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J.Fleng Steffensen, Anthony P. Farrell

Institutions: Aarhus University

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Swimming Performance, Venous Oxygen Tension and Cardiac Performance of Coronary-Ligated Rainbow Trout, *Oncorhynchus mykiss*, Exposed to Progressive Hypoxia

J. Fleng Steffensen and A.P. Farrell†*
Department of Zoophysiology, University of Aarhus, Aarhus, Denmark

ABSTRACT. We performed *in vivo* studies to examine the idea that cardiac work is impaired in rainbow trout (*Oncorhynchus mykiss*) below a certain venous Po_2 threshold. We hypothesized that coronary-ligated fish, swimming continuously at a reasonably high water velocity (1.5 body lengths · s^{-1}) and exposed to progressive hypoxia, would fatigue at higher venous Po_2 and ambient water Po_2 compared with sham-operated fish. However, we found that both the lowest venous Po_2 that supported hypoxic swimming (9.9 torr for coronary-ligated fish and 11.1 torr for sham-operated fish) and the venous Po_2 at fatigue (7.8 torr and 8.6 torr, respectively) were the same for coronary-ligated and sham-operated fish. Also, both groups quit swimming at the same water Po_2, heart rate and hematocrit. Nevertheless, significant differences in cardiac performance did exist between the two groups. Whereas ventral aortic blood pressure (P_{va}) increased significantly with hypoxic swimming in sham-operated fish, there was no such increase in coronary-ligated fish. In addition, cardiac arrhythmias occurred in coronary-ligated fish at fatigue, and these fish were slower to recover from exhaustion. We believe that the venous Po_2 threshold to support cardiac performance in the absence of a coronary supply was between 7.8 and 9.9 torr. Furthermore, we suspect that the low P_{va} in coronary-ligated fish effectively lowered their myocardial O_2 demand. Uncertainty still exists regarding whether or not the venous Po_2 threshold lies between 8.6 and 11.1 torr in sham-operated fish. COMP BIOCHEM PHYSIOL 119A;2:585–592, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. Coronary circulation, hypoxia, heart rate, cardiac performance, swimming performance, venous oxygen tension, blood pressure, coronary ligation

INTRODUCTION

For most species of fish, the heart relies on O_2 contained in the venous blood being pumped through the cardiac chambers (the luminal O_2 supply) (19). However, some fish species supplement this luminal O_2 supply with a coronary circulation, an arterial O_2 supply derived from the gills (6,16,37). Fish that posses a coronary circulation fall into three major groupings: (a) all cartilaginous fishes, (b) teleosts that are tolerant of severe hypoxia, such as eels and carp, and (c) teleosts that are capable of moderate to high levels of prolonged swimming activity such as salmonids, tuna and marlin (6,37).

The phyletic distribution of the coronary circulation suggests that the coronary circulation has a selective advantage both under hypoxic conditions and during prolonged swimming activity. This contention is certainly supported by experimental work. A number of *in vitro* studies suggest that the coronary circulation is needed for maximum (rather than routine) cardiac performance, especially under hypoxic conditions (7,8,11,17). Furthermore, *in vivo* measurements of coronary blood flow in salmonids clearly show that coronary blood flow can increase several-fold during both exercise and hypoxic exposure (2,21,22). An increase in coronary blood flow during swimming would help explain why surgical ablation of the coronary artery reduced maximum prolonged swimming speed in some (14,18), but not all (10), salmonid studies.

Although evidence has accrued regarding the importance of the fish coronary circulation under conditions of hypoxia and activity, a curious finding is that the luminal venous O_2 supply is normally far in excess of the O_2 demands of even the largest and most active fish heart (16). This finding was drawn from a relationship between myocardial power output and myocardial O_2 consumption measured directly in isolated, working hearts from various fish species (7,13,19,26). This relationship indicated that myocardial

Address reprint requests to: Tony Farrell, SFU Dept. of Biological Sciences, Burnaby, British Columbia, V5A 1S6, Canada. Tel. (604) 291-3647; Fax 291-3496.

*Permanent address: Marine Biological Laboratory, University of Copenhagen, DK-3000, Helsingor, Denmark.

†Permanent address: Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia, V5A 1S6, Canada.

