PLOS ONE

Hemoglobin Oxygen Affinity in Patients with Cystic Fibrosis

Dieter Böning[1]*, Angela Littschwager[1], Matthias Hütler[1], Ralph Beneke[1], Doris Staab[2]

1 Institut für Sportmedizin, Charité - Universitätsmedizin Berlin, Berlin, Germany, 2 Klinik für Pädiatrische Pneumologie und Immunologie, Charité - Universitätsmedizin Berlin, Berlin, Germany

Abstract

In patients with cystic fibrosis lung damages cause arterial hypoxia. As a typical compensatory reaction one might expect changes in oxygen affinity of hemoglobin. Therefore position (standard half saturation pressure P_{50}st) and slope (Hill's n) of the O_2 dissociation curve as well as the Bohr coefficients (BC) for CO_2 and lactic acid were determined in blood of 14 adult patients (8 males, 6 females) and 14 healthy controls (6 males, 8 females). While Hill's n amounted to approximately 2.6 in all subjects, P_{50}st was slightly increased by 1 mmHg in both patient groups (controls male 26.7 ± 0.2, controls female 27.0 ± 0.1, patients male 27.7 ± 0.5, patients female 28.0 ± 0.3 mmHg; mean and standard error, overall $p < 0.01$). Main cause was a rise of 1–2 µmol/g hemoglobin in erythrocytic 2,3-biphosphoglycerate concentration. One patient only, clearly identified as an outlier and with the mutation G551D, showed a reduction of both P_{50}st (24.5 mmHg) and [2,3-biphosphoglycerate] (9.8 µmol/g hemoglobin). There were no differences in BCCO_2, but small sex differences in the BC for lactic acid in the controls which were not detectable in the patients. Causes for the right shift of the O_2 dissociation curve might be hypoxic stimulation of erythrocytic glycolysis and an increased red cell turnover both causing increased [2,3-biphosphoglycerate]. However, for situations with additional hypercapnia as observed in exercising patients a left shift seems to be a more favourable adaptation in cystic fibrosis. Additionally when in vivo PO_2 values were corrected to the standard conditions they mostly lay left of the in vitro O_2 dissociation curve in both patients and controls. This hints to unknown fugitive factors influencing oxygen affinity.

Introduction

Cystic fibrosis (CF) is the most frequent genetic disease in Caucasians [1–3]. Mutations on chromosome 7 (location 7q31.2) reduce the effectiveness of the cystic fibrosis transmembrane conductance regulator (CFTR), which is essential for the secretion of chloride (Cl^-) and consequently water in many glands. The clinical manifestation with heaviest impact is the progressive pulmonary disease. Because of the resulting deteriorated lung function in patients with cystic fibrosis causing hypoxia and partly also hypercapnia one might expect compensatory reactions in concentration and oxygen affinity of hemoglobin (Hb) to secure oxygen loading in spite of the reduced oxygen pressure (PO_2) in pulmonary capillaries.

There are various strategies of defense against arterial hypoxia [4–8]. In addition to hyperventilation most healthy humans, except partly Tibetans and Ethiopeans [9–11], react to hypoxia with an increase in Hb concentration ([Hb]) which facilitates sufficient binding of oxygen at lowered PO_2 in the lungs. Furthermore a right shift of the oxygen dissociation curve (ODC) under standard conditions (pH 7.4, PCO_2 40 mmHg, 37°C) partly compensates for the reduced diffusion pressure in the tissues because of the low oxygen saturation (SO_2) in the capillaries; the shift is caused by more 2,3-biphosphoglyrate (BPG) in the red cells. In contrast typical altitude animals like

llamas, guinea pigs and partly birds possess left-shifted ODCs securing oxygen loading in the lungs and rather low [Hb] reducing circulatory resistance. In addition small red blood cells and a dense capillary net in the tissues diminish the diffusion distance and thus compensate for the decreased capillary PO_2 [12]. The human fetus exists also at very low arterial PO_2 but the concentration of the high affinity fetal Hb (HbF) is increased. Recent in vivo determinations of the ODC in adults point to a possible left shift of its upper part at altitude [13,14].

Astonishingly few studies investigated the combined effect of hypoxia and hypercapnia. The fetal conditions with higher arterial PCO_2 than in maternal blood point to an advantage of a left shifted ODC. Moles living in earth holes with reduced air exchange inspire hypoxic/hypercapnic gas and possess also Hb with increased oxygen affinity [15]. Huckauf et al ([16]) describe a left shifted ODC in patients with chronic obstructive lung disease and in a review Morgan [17] mentions that [BPG] is often reduced in critically ill patients.

Patients with cystic fibrosis often show normal or even anemic [Hb] (e. g. [18–20]. Interestingly, however, they may possess an increased red cell volume masked by a concomitant rise in plasma volume [21,22]. Compensatory reactions of oxygen affinity in cystic fibrosis have been investigated rarely. Slight right shifts of the standard ODC, characterized by a rise of P_{50}st and caused by

increased [BPG], were detected by some authors [18,23], while others found unchanged [BPG] or P_{50}st [19,24].

However, there are various additional mechanisms for the regulation of oxygen affinity. Besides phosphates other anions like lactate, chloride and glutathione (e. g. [25,26]) bind to Hb. Depending on the binding site these substances also influence the cooperativity of the subunits visible as change in the slope of the ODC (Hill's n); additionally they may modify the intraerythrocytic pH. The Bohr effect, which in the physiological pH range causes an increase of PO_2 at constant saturation by acidification (essential in working muscles), may vary depending on various factors like oxygen saturation [27,28], type of acid [27,28], substance concentrations and age of the erythrocytes (e. g. [29]); in altitude residents a tendency to lowered Bohr coefficients (BC = ΔlogPO$_2$/ΔpH) has been observed [30,31]. Also sex differences in oxygen binding properties have been described: women [32–34] as well as children [35] tend to higher P_{50}st than men. Finally in vivo variations of the ODC in venous blood of anemic patients as well as of trained subjects have been observed during exercise which were no more detectable after in vitro equilibration of blood [36–38]. The underlying mechanisms are not yet clarified.

Previous studies on oxygen affinity in cystic fibrosis were performed on rather heterogeneous groups of patients. Differences in the severity of the illness are almost inevitable but possible effects of age and sex were not considered. Also control groups were small or not clearly defined or even lacking. To our knowledge neither cooperativity (Hill's n) nor the Bohr effect have ever been studied in cystic fibrosis.

Considering all these factors it seemed worthwhile to perform a systematic study of mechanisms influencing blood O_2 affinity as possible facilitation of oxygen uptake in cystic fibrosis.

Methods

Study Participants

Measurements were performed in 14 adult patients and 14 controls; anthropometric data are presented in Table 1. The patients (8 males, 6 females) showed severely reduced lung function but were in a stable clinical condition. One male subject was bearer of the Class III G551D mutation which is one of five mutations with a frequency >0.1% accounting for 2 to 3% mutations worldwide. It impairs CFTR-mediated Cl^- transport by limiting channel gating at the cell surface [3,39].

