



Effects of Carbon Dioxide and Temperature on the Oxygen-Hemoglobin Dissociation Curve of Human Blood: Implications for Avalanche Victims

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Completely avalanche-buried patients are frequently exposed to a combination of hypoxia and hypercapnia with a risk of normothermic cardiac arrest. Patients with a long burial time and an air pocket are exposed to a combination of hypoxia, hypercapnia, and hypothermia which may lead to the development of the “triple H syndrome”. This specific combination has several pathophysiological implications, particularly on the cardiovascular system and oxygen transport (oxygen supply and oxygen consumption). To examine the effects on hemoglobin oxygen affinity, we investigated venous blood samples from 15 female and 15 male healthy subjects. In a factorial design of four different carbon dioxide partial pressure (PCO₂) levels (20, 40, 60, and 80 mmHg) and five different temperature levels (13.7°C, 23°C, 30°C, 37°C, and 42°C), 30 unbuffered whole blood samples were analyzed in a newly developed *in vitro* method for high-throughput oxygen dissociation curve (ODC) measurements. P50s, Hill coefficients, CO₂-Bohr coefficients, and temperature coefficients were analyzed using a linear mixed model (LMM). Mean P50 at baseline (37°C, 40 mmHg PCO₂) was 27.1 ± 2.6 mmHg. Both CO₂-Bohr ($p < 0.001$) and temperature coefficients ($p < 0.001$) had a significant effect on P50. The absolute CO₂ effect was still pronounced at normothermic and febrile temperatures, whereas at low temperatures, the relative CO₂ effect (expressed by CO₂-Bohr coefficient; $p < 0.001$, interaction) was increased. The larger impact of PCO₂ on oxygen affinity at low temperature may be caused by the competition of 2,3-BPG with PCO₂ and the exothermic binding characteristic of 2,3-BPG. In a model of an avalanche burial, based on published data of CO₂ levels and cooling rates, we calculated the resulting P50 for this specific condition based on the here-reported PCO₂ and temperature effect on ODC. Depending on the degree of hypercapnia and hypothermia, a potentially beneficial increase in hemoglobin oxygen affinity in the hypoxic condition might ensue.

Keywords: oxygen affinity, oxygen dissociation curve, carbon dioxide, temperature, avalanche burial, hypoxia, hypothermia, hypercapnia

INTRODUCTION

A completely avalanche-buried patient who is still able to breathe into an air pocket (any space in front of mouth and nose) may cool down during a long burial time and suffer from cardiovascular and respiratory changes that are not exclusively explainable by the effect of decreased core temperature. In the early phase of burial, acute hypoxia is associated with high carbon dioxide (CO₂) levels due to rebreathing exhaled air (dead space ventilation) (1, 2). Without a sufficient supply of O₂ and removal of CO₂, hypoxia and hypercapnia increase the risk of normothermic cardiac arrest. After a long burial time but with sufficient supply or removal of respiratory gases, the patient is exposed to a combination of hypoxia, hypercapnia, and hypothermia (3). This combination of hypoxia, hypercapnia, and hypothermia has been defined as the triple H syndrome (1). Experimental studies showed that when human participants breathed into snow air pockets, the inspiratory CO₂-fraction rapidly increased and stabilized at a level of approximately 5–6% of CO₂ (1, 4, 5). Despite hypercapnia can speed up the cooling rate (6), the combination of hypothermia with hypoxia and hypercapnia may result in a worse neurological outcome in completely avalanche-buried patients compared to patients suffering solely from hypothermic cardiac arrest (7–9). Such outcome is determined by the temporal sequence of the events, as a decrease in oxygen supply and CO₂ removal usually proceed the development of hypothermia.

The oxygen dissociation curve (ODC) describes the reversible binding of four molecules of oxygen to Hb (10–12). The ODC is usually described by the P50 value, that is, the value of O₂ partial pressure, PO₂ at which 50% of Hb is saturated with oxygen, and by the Hill coefficient (HC), a parameter that describes maximum steepness in the Hill plot and that reflects the cooperativity of ligand binding. Temperature, pH, 2,3-bisphosphoglycerate (2,3-BPG), and PCO₂ are the four main factors that affect the ODC. Based on Severinghaus' equation, ODC should be corrected for the effects of the different factors (11). Specifically, exposing blood to CO₂ results in a decrease of oxygen affinity *via* two ways, first a decrease in pH resulting in a decrease of oxygen affinity (Bohr effect) and second a direct binding to the oxygen-linked CO₂ binding site and carbamino-Hb formation (CO₂ effect) (12, 13). The combined effect with temperature as another independent factor can result in a combination of three of the four main effectors to the ODC. However, the effects of the association of hypercapnia with hypothermia on the oxygen cascade, that is, uptake, transportation, and delivery of oxygen to tissues, defined by the hemoglobin (Hb) oxygen affinity, are not fully understood. Interactions of CO₂ and temperature may affect cellular oxygenation *via* several different pathways. In blood, the solubility of O₂ and CO₂ is affected by temperature and pH. In the tissues and at a cellular level, oxygen consumption and CO₂ and also metabolic acid production decrease at lower temperatures. The possible interaction of CO₂-Bohr coefficient (CO₂-BC), a function of P50 CO₂ dependency, with temperature coefficient (TC), a function of P50 temperature dependency, was studied with contradictory results in humans and animals (14–16).

The aim of this study was to describe single and combined effects of CO₂ and temperature on the ODC in unbuffered whole blood to better understand the pathophysiology of oxygen transport in pathophysiological situations characterized by changes in temperature and levels of respiratory gases.

METHODS

This study was approved by the Ethical Board of the Medical University of Innsbruck (vote nr. 1123/2019) and is registered with clinicaltrials.gov (NCT04041531). Written informed consent was obtained from all participants.

