Blood oxygen transport and depletion in diving emperor penguins

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Summary Statement
Venous hemoglobin saturation profiles reveal a spectrum of cardiovascular dive responses in emperor penguins.

Abstract
Oxygen store management underlies dive performance and is dependent on the slow heart rate and peripheral vasoconstriction of the dive response to control tissue blood flow and oxygen uptake. Prior research has revealed two major patterns of muscle myoglobin saturation profiles during dives of emperor penguins. In Type A profiles, myoglobin desaturated rapidly, consistent with minimal muscle blood flow, and low tissue oxygen uptake. Type B profiles, with fluctuating, and slower declines in myoglobin saturation, were consistent with variable tissue blood flow patterns and tissue oxygen uptake during dives. We examined arterial and venous blood oxygen profiles to evaluate blood oxygen extraction and found two primary patterns of venous hemoglobin desaturation that complemented corresponding myoglobin saturation profiles. Type A venous profiles had saturations that a) increased/plateaued for most of a dive’s duration), b) only declined during the latter stages of ascent, and c) often became arterialized (arterio-venous (a-v) shunting). In Type B venous profiles, variable but progressive hemoglobin desaturation profiles were interrupted by inflections in the profile that were consistent with fluctuating tissue blood flows and oxygen uptake. End-of-dive saturations of arterial and Type A venous saturation profiles were not significantly different, but did differ from those of Type B venous profiles. These findings provide further support that the dive response of emperor penguins is a spectrum of cardiac and vascular...
components (including a-v shunting) that are dependent on the nature and demands of a given dive and even of a given segment of a dive.

Introduction
The management of enhanced oxygen (O\textsubscript{2}) stores in diving mammals and birds underlies their dive capacities, foraging ecology and even their responses to environmental disturbance (Davis, 2014; Ponganis et al., 2011; Williams et al., 2022). Utilization of the respiratory, blood and muscle O\textsubscript{2} stores during dives depends on multiple factors, including the cardiovascular dive response, lung function at depth, the O\textsubscript{2}-hemoglobin (Hb) dissociation curve, locomotory metabolism in muscle and tissue hypoxemic tolerance (Davis, 2014; Ponganis et al., 2011). Importantly, the decreased heart rate (bradycardia) and peripheral vasoconstriction of the dive response reduce cardiac output and redistribute blood flow, resulting in decreased tissue O\textsubscript{2} uptake from blood, conservation of blood O\textsubscript{2}, and, in muscle, greater dependency of aerobic metabolism on myoglobin-bound O\textsubscript{2} (Irving et al., 1941; Scholander, 1940; Scholander et al., 1942).

The intensity of the reduction in heart rate varies with the nature and demands of a given dive or breath hold; heart rate can be variable, only moderately reduced, and modulated with exercise (Butler, 1988; Davis, 2014; Davis and Williams, 2012; Ponganis et al., 2011; Williams et al., 2015). The effects of these variations in heart rates during dives on peripheral blood flow, blood O\textsubscript{2} extraction (arterio-venous (a-v) O\textsubscript{2} differences), and the pattern/rate of myoglobin (Mb) desaturation, although often presumed, have been far less studied. Such investigations are important, however, because the degree, duration and frequency of tissue hypoxia (low O\textsubscript{2} levels) affect dive performance and are critical to mechanisms of extreme hypoxic tolerance that may potentially have translational application to human pathologies (Allen and Vázquez-Medina, 2019; Geiseler et al., 2016; Ponganis, 2019; Tift and Ponganis, 2019). For all these reasons, we examined arterial and venous O\textsubscript{2} profiles in diving emperor penguins (\textit{Aptenodytes forsteri}) to address how the dive response regulates blood O\textsubscript{2} extraction and muscle O\textsubscript{2} utilization in different types of dives. The emperor penguin is ideal for such investigation because it is the only species among diving birds and mammals in which heart rate responses, arterial/venous Hb saturation profiles and muscle Mb saturation profiles have all been documented during dives (Meir and Ponganis, 2009; Meir et al., 2008; Williams et al., 2011).

Our first goal was to determine if venous Hb saturation profiles could be divided into two types that would be consistent with the two predominant and distinct types of myoglobin (Mb) desaturation profiles (Types A and B) previously found in diving emperor penguins (Williams et al., 2011). In Type A dives, Mb desaturated progressively, suggesting minimal to no muscle blood flow (Fig. 1). In contrast, the
slower, fluctuating Mb desaturation profiles of Type B dives suggested some continuous, but variable and/or intermittent muscle blood flow during dives (Fig. 1). Blood O₂ extraction by muscle during Type B dives may be more similar to that in flying and running birds, where blood O₂ extraction and the a-v difference in O₂ content increase, resulting in a lower venous Hb saturation (Butler et al., 1977; Grubb et al., 1983; Hawkes et al., 2014). Type A Mb desaturation dives (20% of dives) were shorter in duration and less than the 5.6-min aerobic dive limit (ADL, dive duration associated with the onset of post-dive blood lactate accumulation (Ponganis et al., 1997; Williams et al., 2011)). Venous Hb saturation profiles that were consistent with Type A and Type B patterns of Mb desaturation would confirm such plasticity in the dive response of emperor penguins.

Based on the two types of Mb saturation profiles and prior studies of Hb saturation profiles of emperor penguins, we hypothesized there would be two main patterns of venous Hb desaturation during dives. In one pattern (Type A), venous Hb desaturation would be minimal until the ascent phase of the dive. In the second pattern (Type B), a decline in venous Hb desaturation would predominate throughout the dive. We postulated that in Type A dives, venous Hb saturation would remain elevated because there would be intense vasoconstriction with little to no muscle blood flow and with minimal blood O₂ extraction by muscle or other tissues during most of the dive (Fig. 2). This pattern is consistent with Type A Mb desaturation profiles (Williams et al., 2011). In addition, in Type A dives, we suspected that a-v shunting could occur and increase venous Hb saturations to near-arterial values. Such arterialized venous blood often occurred during interdive surface intervals and even during some dives of these birds (Meir and Ponganis, 2009).

