al. (46) have observed a reduction in sublingual small vessel density as well as the number of perfused vessels using sidestream darkfield imaging. Using the same method, it was also shown that microvascular perfusion was even further impaired during ultrafiltration (47), with a lesser role for HD *per se* (44). Similarly, a reduced capillary density in the heart of dialysis patients was observed, potentially impairing oxygen supply to cardiomyocytes (48), as well as a reduction in capillary density in the omentum of uremic children (49). The mechanism behind these changes in microvascular function are as yet unknown, although a decreased NO generation (50), lower angiopoietin 2 expression (49), or reduced VEGF expression due to high interstitial sodium levels (51) have been implicated. Importantly, functional abnormalities in microcirculatory function already occur early in the course of renal disease (52).

These findings are in sharp contrast to the condition of the microcirculation in Sherpa, which is characterized by an increase in capillary density and microvascular perfusion, as well as an increase in vasodilator capacity (53), possibly mediated through enhanced NO production. Nevertheless, literature reports on NO metabolites in the blood of Sherpa are equivocal. Horscroft et al. (28) did not observe differences in plasma NO metabolites between Sherpa and acclimatized lowlanders during an identical ascent to EBC in contrast to earlier reports of markedly elevated NO metabolite concentrations in

Tibetan highlanders compared to a cohort of US sea-level residents (54). Nevertheless, NO production increased, concomitantly with markers of oxidative stress, during the acclimatization of lowlanders to high altitude (55), although concentrations of plasma NO metabolites measured were considerably lower than those detected in the Tibetan highlanders. Peculiarly, microcirculatory blood flow in the acclimatized lowlanders correlated inversely with plasma nitrite, a marker of vascular endothelial NO production (56). Capillary density also appears to play a role in the exercise performance of lowlanders at altitude, as sublingual capillary density at sea level was higher in mountaineers who reached the summit of Mount Everest as compared to those who did not (57). As an interesting example of translational physiology, naked mole rats, who live under conditions of reduced ambient oxygen supply in their subterranean burrows can survive 18 minutes of total oxygen deprivation due to fructose-driven glycolysis (58). Moreover, their muscle capillary density is increased and is accompanied by an increase in HIF-1 α and VEGF transcription (59) as well as increased expression of Nrf2 (60).

Tissue oxygen extraction and utilization

Reduced convective oxygen transport is a major determinant in tissue ischemia during HD and is associated with an increased extraction of oxygen at tissue level (61). However, disturbances in cellular energy metabolism, due to a reduction in mitochondrial density, as well as mitochondrial dysfunction, may add to the effects of a reduced tissue oxygen supply in HD patients. Watson et al (62) have observed a reduction in mitochondrial density in skeletal muscle of HD patients compared to healthy age-matched controls, which was not restored following 12 weeks of exercise training. In a recent study, Liu et al (63) reported reduced skeletal muscle expression of mitochondrial-derived peptides and Nrf2 in CKD. Moreover, ATP yield in relation to oxygen flux was reduced in muscle from CKD patients compared to controls, suggesting impaired mitochondrial metabolism due to uncoupling of oxidative phosphorylation (64). In support of this notion, mitochondrial respiration was impaired in uremic rats due to increased uncoupling (65). Therefore, mitochondrial bioenergetics in CKD appear to be characterized by a reduction in mitochondrial density, impaired mitochondrial metabolism due to increased uncoupling and an increase in oxidative stress (66).

However, the adaptation to hypobaric hypoxia in healthy subjects also appears to be different from what was previously assumed. Martin et al. (67) have shown that in acclimatized lowlanders at altitude, systemic oxygen extraction at an altitude of 4.559 meter decreased in comparison to sea level. This was hypothesized to be due to either tissue diffusion limitation of oxygen due to a reduction in the partial pressure gradient from the capillary, a localized mismatch between tissue oxygen demand and microcirculatory blood flow, redistribution of blood from the muscle to vital organs, or an