Inclusion criteria were the age between 18 and 40 years, no recent history of acute or chronic illness, blood loss, or sojourn at high altitude (>3,000 m a.s.l.) within the past 28 days. All participants were at fasting (6 h) before blood collection. Blood was taken from nonsmoking volunteers from an antecubital vein with a minimum of stasis period. The blood samples were split into two aliquots: one was immediately analyzed by a blood gas analyzer (ABL 800 flex, Radiometer, Denmark), and another aliquot of the sample was placed on ice for the ODC experiment and analyzed within 5 h.

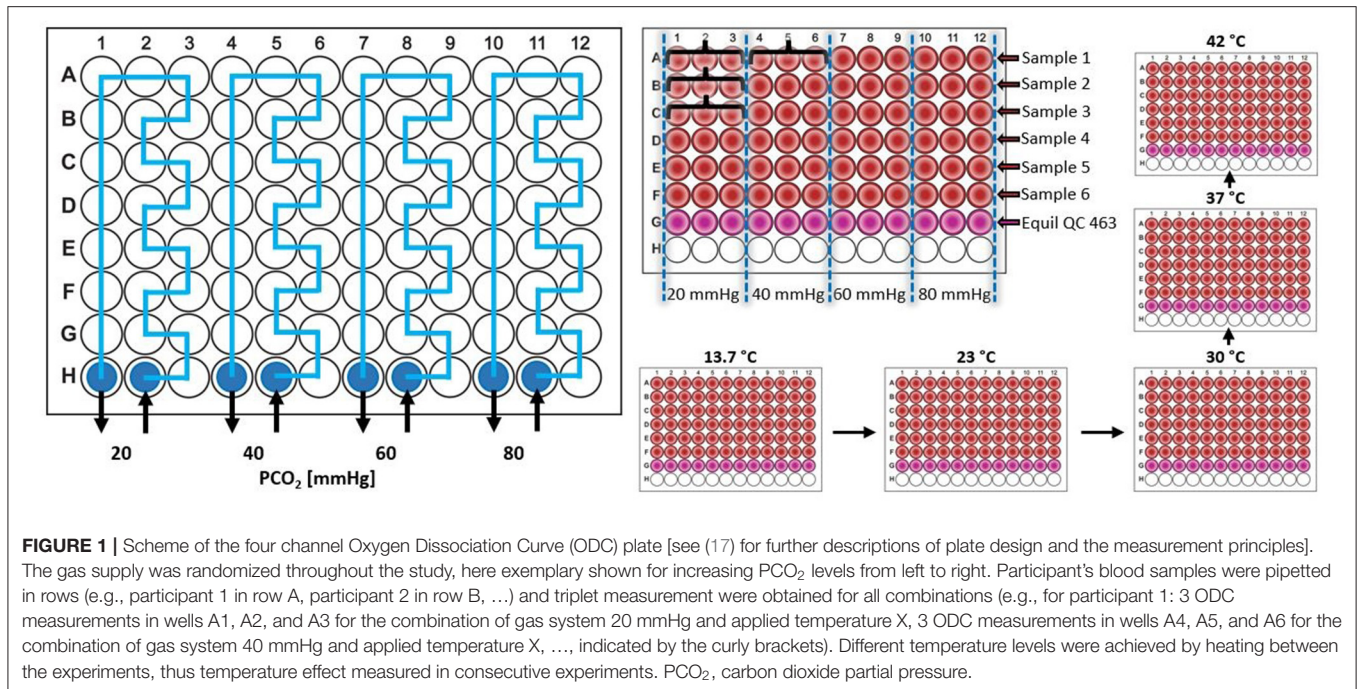
ODC Experiments

Oxygen dissociation curve experiments were performed with a modification of a recently published method (17). In this method, PO₂ is continuously decreased from 140 mmHg to 0 mmHg, and data points of PO₂ and SO₂ are acquired every minute to record the ODC. Within the study setting, different aliquots of the same sample can be simultaneously exposed up to four different gas mixtures. Specifically, four different PCO₂ levels (20, 40, 60, and 80 mmHg) were applied and combined in consecutive measurements with five different temperatures (13.7, 23, 30, 37, and 42°C) that were chosen based on previously publications related to hypothermia and avalanche burial, to maximize comparability and clinical relevance (14–16, 18–20). To quantify the relative effect of temperature (TC) and CO₂ (CO₂-BC) on P50, coefficients were calculated [Eqs. 1, 2; (12)].

$$Eq.1 : CO_2 - BC = \frac{\Delta \log_{10} P50}{\Delta \log_{10} PCO_2}$$

$$Eq.2 : TC = \frac{\Delta \log_{10} P50}{\Delta temperature}$$

In a 4 × 5 factorial design, every blood sample was exposed to all 20 combinations of four PCO₂ levels and five temperature levels, starting with the lowest temperature level, followed by heating the entire experimental setup to the next higher temperature level. To address the increase of water vapor saturation pressure with temperature, gas mixtures were prepared volumetrically from dry, pure gases (CO₂, N₂ and O₂) in gas-tight sampling bags, according to the Magnus equation (21). The temperature management of the fluorescence plate reader instrument (Tecan 200 Infinite Pro, Tecan Genios Corp., Switzerland) ensured correct temperature in the measurement chamber, whereas temperature of the conducting Viton tubes, gas mixtures, humidifiers, and temperature of the whole experimental setup



was monitored and strictly controlled. The supply of the four different gas mixtures to the four gas systems in the four-channel ODC plate was randomized (Figure 1). Up to six participant's blood samples were analyzed per experiment (rows). The average of triplet measurements for every combination and sample (Figure 1) was used for analysis. Additionally, an internal standard Hb solution (Equil QC 463 Level 2, RNA medical, USA) was analyzed in each test to check and adjust for accuracy of the measurements as described before (17).

Statistical Analysis

Student's *t*-test was used to detect sex differences in P50 and HC at baseline. Factors that affect P50, HC, CO₂-BC, and TC were analyzed by means of a linear mixed model (LMM). The factors investigated were the following.