In contrast to Type A venous saturation profiles during dives, we postulated that in other dives (Type B dives) a decline in venous Hb saturation would predominate due to maintenance of continuous or intermittent low muscle blood flow, and variable blood O₂ extraction (Type B venous saturation profile). These patterns are consistent with Type B Mb desaturation profiles (Williams et al., 2011). Although we expected venous Hb saturation would primarily decline in these dives, we also predicted we would find transient fluctuations or interruptions in venous Hb desaturation consistent with the fluctuating declines in Mb saturation observed in muscle during the later segments of Type B saturation profiles. The presence of temporary interruptions in the venous desaturation profiles of Type B dives would provide evidence of a) transient a-v shunting and/or b) decreased O₂ extraction by tissues (secondary to more vasoconstriction and decreased tissue perfusion). Although transient a-v shunting in Type B dives might temporarily elevate venous Hb saturation at any point in the dive, we expected that the venous saturation profile of the entire dive would be dominated by a decline in saturation.
Our next goal was to evaluate differences between Hb saturation profiles. We analyzed arterial Hb saturation profiles in order to provide a baseline with which to compare to venous Hb saturation profiles (Fig. 3). We expected that arterial Hb would remain highly saturated during most of the dive because of continued gas exchange and the compressive hyperoxia that occurs in the air sacs and arterial blood of diving penguins (Meir and Ponganis, 2009; Stockard et al., 2005; Williams et al., 2021). Based on our proposed differences in the peripheral dive responses underlying Type A and B venous saturation profiles (Fig. 2), we suspected that Hb saturations in Type A venous profiles would approach and remain near arterial values during much of the dive. Although initial venous Hb saturations of the two types of saturation profiles were not expected to be different, we hypothesized that the increase from start-of-dive to peak venous saturation would be greater in Type A profiles than in Type B and arterial profiles, as we expected a-v shunting to contribute to the arterialization of Type A Hb saturation profiles. We further hypothesized that end-of-dive and mean Hb saturation values would be significantly different between Type A and Type B, with Type A profiles more similar to arterial profiles.

**Materials and methods**

Analyses were conducted on data previously collected by this laboratory in 2001, 2003-2005, and 2007-2008 from 15 temporarily captive emperor penguins diving freely at an isolated dive hole in McMurdo Sound, Antarctica (Meir and Ponganis, 2009; Ponganis et al., 2009; Ponganis et al., 2007). These data included venous P$_{O_2}$ profiles from ten penguins and arterial P$_{O_2}$ profiles from five penguins (Table S1). Each bird had either venous or arterial data collected; no penguin had both. Body masses ranged from 20 to 30 kg. Arterial and venous Hb saturation profiles were constructed by application of O$_2$-Hb dissociation curves to intradive arterial and venous P$_{O_2}$ profiles obtained with a bio-logging recorder and intravascular P$_{O_2}$ electrode (see above references for details). Depth profiles were obtained with an attached time-depth recorder (TDR). Due to recorder memory constraints in the early 2000s, the majority of P$_{O_2}$ data were collected at 15-s intervals; later data were collected at 5-s intervals. Only dives greater than two min in duration were included to examine the shape of the Hb saturation profile and the pattern of desaturation. Hb saturation profiles were constructed with use of the pH 7.5 O$_2$-Hb dissociation curve because the change in blood pH during a dive is largely unknown. The typical blood pH of resting emperor penguins is 7.5 (Ponganis et al., 2007); blood pH during the first 3.2 min of dives ranged from 7.35 to 7.47 (Ponganis et al., 2009).

Venous Hb saturation profiles were plotted and dive types identified based on classification criteria. For Type A dives, the classification criteria included a) venous saturations that increased and/or plateaued for
most of a dive and that only declined during the latter stages of ascent, or b) venous saturations that did not decline below 90% the entire dive. Type B dives were dominated by progressive declines in venous saturation that were often interrupted by transient increases and or plateaus in saturation that sometimes could even occur at the start of a dive.

Diving behavior parameters, duration and depth, were determined using TDR depth data for all three groups (dives with Type A venous saturation profile, Type B venous saturation profiles or arterial saturation profiles). Dive duration and depth were compared among the three groups (arterial, Type A (venous), Type B (venous)) to determine any differences in dive characteristics by dive group and to test the hypothesis that dives with Type A saturation profiles were shorter than those with Type B profiles.

The three groups of saturation profiles (arterial, Type A venous and Type B venous) were evaluated and compared. Because dive durations varied and we were examining Hb saturation values at points within a dive, we included fraction of dive duration (how far into a dive the event occurred) to better compare events between dives of different durations. For example, 0.1 fraction of dive time would be 1 min for a 10-minute dive or 30 seconds for a 5-minute dive. From these evaluations, we determined the following five saturation values and when they occurred, including the number of minutes into a dive and the fraction of dive duration: (1) start-of-dive, (2) mean, (3) peak, (4) end-of-dive, and (5) the magnitude of change between start-of-dive and peak saturation. In addition, we obtained the times into dive and dive duration fractions until arterial saturation decreased by 2.5%, 5.0% and 10% saturation or the time at which venous saturation decreased by approximately 10% saturation from the peak value. Because depth data were collected at 1-s intervals and the saturation data at 5 to 15-s intervals, data were often not available precisely at the dive’s start or at a given percentage decrease in saturation. Accordingly, the data values closest to the start of a dive and to a given percentage decline from the peak value were used.

Interruptions in desaturation of Type B saturation profiles were classified as either a positive inflection (increase in saturation >4%) or a plateau (desaturation profile dominated by no change or < 4% increase in saturation). The magnitude of change in saturation during an interruption, the time required to reach the peak value of an inflection, and the duration of a plateau in saturation were determined. Depth profile characteristics of the interruption in the dive (descent, ascent, bottom phase, change in vertical direction) were also noted.

Statistics. We used linear mixed-effects models [packages lme4, lmerTest, MuMin, afex, emmeans and pbkrtest (https://cran.r-project.org/web/packages/nlme/index.html) implemented in R (RCoreTeam, 2023) (version 4.2.3; https://www.r-project.org/)] to analyze the data and test hypotheses (Bartoň, 2023; Bates and Maechler, 2009; Halekoh and Højsgaard, 2014; Kuznetsova et al., 2017; Lenth, 2023; Singmann et
al., 2023). In all models, individual bird was included as a random effect to account for repeated sampling. Including both individual bird and year as random effects in the model typically resulted in a lack of convergence in the model, except for the model examining the effect of depth on duration of all dives. Prior to eliminating complex models that did not converge, control parameters were adjusted using R package afex to try to resolve convergence issues. However, these adjustments did not fix convergence issues, likely due to the small sample size and the relationship between year and individual penguin (each penguin was only present during one year). Fixed effects included depth, duration and saturation parameters. All models were fit by maximum likelihood using Akaike’s information criterion (AIC). Models were compared using Chi-squared distributed likelihood ratios and, when two models were not significantly different, the simplest model with the lowest AIC value was selected. The best model was compared to a model without the fixed effect to determine whether the fixed effect was significant using Chi-squared distributed likelihood ratios. P-values were calculated using Satterwaithe’s method to estimate denominator degrees of freedom for t-statistics (Kuznetsova et al., 2017). Marginal and conditional $R^2$ ($R^2_m$ and $R^2_c$, respectively) values were obtained to determine goodness of fit (Nakagawa and Schielzeth, 2013). Residuals were visually assessed to confirm model requirements, including normality and homoscedasticity, for all models. Duration, depth and increase in saturation from start to peak values were log-transformed to meet model requirements. Model assumptions were generally met and any minor deviations should not affect statistical results as mixed effect models are known to be robust to violations of model requirements (Schielzeth et al., 2020). Models were used to assess the effect of the three Hb saturation profile groups on duration, depth and saturation parameters. Differences between the three saturation profile groups (arterial, Type A venous, Type B venous) were compared with Tukey’s post hoc pairwise comparison. Graphics were constructed in Origin (Originlab, Northhampton MA) or R. Data are expressed as mean ± standard error. Significance was set at $p<0.05$.

