

**EFFECTS OF VENTRICULAR HYPERTROPHY ON
THE CORONARY MICROVASCULATURE
IN MALE RAINBOW TROUT**

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Male rainbow trout (*Oncorhynchus mykiss*) experience a dramatic increase in ventricular mass during reproductive maturation (Clark and Rodnick, 1998). This enlargement of the ventricle is associated with systolic hypertension, expanded blood volume, and a selective enlargement of the coronary-perfused epicardium (Clark and Rodnick 1998, 1999). Given the importance of the coronary circulation to the contracting myocardium of mature fish (Steffensen and Farrell, 1997), and the increased work performed by the enlarged ventricle of male rainbow trout (Clark and Rodnick, 1999), one would predict that growth of the coronary vasculature would match that of the epicardium. However, ventricular hypertrophy due to hemodynamic overload in mammals is often characterized by a decrease in capillary numerical density and reduced performance. In this study, we examined whether the coronary circulation expands during sexual maturation to mirror ventricular growth and help support increased cardiac performance. In addition, we used a theoretical analysis to predict whether ischemic zones would develop in the trout myocardium during reproductive maturation.

Methods

Male rainbow trout ($n = 18$, 20-31 months, 49.5 ± 6.2 cm, 1611 ± 407 g) were obtained from a local fish hatchery. Animals were anesthetized, ventricles were excised, and epicardial layers processed for light microscopy. Semi-thin sections (~ 1.0 μm) of cross-sectional tissue were stained with 0.5% toluidine blue and measurements of capillary density were made using the forbidden line rule. Additional fish ($n = 13$, 20-24 months, 46.0 ± 2.3 cm, 1163 ± 150 g) were anesthetized, had their ventricles removed, and the coronary vasculature was cast with Microfil (Carver, MA) and allowed to polymerize. Epicardial samples were fixed, embedded in paraffin, and sectioned at 5 μm . These sections were stained (aqueous PAS and counterstained in 0.025% toluidine blue) and analyzed for additional measurements of capillary density, diffusion distance and capillary segment length. Anatomical diffusion distance in all tissues was estimated using the closest individual method. To determine capillary lumen dimensions and endothelial cell thickness, ultra-thin sections (~ 100 nm) of epicardial tissue in Spurr's plastic were placed on hexagonal copper grids, stained with 2% uranyl acetate and 0.1% lead citrate, photographed, and quantified.

We applied piecewise linear regression analyses to determine if there was a significant relationship between RVM (relative ventricular mass $<0.125\%$ and $>0.125\%$) and capillary density. We chose 0.125% as the break point for our analysis because an RVM above 0.125% describes a ventricle that is at least 30% larger than the normal-sized ventricle of immature males (RVM $\sim 0.09\%$) and reflective of ventricular hypertrophy (Clark and Rodnick, 1998). Least squares regression analysis was used to test for significance between RVM and diffusion distance, capillary lumen dimensions and endothelial cell thickness.

Statistical significance was established at $P = 0.05$. All data are expressed as means \pm S. E.

We used a three dimensional model of O_2 diffusion (Synder, 1988) to estimate tissue PO_2 at a given distance from the capillaries to cardiac myocytes and therefore define effective diffusion distances. This model uses the following equation and variables: $C_L = C_a - A_T A_C^{-1} L V^{-1} Q O_2$; C_L is $[O_2]$ at a given length, L , C_a is arterial $[O_2]$, A_T is the area of tissue being supplied with O_2 , A_C is capillary cross-sectional area, L is the capillary segment length, V is the velocity of blood in the capillary bed, and QO_2 is the O_2 consumption rate of the tissue at 15 °C. C_a , and QO_2 were derived from published values. Since functional demands on the heart of male salmonids increase in response to reproductive maturation (Clark and Rodnick, 1999), we calculated capillary blood velocity according to three reported values of coronary blood flow (0.14, 0.33, and 0.43 $ml\ min^{-1}\ kg\ body\ weight^{-1}$). These values correspond to about 1% of cardiac output at different activity levels.

We also used a two dimensional model (Boag, 1970) to determine how far O_2 could diffuse into the epicardium from the ventricular lumen. Boag's model uses the following equation and variables: $C_0 = C_a - Kb^2 (3D)^{-1}$; C_0 is $[O_2]$ at a given radius, C_a , is arterial O_2 content, K is the O_2 consumption rate, b is the diffusion distance, and D is the O_2 diffusion coefficient. Values for C_a , K , and D were obtained from published data.

Results

After an initial decrease, presumably due to ventricle growth (RVM $>0.10\%$ but $<0.12\%$), capillary density increased to pre-hypertrophy levels (Fig. 1).