- For P50 and HC: sex, age (two levels: <30 and ≥30 years), temperature (13.7, 23, 30, 37, and 42°C), PCO₂ (20, 40, 60, and 80 mmHg), and interactions of temperature with PCO₂, sex with temperature, sex with PCO₂, and sex with age.
- For CO₂-BC: sex, age, temperature, and interactions of sex with temperature and sex with age.
- For TC: sex, age, PCO₂, and interactions of sex with PCO₂ and sex with age.

The covariance structure for the residuals of the LMM was chosen by means of the Schwarz's Bayesian Criterion among diagonal, compound symmetry, unstructured and first-order autoregression. The Holm-Bonferroni method was used to correct *p*-values for multiple comparisons. Considering ΔT as difference of temperature from 37°C (baseline temperature), ΔPCO₂ as difference of PCO₂ from 40 mmHg (baseline PCO₂), and ΔP50 as difference from P50 at 37°C and 40 mmHg, a LMM was also performed to calculate an equation to predict ΔP50

TABLE 1 | Results of blood gas analysis for women and men as mean ± standard deviation (Student's *t*-test).

	All	Women	Men	<i>p</i> -value
pH	7.36 ± 0.03	7.37 ± 0.04	7.35 ± 0.02	0.070
PCO ₂ [mmHg]	46.5 ± 6.5	42.7 ± 5.8	50.6 ± 4.8	<0.001
HCO ₃ [mmol/l]	23.6 ± 1.5	22.9 ± 1.0	24.3 ± 1.6	0.006
Hb [g/dl]	14.9 ± 1.5	13.7 ± 0.8	16.0 ± 1.0	<0.001
Hct [%]	45.6 ± 4.5	42.0 ± 2.4	49.0 ± 3.1	<0.001
Cl ⁻ [mmol/l]	106.8 ± 1.9	107.7 ± 1.5	106.1 ± 1.9	0.017
Glucose [mg/dl]	94.4 ± 6.8	91.9 ± 7.5	95.9 ± 4.6	0.094
Lactate [mg/dl]	9.9 ± 5.4	8.9 ± 5.1	10.9 ± 5.8	0.326
COHb [%]	1.4 ± 0.6	1.4 ± 0.5	1.4 ± 0.6	0.926
MetHb [%]	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.112

PCO₂, carbon dioxide partial pressure; HCO₃, bicarbonate; Hb, hemoglobin concentration; Hct, hematocrit; Cl⁻, chloride concentration; COHb, carboxyhemoglobin; MetHb, methemoglobin.

from ΔT and ΔPCO₂. For the calculation, ΔT and ΔPCO₂ were inserted in the LMM as covariates in a model without intercept.

Data are shown as mean ± standard deviation, whereas LMM estimated means as mean (95% confidence interval; CI). *p* < 0.05 (two-sided) was considered statistically significant. Excel (Microsoft Office 2016, Microsoft Corp.) and SPSS version 25 (IBM Corp., Armonk, NY, USA) were used for data collection, calculation, and analysis.

RESULTS

Thirty participants (15 women and 15 men) were recruited and 30 blood samples were analyzed. Participants' mean age was 30.6 ± 3.9 years. Results of blood gas analysis, measured immediately