**Results**

**Hemoglobin Saturation Profiles**

Venous and arterial saturation profiles were obtained from 102 dives and 64 dives, respectively (Table S1). Review of venous Hb saturation profiles revealed two primary types that met the classification criteria of Type A and Type B (Fig. 4). Of the 102 dives with venous saturation profiles, 31 were classified as Type A and over twice as many dives were classified as Type B (65 dives). Six dives, which did not fit the criteria, were classified as Other and excluded from further analysis.
Diving Behavior

Mean and median durations for the 31 dives with Type A venous saturation profiles were 4.1 ± 0.35 and 3.1 min, while those for the 65 dives with Type B venous saturation profiles were 9.3 ± 0.49 min, and 9.0 min (Fig. S1). Mean and median maximum dive depths of dives with Type A saturation profiles were 27 ± 2.2 and 24 m, and those for dives with Type B saturation profiles were 54 ± 2.9 and 51 m. Maximum duration and depth for Type A dives were 9.1 min and 65 m, respectively and, for Type B dives, 23.1 min and 155 m, respectively. For the 64 dives with arterial saturation profiles, mean and median dive durations were 5.5 ± 0.2 and 5.3 min, respectively. Corresponding mean and median maximum depths were 30 ± 2.2 and 23 m. Maximum dive duration was 11.8 min and maximum depth was 92 m. Regarding the number of dives ≥ 5.6 min, the ADL, Type A had 8 of 31 dives and Type B had 56 of 65 dives. For dives with arterial profiles, 25 of 64 dives were ≥ 5.6 min.

For all dives, depth had a significant effect on duration ($X^2(1)$=10.13, p<0.01) and explained 43% of variation under the model, whereas bird ID and the interaction between depth and year contributed an additional 19% of explanatory power ($R^2_m=0.434$, $R^2_c=0.621$).

In the models assessing the effect of saturation profile group on depth and duration, profile group had a significant effect on both duration ($X^2(1)= 60.34$, p<0.0001) and depth ($X^2(1) = 41.24$, p<0.001) (Fig. 5). Profile group explained 38% of variation in duration and 29% of variation in depth under the model, whereas the effect of individual bird contributed an additional 14% and 35% of explanatory power in models for duration and depth, respectively (duration: $R^2_m = 0.438$, $R^2_c=0.677$, depth: $R^2_m = 0.291$, $R^2_c = 0.641$). Tukey’s post-hoc pairwise comparison demonstrated that there was no significant difference in depth or duration of dives between the arterial and Type A venous groups, but the dives of the Type B venous group were significantly deeper and longer than dives in the Type A venous group and the arterial group (Fig. 5).

Hb Saturation Profiles

Type A venous Hb saturation profiles. In Type A venous saturation profiles, Hb saturation often increased and then plateaued, remaining elevated throughout most of the dive (Fig. 4). Venous saturation increased by 11.4% to a mean peak value of 94.1% within 1.5 min of the start of dive (0.4 ± 0.04 fraction of dive time) (Table 1). The mean net decrease between peak and end-of-dive saturation in all Type A profiles was 19.6 ± 3.9%. Saturation declined by less than 10% from peak values in 14 of the 31 dives (45%). In the other 17 dives, saturation declined greater than 10% from peak values, reaching 83.3 ± 1.4% by 3.2 ±
0.4 min after the start of dive (0.75 ± 0.05 fraction of dive time), and 1.7 ± 0.3 min after the time of peak saturation. Mean end-of-dive saturation of all Type A dives was 74.5% (Table 1).

**Type B venous Hb saturation profiles.** In Type B venous saturation profiles, the profile was dominated by a progressive decline throughout much of the dive (Fig. 4). Type B dive venous saturation profiles increased by 1.8% to a mean peak value of 79.1% within 0.4 min of the start of dive (Table 1). Often the initial saturation was the peak value. In all Type B saturation profiles, saturation decreased ≥ 10%, reaching a mean net decrease between peak and end-of-dive saturation in the 65 Type B profiles of 48.1 ± 3.1%. Saturation declined from peak values to 68.8 ± 1.7% within 1.5 ± 0.2 min (0.18 ± 0.02 fraction of dive time) of the start of dive, and 1.0 ± 0.1 min after the time of peak saturation. Mean end-of-dive saturation of all Type B profiles was 31.1% (Table 1).

Temporary interruptions in the desaturation profiles occurred in 64 of 65 Type B saturation profiles (See e.g., Fig 4). These interruptions in desaturation were either a positive inflection or a plateau in saturation (see methods). Among these 64 dives, 53.8%, 32.3%, and 12.3% had one, two, or three interruptions in desaturation, respectively, for a total of 103 interruptions. 81.6% of the 103 interruptions in desaturation in the Type B profiles were transient positive inflections. The mean increase in saturation of the inflections was 15.1 ± 9.9% and the mean time to reach peak value was 82.1 ± 49.8 s. Plateaus occurred in the remaining interruptions in the desaturation profiles. The mean range of saturation change and duration of plateaus were 2.4 ± 1.5% and 107.4 ± 56.0 s. 66% of all interruptions in venous saturation profiles increased saturation > 10%; the maximum increase in saturation was 46.8%. The initial transient inflection in saturation occurred during descent in 48.4% of all Type B dives, during ascent in 28.1%, and at maximum depth in 23.4%. Among the 64 dives with transient interruptions in venous desaturation, 44.6% of dives had two to three such interruptions in the saturation profile. In these 29 dives, 8 had a later interruption in desaturation at maximum depth. Consequently, maximum depth was associated with an interruption in venous desaturation in 23 of all 64 dives (35.9%).