altered cellular metabolism with reduced oxygen consumption at the mitochondrial level (67). Indeed, in the setting of chronic adaptation to environmental hypobaric hypoxia, both in lowlanders as well as in Sherpa, mitochondrial density is reduced, possibly due to a reduction of PGC under the influence of HIF. The reduced mitochondrial density is, in the case of Sherpa, compensated by an increase in coupling efficacy (8). Another important factor in the mitochondrial adaptation in lowlanders, as well as in Sherpa, is altered substrate utilization at the cellular/mitochondrial level. In lowlanders who ascend to high altitude, clear changes in fatty acid metabolism are observed. In a recent study in 198 healthy subjects before and during ascent to EBC, an increase in free fatty acid (FFA) levels was observed, most likely due to a reduced β -oxidation (68). This is adaptive from an energetic standpoint, as metabolism of carbohydrates needs less O₂ per mol ATP compared to FFA oxidation. Moreover, mitochondrial OXPHOS capacity was suppressed in lowlanders (8), likely through a direct effect of HIF-1 α (69). In Sherpa, a reduction in the capacity for fatty acid oxidation was observed, most likely related to mutation in the peroxisome proliferator-activated receptor A (PPAR-A) gene associated with a decreased expression (28). In contrast to lowlanders at altitude, Sherpa are protected from the accumulation of potentially harmful lipid intermediates, characterized by the ratio of long-chain acylcarnitines to total carnitine, possibly by an increase in gamma oxidation (8). Coupling efficacy of the electron transport chain is improved in Sherpa compared to lowlanders at equivalent altitude (8). Also, muscle phosphocreatinine, a marker of energetic reserve, is higher in Sherpa compared to lowlanders at altitude (8, 28). Sherpa also appear to be protected from cellular oxidative stress in muscle, which is increased in lowlanders at altitude but not in Sherpa (28). Whether there are other mechanisms involved in the prevention of the hypoxia-associated oxidative stress in Sherpa, apart from the reduced accumulation of oxidative lipid remnants and the increased coupling in the electron transport chain, remains to be determined. In skeletal muscle in mice, hypobaric hypoxia increased expression of Nrf2, which was potentiated by HIF1 α (70). Lessons from the animal kingdom indicate that modulation of Nuclear factor erythroid 2-related factor 2 (Nrf2) activity in response to hypoxia is a common solution (71). Thus, comparative studies on muscular Nrf2 expression in Sherpa vs. lowlanders should be of interest to conduct.

Taken together, the adaptation in energetics towards hypoxia in Sherpa is characterized by a reduced capacity for FFA oxidation and a reduction in mitochondrial density, combined with an increased mitochondrial coupling and protection against accumulation of lipid intermediates and oxidative stress (8). This can be summarized as a hypometabolic state with increased mitochondrial efficacy. In direct contrast, the uremic phenotype is characterized by decreased mitochondrial coupling efficacy and increased oxidative stress in muscle (63, 72). Thus, both in HD patients as well as in Sherpa,

mitochondrial density appears to be reduced (66), albeit in response to different mechanisms. In Sherpa, this is an adaptive response to the ambient hypoxia that evolved over many generations, leading to a reduction in cellular oxygen demand while preserving cellular energetics due an increase in coupling efficacy and a preference for carbohydrate oxidation (8). In HD-patients, there is profound mitochondrial biogenesis likely due to uremic toxicity (73), associated by a reduction in the number of mitochondria, a reduction in coupling efficacy and a profound increase in oxidative stress.

Systemic consequences of hypoxia at tissue level

Persistent cellular hypoxia tends to induce oxidative stress and inflammation. Whereas the role of renal hypoxia in progressive kidney damage has been well established (74), less is known about the role of hypoxia in systemic complications of renal failure (see *Figure 2* for its possible consequences). However, in addition to low S_aO_2 levels, also low intradialytic $S_{cv}O_2$ levels were significantly related to mortality. (4, 6).