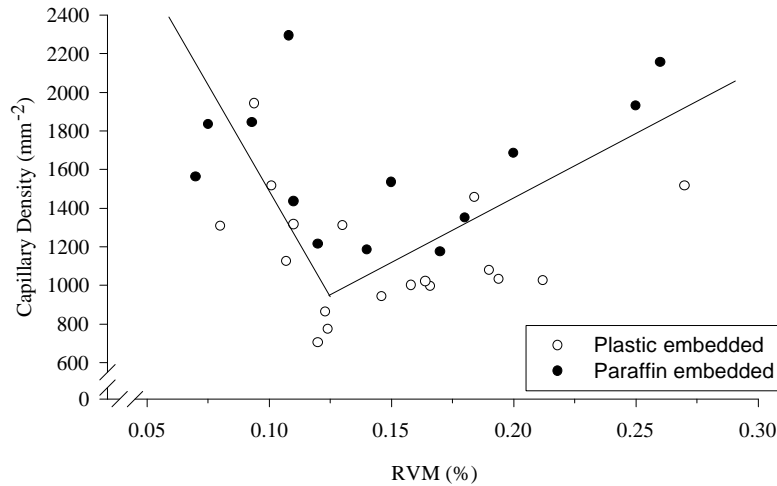


Fig.1. The relationship between RVM and capillary density. Significant relationships existed for RVM < 0.125%: $n = 14$, $y = 2914 - 14673(x)$ and RVM > 0.125%: $n = 17$, $y = 313 - 5398(x)$, overall $P = 0.006$.

Linear regression analysis revealed that there was not a significant relationship between RVM and anatomical diffusion distance ($11.2 \pm 2.4 \mu\text{m}$) in fish with RVM < 0.125 ($P = 0.07$, $r^2 = 0.25$) or in fish with RVM > 0.125% ($P = 0.07$, $r^2 = 0.20$). In addition, RVM and capillary lumen dimensions (major axis = $6.4 \pm 1.2 \mu\text{m}$, $P = 0.14$), were not related, suggesting that diffusion distance and capillary dimensions were conserved during ventricular enlargement. There was a trend for endothelial cell thickness to increase during ventricular enlargement ($P = 0.06$), and this provided direct evidence for capillary proliferation (Zhou et al., 1998). At maximum rates of O_2 consumption ($3.12 \times 10^{-5} \text{ mole O}_2 \text{ L}^{-1} \text{ sec}^{-1} \text{ g ventricle}^{-1}$), maturing male trout (RVM ≥ 0.14) should maintain epicardial O_2 levels well above hypoxic values (Table 1). However, it is unlikely that passive diffusion of O_2 from the endocardium to the epicardium can extend beyond $20 \mu\text{m}$ during maximum rates of oxygen consumption.

Table 1. Epicardial tissue PO₂ at selected diffusion distances (D/2) from the capillary lumen given a capillary segment length of 65 μm. Estimates of tissue PO₂ were made on four different-sized ventricles. For each ventricle, the PO₂ was determined at basal and maximum rates of oxygen consumption. We corrected capillary blood velocity for predicted elevations in coronary blood flow, presumably associated with ventricular hypertrophy and increased cardiac workload. This, in turn, will increase tissue PO₂ at high RVMs. All data are reported in mm Hg.

RVM (%)	Conditio n	D/2 (μm)					
		5	9	11	13	15	17
0.10	Basal	116	109	105	100	95	85
	Maximum	112	101	94	86	76	66
0.14	Basal	111	98	90	81	70	58
	Maximum	104	82	68	51	33	11
0.16	Basal	101	75	57	38	16	0
	Maximum	87	41	10	0	0	0
0.18	Basal	105	84	71	55	38	18
	Maximum	94	58	34	7	0	0

Summary

During ventricular enlargement in male rainbow trout, the epicardium contributes more proportionately to total ventricular mass (up to 75%) than the endocardium, (Clark and Rodnick, 1998) and overall cardiac work increases (Clark and Rodnick 1999). We hypothesized that the coronary circulation plays a more important role in the physiological function of the ventricle as the epicardial layer expands during sexual maturation. Our morphological measurements suggest that adaptive growth of the coronary microcirculation (i.e., capillary angiogenesis) and maintenance of capillary length density helps to promote enhanced physiological performance of the enlarged ventricle of maturing male trout. Indeed, our modeling of O₂ diffusion in the growing trout heart shows that even during enlargement, diffusion of O₂ from the coronary

microvasculature (over an 11 μm distance) should provide adequate aerobic support for maximum contractile performance of the epicardium.

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