**Arterial Hb saturation profiles.** In dives of the arterial saturation profile group, Hb saturation increased after the start of dive by 2.5% over the first minute to a mean maximum value over 99% (Table 1). Arterial Hb saturation typically remained high (95-100%) throughout most of the dive. Arterial saturation only declined significantly during the final ascent phase of the dive (Fig. 4A). Dive duration had a significant effect on the time into dive at which 10% desaturation occurred in these profiles ($\chi^2(1)=87.24$, p<0.001, Fig. 4A). Duration explained 79% of variation under the model, whereas the effect of bird contributed an additional 5% of explanatory power ( $R^2_m=0.793$, $R^2_c=0.846$). The arterial Hb saturation decreased to 96.6 ± 0.1% by 3.8 ± 0.2 min into a dive (0.70 ± 0.02 fraction of dive time, n=63), to 94.5 ± 0.1% at 4.3 ± 0.2 min into a dive (0.77 ± 0.01 fraction of dive time, n=57), and to 89.2 ± 0.1% by 4.8 ±
0.2 min into a dive (0.85 ± 0.01 fraction of dive time, n=53). Mean end-of-dive arterial Hb saturation for all dives was 77.9% (Table 1).

Comparisons among arterial and venous (Type A and B) saturation profile groups

Start-of-dive Hb saturation. In the model assessing the effect of the saturation profile group on start-of-dive Hb saturation, group had a significant effect on saturation ($X^2(2)=13.81$, $p<0.01$, Fig. S2A). Saturation profile group explained 40% of variation in start-of-dive saturation, whereas the effect of individual bird contributed an additional 41% of explanatory power in the model ($R^2_m=0.403$, $R^2_c=0.812$). Tukey’s post-hoc pairwise comparison demonstrated that there was no significant difference in start-of-dive saturation between dives with Type A and Type B venous saturation profiles ($t(157.9) = -1.9$, $p=0.46$), but start-of-dive arterial Hb saturation values were significantly higher than Type A ($t(20.2) = -3.6$, $p<0.01$) and Type B ($t(19.2) = 3.04$, $p<0.05$) venous saturation profiles (Fig. S2A).

Magnitude of increase in saturation from start-of-dive to peak saturation. In the model assessing the effect of saturation profile group on the magnitude of saturation increase from start-of-dive to peak Hb saturation, profile group had a significant effect on the magnitude of saturation increase ($X^2(2)=52.99$, $p<0.0001$, Fig. S2C). Profile group explained 34% of variation in the magnitude of increase in saturation, whereas the effect of individual bird contributed only an additional 6% of explanatory power in the model ($R^2_m=0.342$, $R^2_c=0.402$). Tukey’s post-hoc pairwise comparison demonstrated that there was no significant difference in saturation change (start-of-dive to peak) between arterial and Type B profiles ($t(18.6) = 1.84$, $p = 0.18$), but the increase in saturation (start-of-dive to peak) of Type A profiles was significantly higher than in arterial profiles ($t(26.2) = 4.516$, $p<0.001$) and Type B profiles ($t(120.6) = 7.911$, $p<0.0001$) (Fig. S2C).

Mean Hb saturation. In the model assessing the effect of saturation profile group on mean Hb saturation, profile group also had a significant effect on mean Hb saturation ($X^2(2)=34.23$, $p<0.0001$, Fig. S2B). Profile group and duration explained 56% of variation in mean saturation, whereas the effect of individual bird contributed an additional 31% of explanatory power in the model ($R^2_m=0.573$, $R^2_c=0.896$). Tukey’s post-hoc pairwise comparison demonstrated that there was no significant difference in mean saturation between arterial and Type A profiles ($t(19.6) = -2.3$, $p = 0.08$), but mean saturation values of Type B profiles were significantly lower than Type A ($t(155.3) = 5.4$, $p<0.0001$) and arterial ($t(19.5) = 4.0$, $p<0.05$) mean dive saturation values (Fig. S2C).
End-of-dive Hb saturation. In the model assessing the effect of saturation profile group on end-of-dive Hb saturation, profile group had a significant effect on end-of-dive saturation ($X^2(2) = 19.07$, $p < 0.0001$, Fig. S2D). Profile group and duration explained 67% of variation in end-of-dive saturation, whereas the effect of individual bird contributed an additional 17% of explanatory power in the model ($R^2_m = 0.513671$, $R^2_c = 0.837$). Tukey’s post-hoc pairwise comparison demonstrated that there was no significant difference in end-of-dive saturation between arterial and Type A profiles ($t(20.6) = -1.8$, $p = 0.20$), but mean saturation of Type B profiles were significantly lower than Type A ($t(163.3) = 3.7$, $p < 0.001$) and arterial ($t(20.1) = 3.6$, $p < 0.01$) end-of-dive saturation values (Fig. S2D).

Discussion

Dive behavior

Although the dive behaviors of penguins at the experimental isolated dive hole have been described in prior studies (Ponganis et al., 2007; Ponganis et al., 2001; Ponganis et al., 2003; Ponganis et al., 2000), we first briefly review the dive behavior of the birds to support our comparisons of arterial, venous and muscle O$_2$ profiles from different dive studies, and to remind readers of the similarities and differences of these dives with those at sea. The dives of these penguins at an isolated dive hole were primarily under 50 m, at the shallow but common end of their diving spectrum (30-70% of all dives at sea) (Kirkwood and Robertson, 1997; Kooyman and Kooymen, 1995; Sato et al., 2011). Most dive durations were less than 12 min; there were fifteen dives between 12 and 17 min and one dive of 23.1 min (See suppl. Fig. S1). These durations were similar to those at sea. This pattern of diving was associated with the routine hunting and capture of the sub-ice fish, *Pagothenia borchgrevinki* (Ponganis et al., 2000). At sea, this fish is a minor prey item (Cherel and Kooyman, 1998; Kirkwood and Robertson, 1997). Dive duration at the isolated dive hole correlated with maximum depth but was highly variable, again similar to the prior studies (Fig. S1). Although most dives at sea are less than the ADL, there was a high percentage of dives in the present study that were greater than the 5.6-min ADL. These longer duration dives may be a result of several factors: 1) at the experimental dive hole, birds must dive under the fast ice to find fish and return to the hole to breathe, whereas at sea, they can surface and breathe without a long return since they typically dive in the pack ice or in open water, 2) due to localized depletion of prey fish near the dive hole by a large number of penguins during the season, the birds may have to travel farther underwater to find fish, and 3) unlike the studies at sea (Kooymen and Kooymen 1995, Sato et al 2011), dives less than two min
were not included in the present study; this reduces the number of short duration dives and increases the percentage of longer dives in the present study.

Maximum depths and durations of arterial and venous Type A saturation profile groups were in the same range, but dives in the Type B venous saturation group were deeper and longer. Type A venous Hb desaturation profiles occurred in 32% of all venous profiles, similar to the distribution of Type A and B muscle Mb desaturation dives (Williams et al., 2011). As in the muscle studies, dives with Type A venous profiles were also shorter in duration than those with Type B venous profiles (Fig. 5), and similar in duration to Type A muscle Mb desaturation dives (4.2 min) (Fig. 5) (Williams et al., 2011). The similarities of Type A venous and Type A muscle desaturation dives in dive distribution and dive duration support the hypothesis that the characteristics and intensity of the dive response are the same in both the muscle and venous Type A dives (i.e., little to no muscle blood flow during the dive, primary reliance of muscle metabolism on Mb-O$_2$, and limited tissue O$_2$ extraction by tissues in these dives).