$S_{cv}O_2$ is a composite parameter depending on (upper body) oxygen *supply* (convective oxygen transport), *distribution* (microcirculatory flow), *processing* (mitochondrial function) and oxygen *demand* (75). In a review of studies, mean $S_{cv}O_2$ levels were 1.2 to 8.5% higher than mixed venous oxygen saturations (S_vO_2) (5). One study in non-uremic patients found a mean $S_{cv}O_2$ level of 77% during cardiac catheterization (76). In critically ill patients, $S_{cv}O_2$ levels <60% were associated with increased mortality (77), whereas it has been suggested that levels <65-70% under acute conditions should prompt the clinician to the presence of tissue hypoxia or inadequate perfusion (78). This is of concern as nearly 2/3 of HD patients appear to have $S_{cv}O_2$ levels <60% throughout the course of their dialysis treatment (4). Whereas decreased levels of $S_{cv}O_2$ may indicate global tissue hypoxia (79), the relation between $S_{cv}O_2$ and local tissue hypoxia is not necessarily linear (80). However, Benhamou et al. have observed severe ischemia (defined as a transcutaneous ($TcpO_2$) levels <30 mmHg, and critical ischemia ($TcpO_2$ <10 mmHg) during HD in 47% and 16% of patients, respectively (81). Critical ischemia was only observed in those patients with peripheral vascular disease, showing the importance of the deleterious interaction between pre-existing abnormalities in the vascular tree and the effect of HD. It was also demonstrated that a reduction in cardiac perfusion, leading to myocardial stunning, as well as a reduction in cerebral perfusion are related to organ damage in the long term (12, 82).

There is direct relationship between hypoxia and *inflammation* (83), and both PIH as well as low $S_{cv}O_2$ levels were related to an inflammatory phenotype (4, 6). Tissue hypoxia has strong pro-inflammatory effects. As an extreme example acute mountain sickness complicated by increased vascular permeability result in lung and/or brain edema. On the other hand, inflammation can lead to tissue hypoxia due to tissue oedema

or a reduction in substrate availability due to activation of immune cells. HIF-1 appears to have pleiotropic effects on inflammation, as it activates NF- κ B, a key transcription factor in the regulation of the innate immune response that also has immunoregulatory effects in the adaptive immune response (83).

Hypoxia also has *catabolic* effects, as has been observed in lowlanders ascending to high altitude. In climbers ascending to EBC, a loss of 4.9 kg in body weight has been observed. In mountaineers reaching the summit of Everest, the mean decline amounted to 7.3 kg. The decline was higher for fat-free mass as compared to fat mass. It is likely that inflammation plays a role in the observed changes in body composition, as the decline in fat-free mass was related to the increase in IL-6. It is possible that an increased turnover of muscle protein under these conditions provide critical amino acids not readily available from other sources to enable the synthesis of biomolecules required for successful acclimatization (84). The role of HIF-1 in altitude-related muscle catabolism is yet uncertain (85). In addition, a decrease in the branched-chain amino acid (BCAA) isoleucine was observed during ascent, which could play a role in altitude-associated muscle catabolism next to inflammation. BCAA are essential for mTOR induced protein synthesis. As mTOR levels decrease in the vastus lateralis of humans at altitude (86), ascent to altitude appears to reduce anabolism while increasing catabolism. Interestingly, compared to lowlanders ascending to altitude, Sherpa do not suffer from muscle catabolism, likely a result of their prior adaptation to this environment.

Whether tissue hypoxia plays a direct role in dialysis-associated muscle weakness is yet unknown, although there is substantial evidence that abnormalities in oxygen handling at the mitochondrial level are involved in the pathogenesis of muscle dysfunction. Hypoxia may also induce cellular senescence (87) (88, 89) (90). In dialysis patients, this process may be aggravated by mitochondrial pathology, as a recent study has shown a reduction in tissue expression of mitochondria-derived peptides (MDPs), such as humanin and MOTS-c (63). In combination with a reduction in Nrf2 and systemic inflammation, cellular hypoxia and mitochondrial pathology may also play a role in the accelerated uremic ageing phenotype (63, 91). The role of HIFs in this respect is still uncertain as, given their pleiotropic effects, HIF activation is associated with both increased well as reduced survival in animal models, and can have both pro- and anti-inflammatory effects (89, 92). As intermittent hypoxia exacerbate tumor progression in a mouse model of lung cancer (93) intermittent intra-dialytic hypoxia might explain why HD patients are at increased risk to develop cancer.

