Pressure and volume overloads are associated with ventricular hypertrophy in male rainbow trout

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Clark, Raymond J., and Kenneth J. Rodnick. Pressure and volume overloads are associated with ventricular hypertrophy in male rainbow trout. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R938–R946, 1999.—We investigated whether ventricular hypertrophy in reproductively mature male trout (Oncorhynchus mykiss) is associated with elevated hemodynamic loads. We measured ventral aortic blood pressure, pulse pressure dynamics, and blood volume in cannulated, unanesthetized trout with a wide range of relative ventricle masses (RVM; 0.076–0.199% of body wt). We also investigated in vitro pressure-volume dynamics in the bulbus arteriosus taken from trout with a wide range of RVMs. RVM was positively correlated with peak systolic pressure (SBP), mean blood pressure, and pulse pressure. Diastolic pressure and the absolute duration of arterial systole were similar among all animals, but a lower heart rate and a smaller relative duration of arterial systole were correlated with increasing RVM. Blood volume was expanded up to 34% as ventricles enlarged, and clearance of Evans blue dye was greater at higher SBP. Mass, maximal volume, and the pressure-volume dynamics of the bulbus were similar among all animals, suggesting that the bulbus did not compensate for ventricular enlargement. This conclusion was supported by the elevated maximal rates of arterial pressure development (+dP/dt) and decay (−dP/dt) observed as RVM increased. We concluded that 1) mature trout are hypertensive and hypervolemic, 2) the dynamics of the bulbus may contribute to increased afterload, and 3) these changes in hemodynamic load may promote ventricular hypertrophy.

Oncorhynchus mykiss; cardiac hypertrophy; blood pressure; blood volume; pressure-volume dynamics

CARDIAC HYPERTROPHY and its underlying etiology have been studied intensively in mammals, but until recently it has received little attention in fish. Several recent investigations have established that hypertrophy of the cardia ventricle is associated with reproductive maturation in male, but not female, rainbow trout (Oncorhynchus mykiss) (2, 4, 12). These studies demonstrate that relative ventricle mass [RVM; (ventricle weight/body wt) × 100] is up to threefold greater in mature males with a large gonadosomatic index [GSI; (gonad weight/body wt) × 100] when compared with females or immature males. In mammals, the primary precursor for both pathological and physiological enlargement of the heart is an elevated hemodynamic workload (30). Studies in trout (10, 16) also suggest that cardiac work influences ventricular growth in fish. Because reproductive maturation and spawning activities increase functional demands on the heart of male salmonids (1, 28), it is plausible that hemodynamic overload promotes the dramatic ventricular hypertrophy observed in mature male trout.

Types of hemodynamic overloads leading to cardiac hypertrophy in mammals can be segregated qualitatively into pressure and volume overloads. Defined broadly, a pressure overload is an increase in resistance to ejected blood volume. Although increased vascular resistance is the etiologic factor most commonly cited to explain cardiac hypertrophy in mammals (30), the presence of the gill circuit between the heart and systemic vessels in fish imposes constraints on pressure development during systole. Dampening of the pressure pulse is accomplished by the elastic bulbus arteriosus, which is analogous to the ascending aorta in mammals. Adequate function of the bulbus depends on its distensibility (i.e., fractional change in volume per unit change in pressure) and compliance (i.e., change in volume per unit change in pressure) (9, 11). A reduction in bulbus distensibility or compliance relative to ventricular output could elevate cardiac afterload and lead to ventricular hypertrophy, as occurs with decreased aortic compliance in humans (14).

Volume overload, wherein end diastolic volume is increased, can be invoked by an expanded blood volume (VB). Increased VB could elevate central venous pressure, enhance end diastolic volume, and result in a greater stroke volume (6, 31). Although VB has not been compared previously between mature and immature fish, Franklin and Davie (12) demonstrated that maximum stroke volume increases with ventricular hypertrophy in mature male trout.