The patients were the members of a group with exercise therapy. They usually lived at home but were under continuous supervision by physicians of the pediatric clinic of the faculty. Twice a week they performed a disease status tailored exercise program addressing endurance, strength, coordination and flexibility supervised by staff of the Institute of Sports Medicine and received individual advice for additional daily exercises at home. Occasionally some patients used short term oxygen supplementation, but not on the test day. The nonsmoking controls (6 males, 8 females) were physically active but not specifically or regularly training staff members and students. One female was slightly anemic ([Hb] 11.2 g/dl), but all other measurements yielded clinically normal values within the range of the group. The study protocol was approved by the ethics committee of the faculty (Ethikkommission, Charité – Universitätsmedizin Berlin, Ethikausschuss CBF, No. ek.185-13b) and written informed consent was obtained from all participants.

Study Procedure

The subjects arrived at the laboratory between 9.00 and 10.00 a.m. Lung function (forced vital capacity FVC, forced expiratory

Table 1. Subjects.

	n	Age (years)	Body mass (kg)	Height (cm)	BMI (kg/m²)	FVC (%)	FEV1 (%)	PEF (%)
Controls male	6	28±3	80.4±3.2	185±4	24.2±1.2	93±2	92±3	102±8
Controls female	8	27±2	62.8±3.0	171±3	21.5±0.8	117±4	104±5	98±10
Patients male	8	28±2	59.0±3.0	177±4	18.8±0.6	59±7	38±6	57±5
Patients female	6	29±1	48.8±1.7	162±2	18.6±0.5	56±8	37±6	51±12

Means and standard errors (SE). BMI body mass index, FVC forced vital capacity, FEV1 forced expiratory volume during 1 s; PEF peak expiratory flow. % of expected values for age and sex [40] or of individual FVC. All anthropometrical and lung function values are significantly (P<0.001) reduced in the patients.
doi:10.1371/journal.pone.0097932.t001

volume during 1 s FEV1, peak expiratory flow PEF) was measured with a spirometer system (Oxycon gamma, Mijnhardt, Bunnik, The Netherlands). Percent of expected values for age and sex [40] or of individual FVC are presented in Table 1. Blood was sampled in supine position. Acid base status at 37°C (ABL 500 or 510 with no systematic difference between apparatus, Radiometer Copenhagen, Denmark), oxygenation status (PO_2, SO_2, COHb, MetHb) and [Hb] (OSM 3; Radiometer Copenhagen, Denmark) were measured in heparinized blood samples taken from hyperemized ear-lobes. Values for PO_2 are slightly lower than in arterial blood [41], but this is of negligible importance for saturations above 90% in the flat part of the ODC. Fifty ml of venous blood were drawn without stasis using heparinized vaccutainers and stored in an ice-water mixture. Oxygenation status, [Hb], hematocrit (Hct, microhematocrit method) and [Cl^-] in plasma (EML 100, Radiometer Copenhagen) were determined immediately. Aliquots were deproteinized and stored at -20°C for duplicate measurements of ATP and BPG concentrations (enzymatic kits, Sigma Diagnostics) on the next day.

Five ml each were equilibrated 20 min in sphere tonometers at 37°C with air/CO_2 or nitrogen/CO_2 mixtures (3, 6 or 10% CO_2). Lactic acid (13.5 mmol/l blood) was added to an additional sample equilibrated thereafter with 6% CO_2 in air or N_2. After taking aliquots for additional ATP and BPG measurements 0.2 ml of oxygenated blood were successively added 8 to 10 times to 1 ml deoxygenated blood using 2 connected syringes and mixed. After measurement of SO_2, COHb, MetHb, [Hb], pH, PCO_2 and PO_2, ODCs were drawn in the Hill plot (log SO_2/100-SO_2) versus log PO_2).

Samples of native blood as well as of blood equilibrated with N_2/6% CO_2 and with air/6% CO_2 were centrifuged for 10 min (3500 rpm, 4°C). Part of the red cell sediment was hemolyzed by repeated freezing and thawing and used for measurement of pH and [Cl^-] in the erythrocytes.

Twelve patients (7 males, 5 females) performed an incremental test (initially 0.3 W/kg, plus 0.3 W/kg every 2 min) until exhaustion on a cycle ergometer (Lode Excalibur, The Netherlands) during exercise therapy. Blood gases and lactate concentration (Ebio plus, Eppendorf, Germany) were measured in ear lobe blood and used to calculate P_{50} at exhaustion.

Calculations

The slope n of the oxygen dissociation curves linearized in the Hill plot served as measure of cooperativity. For 5% steps of SO_2 between 15 and 90% logPO_2 values were calculated from the regression equations and the corresponding pH values obtained by interpolation. Comparison of the ODCs for 3 and 10% CO_2 yielded Bohr coefficients for CO_2 (BCCO_2), comparison of the 6% CO_2 and the 13.5 mmol/l lactic acid curves yielded Bohr coefficients for fixed acid (BCLa) at each saturation step. P_{50}st were calculated from the curves of blood equilibrated with 6% CO_2 by use of the corresponding individual BCCO_2. Mean cellular hemoglobin concentrations (MCHC) calculated from [Hb] and Hct were corrected for 2% trapped plasma. [Cl^-]$_{ery}$ were corrected for 10% in the sediment after centrifugation with 3500 rpm; because of the large buffer capacity of red cells this is not necessary for pH$_{ery}$. Electrodes in the electrolyte analyser measure concentrations in water [42]; therefore [Cl^-]$_{ery}$ is given per l cell water. Values for the control subjects coincide with titrimetric measurements [43]. In vitro blood buffer capacities ($-\Delta$[acid]/ΔpH) for CO_2 and lactic acid were calculated from the measurements in the corresponding equilibrated samples.

Statistics

All data are presented as means±standard errors (SE). Dependent on the number of comparisons, t-tests or analysis of variance (ANOVA) were used for significance calculations. The probability that an outlier does not belong to a sample was tested eventually [44]. Differences with P<0.05 were considered as significant.

Results

Anthropometry and Pathology

Table 1 shows marked reduction in both body height and body mass in the patients compared to healthy subjects. Their lung function was substantially impeded by restrictive as well as obstructive damage visible from low vital capacity and expiratory flow (FEV1, PEV); FEV1 ranged between 22 and 74%.

Blood Gases and Acid Base Status

The impaired lung function of the patients caused a reduction of ear-lobe PO_2 and SO_2 (Table 2). Generally these values were also slightly lower in males than in females. Correspondingly, PCO_2 tended to higher values in males and in patients. However, the pH was equal in all subgroups because of non-respiratory compensation visible as increased base excess in males and in patients. Venous blood pH scattered more, but there were also no systematic differences among groups (means between 7.35 and 7.38); red cell pH showed no influence of sex or illness as well (means about 7.16). In vitro buffer capacities of blood tended to higher values in all males; this was significant for acidification with CO_2 as well as lactic acid (both P<0.05; latter not shown in Table 2) in oxygenated blood. Concentrations of COHb (controls male 0.6±0.1%, controls female 0.4±0.1%, patients male 0.6±0.2%, patients female 0.7±0.1%) and MetHb (controls male 0.5±0.1%, controls female 0.6±0.1%, patients male 0.5±0.1%, patients female 0.5±0.1%) were low and not different among groups.