While it is tempting to speculate as to why some dives are Type A and others are Type B, both the current study and the earlier Williams et al. study (2011) have fairly small sample sizes. In the present study of venous Hb saturation profiles, ten birds with 96 dives are represented, with the number of dives per bird ranging from 2 to 19 (Table S1). While Type A dives were shorter and shallower than Type B dives in both studies, the dive profiles were not distinct. Both Type A and Type B dives had intra-dive sub-ice ascents suggestive of foraging for *Pathogenia borchevenki*. Further, in the Mb saturation study, there were no Type A dives greater than the ADL (5.6 min); however, in the current study, over a quarter of Type A dives (8 of 31 ) were equal to or greater than 5.6 min. Finally, there was also not a typical distribution pattern of Type A vs Type B dives. Type A dives were not consistently an initial dive before a series of Type B dives and could occur in a series themselves. Future studies measuring blood O$_2$, as well as accelerometry, in a larger number of dives per bird may be able to provide more insight.

**Study limitations**

One limitation of our interpretation of venous blood O$_2$ profiles is the posterior vena caval location of the O$_2$ electrodes. Although we make inferences as to overall patterns of O$_2$ depletion and muscle perfusion, the venous P$_{O2}$ and Hb saturation data are not mixed venous values and not necessarily indicative of the entire venous system.

Another limitation in data interpretation is the potential decrease in Hb’s affinity for O$_2$ due to a lower (more acidic) pH (the Bohr shift) and/or an increase in temperature throughout the course of a dive.
Because the time course of any pH changes during dives of emperor penguins is not known, we used the pH 7.5 O\textsubscript{2}-Hb dissociation curve to convert P\textsubscript{O2} values to Hb saturations. Temperature correction of the dissociation curve of emperor penguins was not possible because, although arterial and vena caval temperatures increase slightly during dives of penguins at the isolated dive hole (Ponganis et al., 2001; Ponganis et al., 2004; Ponganis et al., 2003), the temperature sensitivity of their O\textsubscript{2}-Hb dissociation curve is not known. As recently reviewed for the Bohr shift in penguins (Meir and Ponganis, 2009; Signore et al., 2021) and for increased temperature in high-flying geese (Meir and Milsom, 2013), we expect that a decrease in pH /or an increase in temperature would decrease Hb’s affinity for O\textsubscript{2} during longer dives, enhancing O\textsubscript{2} delivery to tissues and lowering venous Hb saturation. However, that does not affect our interpretation of blood flow patterns from Hb saturation profiles during dives.

**Arterial saturation profiles**

We begin discussion of saturation profile with the arterial saturation profiles because this analysis is useful for interpreting venous Hb saturation profiles. We also remind readers that penguins were only equipped with one O\textsubscript{2} electrode (Table S1). Arterial and venous data were not collected simultaneously. Despite compression hyperoxia during descent (Stockard et al., 2005; Williams et al., 2021), the initial increase in arterial saturation was minimal and rapid. This was secondary to the shape of the O\textsubscript{2}–Hb dissociation curve, an adequate arterial P\textsubscript{O2} and an already high Hb saturation at the start of the dive (Meir and Ponganis, 2009; Ponganis et al., 2009; Ponganis et al., 2007). As the dive progressed, arterial saturation was well maintained at depth due to continued gas exchange and elevated air sac P\textsubscript{O2} (Figs. 3, 4A). Despite expected decreases in the respiratory O\textsubscript{2} fraction, arterial, as well as air sac, P\textsubscript{O2} were still high due to elevated ambient pressure at depth (Boyle’s Law) (Stockard et al., 2005; Williams et al., 2021). Decline in arterial saturation was dependent on dive duration with the rate of arterial desaturation slowing in longer dives. In the longest (11.8 min) arterial dive, arterial saturation slowly declined, reaching 89.7% at 10.3 min into the dive (Fig. 4A). Arterial saturation did not begin to decline more rapidly until ascent, and especially the latter part of ascent (secondary to a rapid decrease in P\textsubscript{O2} due to a decreased respiratory O\textsubscript{2} fraction (Dalton’s Law) and the decline in ambient pressure (Boyle’s Law) (Stockard et al., 2005; Williams et al., 2021)).

This type of overall high arterial saturation profile at depth has been reported in diving California sea lions and human breath-hold divers (McDonald and Ponganis, 2012; McKnight et al., 2021; Mulder and Schagatay, 2021; Ruesch et al., 2021). The decline in P\textsubscript{O2} at the end of dives appears uneventful in penguins and pinnipeds (McDonald and Ponganis, 2012; Meir and Ponganis, 2009), but, in humans, this
decrease can be associated with shallow water blackout (Lindholm and Lundgren, 2009). In contrast to emperor penguins, sea lions, and humans, arterial saturation in elephant seals declined continuously throughout dives after an early initial peak (Meir et al., 2009). This is most probably secondary to start-of-dive air exhalation, early alveolar collapse, and lack of gas exchange at depth in seals (Fahlman et al., 2009; Kooyman et al., 1970; Kooyman et al., 1971; Kooyman et al., 1973a). All evidence indicates that gas exchange continues to much deeper depths in penguins and sea lions (Kooyman et al., 1973b; Kooyman and Sinnett, 1982; McDonald and Ponganis, 2012; Ponganis et al., 1999).

The analysis of the emperor penguin’s arterial Hb saturation profiles provide two key considerations in the evaluation of venous Hb saturation profiles. First, any large increases in venous saturation during descent and even in other phases of the dive would be more likely secondary to decreased tissue oxygen extraction or to a-v shunts than to the minimal increase observed in arterial saturation. Second, any rapid declines in venous saturation during ascent may be secondary not only to increased tissue O$_2$ extraction, but also, and perhaps more significantly, to the rapid decline observed in arterial saturation at the end of a dive.