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O_2 demand was around 1% of the O_2 contained in the luminal venous blood. Thus, it appears that factors related to the rate of O_2 diffusion to the myocardial muscle, rather than the availability of O_2, represent the main selection pressure for a coronary circulation in fishes.

The coronary circulation preferentially perfuses the outer, compact layer of the ventricle. It would seem, therefore, that the coronary circulation acts primarily to compensate for the large diffusion distance and/or the low O_2 gradient between the luminal blood and the outer myocardium in the large, thick-walled ventricle characteristic of highly active fishes. This advantage would be accentuated in situations where venous Po_2 decreases (*e.g.*, during swimming when tissue O_2 extraction increases, or during environmental hypoxia when arterial saturation decreases). What then follows is that there must be a venous Po_2 threshold, below which the rate of O_2 diffusion from the luminal supply cannot satisfy the rate of myocardial O_2 consumption. In this case, cardiac performance would fail unless there was a supplementary coronary O_2 supply. Jones (28) suggested that 10 torr is the absolute limit at which cardiac cells, in general, can extract O_2. Furthermore, Davie and Farrell (6) suggested that a venous threshold for fish lies between 6–16 torr based on a review of venous Po_2 measurements obtained either during progressive hypoxia or during swimming. Experimental verification of a venous Po_2 threshold is lacking for fish.

Here we report the first *in vivo* study that examines the importance of the venous Po_2 gradient in determining cardiac performance in salmonids. To experimentally create a situation in which the heart was dependent on the luminal O_2 supply, we surgically ligated the coronary artery in rainbow trout. Then, while the fish were swimming, venous Po_2 was progressively reduced using environmental hypoxia. We reasoned that the venous Po_2 threshold would be revealed in a situation where cardiac O_2 demand was elevated (*i.e.*, swimming) and the heart relied only on the luminal O_2 supply (*i.e.*, with coronary ligation). Since we know that hypoxia and anoxia can debilitate maximum cardiac performance in salmonids, and maximum cardiac performance is needed to support maximum sustained swimming activity, we anticipated that fish would fatigue when the venous Po_2 threshold was reached. In addition, we expected that the venous Po_2 threshold would be higher in coronary-ligated compared with sham-operated fish.

MATERIAL AND METHODS

Rainbow trout, *Oncorhynchus mykiss*, (body mass: 483–700 g) were obtained from a local fish farm and were kept indoors at 15°C in 500 litre aquaria supplied with well-aerated tap water. The fish were fed daily and acclimated to laboratory conditions with a 12 hr light:12 hr dark regime for at least 2 weeks.

For the surgical procedures, a fish was anaesthetized in water (buffered MS-222; 1:5,000) and placed on an operating table. The gills were irrigated with water containing diluted anaesthetic (buffered MS222; 1:15,000). In one group of fish (coronary-ligated), the coronary artery was ligated via a small incision in the side of the isthmus, as described by Farrell and Steffensen (18). In sham-operated fish, ligatures were not tied around the coronary artery, but a similar procedure was used to make the incision and dissect the coronary artery free of the ventral aorta. In both groups of fish, the ventral aorta was cannulated for blood pressure (P_{va}) measurements and blood sampling. A polyethylene (PE-50) cannula, with side holes near the tip and fitted with a sharpened stilet, was inserted into the exposed ventral aorta in the direction of the heart. The cannula had been heat-shaped so as to conform to the contours of the isthmus, onto which it was sutured with 4-0 silk thread. A connecting cannula was anchored more securely to the skin near the pectoral fins. The cannula was maintained free of blood clots by filling it with saline containing 5,000 IU · mL^{-1} sodium heparin. The incision in the isthmus was closed with 4-0 silk sutures.

Fish were allowed to recover from surgery for at least 24 hr before being transferred to the respirometer, where overnight habituation occurred at a water velocity of around 0.2 body lengths (BL) · s^{-1} under normoxic conditions (water $Po_2 > 140$ torr). The experiments were performed in a modified-Brett swimming respirometer (34). Water Po_2 in the respirometer was controlled to preset values (±1 torr). The water was deoxygenated with compressed nitrogen gas. The experimental temperature was 15 ± 0.5°C. Swimming speed (BL · s^{-1}) was corrected for solid blocking effect of the fish (3).