Blood Composition

[Hb] and Hct were higher in males than females, but there were no significant differences between controls and patients (Table 3). In the male patients [Hb] was negatively correlated with FEV1 (r = -0.762, P<0.05). MCHC, however, was slightly but significantly lowered in patients. They showed also slightly decreased [Cl^-] in plasma and red cells. [BPG] and [ATP] were significantly increased in the patients inspite of very low [BPG] (9.8 µmol/gHb) in the subject with the G551D mutation. Additionally there was a sex difference for [BPG] with higher concentrations in females. After equilibration [BPG] and [ATP] tended to slightly higher values (0.9 and 0.2 µmol/gHb on average, respectively, not significant) compared to native blood.

Oxygen Dissociation Curves

In the Hill plot (Fig. 1) all curves were linear (correlation coefficients better than 0.98, not corrected for the slightly decreasing pH with rising saturation) and the slopes amounted to approximately 2.6 with very little scattering in all groups (Table 4).

The standard half saturation pressures (Table 4) corresponded to known normal values in the controls. In patients P_{50}st was significantly increased by 1 mmHg (with slightly but not significantly higher values for females). When corrected to arterialized pH and PCO_2, all means were 0.8 mmHg lower. The patient with the G551D mutation presented a markedly lowered P_{50}st of 24.5 mmHg (arterialized blood 23.3 mmHg) clearly identified as

Table 2. Blood gases and acid-base status.

	n	PO$_{2art}$	SO$_{2art}$	PCO$_{2art}$	pH$_{art}$	SBE	Buffer Cap
		mmHg	%	mmHg		mmol/l	mmol/l
Controls male	6	91.4±3.0	95.9±0.3	38.0±1.6	7.430±0.006	1.3±0.7	29.9±1.2
Controlsfemale	8	99.3±1.2	96.8±0.2	34.4±1.6	7.430±0.008	−0.9±0.7	25.2±1.0
Patients male	8	64.9±3.5	91.2±1.5	41.7±1.8	7.423±0.008	3.4±0.5	28.9±0.7
Patients female	6	70.5±2.7	93.3±0.7	37.2±2.0	7.427±0.012	1.4±0.7	26.8±1.0
Anova sex		a		a		b	a
illness		c	d			d	

Means ± SE. art measurements in ear lobe blood. Standard base excess (SBE, 100% SO$_2$, standardized [Hb] of 5 g/dl, [71]) measured in venous blood. Buffer Cap: in vitro buffer capacity for CO$_2$ in oxygenated blood (-Δ[HCO$_3$ $^-$]$_{plasma}$/Δ pH). ANOVA: a P<0.05, b P<0.01 or better for differences between males and females, c P<0.05, d P<0.01 or better for differences between controls and patients.
doi:10.1371/journal.pone.0097932.t002

Table 3. Substance concentrations in venous blood.

	n	[Hb]	Hct	MCHC	[Cl$^-$]$_{plasma}$	[Cl$^-$]$_{ery}$	[BPG]$_{ery}$	[ATP]$_{ery}$
		g/dl	%	g/dl	mmol/l	mmol/l H$_2$O	µmol/gHb	µmol/gHb
Controls male	6	15.4±0.4	45.7±0.8	33.8±0.5	103.3±0.5	73.9±1.1	13.3±1.2	4.1±0.3
Controls female	8	12.8±0.4	39.6±0.2	32.8±0.3	103.2±0.4	76.0±1.5	14.6±0.8	4.2±0.2
Patients male	8	14.7±0.4	46.5±1.5	31.9±0.4	99.6±0.5	71.9±1.9	14.1±0.8*	4.6±0.1
Patients female	6	12.9±0.4	42.0±1.3	31.8±0.6	100.3±1.2	73.0±1.3	16.7±1.5	4.3±0.3
Anova sex		a	a				a	
illness				c	d	c		d

Means ± SE. Significance levels indicated like for Table 2. MCHC and [Cl$^-$]$_{ery}$ corrected for 2% and 10% trapped plasma, respectively. *14.8±0.5 without the patient with the G551D mutation.
doi:10.1371/journal.pone.0097932.t003
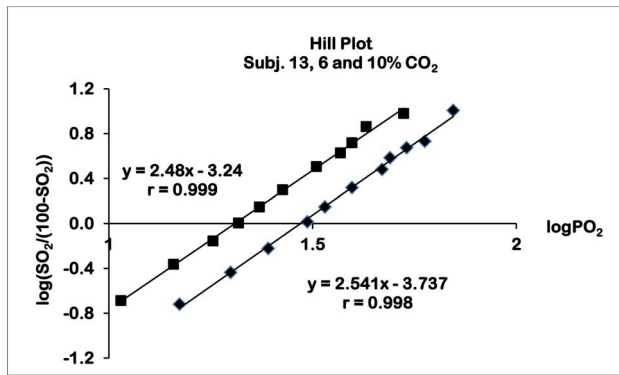
Figure 1. Two oxygen dissociation curves of one subject in the Hill plot.
doi:10.1371/journal.pone.0097932.g001

an outlier ($P<0.02$). Without the latter value mean P_{50}st for the male patients rose by 0.4 mmHg.

Regression analysis including all subjects yielded a significant relation between [BPG] in the equilibrated samples and P_{50}st (Fig. 2). The male patient with the extremely low P_{50}st value fell, however, far outside of his group with a correspondingly low [BPG].

Bohr Coefficients

The Bohr coefficients (Fig. 3) for CO_2 corresponded to published data: The value was about -0.5 and decreased numerically with higher saturations ($P<0.01$ for all subjects); there was also a tendency to lower values in women. No influence of the disease was visible. The Bohr coefficients for lactic acid (Fig. 4) were generally lower numerically than for CO_2 in all groups up to 45% saturation (-0.40 to -0.45). Differences between males and females at higher SO_2 disappeared in the patients (interaction sex-illness $P<0.01$). Among the patients the subject with the G551D mutation presented the highest BC for both acids between 70 and 90% SO_2 (approx. -0.54).

Exercise Tests in Patients

At exhaustion SO_2 dropped in all patients resulting from reduced PO_2 and both respiratory and non-respiratory acidosis which caused a rise of P_{50} (Table 5). Again the subject with the G551D mutation showed the lowest P_{50} value (29.6 mmHg).

In vivo Effects

When the PO_2 values in non-equilibrated venous blood (fresh or stored in ice until measurement) were corrected with the corresponding Bohr coefficients ($BCCO_2$) to pH 7.4, they should have fallen on the individual standard ODC. However, in the range between 45 and 90% SO_2 there was a tendency for a deviation to the left (Fig. 5) in controls (-1.8 ± 0.4 mmHg, $P<0.05$) as well as in patients (-2.2 ± 0.4 mmHg, $P<0.001$). Some samples with higher values of SO_2 were not considered, because the BCs were not measured for $SO_2>90\%$. In addition there is large scattering of PO_2 in the flat part of the ODC. There was no correlation between PO_2 differences and [BPG] differences for native and equilibrated blood.