**Venous saturation profiles**

*Type A Hb saturation profiles are consistent with Type A Mb saturation profiles.* Provided the posterior vena caval Hb saturation is representative of all venous blood, the magnitude and time course of desaturation of muscle Mb and arterial Hb and venous Hb in Type A dives are consistent with the hypothesis of simultaneous a-v shunting and regional vasoconstriction to tissues such as muscle during most of duration of a dive with a Type A venous (and muscle) saturation profile (Fig. 6A). During the first four min of the dive in Fig. 6A, the calculated a-v difference in Hb-bound O$_2$ was less than 2.5 ml O$_2$ dl$^{-1}$, more than 50% less than the resting value and consistent with this hypothesis of simultaneous a-v shunting and regional vasoconstriction to tissues. The decline in venous saturation towards the end of the dive reflects at least partially the simultaneous decline in arterial saturation during ascent. If a-v shunting decreases during ascent, that decline in venous saturation during ascent may also be secondary to increased tissue perfusion and blood O$_2$ uptake by tissues, especially muscle (early re-loading of O$_2$-depleted Mb (Thompson and Fedak, 1993)). However, the similarities between Type A and arterial end-of-dive Hb saturation values suggest the decline in venous saturation is predominantly a result of the decline an arterial Hb saturation rather than increased O$_2$ uptake by muscle, which is again consistent with Type A Mb saturation profiles (Williams et al., 2011).
Similarity of Type A and arterial saturation profiles support a-v shunting. Type A venous Hb saturation profiles, characterized by an increasing saturation or plateau during most of the dive, were more similar to arterial Hb saturation profiles than Type B Hb saturation profiles (Fig. 4). The start-of-dive saturation was not significantly different between Type A and Type B profiles and the differences were partially attributable to individual bird differences, rather than primarily differences in Hb saturation profile group. However, the saturation increase to peak venous values in Type A profiles was more than 5x greater than that in Type B dives and driven by saturation profile group, not individual differences. This increase in saturation was also over 4x greater than in the aorta, resulting in Type A saturation levels approaching arterial. Further, the Type A peak saturation (94.1%) was much greater than the 72% saturation calculated on the basis of measured arterial and venous O\textsubscript{2} contents at rest and also greater than the 78% value estimated with a venous blood O\textsubscript{2} content calculation of arterial O\textsubscript{2} content at 99% saturation – 5 ml O\textsubscript{2} dl\(^{-1}\) (Kooyman and Ponganis, 1998; Ponganis et al., 2007). Such a large increase in venous saturation is most consistent with decreased tissue O\textsubscript{2} uptake and probably utilization of a-v shunting. The elevated heart rates that occur during this phase of the dive (Meir et al., 2008; Wright et al., 2014) could provide the blood flow to support such shunting. Because arterial blood is oxygenated and stroke effort is highest during this phase of the dive (Meir et al., 2008; van Dam et al., 2002; Williams et al., 2012), hypometabolism secondary to hypoxia or decreased locomotory effort seems unlikely. Rather, decreased regional tissue perfusion and a-v shunting appear more plausible. The lack of significant difference in mean dive Hb saturation between Type A venous profiles and arterial profiles also support the use of a-v shunting.

Decreases in Type A venous saturation profiles were slow, were often less than 10% in magnitude, and, similar to arterial saturation profiles, only occurred more rapidly during late ascent. Type A end-of-dive saturation was also not significantly different from the corresponding arterial value. These similarities argue that a-v shunting may continue throughout most if not all of a dive with a Type A profile, and that end-of-dive decreases in arterial saturation contribute significantly to the corresponding declines in venous saturation. A-v shunting during a dive would have implications not only for O\textsubscript{2} store management, but also for nitrogen uptake/distribution during dives (Fahlman et al., 2007). The relatively high arterial and Type A venous end-of-dive saturations even in dives beyond the ADL also support the concept that the rise in post-dive blood lactate concentration at the ADL is primarily due to depletion of the muscle O\textsubscript{2} store and the onset of muscle lactate accumulation during the dive (Williams et al., 2011). Although depletion of the respiratory and blood O\textsubscript{2} stores are highly variable, these O\textsubscript{2} depots are typically not completely depleted at the ADL and or in many dives beyond the ADL (Meir and Ponganis, 2009, Stockard et al. 2005, Williams et al., 2021).
Type B Hb saturation profiles are consistent with Type B Mb saturation profiles. Type B venous Hb saturation profiles were dominated by an overall progressive decline in saturation, which is consistent with slower saturation decline in Type B Mb saturation profiles (Figs 2 and 3). Saturation sometimes increased at the start of the dive, but, often, the initial saturation was the peak value. The average increase in peak saturation in Type B profiles was about one-fifth that in Type A profiles. The rate of decline of saturation from peak values and the magnitude of decline between peak and end-of-dive saturations in Type B profiles were also faster and greater than those in Type A venous saturation profiles. All this evidence supports the hypothesis that there is greater tissue perfusion, blood O\textsubscript{2} extraction, and blood O\textsubscript{2} supplementation of muscle metabolism in dives with Type B venous saturation profiles, consistent with Type B Mb saturation profiles.

The transient but sometimes prolonged interruptions in the Type B venous Hb saturation profiles are also consistent with Type B muscle Mb desaturation profiles observed by Williams et al. (2011) and with the hypothesis that muscle blood flow can be variable during a dive. In Type B Mb saturation profiles, the decline in Mb saturation was slow, could plateau mid-dive, and often progressed more rapidly and completely during the latter segments of long dives. Those findings in muscle are consistent with the predominant decreasing venous saturation profiles and the interruption points in venous saturation observed in Type B venous profiles in this study (Figs. 4, 6B).

Transient interruptions in Type B profiles further support the plasticity of the dive response and a-v shunting. Although large declines in saturation dominated Type B venous profiles, the progressive declines were often interrupted by transient changes (increases and/or plateaus) in venous saturation in 98% of dives with Type B profiles (Fig. 4). Transient interruptions were usually associated with a change in vertical direction, and could occur during descent, ascent, and at maximum depth. We propose that such interruptions in venous Hb desaturation are secondary to changes in peripheral vascular regulation and/or heart rate, and represent times where tissue O\textsubscript{2} uptake is decreased by regional vasoconstriction and/or by utilization of a-v shunts. The neuroregulatory mechanism responsible for such changes in these cardiovascular responses is unclear.

As illustrated in the 9.9- min dive of EP 17 (Fig. 6B), the prolonged inflection in the latter half of the saturation profile included a large increase and then decline in venous saturation. We hypothesize that this inflection is secondary to vasoconstriction to tissues (including muscle) and simultaneous a-v shunting. The end result would be a large increase in venous saturation during ascent that only declines in final ascent due to the decline in arterial saturation (Fig. 6B). In other words, during this phase of the dive,
peripheral vasoconstriction isolates muscle and/or other tissues from blood, while simultaneous a-v shunting delivers more oxygenated blood to the vein, but, in the final phase of the dive as arterial blood desaturates, so too does the venous blood.