The purpose of this study was to ascertain whether ventricular hypertrophy in male trout is associated with an increased hemodynamic load. Using male trout with a broad range of RVMs (representing increasing degrees of hypertrophy), we measured blood pressure and pulse pressure dynamics in the ventral aorta, V6 with the use of Evans blue dye (EBD), and the pressure-volume dynamics of the bulbus arteriosus. We describe relationships between these hemodynamic variables and the relative mass of the ventricle. Our observations indicate that ventricular hypertrophy in reproductively mature male trout is associated with hypertension and hypervolemia, and the pressure-volume dynamics of the bulbus do not compensate for ventricular enlargement. Furthermore, our data demonstrate that higher blood pressure has important implications for estimations of blood volume.

MATERIALS AND METHODS

In Vivo Measurements of Hemodynamics in Trout

Animals. Rainbow trout [n = 16, body wt = 752.9 ± 115.1 g (mean ± SE), fork length = 37.7 ± 2.6 cm, 14–17 mo old] were...
supplied by a commercial hatchery (Clear Springs Foods, Buhl, Idaho). The animals were housed outdoors in concrete raceways supplied with constant 14 ± 1°C spring water and fed daily with a commercial trout pellet. All experiments took place between September and December of 1997.

Surgical procedures. Individual fish were netted and transferred to an oxygenated anesthetic bath containing 1% NaCl (to reduce osmotic stress), 5.0 mM sodium bicarbonate, and 0.02% MS-222 (Crescent Chemical, Phoenix, AZ). Upon loss of locomotory ability, fish were weighed and placed upright in a holder made from 8-in. polyvinyl chloride (PVC) pipe split lengthwise. Animals were kept moist throughout surgery, and constant retrograde irrigation of the gills was provided (8.6 l/min) by means of a recirculating, oxygenated solution (−7°C) containing 1% NaCl, 5.0 mM sodium bicarbonate, and 0.006% MS-222.

A Dr. M. Moto-Tool (model 3801; Emerson Electric, Racine, WA) fitted with a 3.3-mm-diameter stainless steel grinder was used to smooth the teeth on the tongue to prevent abrasion of the cannula. We cannulated the ventral aorta (VA) (using slight variations of the procedures presented in Gamperl et al. (13). Cannulas (PE-50 Intramedic; Clay Adams, Parsippany, NJ) were cut to a constant length (0.8 m), and a small bubble was made 1.5 cm from the tip of each cannula to assist in securing it to the animal. We used a drilling motion to insert a beveled (60°), 12-mm-long, 23-gauge needle into the isthmus between the first and second gill arch. When resistance ceased, the needle was removed, and a heparinized cannula fitted with an indwelling stainless steel wire (18-gauge guitar string) was inserted immediately. After insertion, the wire was removed slowly, and the cannula was positioned to maximize blood flow. We cleared blood from the cannula by injecting ~0.5 ml of filtered (0.22 μm), sterile 1% NaCl containing 100 U/ml lithium heparin. The cannula was connected to a blood pressure analyzer (see Measurement of blood pressure) to facilitate final positioning of the cannula within the VA and to monitor blood pressure during the remainder of surgery. The cannula was secured to the tongue with ligatures (4-0 coated Vicryl and curved RB-1 needle; Ethicon, Somerville, NJ) applied immediately anterior and posterior to the bubble. A third ligature was applied posterior to the tip of the tongue to prevent cannula movement with respiratory movements or swimming. A 12-gauge steel needle was used to insert a 2-cm-long heat-flared sleeve (PE-205, Intramedic) through the bottom of the mandible at a position slightly rostral to the tip of the tongue and 0.5 cm lateral from the midline of the jaw. The cannula was disconnected from the blood pressure monitor, exteriorized through the sleeve, and cleared with 0.5 ml heparinized saline, and the end was tied. Total time under anesthesia for each fish was 10–20 min.