The experimental protocol consisted of the following steps. The experiment began by increasing the swimming velocity to 0.5 BL · s^{-1}. Heart rate, P_{va}, venous Po_2, haematocrit and water Po_2 were measured after 20 min at this swimming velocity. Swimming velocity was then increased to 1.5 BL · s^{-1} and, after a further 20 min, the above measurements were repeated. Water Po_2 was then reduced in a step-wise fashion using 15 min intervals at 100, 80, 60, 50, 40 and 30 torr, or until the fish fatigued. Heart rate, blood pressure, venous Po_2, haematocrit and water Po_2 were measured at the end of each hypoxic period and also when fish became exhausted. After exhaustion, the swimming velocity was immediately reduced to 0.5 BL · s^{-1} and the water Po_2 was increased. When the water Po_2 had reached 100 torr (approximately 15–20 min after exhaustion), the above measurements were repeated.

The threshold water Po_2 was calculated based on the formula for critical swimming speed (4):

$$\text{Threshold water } Po_2 = Po_{2i} + [(t_i/t_{ii})\ \Delta\ Po_2]$$

where Po_{2i} = the lowest water Po_2 (torr) maintained for a 15 min period, $\Delta\ Po_2$ = oxygen tension increment (torr), t_i = the time (min) the fish swam before fatigue at the low-

est O_2 tension, t_{ii} = the 15 min swimming interval. This formula interpolates for those fish that fatigued within a 15-min. hypoxic period. Between each hypoxic step there was an additional 5 min period of unstable water Po_2 while the Po_2 was being reduced to a new level. This period was ignored in the calculation of the threshold water Po_2 and resulted in a minor under estimate. Following each experiment the fish was killed by a sharp blow to the head and the coronary ligation confirmed by visual inspection. The atrium and ventricle were removed and weighed.

Ventral aortic pressure (P_{va}) was monitored with a Statham GP-23 pressure transducer connected a Data Translation DT2801 interface board and a personal computer. Labtech Notebook programs were used for process control, data acquisition and data processing (*i.e.*, monitoring and control of water Po_2, monitoring and correction of swimming speed and water temperature, monitoring and calculation of a mean P_{va}, and calculation of cardiac frequency from 60-s time intervals). Venous Po_2 and hematocrit (Hct) were measured using 0.5 mL blood samples drawn from the ventral aorta. Radiometer E-5046 oxygen electrodes fitted in a thermostatted Radiometer D-616 cuvette and connected to a Radiometer PHM 73 Acid-Base Analyzer were used to measure Po_2 in the blood and water.

Statistically significant differences ($p < 0.05$) were determined using the Student's unpaired t-test and ANOVA where appropriate. Values are presented as mean ± SD for N fish.

RESULTS

The body mass (580 ± 63 g, N = 9 vs 612 ± 56 g, N = 6), ventricular mass (0.592 ± 0.144 g, N = 8 vs 0.790 ± 0.208 g, N = 5) and atrial mass (0.103 ± 0.032 g, N = 8 vs 0.106 ± 0.029 g, N = 5) were not significantly different in sham-operated and coronary-ligated fish, respectively.

Sham-operated fish swimming at 1.5 BL · s^{-1} usually fatigued when water Po_2 was reduced to either 50 or 40 torr. The threshold water Po_2 for sham-operated fish (45.7 ± 4.8 torr, N = 9) was not significantly different compared with coronary-ligated fish (48.6 ± 7.8 torr, N = 6)(Table 1).

Venous Po_2 values in fish swimming slowly at 0.5 BL · s^{-1} in normoxic water were similar in sham-operated and coronary-ligated fish (36.4 ± 6.0 torr, N = 9 vs 33.8 ± 9.3 torr, N = 6) (Fig. 1). Likewise, P_{va} and Hct were not significantly different between the two groups during normoxic swimming at 0.5 BL · s^{-1} (Fig. 2, Table 1). When swimming speed was increased to 1.5 BL · s^{-1} at ambient water Po_2, venous Po_2 decreased significantly in both fish groups (Fig. 1). This decrease was greater in the coronary-ligated group, resulting in a significantly lower venous Po_2 (17.0 ± 7.1 torr) compared with sham-operated fish (venous Po_2 = 26.8 ± 5.0 torr).