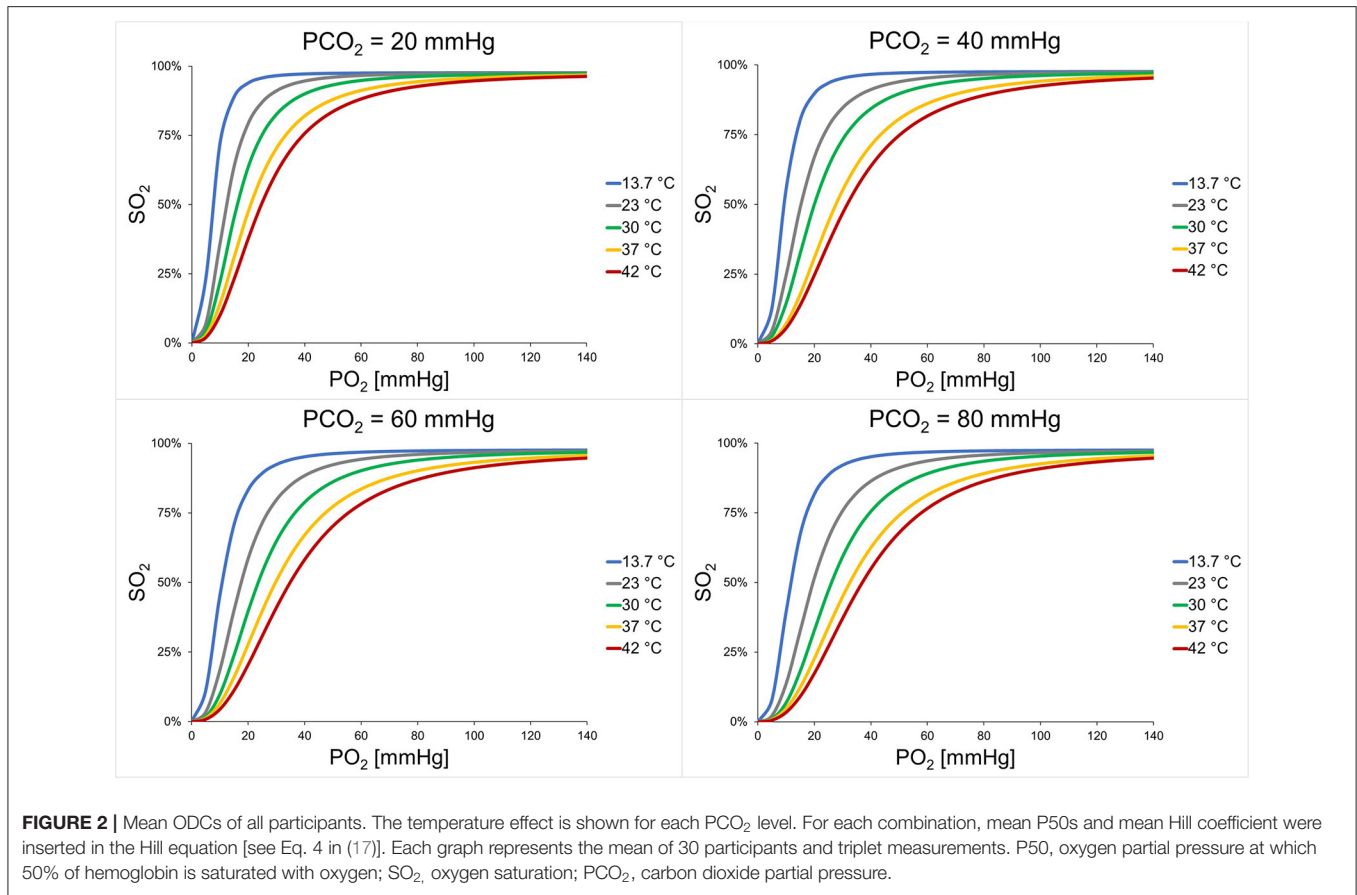


TABLE 2 | *p*-Values of the factors inserted in the LMMs.

Dependent variable	Intercept	Sex	Age	Temperature	PCO ₂	Temperature * PCO ₂	Sex * Temperature	Sex * PCO ₂	Sex * Age
P50	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	1.000	1.000
Temperature coefficient	<0.001	0.576	1.000	–	0.034	–	–	1.000	0.543
CO ₂ -Bohr coefficient	<0.001	1.000	0.063	<0.001	–	–	0.554	–	1.000
Hill coefficient	<0.001	0.002	1.000	<0.001	<0.001	0.002	1.000	0.096	1.000

The *p*-values are corrected by means of the Holm–Bonferroni method. P50, oxygen partial pressure at which 50% of hemoglobin is saturated with oxygen; PCO₂, carbon dioxide partial pressure. * An asterisk between two factors indicates the effect of interaction of the two factors. - A dash indicates that the factor was not inserted in the LMM.

after blood sampling, are shown in **Table 1**. At baseline (37°C and 40 mmHg PCO₂), P50s in women were not significantly higher than in men (28.0 ± 2.8 and 26.2 ± 2.2 mmHg; *p* = 0.061) and HCs were 2.53 ± 0.14 and 2.56 ± 0.16 (*p* = 0.641), respectively.

Factors Affecting P50

Mean ODCs in relation to different PCO₂ and temperature levels are shown in **Figure 2**. A significant effect of PCO₂, temperature, and sex on P50 could be detected (*p* < 0.001 for all parameters; **Table 2**). The effect of the interaction of temperature and PCO₂ on P50 was also significant (*p* < 0.001). At temperatures lower than 37°C, PCO₂ increased P50 in an almost linear relationship, whereas at higher temperatures, this linearity was lost (**Figure 3**). An effect of age and of the interaction of sex and temperature on P50 was also significant (*p* < 0.001 and *p* = 0.001, respectively).

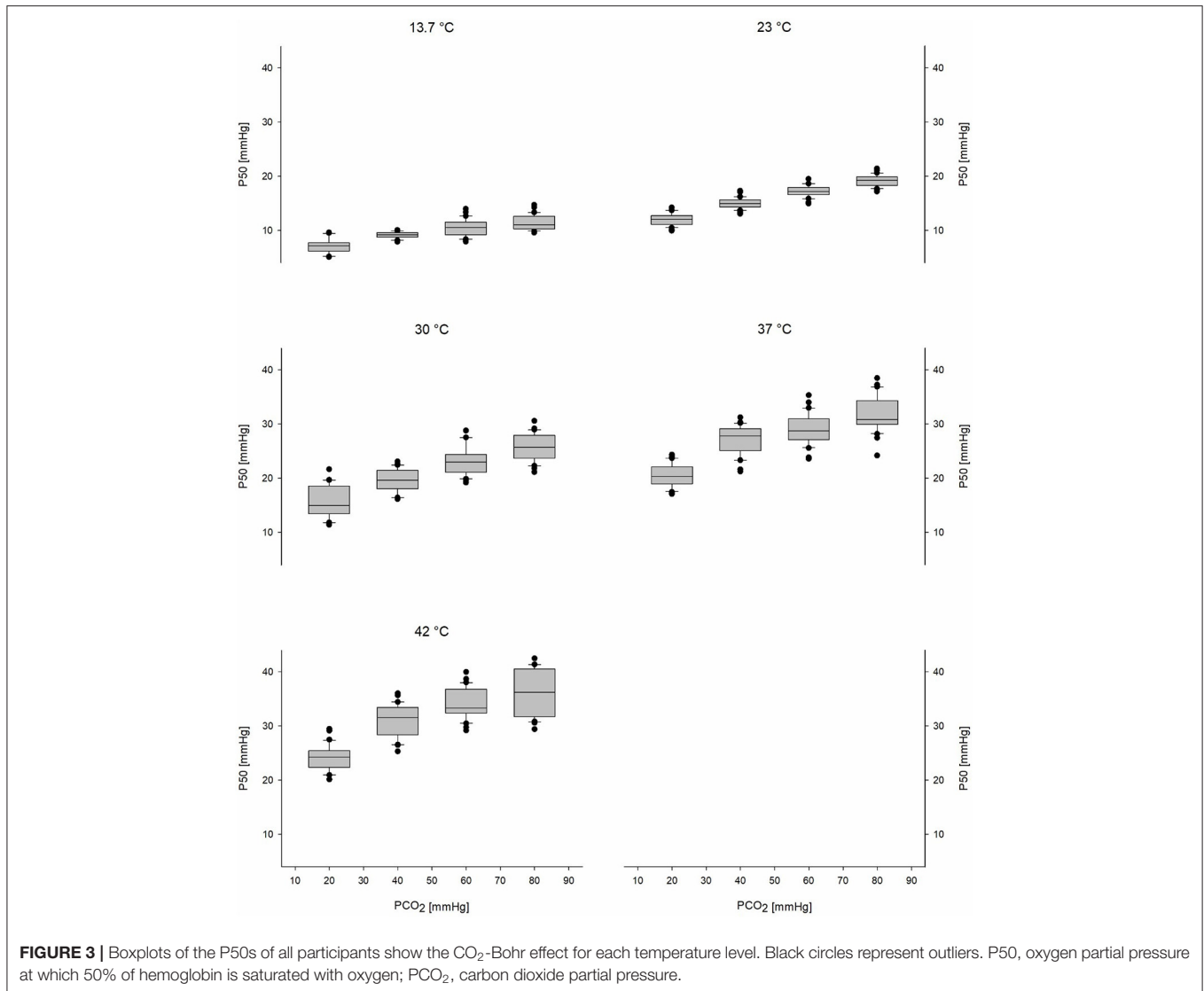
The estimated means of P50 were higher for participants <30 years in comparison with participants ≥30 years old [21.3 (95% CI 21.1–21.5) vs. 20.7 (95% CI 20.5–20.9)]. The effect of the interaction of temperature and PCO₂ on P50 was quantified in Eq. 3 and plotted in **Figure 4**.