Discussion

Synopsis of Results

Our results confirm former investigations that there is a small right shift of the standard ODC in most patients with cystic fibrosis probably caused by slightly increased intraerythrocytic concentrations of organic phosphates [18,23]. This is accompanied by a constant slope of the ODC and only small changes of the Bohr coefficients. In spite of the lacking hypocapnia this reaction is similar to the typical human acclimatization to altitude but seems to be attenuated. During exercise the right shift of the ODC is enforced by hypercapnia in CF patients and a clear drawback for arterial oxygen loading. Interestingly there seems to exist an additional mechanism in controls as well as in patients: The in vivo standard ODC falls slightly left of the in vitro curve.

Blood Gases and Acid Base Status

The deterioration of lung function in the patients results in hypoxia visible in arterialized blood; a tendency to a slightly higher PCO_2 than in the controls is not significant probably because of the low number of measurements and the resting situation; when exercising the increase in PCO_2 is more marked. Measurements in 69 patients in our laboratory showed corresponding results; $PaCO_2$ increased with the severity of the illness at rest as well as during exercise [45]. This is different to healthy subjects who always show a decrease of $PaCO_2$ at high work load. The arterial oxygen saturation in patients at rest is as low as in highlanders [46,47] living 2600 m above sea level (inspiratory PO_2 approx. 120 mmHg). But in spite of a similar reduction of spirometric values the female patients show higher PO_2 and lower PCO_2 than the male patients like their healthy counterparts. Probably the long-known stimulation of respiratory brain centres by female

Table 4. Characteristics of the oxygen dissociation curves.

	n	P_{50}standard mmHg	Hill's n 6% CO_2
Controls male	6	26.7±0.2	2.63±0.02
Controls female	8	27.0±0.1	2.56±0.02
Patients male	8	27.7±0.5*	2.57±0.03
Patients female	6	28.0±0.3	2.61±0.03
Anova	sex		
	illness	d	

Means ± SE. Significance levels indicated as for Table 2. P_{50}standard: standard half saturation pressure calculated from the curves of blood equilibrated with 6% CO_2.*28.1±0.3 mmHg without the patient with the G551D mutation.
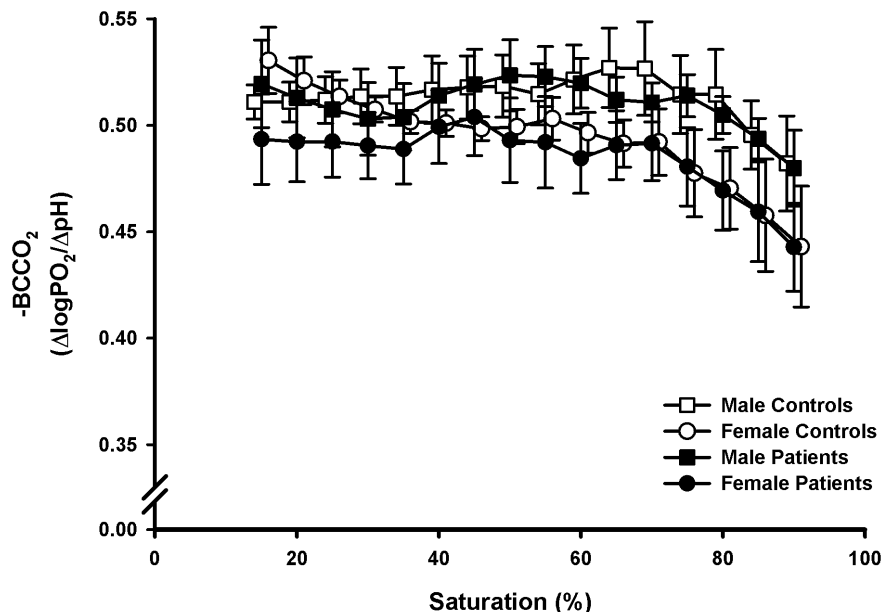doi:10.1371/journal.pone.0097932.t004
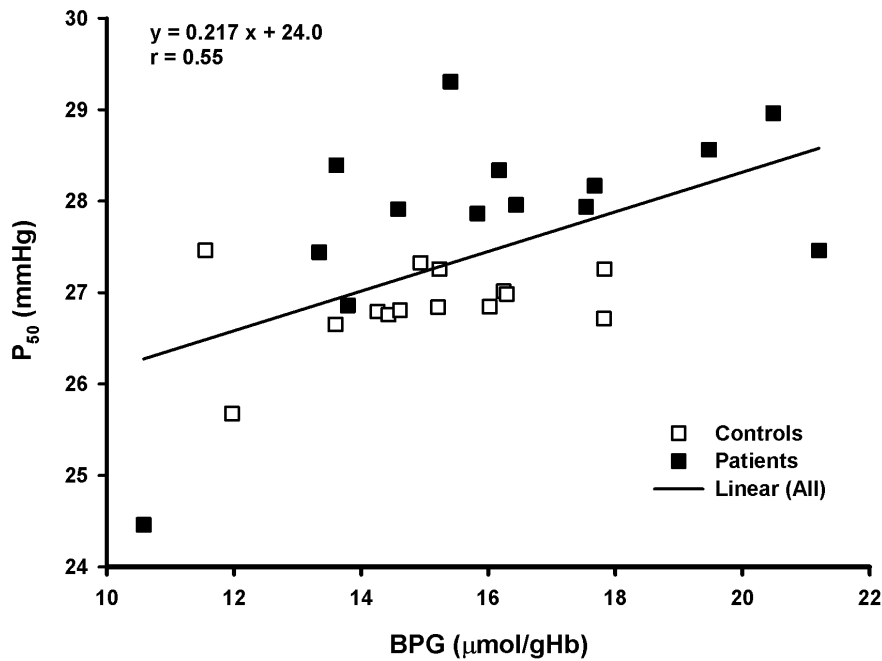
Figure 2. Dependence of P_{50}st on BPG concentration (means of equilibrated samples). Regression line for all values, correlation coefficient r different from zero (P<0.01).
doi:10.1371/journal.pone.0097932.g002

hormones important for fetal oxygen supply is the cause (reviewed in [47]). The fact that arterialized pH is equal in all subgroups in spite of differences in PCO_2 demonstrates the importance of acid-base homeostasis for physiological functions. Non-respiratory compensation is mainly done by renal excretion/reabsorption of bicarbonate. In the patients the osmotic effect of the rise of $[HCO_3^-]$ is counteracted by a decrease of $[Cl^-]$. Also the loss of chloride via sweat glands might play a role. The slightly increased in vitro buffer capacity in both male groups is obviously caused by

the higher Hb concentration. In cystic fibrosis the slight rise in bicarbonate concentration as well as the possibly increased Hb mass [21,22] help to attenuate the extracellular pH changes during exercise [48] caused by CO_2 retention.

