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Increases in Arterial Blood Oxygen During Exercise in the Lemon Shark (*Negaprion brevirostris*)

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Summary. Polyethylene cannulae were implanted in pre- and post-branchial blood vessels allowing non-stressful blood sampling over a variety of activity ranges in an active tropical elasmobranch, the lemon shark (*Negaprion brevirostris*). The P_{50} was found to be 11.8 Torr at 24 °C and pH of 7.7. A Bohr shift of −0.36 was also found. Blood P_{O_2} and oxygen content were measured during rest, routine swimming, and exercise in unanesthetized, free swimming juveniles. Under all conditions venous oxygen levels were low with venous P_{O_2} of 7.1 ± 2.7 Torr, and venous oxygen content ($C\bar{v}_{O_2}$) of 0.56 ± 0.4 vol%. However, a large variability was found in arterial blood measurements. Arterial P_{O_2} ranged from 7 to 80 Torr, while arterial oxygen content (Ca_{O_2}) varied from 1.6 vol% to 6.8 vol% among ten experimental animals. A significant increase in arterial P_{O_2}, oxygen content, and hematocrit was noted during increased activity. Since the venous system provides little or no oxygen reserve, increased oxygen extraction from the blood (Ca_{O_2}–$C\bar{v}_{O_2}$) appears to be met by an increase in Ca_{O_2} rather than a decrease in $C\bar{v}_{O_2}$. Mechanisms to accomplish this may include increasing hematocrit and vascular gill shunts.

Introduction

Sharks are among the largest and most active tropical fishes. However, the respiratory physiology of sharks has been studied in only a few temperate water species and the results suggest these animals are rather ineffective in providing oxygen to meet metabolic demand, especially during exercise (Piiper et al. 1977).

This contrasts sharply with what is known in teleosts. Active fish, such as salmonids, often chosen as experimental animals, have a high aerobic capacity (Stevens and Randall 1967a, b; Randall et al. 1967; Kiceniuk and Jones 1977). Depending on temperature, sockeye salmon (*Oncorhynchus nerka*), for instance, increase their oxygen consumption in response to exercise, from five to fifteen times their basal level (Brett 1964, 1965, 1972). Such large aerobic scopes indicate highly efficient oxygen extraction and delivery systems.

It is possible that shark respiratory studies have been biased towards tractable, and perhaps unrepresentative species. Active species are particularly difficult to maintain in good condition in captivity resulting in many experiments having been done on sluggish dogfish-type sharks (Hughes and Umezawa 1968; Grigg 1970; Piiper et al. 1977; Taylor et al. 1977; Brett and Blackburn 1978). Only one study focused on the shark's respiratory adjustments to increased activity (Piiper et al. 1977).

Aquarium techniques developed by Gruber (1980) have enabled workers to utilize an active, tropical elasmobranch, the lemon shark (*Negaprion brevirostris*), in physiological studies. The family Carcharinidae, to which it belongs, is the most specious family of the suborder Galeomorpha, occurs in all the world's oceans, and may be judged one of the more successful group of sharks (Garrick 1967; Moss 1972).

A study of the respiratory system of an active shark's response to exercise is therefore of some interest. Such an investigation should also have value from a comparative aspect, as sharks and teleosts vary in potentially fundamental ways in the morphology of their circulatory and respiratory systems.

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Materials and Methods

Eight juvenile lemon sharks *(Negaprion brevirostris)* ranging in weight from 2.0 to 3.4 kg were used in this study. The animals were caught in the upper Florida Keys by cast netting or with rod and reel with equipped barbless hooks. The sharks were then transported to holding facilities at the University of Miami's Rosenstiel School of Marine and Atmospheric Science on Virginia Key. The animals were maintained for at least two months in 1,000 l recirculating aquaria at a temperature of 25 ± 1 °C and a salinity of 31 ± 1‰. Following cannulation, the sharks were transferred to smaller, 500 l aquaria where they were held individually throughout the course of the experiment.

Sharks were anesthetized with MS 222 (tricaine methanosulfate) and cannulated without artificial ventilation, since the procedure took less than fifteen minutes. Following surgery the sharks were placed in smaller aquaria and allowed to recover for at least 24 h before experiments were performed.