During progressive hypoxia, venous Po_2 decreased significantly in both fish groups, but more so in the sham-operated fish (Fig. 1). As a result, there was no significant difference between the two venous Po_2 values at any level of hypoxia, including when the fish fatigued. At fatigue, the venous Po_2 values were 8.6 ± 2.1 torr in sham-operated fish and 7.8 ± 2.5 torr in coronary-ligated fish. The lowest venous Po_2 that supported hypoxic swimming was 9.9 torr for coronary-ligated fish and 11.1 torr for sham-operated fish (Table 1).

The significant differences between fish groups for heart rate and P_{va} during normoxic swimming were unexpected. Heart rate was significantly lower in sham-operated fish (54.2 ± 16.4 beats · min^{-1}) compared with coronary-ligated (73.0 ± 7.2 beats · min^{-1}) during normoxic swimming at 0.5 BL · s^{-1} (Table 1, Fig. 3). When swimming speed was increased to 1.5 BL · s^{-1} in sham-operated fish, both heart rate and P_{va} increased (to 63.9 ± 14.1 beats · min^{-1} and 69.4 ± 10.9 cmH_2O, respectively, Figs 2 and 3). In contrast, heart rate and P_{va} (68.6 ± 18.6 beats · min^{-1} and 51.9 ± 10.3 cmH_2O, respectively) were unchanged in the coronary-ligated group (Figs 2 and 3). As a result, P_{va} but not heart rate was significantly different in coronary-ligated fish during prolonged normoxic swimming at a moderately high velocity.

During progressive hypoxia, heart rate decreased and Hct increased in both groups. At fatigue, heart rate and Hct were the same in both fish groups (Fig. 3, Table 1). However, all coronary-ligated fish displayed cardiac arrhythmias at fatigue (Fig. 4), unlike the sham-operated fish.

At fatigue, P_{va} was significantly different between the two groups (Fig. 2). P_{va} in sham-operated fish, already elevated by swimming, increased numerically from 69.4 ± 10.9 cmH_2O to 77.2 ± 12.6 cmH_2O. In contrast, P_{va} in coronary-ligated fish was significantly lower (37%) at fatigue (48.5 ± 8.7 cmH_2O). Thus, despite the similar swimming challenges, water Po_2 values and venous Po_2 values for the two groups, the lower P_{va} and cardiac arrhythmias in coronary-ligated rainbow trout at fatigue point to a severe cardiac dysfunction not present in the sham-operated fish. Whereas sham-operated fish increased P_{va} as venous Po_2 decreased (Fig. 5), coronary-ligated fish were unable to increase P_{va}.

Recovery of the sham-operated group was generally faster than that in the coronary ligation group as judged by the rapidity with which fish reoriented themselves, resumed swimming at 0.5 BL · s^{-1}, and recovered their cardiovascular status. At a water Po_2 of 100 torr, some 10–15 min after fatigue, heart rate was 27% higher in sham-operated fish (80.1 ± 9.3 beats · min^{-1}) compared with coronary-ligated fish (63.0 ± 25.5 beats · min^{-1}) (Table 1). This difference was not statistically significant due to the unusually high variance for heart rate in coronary-ligated fish that resulted from arrhythmias persisting into the recovery period. Venous Po_2 also recovered faster in the sham-operated group, as indicated by a significantly higher venous Po_2 (19.0 ± 3.2 torr) compared with coronary-ligated fish (13.9 ± 3.8 torr) (Table 1). Furthermore, P_{va} remained significantly

TABLE 1. Summary of data from control and coronary-ligated fish swimming and exposed to hypoxia