Blood Composition

Hb concentrations showed typical sex differences but no sign of anemia in the patients. The latter might be expected in CF because of frequent problems with iron resorption. However, in

Figure 3. Saturation-dependent Bohr coefficients for acidification with CO_2 (BCCO$_2$). Means and standard errors.
doi:10.1371/journal.pone.0097932.g003
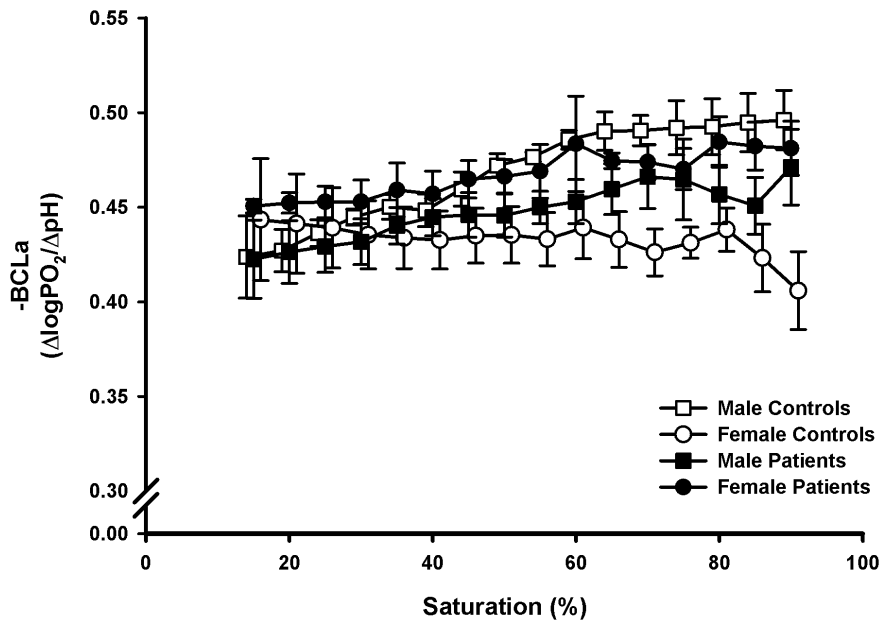
Figure 4. Saturation-dependent Bohr coefficients for acidification with lactic acid (BCLa). Means and standard errors.
doi:10.1371/journal.pone.0097932.g004

our patients iron metabolism was routinely checked and deficiency was treated. One explanation for normal [Hb] in other studies might be the counteracting effects between iron deficiency and hypoxia [49]. Christoforou et al. [19] described a negative correlation of [erythropoietin] with FVC and FEV1. Such a dependency is probably the cause of the correlation between [Hb] and FEV1 in our male patients. Some authors [21,22] have even observed an increase in red cell volume in CF probably stimulated by erythropoietin which might be explained as a typical hypoxia reaction. However, only in a fraction of the corresponding studies [19,20,50,51]http://www.ncbi.nlm.nih.gov/ pubmed?term = Mc-Colley%20SA%5BAuthor%5D&cauthor = true&cauthor_-uid = 21365780 erythropoietin concentration was increased. Own unpublished measurements support the idea of chronic stimulation of erythropoesis in CF patients based on elevated erythropoietin as well as soluble transferrin receptor concentrations in a cohort of 79 CF patients. Also a low MCHC like in the patients is often related to an increased water content typical for young erythrocytes. Furthermore in patients the high level of the soluble transferrin receptor [20] might be indicative for an increased red cell production and thus a reduced erythrocytic age. However, also a link between CFTR and the function of the hypoxia inducible factor has been put forward [52] which may serve as one potential reason for a lack of increased [Hb] in CF patients.

Factors possibly increasing [BPG] and [ATP] are low SO_2 (reducing product inhibition because of BPG binding to Hb) and alkalosis (stimulating glycolysis and thus BPG synthesis). A probable explanation for the rather small increase of [BPG] and P_{50}st in CF compared to highlanders with similarly lowered arterial SO_2 and equal pH at rest (e. g. 18 µmol/g Hb in [30]) might be the different effect of physical activity: CO_2 retention causes respiratory acidosis already during moderate physical activity in the patients while highlanders effectively hyperventilate at each exercise level. In the present patient group with normal daily life and exercise therapy physical activity was obviously a factor of some importance. Additionally a low red cell age as suggested above might lead to elevated [BPG] as well as [ATP]

because of high enzymatic activity [29]. The low [BPG] in the patient with the G551D mutation possibly results from changed enzyme activities because no differences in erythrocyte physiology were detectable. CFTR is incorporated into the red cell membrane (e. g. [53]), but a relation to BPG metabolism remains speculative.

[Cl^-] in red cells in part follows passively changes in plasma [Cl^-] and therefore is lowered in patients. Generally the marked concentration difference results from the high erythrocytic content of non-diffusible anions (Hb^- and organic phosphates) causing a Donnan equilibrium. Cl^- crosses the cell membrane mainly through band 3 channels. The reduction of the number of CFTR molecules in patients (e. g. [53]) does not affect this exchange [24]. Cl^- concurs with BPG for the same binding sites on Hb [54] but its affinity is lower and the small decrease of its concentration in CF is compensated for by increased [BPG].

Oxygen Dissociation Curves

The P_{50}st values of controls scatter around the normal mean value (approximately 27 mmHg) without significant sex differences. The generally higher P_{50}st in patients results from the increased [BPG] (change approx. 0.6 mmHg per µmole BPG/gHb according to [55]) while ATP plays only a minor role because of complexing with Mg^{++}. This corresponds to the typical chronic hypoxic reaction of most humans. It allows to extract more oxygen in the tissue capillaries without lowering the diffusion pressure, but it is not helpful for oxygen loading in pulmonary capillaries. In highlanders with similar reduction of arterial SO_2 P_{50}st scatters around 30 mmHg [30]. In both healthy subjects and patients the reaction (affinity change, increased ventilation and partly stimulated erythropoesis) is a sufficient compensation of moderate hypoxia at rest but maximal performance capacity is reduced. The left shift of the ODC in moles [15] living and working under comparable conditions (inspiring air with reduced O_2 and increased CO_2 content) as the patients is more reasonable but is rare in humans. Under extreme acute conditions (above 6000 m of altitude) healthy mountaineers lower their P_{50} by extreme hyperventilation [56] which is not a sustainable option for CF-

Table 5. Blood gases, acid-base status and P_{50} of patients at maximal exercise.

	n	Powermax	PO_{2art}	SO_{2art}	PCO_{2art}	pH_{art}	[Lactate]	P_{50}
		Watt/kg	mmHg	%	mmHg		mmol/l	mmHg
Patients male	7	2.2±0.3	58.9±5.4	80.1±3.1	48.3±4.0	7.240±0.018	7.9±1.1	33.1±0.9
Patients female	5	1.8±0.1	55.6±2.1	82.6±4.0	51.2±3.6	7.227±0.031	8.8±0.6	33.7±0.9