A dorsal aortic cannula was implanted using a procedure modified from Soivio and Oikari (1976; Fig. 1). A 5 cm length of P.E. 160 polyethylene tubing was inserted dorsal-ventrally through the chondocranium by way of a 13 gauge hypodermic needle. Two soft plastic retaining rings 1.0 cm in diameter were threaded over each end of the tube. A hot metal probe was used to flare each end of the tubing to keep the retaining rings in place and to insure the collar assembly would be secured firmly. The cannula consisted of P.E. 50 polyethylene tubing with the tip drawn out to a smaller diameter by warming over a low flame. Approximately 10 cm from the tip, a bulge in the wall was formed by heating and the cannula permanently bent at a 90° angle. A piece of stainless steel wire was threaded into the cannula to give it rigidity sufficient to puncture the dorsal aorta.

Beginning at a point $^2/_3$ of the distance between the collar and the first branchial artery, the tip of the cannula was threaded subdermally along the midline of the roof of the mouth. When the aorta was punctured, the wire was withdrawn and the cannula threaded an additional five to six cm into the aorta to insure that the blood sampled had passed through all branchial arteries. The free end of the cannula was passed through the collar assembly and pulled through the top of the head. No sutures were necessary in this procedure as the bulge in the cannula was too big to pass through the collar. Ventral aortic cannulations were performed in a similar manner. The cannula was implanted in the ventral aorta and led to the outside through the side of the tongue and lower jaw.

Placement of the cannulae was confirmed by x-ray photography immediately after surgery and by dissection at the conclusion of the experiment.

The free ends of the cannulae trailed behind the animal allowing nonstressful blood sampling while the animal had complete freedom of movement. The cannulae were flushed daily with 1.0 mg heparin per ml elasmobranch Ringer's solution (Kline et al. 1978). In most cases this preparation kept the cannulae patent for over three months.

Hematocrit (Hct), oxygen partial pressure (P_{O_2}), oxygen content (C_{O_2}), and pH were assessed for each blood sample. A Radiometer PHM 72/MkII blood gas analyzer was used to measure P_{O_2} and pH; oxygen content was determined by the Tucker method (Tucker 1967). Blood sampled from three animals was pooled for determination of the oxygen dissociation curves. Five ml of whole blood combined with 0.7 ml of Tris buffer were tonometered for one hour with gases of known P_{O_2} mixed by a Wösthoff gas mixing pump. All measurements were made at 24 °C. Hemoglobin bound oxygen was calculated by using the coefficient of solubility ($\alpha = 0.026$) determined at 20 °C for the Port Jackson shark (Grigg 1974).

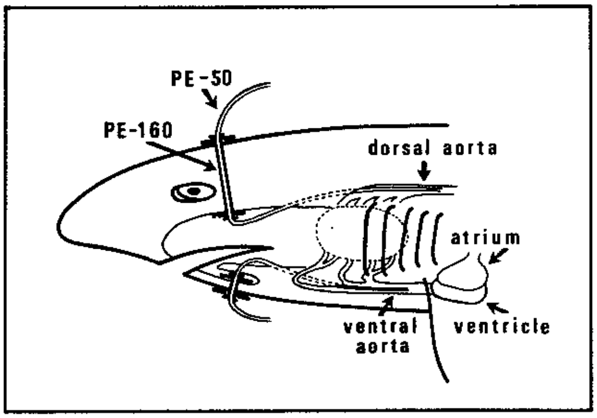

Fig. 1. Site and mode of attachment of the dorsal aortic and ventral aortic cannulae in the lemon shark

Blood gas measurements were made on animals in three activity states: resting, routine swimming, and exercise. A resting state was achieved by allowing the animal to sit undisturbed on the bottom of the tank actively pumping water over its gills for at least five minutes before blood was sampled. Routine swimming was defined as a slow, sustained, but relaxed swimming rate. The exercise state was achieved by forcing the shark to swim at a higher, easily sustainable pace by occasional prodding for a 5 min period.