Control fish						Coronary-ligated fish				
Water Po_2, torr	Heart rate, min^{-1}	Blood pressure, cmH_2O	Hct, %	Venous Po_2, torr	Swim speed, $BL.s^{-1}$	Water Po_2, torr	Heart rate, min^{-1}	Blood pressure, cmH_2O	Hct, %	Venous Po_2, torr
145.5	54.2	48.7	20.5	36.4	0.5	143.7	**73.0**	43.8	20.7	33.8
6.8	16.4	4.8	4.4	6.0		3.8	7.2	6.1	2.5	9.3
9	9	9	9	9		6	6	6	5	6
139.4	63.9	69.4	20.9	26.8	1.5	141.7	68.6	**51.9**	23.3	**17.0**
7.7	14.1	10.9	5.4	5.0		3.6	18.6	10.3	2.1	7.1
9	9	9	5	9		6	6	6	5	6
98.9	62.2	69.4	23.3	20.9	1.5	99.5	68.4	54.2	22.5	16.7
1.0	14.7	7.6	13.7	5.5		1.1	18.9	8.3	3.3	6.0
9	9	9	2	9		6	6	6	5	6
80.6	62.4	68.6	22.8	19.9	1.5	79.6	63.7	54.5	23.4	16.0
1.1	14.2	7.8	1.3	4.2		1.2	23.1	7.5	2.7	6.2
9	9	9	5	9		6	6	6	5	6
60.2	52.9	78.2	26.0	14.3	1.5	60.7	61.4	**57.5**	26.1	13.6
1.4	6.1	12.1	1.4	2.9		1.3	19.5	9.5	5.5	4.6
9	9	9	2	9		6	6	6	5	6
50.1	45.7	77.8	28.2	11.1	1.5	50.7	53.2	**52.5**	26.1	9.9
1.4	7.0	9.9	8.9	2.0		1.4	15.2	8.8	6.9	4.0
9	9	9	8	9		6	6	6	5	6
40.5	37.2	79.7	30.3	8.4	1.5	40.5	56.3	**50.0**	28.0	9.0
1.0	6.0	15.0	9.0	2.0		0.2	22.7	4.7	6.6	2.0
7	7	7	6	7		3	3	3	3	3
32.1	39.4	82.8	25.5	6.8	1.5	32.0	41.5	39.8	22.0	5.5
0.5	15.1	2.5	2.1	0.4						
2	2	2	2	2		1	1	1	1	1

Mean value when fish fatigued						Mean values of fish during recovery					
Water Po_2, torr	Heart rate, min^{-1}	Blood pressure, cmH_2O	Hct, %	Venous Po_2, torr	Threshold water Po_2, torr	Threshold water Po_2, torr	Water Po_2, torr	Heart rate, min^{-1}	Blood pressure, cmH_2O	Hct, %	Venous Po_2, torr
40.8	38.6	77.2	25.6	8.6	45.7	48.6	44.6	49.3	**48.5**	28.1	7.8
6.4	6.8	12.6	9.3	2.1	4.8	7.8	8.3	18.6	8.7	6.7	2.5
9	9	9	8	9	9	6	6	6	6	5	6
100.0	80.1	66.9	24.7	19.0	recovery	—	99.7	63.0	**52.5**	21.7	**13.9**
0.3	9.3	10.8	14.0	3.2	0.5 $BL.s^{-1}$		1.7	25.5	8.2	13.3	3.8
9	9	9	5	8			6	6	6	5	6

Values are mean ± standard deviation and N. Mean values in bold type indicate significant differences between the control and coronary-ligated fish ($P < 0.05$).

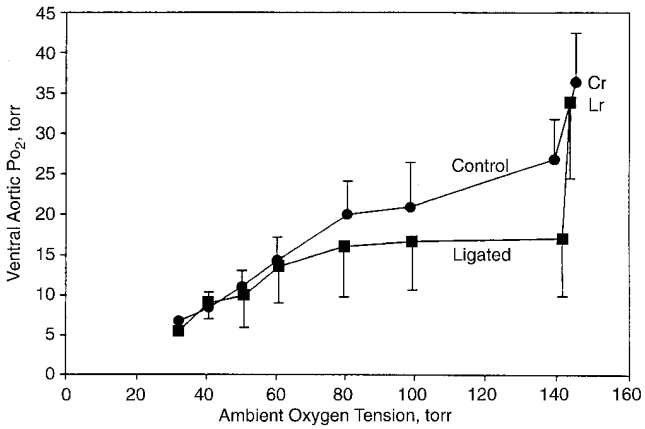

FIG. 1. Venous oxygen tension (venous Po_2, torr) at 0.5 BL · s^{-1} in control (Cr) and ligated (Lr) fish, during normoxic swimming at 1.5 BL · s^{-1}, and during progressive hypoxia while swimming at 1.5 BL · s^{-1}. Values are mean ± standard deviation.