$$Eq.3: \Delta P50 = 0.733 \times \Delta T + 0.214 \times \Delta PCO_2 - 0.002 \times \Delta PCO_2^2 + 0.004 \times \Delta T \times \Delta PCO_2$$

$$\Delta P50 = P50 - P50_{(PCO_2=40mmHg; temperature=37^\circ C)}; \Delta PCO_2 = PCO_2 - 40 \text{ mmHg and } \Delta T = temperature - 37^\circ C.$$

Factors Affecting CO₂-Bohr Coefficient

With LMM, an effect of temperature on CO₂-BC could be detected (*p* < 0.001; **Table 2**). CO₂-BCs progressively decreased



with the increase of temperature (**Figure 5A**). The differences were significant between 13.7 and 37°C ($p = 0.001$), 13.7 and 42°C ($p < 0.001$), and also between 23 and 42°C ($p = 0.044$) and between 30 and 42°C ($p = 0.001$).

Factors Affecting Temperature Coefficient

Only an effect of PCO₂ ($p = 0.034$; **Table 2**) was detected on TC. There was an indication that TCs at 40 mmHg (baseline PCO₂ level) were higher than at 80 mmHg PCO₂ ($p = 0.051$; **Figure 5B**).

Factors Affecting Hill Coefficient

There was an effect on HC by PCO₂, temperature, sex, and the interaction of temperature and PCO₂ ($p \leq 0.002$ for all parameters; **Table 2**). HCs were different at different P50s (**Figure 6A**) with a typical flattening of the ODC when shifted to the right. Mean HCs changed in relation to the different combinations of PCO₂ and temperature (**Figure 6B**). Overall,

HCs were higher at PCO₂ 80 mmHg compared to PCO₂ 60 mmHg for a specific temperature (i.e., a right-shifted, but steeper ODC). The estimated mean of HC at 60 mmHg was significantly lower in comparison with 20, 40, and 80 mmHg [2.73 (95% CI 2.66–2.80) at 20 mmHg; 2.69 (95% CI 2.65–2.72) at 40 mmHg; 2.60 (95% CI 2.56–2.63) at 60 mmHg; and 2.72 (95% CI 2.68–2.75) at 80 mmHg] (i.e., an extended ODC shape).

DISCUSSION

We describe a significant interaction of the CO₂-Bohr effect and the temperature effect on the ODC in unbuffered whole blood samples. At temperatures lower than 37°C, the effect of temperature seems to outweigh the effect of even high CO₂ levels. There was a left-shifted ODC that could facilitate oxygen uptake in the lungs but might possibly limit deoxygenation at tissue level. Such modification could be protective against excessive hypoxia in a state of hypercapnia and hypothermia, as in the triple H

syndrome. At temperatures higher than 37°C and high CO₂ levels, the effect of CO₂-BC on the ODC decreased. Such findings may be due to an excess of respiratory gases (i.e., saturation effect). An increase in PCO₂ up to 80 mmHg resulted in right-shifted but also slightly steeper ODCs compared to those at PCO₂ 60 mmHg, indicated by higher HCs, for example, at 42°C. Due to the great extent of both effects, this finding might be of clinical impact for the oxygen transport from the lungs to the tissue in hypoxic situations where both parameters are out

of physiological range and pulmonary oxygen supply, oxygen transport capacity, or cardiac output is deteriorated (e.g., critical illness, acute respiratory distress syndrome, COVID-19, sepsis, and hypothermic major trauma).