Means ± SE. Measurements in ear lobe blood. P_{50} for actual pH. All differences to rest significant for all patients as well as subgroups except SO_2 for subgroup patients female (P<0.054).
doi:10.1371/journal.pone.0097932.t005
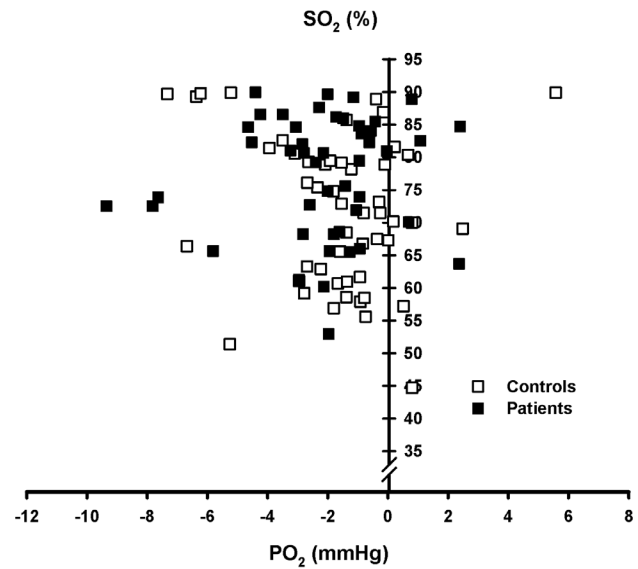
Figure 5. Deviation of in vivo PO_2 corrected to pH 7.4 from the corresponding individual standard ODC between 40 and 90% in controls and patients.
doi:10.1371/journal.pone.0097932.g005

patients. For chronic acclimatization a reduction of [BPG] would be more appropriate. Surprisingly the male patient with the G551D mutation showed such an effect. One can estimate that a reduction of $P_{50}st$ like in his blood would raise the arterial SO_2 at exhaustion in the male patients by 4%.

Interestingly in the 3 papers with $P_{50}st$ measurements in CF patients single low $P_{50}st$ values between 23.5 and 25.5 mmHg can be found [18,19,23]. This points to a special form of hypoxia acclimatization in some patients similar to that in moles.

The magnitude of a change in $P_{50}st$ may reflect further compensating mechanisms. Rosenthal et al [18] showed that $P_{50}st$ is negatively correlated with systemic oxygen delivery which depends on arterial SO_2, [Hb] and cardiac output. This means that low [Hb] or cardiac output favor a rise of $P_{50}st$. Indeed Arturson [57] described a $P_{50}st$ increase with falling [Hb] in chronic pulmonary insufficiency. The present CF patients were not anemic. This might also explain why we observed a small tendency rather than a substantial change in $P_{50}st$.

Hill's n did not deviate much from the usually expected value of 2.7 for HbA (e.g. [55]). BPG binds to Deoxy-Hb only which may therefore increase Hill's n with rising concentration. A sligtly higher n in highlanders [30] and anemic patients [58] might be explained by this mechanism. However, the [BPG] differences between controls and patients in this study are too small to cause measurable effects.

Bohr Effect and Exercise

Similar like in altitude inhabitants [30,31] the Bohr coefficients are little changed in patients with cystic fibrosis. The coefficients for CO_2 correspond to published values [27,28,33]. They are large (numerically) at low saturation because of oxygenation dependent binding of carbamate in addition to H^+ effects during acidification with CO_2. They are lower at very high saturation; the Bohr effect disappears when all Hb molecules are in the R (relaxed) state. Anions (Cl^-, La^- and BPG) compete with CO_2 at the terminal valines (e. g. [25]). Therefore $BCCO_2$ increases at low [BPG]; this might be the cause for the rather high value in the patient with mutation G 551D. The fixed acid Bohr coefficients are small

especially at low saturation compared to $BCCO_2$. The slight unexplained influence of CF on BCLa plays only a very modest role, because the peak lactic acid concentration during exercise is rather low in the patients (Table 5) compared to healthy subjects (e. g. [59]). In the lungs the Bohr effect of CO_2 is helpful for oxygen loading, when CO_2 leaves the blood, especially during hyperventilation with resulting hypocapnia during heavy exercise. In the patients with exercise hypercapnia and mostly high P_{50}st, however, an increase of $BCCO_2$ would be detrimental in this situation.

In vivo Effects

The left shift of the in vivo PO_2/SO_2 pairs relative to the in vitro standard ODCs is on an average modest (approx. 2 mmHg) but 17 differences amount to more than 4 mmHg. Differences in ODCs as well as BCs between fresh blood immediately after sampling and blood after equilibration in tonometers have occasionally been observed (e. g. [38,60]). Concentration changes of BPG, ATP, Cl^-, nitrocompounds or glutathione are possible causes. The means of [BPG] and [ATP] increase slightly but not significantly after equilibration compared to fresh venous samples explaining only 0.7 mmHg of the difference at 50% SO_2. Intraerythrocytic [Cl^-] changes are larger for a given ΔpH in vivo than in vitro resulting from exchange with the interstitial fluid [59]. Because of the opposite effects of SNO-Hb and Hb[FENO] on oxygen affinity [61] NO usually exerts no measurable influence on the ODC neither in vitro nor in vivo if no methemoglobin is formed [62–65]. In our experiments MetHb was stable. For an allosteric effect intraerythrocytic [NO] is by far too low even in Tibetans who present very high values [66]. In contrast to NO glutathione is present in millimolar concentrations in the red cells [67] and binds to oxy-Hb thus shifting the curve to the left and reducing the Bohr effect [26,68]. But a marked deficiency of extra- and intracellular glutathione possibly including erythrocytes in CF patients has been suggested [69] which might be related to the disturbed function of CFTR as glutathione transporter [52]. Very recently also an effect of glutamate on P50 was observed [70]; this substance binds to Ca^{++} channel proteins in the cell membrane and may be interchanged with muscle fibres. Thus at the moment a clear cause for the in vivo – in vitro difference of PO_2 remains unknown, but apparently it rises with

SO_2. This produces a left shift of the in vivo ODC between 50 and 90% SO_2, which is an advantage for oxygen loading. Recently similar results were found at 3600 m of altitude [13,14]. Interestingly at low saturations "standardized" in vivo PO_2/SO_2 pairs tend to lie right of the in vitro curve, especially in venous blood returning from exercising muscles [36–38,55,60]. The result of this opposite changes is a markedly steepened complete in vivo oxygen dissociation curve probably in healthy subjects as well as in CF patients. Such a property is favorable for both loading and unloading of O_2 in lungs and consuming tissues.

Conclusions

The majority of the patients with cystic fibrosis in our study react to the problem of pulmonary oxygen uptake like man at altitude with a small right shift of the in vitro ODC caused by increased organic phosphate concentrations in the red cells. This improves oxygen diffusion into the consuming tissues, but is a drawback for arterialization. Healthy subjects can compensate this by hyperventilation thus reducing arterial PCO_2 with resulting left shift of the ODC during oxygenation. This is not possible for CF patients especially when CO_2 production is increased during exercise. A probably more appropriate left shift by reduction of [BPG] was observed in one patient with the G551D mutation. Also in other papers occasional left shifts can be detected. Whether this is a genetic effect, remains an intriguing question. The slope of the in vitro ODC and the Bohr coefficients were not markedly affected by the disease. Under in vivo conditions, there is a tendency for a left shift of the upper part of the ODC in both healthy controls and patients pointing to unknown affinity modifying factors which improve oxygen loading in the lungs.

Author Contributions

Conceived and designed the experiments: DB MH RB DS. Performed the experiments: AL MH RB. Analyzed the data: DB AL MH RB DS. Wrote the paper: DB AL MH RB DS. Designed the methods used for measurement and evaluation of oxygen dissociation curves: DB MH.