Hypoxia may also play a role in *short-term complications*, such as intra-dialytic hypotension. Tissue or RBC ischemia can lead to increased ATP degradation and the subsequent release of the vasodilator adenosine (94, 95), whereas extracellular adenosine production is also stimulated by HIF (83). The increased plasma adenosine

levels in patients with sudden intra-dialytic hypotension, and the preventive effects of an adenosine antagonist on blood pressure decline during dialysis, supports such a mechanism (96, 97).

Hypoxia, the red blood cell and the redox interactome

Intracellular oxidative stress triggered by hypoxia may also affect erythrocyte life span, although there is still controversy as to whether hypoxia leads to inhibition or stimulation of eryptosis (erythrocyte cell death) (98, 99). This might also depend on other circumstances, as uremia increases the negative effects of hypoxia on erythrocyte life span (99). Due to the so-called erythrocrine function, this aspect may be of even greater relevance than initially assumed, as intact erythrocytes play an important role in the vascular physiology via the presence of NO synthase (95, 100), whereas in contrast, release of the intracellular components by rupture or damage of red blood cell membranes may lead to scavenging of NO. Erythrocytes appear to play many more roles in maintaining bodily homeostasis than originally assigned to their function (i.e. that of a conduit for gas exchange and transport between lung and peripheral tissues). To this end, RBC may be particularly important for the exchange of redox-active molecules from one part of the body to another. We should not forget that oxygen transport and reactive oxygen species (ROS) production/control is just one of several aspects of what has become known as the 'reactive species interactome' (101). In addition to oxygen-related molecules this system comprises nitrogen and sulfur-related species such as the NO and hydrogen sulfide (H₂S)/polysulfides, the production of which represents the cellular interface between the internal and external milieu that allows cells and whole organisms to sense and adapt to metabolic and environmental stressors. For example, the gasotransmitter H₂S serves as an oxygen sensor and likely mediates hypoxic pulmonary vasoconstriction and systemic vasodilation(102). In addition, H₂S appeared to be cardioprotective in ischemia reperfusion injury (102). Little is known about the behavior or reactive sulfur species (RSS) at altitude. A recent study showed a consistent rise in total free thiols, which possibly serve as an antioxidant buffer, in healthy volunteers after ascent to 4.559 meters, but no consistent changes in other ROS or reactive nitrogen species (RNS) (103).

These transmitters are embedded in an even wider paradigm defined as the 'redox interactome' including ROS, RNS, and RSS, which is one of the most fundamental regulatory system that underpins physiological function and connects all living organisms to their environment (104). This provides an explanation for the close relationship between intermediary metabolism and mitochondrial function. Since this concept of the chemical interconnectedness of multiple reactive species has been introduced to biology only recently, not much is known about the significance of individual constituents for

acclimatization/adaptation to hypoxia. However, it offer an attractive new paradigm for exploration to better understand the dichotomy of regulation of oxygen homeostasis in HD patients and well-adapted populations, such as the Sherpa.

Therapeutic implications

Therapeutic interventions can be divided into non-pharmacological and pharmacological approaches. The main goal of possible *non-pharmacological* approaches is to prevent large changes in perfusion pressure and of intra-dialytic hypotension (105), and to prevent hypoxemia related to fluid overload (18). Modification of the dialysis schedule might also preserve tissue perfusion during HD; to this end, an increase in dialysis treatment time with the resulting reduction in ultrafiltration rate as well as cool dialysis both reduced cardiac stunning (106). The same holds true for prescription of cool HD, which was also associated with fewer white matter lesions after a one-year follow up period as compared to standard temperature dialysis (82). Of interest, the oxygen extraction ratio (the difference between S_{aO_2} and S_{cvO_2}) was lower during fluid removal with isolated ultrafiltration compared to HD (61), which is notable because the hemodynamic profile between isolated ultrafiltration and cool dialysis is comparable (107). Whether adaptations to low atmospheric oxygen levels also leads to an improved tolerance for intradialytic hypoxia, e.g. by activation of the NO system, VEGF or HIF (11) and if these mechanisms play a role in the improved survival of HD patients at altitude is a relevant topic for future study. DAH might be treated by supplemental oxygen (15) but whether this strategy improves (surrogate) outcomes has not been assessed yet. However, translating data from ICU suggest that this is not necessarily the case (108).