Our data showed that CO₂-Bohr effect decreased with increasing temperatures from hypothermic to normothermic conditions which is supported by some of the available literature. Differences in the methodological approaches and results in studies investigating the interaction of the CO₂-Bohr and temperature effect in human and animal studies caused inconsistencies. In 1961, Callaghan studied blood of dogs under hypothermic conditions (15, 23, 30, and 37°C) at various CO₂ levels (20, 40, 60, and 70 mmHg) with a self-constructed instrument (16). When analyzing their published data using Eq. 1, CO₂-Bohr effect showed a decrease with increasing temperatures from hypothermic to normothermic conditions. Such results are consistent with our data in humans. In 1977, Hlastala studied the interactions between all four main parameters affecting ODC on 93 humans (14) using an instrument presented by Duvelloy (22). They also studied different combinations of temperature (23, 30, 37, and 44°C) and CO₂ levels (2.5, 5.8, and 8.5%). Hlastala reported an even increased CO₂-Bohr effect with increasing temperature from hypothermic to normothermic conditions. In 1980, Reeves also studied temperature levels from 13 to 43°C and CO₂ levels from 1 to 8% (15) using a self-constructed instrument for rapid ODC measurements in a blood film (23). Reeves reported neither variations in the shape of the ODC with temperature nor an interaction of CO₂-Bohr effect with temperature and also rejected the finding reported by Hlastala that the TC varies with oxygen saturation (15). Overall, we assume that the main reason for the contradictory results is the different approaches in calculating the CO₂-BC. Hlastala calculated CO₂-BC using pH and base excess. pH and base excess are not only difficult to be exactly measured in a blood film, but pH

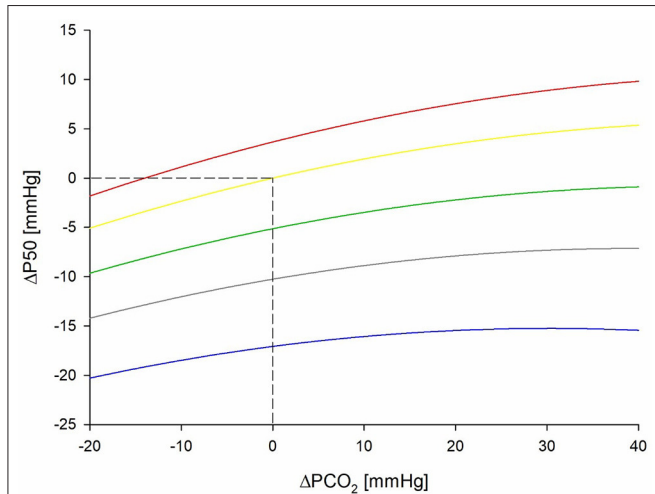


FIGURE 4 | Graphical presentation in form of a nomogram for the equation of ΔP_{50} dependency to ΔPCO_2 and different levels of temperature. ΔPCO_2 denotes difference from 40 mmHg (baseline PCO₂) and ΔP_{50} denotes difference from P₅₀ at 37°C (baseline temperature) and 40 mmHg. Blue line represents 13.7°C, gray line 23°C, green line 30°C, yellow line 37°C, and red line 42°C. P₅₀, oxygen partial pressure at which 50% of hemoglobin is saturated with oxygen; PCO₂, carbon dioxide partial pressure.

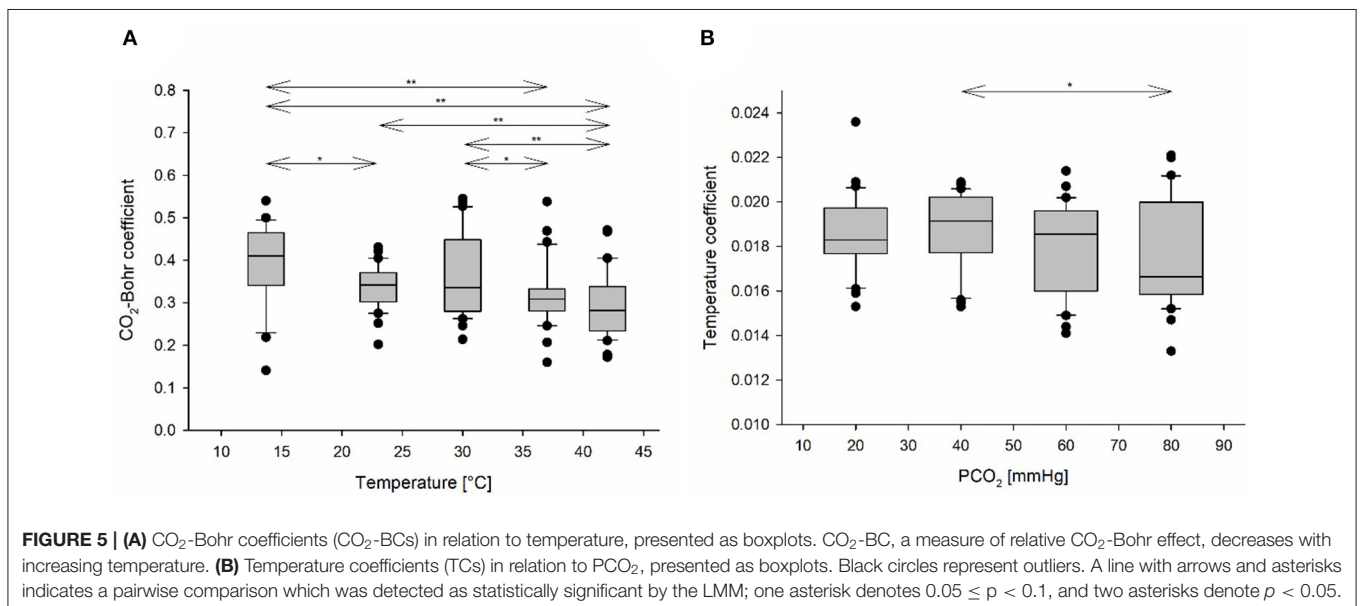
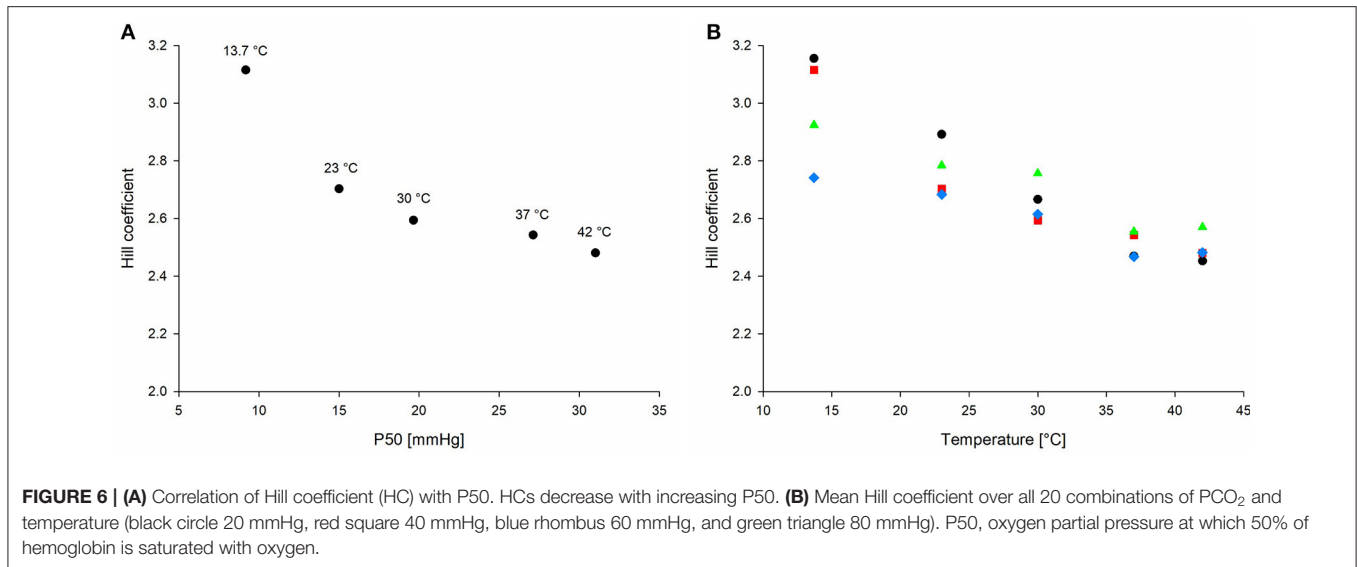