References

1. Cohen TS, Prince A (2012) Cystic fibrosis: a mucosal immunodeficiency syndrome. Nat Med 18: 509–519.
2. Zielenski J (2000) Genotype and phenotype in cystic fibrosis. Respiration 67: 117–133.
3. Derichs N (2013) Targeting a genetic defect: cystic fibrosis transmembrane conductance regulator modulators in cystic fibrosis. Eur Respir Rev 22: 58–65.
4. Baumann R, Bartels H, Bauer C (1987) Blood oxygen transport. In: Fishman AP, Cherniak NS, Widdicombe JG, Geiger SR, editors. Handbook of Physiology Sect. 3. Washington DC: Am. Physiol. Soc. 147–172.
5. Samaja M, Crespi T, Guazzi M, Vandegriff KD (2003) Oxygen transport in blood at high altitude: role of the hemoglobin-oxygen affinity and impact of the phenomena related to hemoglobin allosterism and red cell function. Eur J Appl Physiol 90: 351–359.
6. Weber RE (2007) High-altitude adaptations in vertebrate hemoglobins. Respir Physiol Neurobiol 158: 132–142.
7. Storz JF, Moriyama H (2008) Mechanisms of hemoglobin adaptation to high altitude hypoxia. High Alt Med Biol 9: 148–157.
8. Winslow RM (2007) The role of hemoglobin oxygen affinity in oxygen transport at high altitude. Respir Physiol Neurobiol 158: 121–127.
9. Morpurgo G, Arese P, Bosia A, Pescarmona GP, Luzzana M, et al. (1976) Sherpas living permanently at high altitude: a new pattern of adaptation. Proc Nat Acad Sci USA 73: 747–751.
10. Beall CM, Decker MJ, Brittenham GM, Kushner I, Gebremedhin A, et al. (2002) An Ethiopian pattern of human adaptation to high-altitude hypoxia. Proc Natl Acad Sci U S A 99: 17215–17218.
11. Beall CM (2007) Two routes to functional adaptation: Tibetan and Andean high-altitude natives. Proc Natl Acad Sci U S A 104 Suppl 1: 8655–8660.
12. Jürgens KD, Pietschmann M, Yamaguchi K, Kleinschmidt T (1988) Oxygen binding properties, capillary densities and heart weights in high altitude camelids. J Comp Physiol B 158: 469–477.
13. Balaban DY, Duffin J, Preiss D, Mardimae A, Vesely A, et al. (2013) The in-vivo oxyhaemoglobin dissociation curve at sea level and high altitude. Respir Physiol Neurobiol 186: 45–52.
14. Böning D, Pries AR (2013) Unknown in vivo factors influencing the oxygen dissociation curve? Respir Physiol Neurobiol 188: 79–80.
15. Campbell KL, Storz JF, Signore AV, Moriyama H, Catania KC, et al. (2010) Molecular basis of a novel adaptation to hypoxic-hypercapnia in a strictly fossorial mole. BMC Evol Biol 10: 214.
16. Huckauf H, Schäfer JH, Kollo D (1976) Oxygen affinity of haemoglobin and red cell acid-base status in patients with severe chronic obstructive lung disease. Bull Europ Physiopath Resp 12: 129–142.
17. Morgan TJ (1999) The oxyhaemoglobin dissociation curve in critical illness. Crit Care Resusc 1: 93–100.
18. Rosenthal A, Khaw KT, Shwachman H (1977) Hemoglobin-oxygen equilibrium in cystic fibrosis. Pediatrics 59: 919–926.
19. Christoforou E, Papassotiriou I, Skarmoutsou C, Doudounakis S, Stamoulakatou A, et al. (2005) Soluble transferrin receptors and tissue oxygenation in non-anaemic cystic fibrosis patients. J Cyst Fibros 4: 151–156.
20. Vichinsky EP, Pennathur-Das R, Nickerson B, Minor M, Kleman K, et al. (1984) Inadequate erythroid response to hypoxia in cystic fibrosis. J Pediatr 105: 15–21.
21. Rosenthal A, Button LN, Khaw KT (1977) Blood volume changes in patients with cystic fibrosis. Pediatrics 59: 588–594.

22. Wagener JS, McNeill GC, Taussig LM, Corrigan JJ, Lemen R (1983) Ferrokinetic and hematologic studies in cystic fibrosis patients. Am J Pediatr Hematol Oncol 5: 153–160.

23. Orzalesi MM, Motoyama EK (1973) Blood oxygen affinity in children with cystic fibrosis. Am Rev Respir Dis 107: 928–931.

24. Berghout AG, Bender SW (1984) Anion transport and 2,3-diphosphoglycerate in cystic fibrosis red blood cells. Pediatr Res 18: 1017–1020.

25. Böning D, Schünemann HJ, Maassen N, Busse MW (1993) Reduction of oxylabile CO_2 in human blood by lactate. J Appl Physiol 74: 710–714.

26. Craescu CT, Poyart C, Schaeffer C, Garel MC, Kister J, et al. (1986) Covalent binding of glutathione to hemoglobin. II. Functional consequences and structural changes reflected in NMR spectra. J Biol Chem 261 (31): 14710–14716.

27. Garby L, Robert M, Zaar B (1972) Proton- and carbamino-linked oxygen affinity of normal human blood. Acta Physiol Scand 84: 482–492.

28. Meier U, Böning D, Rubenstein HJ (1974) Oxygenation dependent variations of the Bohr coefficient related to whole blood and erythrocyte pH. Pflügers Arch 349: 203–213.

29. Schmidt W, Böning D, Braumann KM (1987) Red cell age effects on metabolism and oxygen affinity in humans. Respir Physiol 68: 215–225.

30. Schmidt W, Dahners HW, Correa R, Ramirez R, Rojas J, et al. (1990) Blood gas transport properties in endurance-trained athletes living at different altitudes. Int J Sports Medicine 11: 15–21.

31. Morpurgo G, Battaglia P, Bernini L (1970) Higher Bohr effect in Indian natives of Peruvian Highlands as compared with Europeans. Nature (Lond) 227: 387–388.

32. Humpeler E, Vogel S (1977) Oxygen affinity of hemoglobin in postmenopausal women. Pflügers Arch 372: 287–290.

33. Böning D, Draude W, Trost F, Meier U (1978) Interrelation between Bohr and temperature effects on the oxygen dissociation curve in men and women. Respir Physiol 34: 195–207.

34. Humpeler E, Amor H (1973) Sex differences in the oxygen affinity of hemoglobin. Pflügers Arch 343: 151–156.

35. Morse M, Cassels DE, Holder M, O'Connell E, Swanson A (1950) The position of the oxygen dissociation curve of the blood in normal children and adults. J Clin Invest 29: 1091–1097.

36. Rasmussen B, Klausen K, Clausen JP, Trap-Jensen J (1975) Pulmonary ventilation, blood gases and blood pH after training of the arms or the legs. J Appl Physiol 38: 250–256.

37. Sproule BJ, Mitchell JH, Miller WF (1960) Cardiopulmonary physiological responses to heavy exercise in patients with anemia. J Clin Invest 39: 378–388.