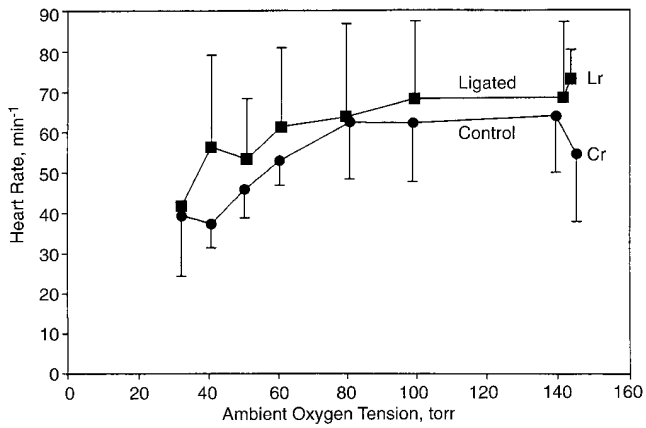

FIG. 3. Routine heart rate (min^{-1}) at 0.5 BL · s^{-1} in control (Cr) and ligated (Lr) fish, during normoxic swimming at 1.5 BL · s^{-1}, and during progressive hypoxia while swimming at 1.5 BL · s^{-1}. Values are mean ± standard deviation.

higher in sham-operated fish (66.9 ± 10.8 cmH_2O) compared with coronary-ligated fish (52.5 ± 8.2 cmH_2O) (Table 1).

DISCUSSION

We are not aware of venous Po_2 measurements under experimental conditions similar to those used here (swimming with progressive hypoxia). Therefore, comparisons have to be limited to the few studies that have measured venous Po_2 during either swimming or hypoxia, but not in combination. These comparisons are generally favorable. In the present study, venous Po_2 for sham-operated fish decreased from 36 torr under normoxic conditions to 8 torr at fatigue under hypoxic conditions. Venous Po_2 values of 16 torr and 9 torr were reported at fatigue with normoxic swimming in rainbow trout (30) and leopard shark (31), respectively. Similar venous Po_2 values were reached with progressive hypoxia experiments in rainbow trout [7 torr (27)], dogfish [7 torr (33)] and eel [6 torr (20)]. While lower venous Po_2 values are reported for progressive hypoxia compared with normoxic swimming, this situation is perhaps not surprising because myocardial O_2 demand is lower during hypoxia compared with swimming. In perfused fish hearts, where the oxygen carrying capacity of the saline is much lower than that of blood, resting cardiac performance began to decline when the saline Po_2 was decreased to around 10 torr (8,9) whereas maximum cardiac performance decreased at a saline Po_2 of 40 torr (17).

Our finding that the lowest venous Po_2 for hypoxic swimming without fatigue was 9.9 torr for coronary-ligated fish

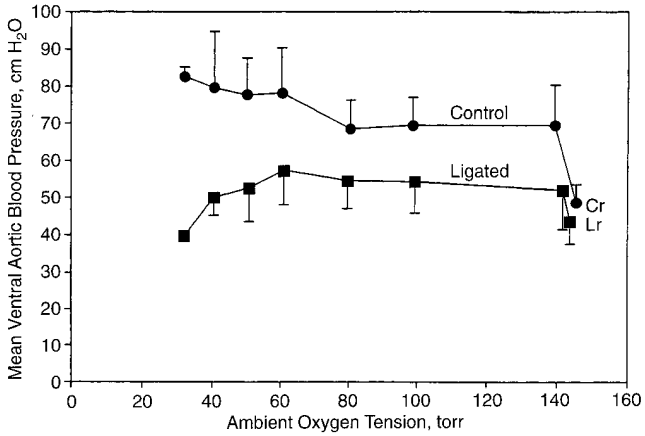

FIG. 2. Mean ventral aortic pressure (P_{va}, cmH_2O) at 0.5 BL · s^{-1} in control (Cr) and ligated (Lr) fish, during normoxic swimming at 1.5 BL · s^{-1}, and during progressive hypoxia while swimming at 1.5 BL · s^{-1}. Values are mean ± standard deviation.