FIGURE 5 | (A) CO₂-Bohr coefficients (CO₂-BCs) in relation to temperature, presented as boxplots. CO₂-BC, a measure of relative CO₂-Bohr effect, decreases with increasing temperature. **(B)** Temperature coefficients (TCs) in relation to PCO₂, presented as boxplots. Black circles represent outliers. A line with arrows and asterisks indicates a pairwise comparison which was detected as statistically significant by the LMM; one asterisk denotes 0.05 ≤ p < 0.1, and two asterisks denote p < 0.05.



itself is temperature-dependent by definition (24). In our study, only PCO₂ values were used for calculations. Unfortunately, raw data are not published by Hlastala, so a direct comparison by recalculating their results using our equation is not possible. Hlastala also used an oxygenation protocol, whereas both Callaghan (15) and our group worked with deoxygenation for the determination of ODCs (17). As the solubility of CO₂ increases with a decrease of temperature, and CO₂ and O₂ are competing agents in binding to hemoglobin (Haldane effect), the way of the oxygen ramp might be of importance as this could interfere with the measurement results. Both methods are working with blood films, and thus, the impact of solubility changes due to temperature changes should be neglectable. Our method might be of advantage for clinical insights when compared to an oxygenation protocol. It simulates more the pathological changes, as at the beginning, full saturation is expected.

Our data showed that the effect of CO₂ was diminished at higher CO₂ levels and the relative effect, expressed by the CO₂-BC, decreased with increasing temperature. Benesch showed that the binding of 2,3-BPG to hemoglobin is an exothermic reaction (25), reducing the amount of 2,3-BPG bound to hemoglobin at higher temperatures. CO₂ binding to hemoglobin and carbamino group formation is favored, as 2,3-BPG and CO₂ are competing agents to the binding to Hb (13, 14). Such data suggest that there is a strong competition between CO₂ and 2,3-BPG at lower temperatures, resulting in a strong effect of PCO₂ on P50. Conversely, at higher temperatures (e.g., 42°C), the majority of 2,3-BPG is dissociated from hemoglobin, lowering the impact of the competition with CO₂, and emphasizing the effect of lower PCO₂ levels (e.g., 20 mmHg or 40 mmHg). A further increase of PCO₂ (60 mmHg or 80 mmHg) seems to cause a reduction of the impact on P50, because the N-terminal binding sites of the Hb β -chains may be already occupied by CO₂ (26). Christiansen described that at lower PCO₂ levels, CO₂ uptake is

facilitated, whereas at higher PCO₂ levels, the curve flattens and may indicate a further increase of CO₂ uptake only by physically solved CO₂ (26). As Hb binding sites for CO₂ are limited by the amount of Hb, we assume that at some point, a state of saturation in the combination of CO₂ and O₂ binding to Hb exists, and a further increase in PCO₂ does not lead to an additional right-shift of the ODC. The human body might not benefit of an improved oxygen delivery to the tissue at P50 levels exceeding 40 mmHg. If the oxygen affinity of Hb is too low, a sufficient binding and thereby transportation of oxygen cannot be provided any more.

Böning also reported an interaction of the Bohr effect and TC and a sex dependency of both effects regarding P50 (27). Our data also show that the temperature effect on P50 is sex-dependent, but the difference almost disappeared at temperature <23°C.

When oxygen content in the inspiratory air is limited, a left-shifted ODC, which increases oxygen uptake in the lungs, is beneficial. For instance, P50 in humans exposed to extreme altitude is reduced due to hypocapnia, resulting in increased SO₂. In the placenta, PaO₂ is very low (30 mmHg) and ODC of the fetus is shift to the left due to fetal hemoglobin (P50 about 20 mmHg) (28). Such status can be observed also in accidentally hypothermic patients and can explain better outcome after hypothermic cardiac arrest. Completely avalanche-buried patients with long burial can develop accidental hypothermia, but they are also exposed to a combination of hypoxia and hypercapnia that can lead to a worse outcome (2, 24). Human experimental studies showed that smaller air pockets and higher density of the surrounding avalanche debris can impair respiratory gas supply or removal and lead to a more severe level of hypoxia and hypercapnia (1, 4, 5). The effects of the combination of the three factors could only be tested in animal models (29, 30). A porcine study on the triple H syndrome experimentally showed a decrease in brain tissue PO₂, cerebral venous SO₂ and regional cerebral SO₂ at a core

TABLE 3 | A model for P50 in an avalanche burial model.