**A-V blood O$_2$ differences further support the plasticity of the dive response.** The constant arterial Hb saturation profile during most of a dive’s duration provides a valuable reference for interpretation of venous Hb saturations and calculation of arterio-venous (a-v) O$_2$ difference estimates. The venous Hb saturation profiles and estimated a-v O$_2$ content differences can serve as an index of tissue blood flow and the magnitude of blood O$_2$ extraction by tissues. It is notable that during the earlier portions of dives, times at which arterial Hb saturations are typically 95-100%, there was a wide range of venous Hb saturations and calculated a-v differences in Hb-bound O$_2$ content (Figs. 6A, 6B, 7). Dives with Type A venous profiles typically had very small a-v O$_2$ differences, consistent with Type A Mb-O$_2$ profiles, peripheral vasoconstriction, minimal blood O$_2$ extraction by tissue, and possible a-v shunting (Figs. 4A, 7).

In contrast, Type B venous Hb saturation profiles, in general, had a wide range of saturations during the first two min of dives, consistent with the Type B Mb desaturation profiles in Williams et al. (2011) and supportive of the concept of a spectrum of cardiovascular responses and muscle blood flow patterns during dives with Type B venous profiles (Fig. 4). Small a-v O$_2$ differences during early descent occurred in some Type B profiles (Fig. 7), which are consistent with our hypotheses of variable muscle blood flow and O$_2$ extraction patterns in dives with Type B venous profiles.

In other Type B venous profiles, Hb saturations during descent were as low as 10 to 40% (Fig. 4). In these types of dives, the calculated a-v differences in Hb-bound O$_2$, based on an arterial saturation of 95% and venous saturation profiles during the first two min of a dive, were as high as 19 ml O$_2$ dl$^{-1}$ (Fig. 7), almost four times the measured value in emperor penguins at rest (Ponganis et al., 2007). In comparison, the a-v O$_2$ content differences in flying pigeons, running emus and running bar-headed geese, all exercising at high intensity, were about 10 ml O$_2$ dl$^{-1}$ (Butler et al., 1977; Grubb et al., 1983; Hawkes et al., 2014). In the running dog at maximum O$_2$ consumption, the a-v O$_2$ difference was 15 ml O$_2$ dl$^{-1}$ (Taylor et al., 1987). Given the low average locomotory muscle oxygen consumption (12.4 ml O$_2$ kg$^{-1}$ muscle min$^{-1}$) during dives (Williams et al., 2011), the extremely low venous Hb saturations and large a-v O$_2$ differences during these particular Type B dives suggest a) muscle oxygen consumption (stroke rate, work effort) may have been higher than average, b) vasoconstriction may have been less and muscle blood flow greater than average, and/or c) Mb may not have been completely saturated prior to diving.
(see Scholander et al. (1942)). In addition, the possibility of anemia (low Hb content) or unusually low arterial Hb saturations in these particular birds cannot be ruled out. Clearly, simultaneous measurements of arterial and venous Hb saturation profiles and venous Hb and muscle Mb saturation profiles and stroke effort are desirable. Nonetheless, the broad range of calculated a-v O$_2$ differences and venous Hb desaturation profiles observed within and among different dives, were consistent with the Mb desaturation profiles of Williams et al. (2011) and the wide end-of-dive range in air sac and blood O$_2$ contents (Meir and Ponganis, 2009; Stockard et al., 2005; Williams et al., 2021). All these findings support the concept of a spectrum of both heart rate and peripheral vascular responses in dives of emperor penguins.

**Summary**

On the basis of Mb saturation profiles and heart rate profiles, both the cardiac and peripheral vascular components of the dive response have been considered quite “plastic” and dependent on the nature of a given dive in emperor penguins (Meir et al., 2008; Williams et al., 2011; Wright et al., 2014). The venous saturation profiles in this study support this concept of plasticity in the dive response, including not only heart rate but also the peripheral vascular component. Accordingly, although there are distinct differences, we emphasize that the dive response and resulting Hb and Mb saturation profiles in emperor penguins cannot even be simply characterized into only Types A and B. Rather, the dive response is a spectrum of cardiac and vascular components dependent on the nature and demands of a given dive and even of a given segment of a dive.

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**Competing interests**

The authors declare no competing or financial interests.
Author contributions

Conceptualization: PJP, CLW; Methodology: PJP, CLW; Software: NA. Validation: NA. Formal analysis: PJP, CLW, JMKB; Investigation: PJP, CLW. Resources: PJP; Data curation: PJP; Writing – original draft: PJP; Writing – review & editing: CLW, JMKB, PJP. Visualization: KMKB. Project administration: PJP; Funding acquisition: PJP.

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Data availability

Data are available from Dryad

References


Fig. 1. Representative Type A and Type B myoglobin (Mb) saturation profiles during dives of emperor penguins (Williams et al., 2011). Type A Mb saturation profiles were characterized by a rapid decline in saturation consistent with minimal muscle blood flow during the dive. Type B saturation profiles were characterized by a slower, fluctuating decline, and sometimes by a plateau in saturation. Type B Mb saturation profiles were considered the result of variable and intermittent muscle blood flow during dives, including periods of minimal muscle blood flow as evidenced by the more rapid decline in Mb saturation in the final segment of this Type B Mb saturation profile.
Fig. 2. Hypothetical arterial, venous and muscle saturation profiles and blood flow patterns in dives with a Type A muscle myoglobin (Mb) saturation profile (Type A dive) and in dives with a Type B muscle Mb saturation profile (Type B dive). In the Type A dive (minimum muscle blood flow and utilization of an arterio-venous (a-v) shunt), venous saturation remains high during most of the dive, but parallels the decline in arterial saturation during ascent. In contrast, in the Type B dive, maintenance of some, but variable muscle blood flow allows for blood O_2 extraction by muscle, a slower decline in muscle saturation, and a decline in venous saturation. However, at about 6-7 min into the dive, a postulated decrease in muscle blood flow and utilization of an a-v shunt allows for a more rapid decline in muscle saturation and an increase in venous saturation during the ascent phase of the dive.
Fig. 3. Arterial partial pressure of O$_2$ (P$_{O2}$), arterial hemoglobin (Hb) saturation, and depth profiles of a 9.8-min, 59-m dive of EP1 2008. Saturation was maintained above 95% until the final two min of the dive (the latter half of ascent). Adapted from Meir and Ponganis (2009).
Fig. 4. A. Representative arterial and Type A and B venous hemoglobin (Hb) saturation profiles from multiple penguins for dives of 2.2 to 12.3 min, and 11 to 155 m. Regardless of dive duration or maximum depth, arterial saturations remained above 95% until the ascent phase of the dive. In Type A venous profiles, Hb saturations typically remained elevated during most of the dive (until the ascent). The Hb saturations were often arterialized (> 90%). In Type B venous profiles, Hb saturations were more varied, often were much lower than in Type A dives, and had common interruptions (inflections and plateaus) in the desaturation profile. B. Venous partial pressure of O₂ (PₐO₂), venous Hb saturation, and depth profiles of two dives with Type A venous profile and three dives with Type B venous profiles in EP19 2004. C. Venous partial pressure of O₂ (PₐO₂), venous Hb saturation, and depth profiles of one dive with a Type A venous profile and two dives with Type B venous profiles in EP05 2003.
Fig. 5. Box plots of dive duration and maximum dive depth in dives with arterial, Type A venous, and Type B venous saturation profiles. Dives with Type B saturation profiles were significantly longer and deeper (p<0.05) than dives with either Type A venous profiles or with arterial saturation profiles, but the latter two groups did not differ significantly (Tukey’s post hoc pairwise comparison). Within each panel, different letters represent a significant difference between the dive groups. N = 15 penguins, 160 dives for all analyses. The gray box ( ) indicates the upper and lower quartiles and includes the median line. Whiskers ( ) extend to 1.5x the interquartile range and outliers are indicated by diamonds (♦). Mean values are represented by a red star (★).
Fig. 6. Composite comparisons of arterial and venous hemoglobin (Hb) saturation profiles and muscle myoglobin (Mb) saturation profiles in shallow dives of similar durations from different penguins. Myoglobin saturation profiles adapted from Williams et al. (2011). A. Type A venous Hb and muscle Mb saturation profiles. B. Type B venous Hb and muscle Mb saturation profiles.
Fig. 7. Calculated arterio-venous (a-v) differences in hemoglobin (Hb)-bound O$_2$ during the first two min of a dive with a Type A venous Hb saturation profile and three dives with Type B venous Hb saturation profiles demonstrate the range of blood O$_2$ extraction during early descent. Dives with Type A profiles were characterized by minimal blood O$_2$ extraction while dives with Type B profiles had variable blood O$_2$ extraction during early descent. Based on venous saturation profiles selected from Figs. 4B and C, an assumed arterial Hb saturation of 95% during this phase of the dive (see Fig. 1), a Hb concentration of 18 g dl$^{-1}$, and 1.34 ml O$_2$ g$^{-1}$ Hb at full saturation.
Table 1. Arterial, Type A venous and Type B venous hemoglobin saturation values during dives and the number of minutes (min) and dive fraction to peak saturation. Saturation values (mean ± s.e.m.) were calculated with pH 7.5 dissociation curve.