Results

The oxygen dissociation curves in Fig. 2 were established at 24 °C, and P_{CO_2} approaching 0 (<0.5 Torr). When log P_{50} was plotted against pH over the range of 7.2 to 8.2 (Fig. 3) a line was found with the equation:

$$\log P_{50}\ (\text{Torr}) = -0.36\ (\text{pH}) + 3.81$$

$$n = 12;\ r > 0.96.$$

The fixed acid Bohr effect (log P_{50}/pH) of lemon shark blood is −0.36. Assuming the specific effect of CO_2 over the range of 2 Torr to <5 Torr would be negligible, the equation predicts the oxygen partial pressure at the point of fifty percent saturation (P_{50}) and the physiological pH (7.7) to be 11.8 Torr.

A wide range in hematocrit (13–26%, $\bar{x} = 16.1 \pm 1.5$ (S.E.)%) and hemoglobin bound oxygen in fully saturated blood (4.3–7.7 vol%, $\bar{x} = 4.8 \pm 1.8$ (S.E.) vol%) was measured. A significant correlation ($r > 0.96$) between both parameters was described by the equation:

$$\text{Total Hb} - O_2\ (\text{vol\%}) = 0.302 \times \text{Hct}\ (\%).$$

The arterial and venous blood data are shown in Figs. 4 and 5 and in Table 1.

No significant differences were found between the parameters measured during the resting and routine swimming state. They were therefore considered as one group in comparison with exercise values for sta-

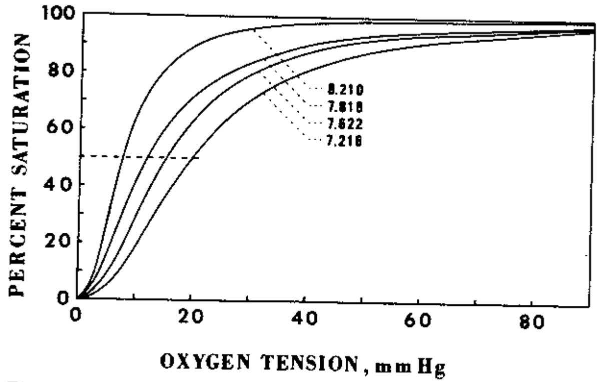

Fig. 2. Oxygen dissociation curves of juvenile lemon shark whole blood at four pH values (24 °C, 0 mm Hg, 15% Tris buffer)

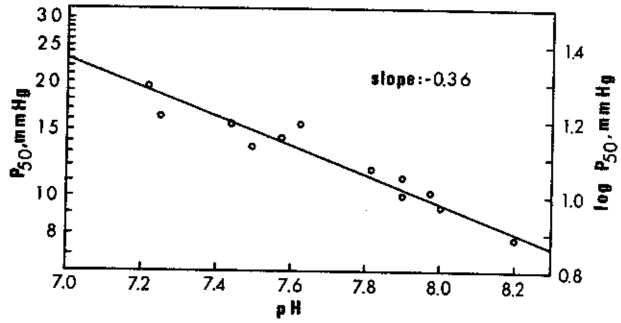

Fig. 3. The Bohr effect of lemon shark blood. Regression line corresponds to the equation:

$\log P_{50} = -0.36\,(\mathrm{pH}) = 3.81$

$n = 12$; (from 9 animals) $r > 0.96$

Table 1. Arterial and venous blood P_{O_2}, oxygen content, pH, and hematocrit content of the lemon shark in different activity states (mean ± S.E.)

	P_{O_2} (Torr)	C_{O_2} (vol%)	pH	Hct (%)	n
Venous	7.1 ±2.7	0.56±0.4	7.54±0.07	12.2±2.7	7
Arterial					
Rest routine	32.5 ±15*	3.06±1.1*	7.72±0.03	14.9±2.6*	10
Exercise	45.35±19.9	4.02±2.0	7.71±0.06	16.3±3.6	10

* $P < 0.05$

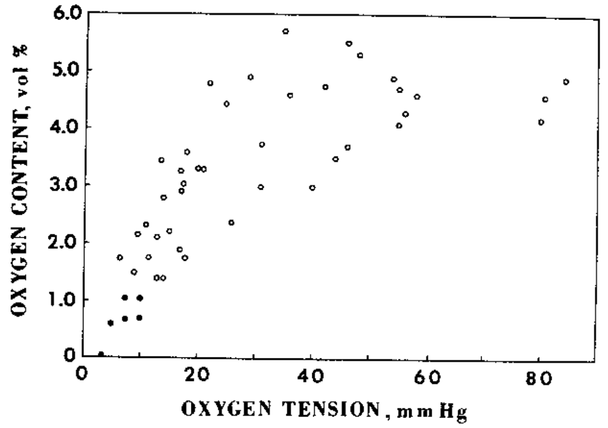

Fig. 4. Oxygen content of arterial (*open circles*) and venous (*closed circles*) blood at *in vivo* P_{O_2} tensions