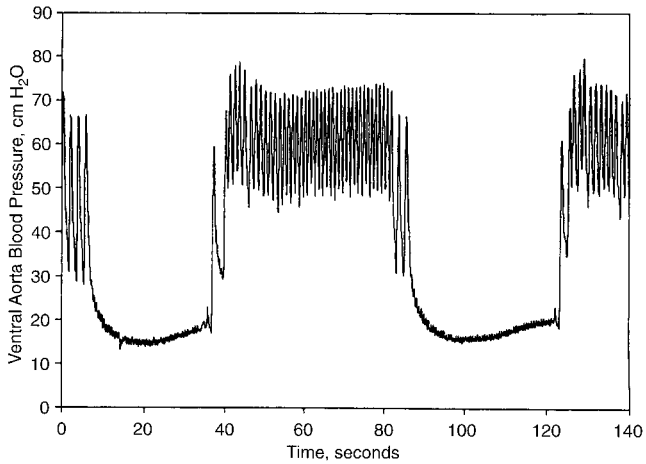

FIG. 4. An example of ventral aorta blood pressure from a coronary-ligated fish at fatigue illustrating an example of severe cardiac arrythmia.

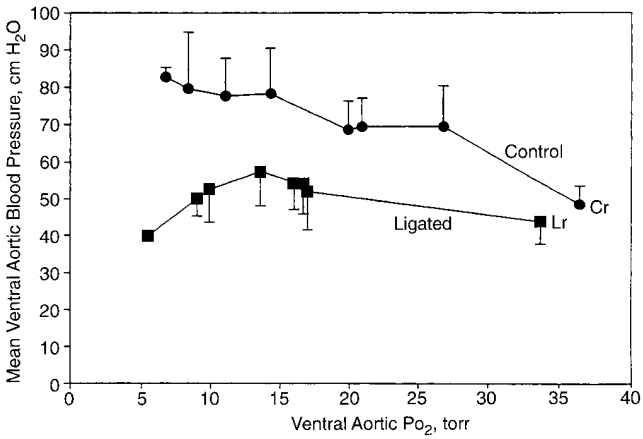

FIG. 5. A plot of mean ventral aortic pressure (P_{va}, cmH_2O) as a function of venous Po_2 (torr). The comparison illustrates that the divergence between the ventral aortic blood pressure in control and coronary-ligated fish increased with decreasing venous Po_2. Values are mean ± standard deviation.

and 11.1 torr for sham-operated fish is certainly consistent with Jones's (28) prediction of 10 torr for the threshold O_2 tension for cardiac cells. Our primary assumption in the present study was that fatigue during hypoxic swimming is associated with cardiac failure that in turn decreases O_2 delivery to locomotory muscles. This assumption was certainly valid for the experiments with coronary-ligated fish, because cardiac failure clearly occurred at fatigue. The observed cardiac arrhythmia's and lower P_{va} at fatigue in coronary-ligated fish are most readily explained by a limited myocardial O_2 supply to the outer compact 30–40% of the rainbow trout ventricle (11,12). Therefore, we can conclude with some measure of certainty that the venous Po_2 threshold to support cardiac performance in the absence of a coronary supply was between 7.8 and 9.9 torr.

The observed reduction in P_{va} in coronary-ligated fish at fatigue is consistent with the known effects of hypoxia on the performance of fish hearts. It is well known that hypoxia reduces maximum isometric force developed by cardiac muscle strips in fish (25). During progressive hypoxia in whole heart preparations, this negative inotropic effect was manifest first as decreased power output and secondarily as decreased cardiac output (17). A related finding is that the pressure generating ability of the hypoxic fish hearts can be restored by initiating coronary perfusion with oxygenated red blood cells (7,8). Collectively, these studies clearly point to the pressure generating ability of the rainbow trout being more sensitive to hypoxia than the flow generating ability. Indeed, even anoxic rainbow trout hearts can maintain a resting level of cardiac output provided pressure generation is at a sub-physiological level (1). Figure 5 shows the degree to which the pressure-generating ability of the heart in coronary-ligated fish was compromised as venous Po_2 decreased. Thus, we conclude that at fatigue the 37% lower P_{va} in coronary-ligated fish most likely reflects hypoxic impairment of the coronary-ligated heart such that it cannot generate normal arterial blood pressures.

In the sham-operated fish we did not observe cardiac abnormalities at fatigue and so the assumption that there was cardiac fatigue is untested. As a result there is some uncertainty whether or not the venous Po_2 at fatigue for sham-operated fish (between 8.6 and 11.1 torr) is indeed a venous Po_2 threshold.