Next to specific dialysis-related interventions to prevent hypoperfusion-related tissue hypoxia and the use of iron and/or erythropoietin to maintain hemoglobin levels within target ranges, preliminary data suggest that preconditioning, such as by intra-dialytic exercise training, might improve cardiac stunning (109). Although the use of remote ischemic preconditioning (RIPC) on organ injury has yielded promising results (110), it is unclear whether RIPC or intermittent hypoxic preconditioning alleviates organ injury or improves outcome in dialysis patients. There is an apparent paradox between the deleterious effects of intra-dialytic hypoxemia and hypoxia on the one hand and the adaptive responses in Sherpa and the preventive effects of RIPC and hypoxic preconditioning on the other hand. However, one should not forget that dialysis, RIPC and hypoxic preconditioning are short-term phenomena whereas the adaptation of Sherpa has evolved over many generations. Still, various important aspects of the response to hypoxia appear to be comparable in different conditions, which is not surprising in view of the fact that the response to hypoxic stress in oxygen-dependent animals has evolved over at least 600 million years (101).

The adaptive responses to preconditioning, as well as to hypobaric hypoxia include stimulation of HIF-1, the NO-system and VEGF, which may increase neoangiogenesis (111, 112). These adaptive responses may be blunted in dialysis patients, e.g. due to inhibition of the NO-system by endogenous inhibitors, such as asymmetric dimethylarginine (113) or compromised renal L-arginine synthesis/reabsorption (114). Secondly, the severity of tissue hypoxia in dialysis patients may be larger because of preexisting cardiovascular disease and capillary rarefaction and therefore induce tissue damage, which is in contrast to the hormetic effects of preconditioning that evoke a protective response without inducing tissue damage (115). Intradialytic hypoxemia and hypoxia, although aggravated by the dialysis treatment, may therefore also be a sign of underlying disease states which in themselves are risk factors for mortality and a chronic hypoxic state. For instance, whereas patients with cardiac stunning are significantly more inflamed (116) a significant percentage of patients with PIH already had low S_aO_2 levels before the start of dialysis (6).

The possible effects of *pharmacological methods* can be divided into attempts to improve microvascular structure and function, to improve mitochondrial efficacy, and/or to reduce hypoxic oxidative stress. As abnormalities in NO metabolism might be involved in the pathogenesis of microvascular dysfunction in HD-patients and because Tibetans have adaptive changes in the microcirculation, which are associated with increased NO generation, supplementation therapy with NO donors would appear to be a potentially promising strategy (117-119). However, earlier attempts to improving microvascular function by dietary means, e.g. the use of supplementary nitrate (in the form of beetroot juice) has not been proven effective yet. In a field study performed at 4.559 m, nitrate supplementation did not improve sublingual blood flow (120). To the best of our knowledge, the effect of NO donors has not been tested yet in uremic patients.

Nrf2 agonists could be used to reduce hypoxia induced oxidative stress (121) although their safety profile needs to be established (122) (88). Interestingly, Nrf2 agonists have been observed to prevent the increased vascular permeability associated with acute mountain sickness (123). Moreover, δ -opioid receptor signaling protects against hypoxic injury via increased Nrf2 protein expression (124). Notably, many Nrf2 agonists are naturally occurring bio-active entities derived from the microbial processing of nutritionally-derived (poly)phenolic acids, which offers a potential avenue to mitigate the effects of hypoxia (91, 121, 125).