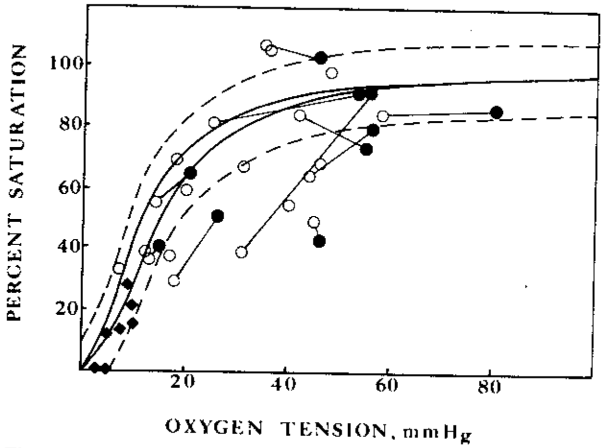

Fig. 5. Percent saturation of arterial (*open and closed circles*) and venous (*closed diamonds*) blood at in vivo P_{O_2} tensions. Individual examples of paired pre-exercise (*open circles*) and exercise (*closed circles*) values are connected by a *solid line*. Oxygen dissociation curves at pH's of 7.7 and 7.8 are drawn in for reference with one standard deviation for each curve represented by *broken lines*

tistical analysis. On a number of occasions, blood measurements were taken in resting and routine-swimming animals without a subsequent exercise period. This general group was considered separate from those resting-routine values determined immediately prior to an exercise experiment. The paired values (pre-exercise and exercise) appear in Fig. 5 as data points connected by a solid line. The remaining arterial values in the figure are members of the general group.

Arterial P_{O_2}'s in the general rest-routine group were widely scattered from 7 to 80 Torr, averaging 30.0 ± 4.6 (S.E.) Torr. Arterial oxygen content of the entire group ranged between 1.2 vol% and 6.8 vol% averaging 3.15 ± 0.02 (S.E.) vol%. Mean pH and hematocrit were 7.71 ± 0.03 (S.E.) and 15.7 ± 1.2 (S.E.) %, respectively. Mixed venous oxygen partial pressure ($P\bar{v}_{O_2}$) varied between 0 and 10 Torr with an average of 7.1 ± 2.7 (S.E.). Oxygen content of venous blood averaged 0.56 vol%, while the pH averaged 7.54.

The means of the paired pre-exercise and exercise blood parameters are presented in Table 1. A significant increase can be seen in arterial P_{O_2}, oxygen content, and Hct during exercise ($P < 0.05$). Arterial oxygen partial pressure rose 40% while oxygen content increased by 31%. A 10% rise in hematocrit was also noted. There was no change in pH however, indicating that during exercise, the sharks were well within their aerobic scope and were probably not utilizing anaerobic metabolic pathways.

Discussion

There are several interesting features concerning the blood/oxygen combining properties and the internal operating oxygen partial pressure of the lemon shark.

Lemon sharks have a high oxygen affinity blood ($P_{50} = 11.8$ Torr) when compared to many other elasmobranchs which characteristically range from 17 to 30 Torr (Manwell 1963; Lenfant and Johansen 1966; Cameron et al. 1971; Hughes 1978). Piiper and Schumann (1967) noted an affinity similar to ours in *Scyliorhinus stellaris* but recorded at a lower temperature (17 °C). In teleosts, high blood oxygen affinity has been associated with low external P_{O_2}; the adult lemon shark, however, is a pelagic animal. It is interesting to note that the lemon shark has a higher affinity blood than the more sluggish dogfish sharks while it has been observed that in teleosts the more active fish (trout and mackerel) often appear to have a lower oxygen affinity (high P_{50}) than the less active fish (Riggs 1970). Possibly, the low P_{50} may be an adaptation to the animal's own internal hypoxic state. At low P_{O_2}'s the higher affinity blood will have a higher capacitance coefficient (Dejours 1975) than low affinity blood resulting in an increased difference between arterial-venous oxygen content at low P_{O_2}'s. A high affinity blood may therefore be advantageous in delivering more oxygen under these circumstances.