<table>
<thead>
<tr>
<th>O₂ Sampling Site</th>
<th>Start-of-dive Saturation</th>
<th>Peak Saturation</th>
<th>Saturation Change from Start-of-dive to Peak</th>
<th>End-of-dive Saturation</th>
<th>Mean Dive Saturation</th>
<th>Time To Peak Saturation</th>
<th>Dive Fraction to Peak Saturation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arterial</td>
<td>96.7 ± 1.6</td>
<td>99.1 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>77.9 ± 1.4</td>
<td>95.3 ± 0.3</td>
<td>1.1 ± 0.03</td>
<td>0.20 ± 0.01</td>
</tr>
<tr>
<td>Type A venous</td>
<td>82.7 ± 2.2</td>
<td>94.1 ± 0.8</td>
<td>11.4 ± 2.1</td>
<td>74.5 ± 3.8</td>
<td>86.5 ± 1.6</td>
<td>1.5 ± 0.2</td>
<td>0.40 ± 0.04</td>
</tr>
<tr>
<td>Type B venous</td>
<td>77.4 ± 1.5</td>
<td>79.1 ± 1.5</td>
<td>1.8 ± 0.3</td>
<td>31.1 ± 3.3</td>
<td>54.5 ± 2.5</td>
<td>0.4 ± 0.1</td>
<td>0.05 ± 0.01</td>
</tr>
</tbody>
</table>
Fig. S1. Dive depth compared with dive duration for dives in the three hemoglobin saturation profile groups: Arterial saturation profile (Arterial, maroon), Type A venous saturation profile (Type A, black) and Type B venous saturation profile (Type B, gray). For all dives, depth had a significant effect on duration ($X^2(1)=10.13, p<0.01$). n= 15 penguins, 160 dives.
Fig. S2. Box plot charts showing significant differences among different types of dives (arterial, Type A venous, and Type B venous) for the start-of-dive hemoglobin (Hb) saturation (A), the increase in Hb saturation from the start of dive to the maximum Hb saturation (B), the mean Hb saturation for the dive (C), and the end-of-dive Hb saturation (D). The letters above box plots represent the results of pairwise comparisons using Tukey’s method. Within each panel, different letters represent a significant difference between the dive types. N = 15 penguins, 160 dives for all analyses. The gray box ( ) indicates the upper and lower quartiles and includes the median line. Whiskers ( ) extend to 1.5x the interquartile range and outliers are indicated by diamonds (♦). Mean values are represented by a red star (★).
Table S1. Year of measurements, penguin identification (ID), number of dives used in analyses, mean and maximum durations of dives in minutes (min), type of oxygen (O₂) sampling site (arterial or venous), and number of Type A or Type B dives identified.

<table>
<thead>
<tr>
<th>Year</th>
<th>Penguin ID</th>
<th># Dives</th>
<th>Duration (min) (mean, max)</th>
<th>O₂ Sampling Site</th>
<th># Type A</th>
<th># Type B</th>
</tr>
</thead>
<tbody>
<tr>
<td>2001</td>
<td>37</td>
<td>9</td>
<td>5.1, 8.6</td>
<td>Venous</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>2001</td>
<td>36A</td>
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<td>4.4, 5.3</td>
<td>Arterial</td>
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<td>NA</td>
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<tr>
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<td>2</td>
<td>1</td>
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<td>Arterial</td>
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<td>NA</td>
</tr>
<tr>
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<td>4</td>
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<td>6.1, 6.9</td>
<td>Venous</td>
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<td>0</td>
</tr>
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<td>5</td>
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<td>Venous</td>
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<td>6</td>
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<td>7</td>
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<td>10</td>
<td>0</td>
</tr>
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<td>Venous</td>
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<td>2</td>
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<td>5</td>
<td>10.5, 13.0</td>
<td>Venous</td>
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<td>5</td>
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<td>11.3, 12.0</td>
<td>Venous</td>
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<td>2</td>
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<td>48</td>
<td>18</td>
<td>9.3, 13.1</td>
<td>Venous</td>
<td>0</td>
<td>18</td>
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<tr>
<td>2007</td>
<td>8</td>
<td>12</td>
<td>5.0, 8.7</td>
<td>Arterial</td>
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<td>NA</td>
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<tr>
<td>2007</td>
<td>10</td>
<td>32</td>
<td>5.2, 8.0</td>
<td>Arterial</td>
<td>NA</td>
<td>NA</td>
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<tr>
<td>2008</td>
<td>1</td>
<td>13</td>
<td>7.4, 11.7</td>
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