In dialysis patients, prolyl hydroxylase 2 (PHD2) inhibitors, which stabilize HIF levels, have been used successfully in the treatment of renal anaemia. HIFs might also reduce oxidative stress and increase angiogenesis (26, 126). However, HIFs have highly pleiotropic effects and have been implicated in both aggravation as well as prevention of tissue effects in organ diseases (126). In cell cultures, PHD2 inhibitors improved

mitochondrial efficacy, reduced oxidative stress and increased Nrf2 expression (127) and protected human epithelial cells and murine kidney from hypoxic injury (128). HIF1 α also increases neo-angiogenesis through VEGF in order to increase the oxygenation of tissues and has been proposed to prevent endothelial and vascular smooth muscle senescence. Still, HIFs may also increase the expression of pro-inflammatory cytokines under hypoxic conditions (27). Data on HIF expression in uremic models are relatively scarce. On the one hand, HIF 1 α expression in transplant biopsies is associated with early recovery of graft function (129). On the other hand, both HIF1 α and VEGF increased in the peritoneal effluent of PD patients with ultrafiltration failure and might be involved in the pathogenesis of peritoneal fibrosis (130). Future trials of HIF enhancing medications, such as PHD2 inhibitors, are needed to investigate whether, in addition to their role in the treatment of renal anemia, they also have preventive effects against hypoxia related tissue injury, inflammation as well as senolytic effects.

Conclusions

In HD patients, both a reduction in S_aO_2 and $S_{cv}O_2$, reflecting respectively hypoxemia as well as a mismatch between oxygen demand and delivery, are associated with an increased mortality risk. Abnormalities along the entire oxygen cascade can be present in this vulnerable patient group. In addition to the decline in diffusive and convective oxygen transport from the lungs to the arterial tree this includes abnormalities in microcirculatory function as well as a decrease in mitochondrial efficacy, impaired tissue oxygen supply, and oxygen handling at the cellular level which may lead to an increase in oxidative stress. Although current evidence is scarce, tissue hypoxia may also play a role in the pathogenesis of systemic inflammation, dialysis-related hypotension and senescence. The apparent alterations in the oxygen cascade of HD patients is in sharp contrast to the adaptation of bodily physiology to hypobaric hypoxia in Tibetans and Sherpa, which is characterized by an increase in capillary density and an increase in bioenergetic efficacy at the mitochondrial level. In addition, the hypobaric hypoxia in these populations is not accompanied by an increase in oxidative stress, and their erythropoietic response is blunted compared to other populations living at high altitude for shorter periods of time. Prevention of tissue hypoxia in dialysis patients currently focuses on optimizing the hemodynamic response during HD while maintaining hemoglobin levels within acceptable limits. Whether pharmacological interventions, such as nitrate supplementation, Nrf2 agonists or PHD2 inhibitors, improve tissue hypoxia and its associated consequences should be tested in future clinical trials. Detailed metabolic phenotyping of patients dialyzed at high altitude dialysis centers may provide important insight into the link between mechanisms of adaptations to hypoxia and clinical outcome.

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Table 1. The oxygen cascade: Comparison between dialysis patients, healthy lowlanders after acclimatization to high altitude and Sherpa/Tibetan highlanders

	Dialysis patients	Altitude Acclimatization	Sherpa
<i>Ambient O₂</i>	=	-	-
<i>A-a gradient</i>	+	=	-
<i>S_aO₂</i>	-	-	-
<i>Sleep apnea/ Periodic breathing</i>	+	+	-
<i>Hemoglobin</i>	-	+	=
<i>2,3 DPG level</i>	-	+	+
<i>CO</i>	-	+	+
<i>Capillary density</i>	-	=	++
<i>Mitochondrial number</i>	-	-	-
<i>Coupling efficacy</i>	-	=/+	++

Abbreviations: A-a gradient= alveolar to arterial gradient; *S_aO₂*= arterial oxygen saturation; CO=cardiac output

Figure 1. Underlying and compensatory mechanisms to hypoxemia and tissue hypoxia in dialysis patients and Sherpa

dialysis patients

diffusion

sleep apnea

restrictive lung

Figure 2. Possible causes and consequences of tissue hypoxia in dialysis patients