The Bohr shift of −0.36 measured in the lemon shark is similar to that found in *Squalus suckleyi* (−0.34) by Manwell (1958, 1963) and *Raja binoculata* (−0.32) (Hughes 1978).

The total oxygen carrying capacity of fully saturated lemon shark blood is comparable to values reported for other elasmobranchs (Lenfant and Johansen 1966; Piiper and Schumann 1967; Hughes 1978). Compared to teleosts, however, it is low. In general, benthic or less active animals have blood oxygen capacities from 7 to 14 vol%, while more active teleosts may range between 11 to 27 vol% (Grigg 1974).

It would appear the lemon shark has a venous oxygen content approaching zero. The arterial oxygen content is much less than fully saturated (60–80%) and increases on activity. Figure 5 graphs the arterial and venous P_{O_2} *versus* percent saturation. Oxygen dissociation curves at pH 7.7 to 7.8 are drawn in for reference. The data broadly follows the oxygen dissociation curves. The venous values are clustered at the lower left hand corner while the arterial values are scattered along the rest of the curve. Individual animals going from rest-routine swimming to an exercise state show a tendency to 'walk-up' the curve. Interestingly, under these circumstances the oxygen dissociation curve functions to load at different P_{O_2}'s in response to demand, in contrast to the usual situation where loading is fixed and the curve tends to unload with increased demand.

A comparison of arterial and venous oxygen content and O_2 partial pressure in other elasmobranchs and teleosts is made in Table 2. In general, they have a high arterial and venous oxygen content. On activity, the typical response is to increase oxygen delivery to the tissues through increased oxygen extraction from the blood by drawing on the venous reserve. Table 3 compares the responses to exercise in an aerobic teleost, the rainbow trout (*Salmo gairdneri*), an elasmobranch, the larger spotted dogfish (*Scyliorhinus stellaris*), and the lemon shark.

In the rainbow trout, increased activity results in a substantial decrease (53%) in venous oxygen content, while changes in arterial P_{O_2} (−6%) and C_{O_2} (−13%) are small. A similar pattern is apparent in the dogfish, where on activity, venous oxygen content decreases by 55%. An indication of the dogfish's inability to handle increased oxygen demand is reflected in the 25% fall in arterial oxygen content. In marked contrast to the previous two cases, the lemon shark has no venous reserve, therefore, on increased demand, arterial oxygen content rises by 31%.

The results (increased oxygen supply to the tissues) are similar but arrived at by opposite approaches. Increased demand can also be met by increases in hematocrit. A rise in hemoglobin concentration during exercise has been documented in many teleosts (Black et al. 1959, 1962, 1966; Stevens 1968; Cameron 1970; Yamamoto et al. 1980), but not in elasmo-

Table 2. Arterial and venous P_{O_2} and oxygen content reported for other elasmobranchs and teleosts

	T (°C)	Pa_{O_2} (Torr)	Ca_{O_2} (vol%)	$P\bar{v}_{O_2}$ (Torr)	$C\bar{v}_{O_2}$ (vol%)	Reference
Heterodontus portjacksoni	20	82	4.8	22.5	2.9	Grigg (1974)
Squalus acanthias	3	104	3.85	18	1.26	Robin et al. (1965)
Squalus suckleyi	11	69	3.55	8.1	1.5	Lenfant and Johansen (1966)
Squalus suckleyi	10	77	–	13	–	Hanson and Johansen (1970)
Squalus suckleyi	10	83	–	10	–	Hanson and Johansen (1970)
Squalus suckleyi	9	104	–	14	–	Cameron et al. (1971)
Scyliorhinus stellaris	17	81	4.5	11	1.5	Piiper and Schumann (1967)
Scyliorhinus stellaris	16	49	4.1	10	1.1	Baumgarten-Schumann and Piiper (1968)
Scyliorhinus stellaris	18	64	3.6	8–12	1.2	Piiper et al. (1977)
Scyliorhinus canicula	12	90.4	–	–	–	Butler and Taylor (1971)
Scyliorhinus canicula	7	90.9	4.0	21.3	2.1	Butler and Taylor (1975)
Scyliorhinus canicula	12	114.4	4.7	34.5	2.7	Butler and Taylor (1975)
Scyliorhinus canicula	17	97.6	4.7	32.9	2.7	Butler and Taylor (1975)
Scyliorhinus canicula	15	64.8	3.9	23.5	2.1	Taylor et al. (1977)
Scyliorhinus canicula	15	95	4.4	23	1.6	Short et al. (1979)
Raja ocellata	10.5	70	–	14	–	Dill et al. (1932)
Dasyatis sabina	25	90	–	14.2	–	Cameron et al. (1971)
Salmo gairdneri	4–8	85	–	19	–	Stevens and Randall (1967a, b)
Salmo gairdneri	10	137	10.3	33.3	7.0	Kiceniuk and Jones (1977)
Tinca tinca	13	36	7.5	7.0	3.1	Eddy (1974)
Cyprinus carpio	10	8	5.2	–	2.5	Garcy (1967)
Negaprion brevirostris	24	32.5	3.06	7.1	0.56	Present study