	PCO ₂		
	41 mmHg	47 mmHg	75 mmHg
28°C	20.7	21.7	24.3
34°C	25.1	26.2	29.5
36.4°C	26.9	28	31.6

Calculations based on mean P50 of this study (P50 = 27.1 mmHg) with PCO₂ levels (4) and cooling rates (32–34) from other studies, respectively. Temperature is denoted in columns and PCO₂ in rows. P50, oxygen partial pressure at which 50% of hemoglobin is saturated with oxygen; PCO₂, carbon dioxide partial pressure.

temperature of 28°C. The reduced FiO₂ and increased CO₂ caused a mismatch between metabolic oxygen consumption and oxygen delivery (30). In sole deep hypothermia, cerebrovascular reactivity (cerebral autoregulation) was impaired in pigs (31). Interestingly, whereas mean arterial pressure and cerebral perfusion pressure were decreased at 28.8°C, brain oxygenation was increased (31). Our study suggests that there can be an ODC shift to the left in spite of moderate elevated CO₂ level, due to hypothermia.

Based on measured CO₂ levels from a human experimental study (4) and cooling rates from observational studies of avalanche victims (32–34), the expected P50s in avalanche-buried victims were simulated in a model (Table 3). The calculated P50 (Table 3, P50 at 36.4°C and PCO₂ of 47 mmHg) suggests an increased tissue oxygen unloading that might contribute to outcome (e.g., better cerebral oxygenation). Such status could be found in the early stage of triple H syndrome (5) when the patients is still not hypothermic. In the presence of an air pocket and sufficient exchange of respiratory gases, arterial PCO₂ might remain stable, whereas the progressive cooling could allow a further left-shift (i.e., a core temperature <35°C; Table 3, P50 at 34°C or 28°C and PCO₂ of 47 mmHg). In case of insufficient exchange of respiratory gases, there could be higher PaCO₂ levels (e.g., 75 mmHg, see Table 3) without a left-shift.

In other hypothermic patients, oxygen consumption is decreased and thereby also CO₂ production. The relevance of the interaction of PCO₂ and temperature may thus also have an impact of the fields of therapeutic hypothermia, ARDS, pulmonary oedema, in cardio-anesthesiology, and intensive care medicine. In hypothermic circulatory arrest, acid-base management is based on two different approaches: pH-stat or alpha-stat. Whether one of the two approaches is superior to the other is under debate, yet an age-dependent approach seems preferable (35). The present findings of an interaction effect of PCO₂ and temperature on the ODC might add a small piece of the puzzle in this debate. In an in-hospital setting, P50 as a therapeutic target plays nowadays a tangential role, despite possible treatment options (36, 37).

Limitations

CO₂-BC was established as a function of PCO₂, as pH is temperature-dependent and hard to measure in a small volume of

blood sample, whereas PCO₂ is a parameter measured routinely *in vivo* in a clinical setting. Due to the small volumes of blood (15 µl) in single cell layer films, there was neither a pH measurement nor a measurement of base excess feasible. Thus, a definition and calculation of CO₂-BC based on pH and base excess could not be carried out, which results in a limitation of comparability to other published data (14). The supply of the different PCO₂ levels to the 3H plate was randomized, but we started always with the lowest temperature and then increased the temperature for practical reasons. Thus, at lower temperature, the blood was fresher than at higher temperature. This possible bias should be neglectable as the correct storage of the blood samples on ice was a main focus and the metabolic parameters were shown to remain stable within hours after blood sampling (17). The presented equation that can be used for exact P50, PCO₂, and temperature correction is obtained from one sample of participants ($n = 30$), and another independent study evaluating this interaction is necessary to validate the proposed equation. In this study, the interactions of CO₂-BC and TC were studied in an *in vitro* experiment. Direct transfer of study findings in an *in vivo* scenario should be done cautiously. Due to the difficulties in feasibility and transfer from animal studies (38–41), the *in vitro* ODC determination is advantageous under these conditions. Further studies are needed to investigate the reasons for the here-reported interaction of CO₂-Bohr effect with temperature effect. The saturation dependency of Bohr effect (42) was not accounted for in this study.

CONCLUSION

We describe a significant interaction of the CO₂-Bohr effect and the temperature effect on the ODC in unbuffered whole blood samples. At temperatures lower than 37°C, the effect of temperature seems to outweigh the effect of even high CO₂ high levels. There was a left-shifted ODC that could result in an increased oxygen uptake in the lungs. At temperatures higher than 37°C and high CO₂ levels, the effect of CO₂-BC on the ODC decreased. Depending on the degree of hypercapnia and hypothermia in a completely avalanche-buried patient, there could be a left-shift of the ODC with a potential benefit when oxygen content in the inspiratory air is limited (i.e., hypoxia and hypercapnia) and oxygen consumption of the brain is decreased (i.e., hypothermia). Further studies are needed to investigate *in vivo* the current *ex vivo* results and the systemic effects of the reported interaction effect of CO₂-Bohr effect and TC, and the clinical use of P50 could allow to better triage avalanche.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethikkommission der Medizinischen Universität Innsbruck, Innrain 43, Innsbruck, Austria. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SW prepared original draft. SW and TDC conducted data curation, formal analysis, and visualization. HB, MS, TH, and GS have done supervision and project administration. All authors performed conceptualization, methodology, validation,

involved in writing, reviewing, editing, and approved the submitted version.

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