38. Böning D, Schweigart U, Tibes U, Hemmer B (1975) Influences of exercise and endurance training on the oxygen dissociation curve of blood unter in vivo and in vitro conditions. Eur J Appl Physiol 34: 1–10.

39. Barrett PM, Alagely A, Topol EJ (2012) Cystic fibrosis in an era of genomically guided therapy. Hum Mol Genet 21: R66-R71.

40. Quanjer PH, Tammeling GJ, Cotes JE, Fabbri LM, Matthys H, et al. (1993) Symbols, abbreviations and units. Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Eur Respir J Suppl 16: 85–100.

41. Hughes JM (1996) Blood gas estimations from arterialized capillary blood versus arterial puncture: are they different? Eur Respir J 9: 184–185.

42. Ben Rayana MC, Burnett RW, Covington AK, D'Orazio P, Fogh-Andersen N, et al. (2008) IFCC guideline for sampling, measuring and reporting ionized magnesium in plasma. Clin Chem Lab Med 46: 21–26.

43. Böning D, Tibes U, Schweigart U (1976) Red cell hemoglobin, hydrogen ion and electrolyte concentrations during exercise in trained and untrained subjects. Eur J Appl Physiol 35: 243–249.

44. Diem K, Lentner C (1969) Wissenschaftliche Tabellen. Geigy, AG, Basel.

45. Uhle MJ (2004) Zur Nutzbarkeit ventilatorischer Messverfahren in der Diagnostik der submaximalen Leistungsfähigkeit bei Patienten mit Zystischer Fibrose (About the usability of ventilatory measuring methods in diagnosis of submaximal exercise performance capacity in patients with cystic fibrosis) [dissertation]. Charité - Universitätsmedizin Berlin.

46. Böning D, Rojas J, Serrato M, Ulloa C, Coy L, et al. (2001) Hemoglobin mass and peak oxygen uptake in untrained and trained residents of moderate altitude. Int J Sports Med 22: 572–578.

47. Cristancho E, Reyes O, Serrato M, Mora MM, Rojas JA, et al. (2007) Arterial oxygen saturation and hemoglobin mass in postmenopausal untrained and trained altitude residents. High Alt Med Biol 8: 296–306.

48. Böning D, Rojas J, Serrato M, Reyes O, Coy L, et al. (2008) Extracellular pH defense against lactic acid in untrained and trained altitude residents. Eur J Appl Physiol 103: 127–137.

49. O'Connor TM, McGrath DS, Short C, O'Donnell MJ, Sheehy M, et al. (2002) Subclinical anaemia of chronic disease in adult patients with cystic fibrosis. J Cyst Fibros 1: 31–34.

50. Fischer R, Simmerlein R, Huber RM, Schiffl H, Lang SM (2007) Lung disease severity, chronic inflammation, iron deficiency, and erythropoietin response in adults with cystic fibrosis. Pediatr Pulmonol 42: 1193–1197.

51. Watts KD, McColley SA (2011) Elevated vascular endothelial growth factor is correlated with elevated erythropoietin in stable, young cystic fibrosis patients. Pediatr Pulmonol 46: 683–687.

52. Duranton C, Rubera I, Cougnon M, Melis N, Chargui A, et al. (2012) CFTR is involved in the fine tuning of intracellular redox status: physiological implications in cystic fibrosis. Am J Pathol 181: 1367–1377.

53. Schillers H (2008) Imaging CFTR in its native environment. Pflügers Arch 456: 163–177.

54. Rollema HS, De Bruin SA, Janssen LHM, Van Os GAJ (1975) Effect of potassium chloride on Bohr effect of human hemoglobin. J Biol Chem 250: 1333–1339.

55. Braumann KM, Böning D, Trost F (1979) Oxygen dissociation curves in trained and untrained subjects. Eur J Appl Physiol 42: 51–60.

56. West JB, Schoene RB, Milledge JS (2007) High Altitude Medicine and Physiology. London: Hodder Arnold.

57. Arturson G (1971) Changes in blood oxygen affinity during anaemia and cardiac and pulmonary insufficiency. Acta Anaesth Scand 45: 39–44.

58. Böning D, Enciso G (1987) Hemoglobin-oxygen affinity in anemia. Blut 54: 361–368.

59. Böning D, Klarholz C, Himmelsbach B, Hütler M, Maassen N (2007) Causes of differences in exercise-induced changes of base excess and blood lactate. Eur J Appl Physiol 99: 163–171.

60. Braumann KM, Böning D, Trost F (1982) Bohr effect and slope of the oxygen dissociation curve after physical training. J Appl Physiol Respir Environ Exerc Physiol 52: 1524–1529.

61. Mairbäurl H, Weber RE (2013) Oxygen transport by hemoglobin. Compr Physiol 2 : 1463–1489.

62. Head CA, Brugnara C, Martinez-Ruiz R, Kacmarek RM, Bridges KR, et al. (1997) Low concentrations of nitric oxide increase oxygen affinity of sickle erythrocytes in vitro and in vivo. J Clin Invest 100: 1193–1198.

63. Hrinczenko BW, Alayash AI, Wink DA, Gladwin MT, Rodgers GP, et al. (2000) Effect of nitric oxide and nitric oxide donors on red blood cell oxygen transport. Br J Haematol 110: 412–419.

64. von Pickardt B (2003) Wirkung NO-freisetzender Verbindungen auf die Sauerstoffbindungskurve (Effect of NO liberating substances on the oxygen dissociation curve) [dissertation]. Freie Universität Berlin.

65. Gladwin MT, Schechter AN, Shelhamer JH, Pannell LK, Conway DA, et al. (1999) Inhaled nitric oxide augments nitric oxide transport on sickle cell hemoglobin without affecting oxygen affinity. J Clin Invest 104: 937–945.

66. Erzurum SC, Ghosh S, Janocha AJ, Xu W, Bauer S, et al. (2007) Higher blood flow and circulating NO products offset high-altitude hypoxia among Tibetans. Proc Natl Acad Sci U S A 104: 17593–17598.

67. Hütler M, Pollmann C, Beneke R, Leithauser R, Böning D (2000) Measurable amount of glutathione in blood is influenced by oxygen saturation of hemoglobin. Clin Chim Acta 301: 213–217.

68. Smith JE, Lee M, Agar S, Du D (1976) Oxygen-hemoglobin equilibrium of normal and glutathione- deficient sheep. Am J Vet Res 37: 1135–1137.

69. Hudson VM (2001) Rethinking cystic fibrosis pathology: the critical role of abnormal reduced glutathione (GSH) transport caused by CFTR mutation. Free Radic Biol Med 30: 1440–1461.

70. Makhro A, Hanggi P, Goede JS, Wang J, Bruggemann A, et al. (2013) N-methyl-D-aspartate receptors in human erythroid precursor cells and in circulating red blood cells contribute to the intracellular calcium regulation. Am J Physiol Cell Physiol 305: C1123-C1138.

71. Christiansen TF (1981) An algorithm for calculating the concentration of the base excess of blood. In: Siggaard-Andersen O, editors. Proceedings of the IFCC Expert Panel on pH and blood gases held at Herved Hospital 1980. Copenhagen: Radiometer A/S. 77–81.