Table 3. Changes in arterial and venous blood P_{O_2} and oxygen content on increased activity in the dogfish (*Scyliorhinus stellaris*), rainbow trout (*Salmo gairdneri*), and the lemon shark (*Negaprion brevirostris*) (mean ±S.E.)

	Pa_{O_2} (Torr)	Ca_{O_2} (vol%)	Pv_{O_2} (Torr)	Cv_{O_2} (vol%)	Reference
S. stellaris					
Rest	64 ±1.1	3.6	–	1.2	Piiper et al. (1977)
Exercise	57 ±6.5	2.7	–	0.53	
Salmo gairdneri					
Rest	137 ±4.2	10.3±0.5	33.3±6	7.04±0.67	Kiceniuk and Jones (1977)
Exercise	123.3±4.2	8.95±0.5	23.5±2.1	3.33±0.4	
Negaprion brevirostris					
Rest	32.5±15	3.06±1.1	7.1±2.7	0.56±0.4	Present study
Exercise	45.4±20	4.02±1.3	–	–	

branchs. Water loss from the blood or increased erythrocyte supply from the spleen have both been hypothesized to account for this change. Yamamoto et al. (1980) estimated that 40% of the hematocrit increase in an exercised yellowtail (*Seriola quinqueradiata*) was explained by an elevated red blood cell count, while another 35% was due to water shift out of the plasma. Whatever the cause, as a temporary response to activity, this adjustment may be underestimated. Possibly the normal hematocrit is the most efficient to transport oxygen. During emergencies, transient hematocrit increases may occur as the major concern in this circumstance is maximizing oxygen transport (Lutz 1982).

Increased oxygen content of the blood leaving the gill could be achieved by a blood shunt. Although secondary lamellae bypass shunts have been hypothesized in eels (Steen and Kruysse 1964) and other teleosts (Richards and Fromm 1969), as well as dogfish sharks (Piiper and Schumann 1967) they have been dismissed by many authors (Vogel 1978; Vogel et al. 1974, 1976; Cooke 1980; Olson and Kent 1980) as being unlikely. However, the diverse and complicated nature of the interconnecting arteries and sinuses is such that blood shunting between respiratory and nutritive pathways should not be ruled out for some species. Some degree of 'effectual' blood shunting can also be achieved by differential blood flow through intralamellar pathways (Bettex-Galland and Hughes 1972; Smith and Johnson 1977; Holbert et al. 1979).

What would be the possible uses of such systems? Most described species, the highly aerobic trout for instance, maintain high arterial P_{O_2} and have a substantial venous O_2 reserve. Advantages are found in having a high blood to tissue P_{O_2} gradient and an oxygen reserve available for immediate use. However, a drawback is having to continuously maintain a high secondary lamellar blood flow and the possible osmotic and solute cost. The lemon shark (and possibly others) on the other hand may keep blood flow through oxygen exchanging secondary lamellae to the minimum sufficient to meet oxygen requirements. This presumably reduces the cost of regulating unwanted diffusion of metabolites and ions through the lamellae while allowing the blood to perform other transport functions not directly related to oxygen uptake. This, however, results in a low internal P_{O_2} and no oxygen reserve.

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