We predicted that the venous Po_2 at fatigue would be higher in coronary-ligated rainbow trout receiving only a luminal myocardial O_2 supply. This was not the case, despite the fact that both groups of fish were swimming at the same velocity and at the same level of hypoxia (here we take this to mean that the locomotory muscles of the fish had a similar level of O_2 demand as well as the fish having a similar level of O_2 supply from the water). Perhaps, the simplest explanation for these observations is that factors other than cardiac fatigue caused the sham-operated fish to stop swimming, and the venous Po_2 threshold for these fish lies below that measured here. However, this interpretation ignores the cardiovascular compensations that were observed in the coronary-ligated fish and so further analysis is needed. The following analysis centres around possible differences in the levels of myocardial O_2 demand at fatigue.

Myocardial O_2 demand is directly related to cardiac power output, the product of cardiac output and P_{va}. Of the two variables needed to estimate myocardial O_2 demand, we only measured P_{va} and therefore assumed that cardiac output would be the same in both fish groups provided Hct, swimming speed and water Po_2 were the same (as was the case here). Several *in vivo* studies with salmonids suggest that cardiac output reaches its maximum level at U_{crit} (24,30,35,36) and that further increases in cardiac output are not possible even with anemic and hypoxic states (5,24,29). Even though maximum cardiac output values determined with *in situ* rainbow trout hearts are somewhat higher than those measured *in vivo* (15,30,32,35), this anomaly may arise simply because there are constraints on cardiac output *in vivo*, such as limited venous return, that are not present *in situ*. The rainbow trout used in the present study swam at about 70% of U_{crit}, and this challenge should have increased cardiac output close to its maximum level (30,35). A further expectation was that cardiac output would reach its maximum at some point during progressive hypoxia, since cardiac output increases slightly in hypoxic rainbow trout (27,38). However, the danger in these assumptions is that, if cardiac output was not at its maximum level for in sham-operated fish, the possibility exists that coronary-ligated fish compensated with a higher cardiac output. While we cannot exclude this possibility, we think it unlikely for several reasons. First, coronary ligation in itself does not alter resting cardiac output in rainbow trout (23). Second, swimming speed (and presumably O_2 consumption), heart rate, Hct (and presumably arterial O_2 con-

tent), and venous P_{O_2} (and presumably tissue O_2 extraction) were all similar at fatigue for sham-operated and coronary-ligated fish. Third, a higher cardiac output in coronary-ligated fish could lead to higher blood pressure (without compensatory vasodilatation), whereas P_{va} was actually lower in coronary-ligated fish.

If we assume that cardiac output was the same in both groups of fish at fatigue, we can conclude that the hypoxic impairment of the myocardium that led to a 37% lower P_{va} in coronary-ligated fish would reduce myocardial O_2 demand by 37%. This reduced myocardial O_2 demand would then preclude the need for a higher threshold venous P_{O_2} at fatigue in coronary-ligated fish, and account for the present observations. Measurements of cardiac output are needed to confirm this speculation.

Although some uncertainty remains about the true venous P_{O_2} threshold in sham-operated fish, it seems reasonable to conclude that the venous P_{O_2} threshold in swimming rainbow trout lies very close to the predicted O_2 threshold of 10 torr for cardiac cells in general (28). Furthermore, observations with coronary-ligated fish show that, when a venous P_{O_2} threshold is reached, the presence of a coronary circulation would prevent cardiac arrhythmias and promote faster recovery. This means that in fish species lacking a coronary circulation, it may be of paramount importance to keep venous P_{O_2} above this threshold level. Indeed, the heart is the last major organ to receive its O_2 supply from the circulatory system, and so venous P_{O_2} may well be "the tail that wags the dog" in terms of sustaining circulation in hypoxic fish. This also means that shifts in the O_2 dissociation curve could be important in setting venous P_{O_2} thresholds.

In summary, the cardiac dysfunction associated with fatigue in coronary-ligated rainbow trout during hypoxic swimming was taken to indicate that a venous P_{O_2} threshold had been reached below 9.9 torr. The lower P_{va} at fatigue was consistent with the myocardium becoming hypoxic at fatigue. Our working hypothesis that the venous P_{O_2} at fatigue would be higher in coronary-ligated fish compared with sham-operated fish proved incorrect.

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