Fish were placed into holding tubes manufactured of PVC pipes (8 in. diameter, 30 in. long) with rigid plastic mesh (30 × 30 mm lattice) covering the ends and narrow slits cut into the tops. Tubes were immersed in concrete raceways and oriented so that fish faced upstream into flowing (3.7 cm/s), 14°C spring water. Cannulas were then exteriorized from the tube through the slits. Fish always recovered equilibrium and swimming ability within 10 min and were subsequently able to maintain position in the tubes with minimal exertion. Preliminary results indicated that 10 h after surgery was sufficient time for stabilization of blood pressure. We permitted fish to recover for 13–18 h before blood pressure measurements and 18–32 h before blood volume determinations.

Measurement of blood pressure. Cannulas were flushed with 0.3 ml of sterile, filtered (0.22 μm) 1% NaCl containing 100 U/ml lithium heparin and then connected via a 23-gauge needle hub to a blood pressure analyzer and transducer (DigiMed BPA 200a; Micro-Med, Louisville, KY). The DigiMed BPA 200a facilitated high-resolution recordings because minimal fluid flow (~0.02 ml/mmHg) across the transducer element was required for detection. The analyzer and chart recorder were calibrated daily with a water-filled manometer. Pressure tracings were recorded for 1 min and monitored for 5 min on a model L45 two-channel flatbed recorder (IITC, Woodland Hills, CA). Recordings were made at chart speeds of 2 and 10 mm/s (Fig. 1). Pressures were recorded for each animal at ~13 and ~22 h post-surgery.

Hemodynamic variables. Peak systolic blood pressure (SBP), end diastolic pressure (DBP), pulse pressure (PP), heart rate (HR), and absolute and relative duration of arterial systole were determined directly from chart recordings by means of a digital caliper (Ultra-Cal Mark III; Fowler, Newton, MA). The highest and lowest points on each pressure spike were used to determine SBP and DBP, respectively. Preliminary analyses indicated that deriving mean arterial blood pressure (MAP) with a standard mammalian calculation [MAP = DBP + 1/3(SBP − DBP)] overestimated MAP. Therefore, MAP was derived by digitizing pressure tracings on a Summagraphics digitizing tablet (Sammagraphics, Houston, TX) and measuring area under the curve with SigmaScan software (Jandel Scientific, San Rafael, CA). Maximum rates of arterial pressure development (+dP/dt) and decay (−dP/dt) were determined from tangents drawn to the rising and falling phases, respectively, of pressure tracings.

Determination of blood volume and Evans blue kinetics. Within 30 min of final blood pressure measurements, 0.5 ml of blood was withdrawn via the VA cannula of each fish and used to determine hematocrit and to establish a plasma reference. Only animals with a hematocrit > 20% were included in the study. EBD (T-1824; 7.0 mg/ml in sterile, filtered 1% NaCl) was injected (1.0 ml/kg body wt) into each animal via the VA cannula, and the cannula was flushed with 0.25 ml sterile, filtered saline containing 10 U/ml lithium heparin. Blood samples (0.5 ml) were withdrawn every 15 min for 90 min. Each sample was placed into heparinized (20 μl of 10 U/ml lithium heparin) 1.5-ml polypropylene microcentrifuge tubes and centrifuged at 600 g for 1 min. Plasma was removed and frozen immediately in cryovials at the temperature of liquid nitrogen. The pellet containing blood cells was resuspended gently in a volume of sterile, filtered saline equal to the

Fig. 1. Representative tracings of blood pressure (BP) in ventral aorta (VA) of unanesthetized, unrestrained male rainbow trout. Pictured are examples of BP tracings from an immature trout (top) with normal ventricle [relative ventricle mass (RVM) = 0.078] and a sexually mature trout (bottom) exhibiting ventricular hypertrophy (RVM = 0.199). Note change in time scale halfway through tracing.
volume of plasma removed, and the resuspended cells were reinjected into the animal via the cannula. Hematocrit was determined again at the final sample.

To determine the concentration of EBD at each time point, plasma samples (and plasma controls) were diluted 1:10 with 1% NaCl, and the absorbance at 600 nm was measured spectrophotometrically. We converted each absorbance to a concentration of dye (mg/ml plasma) by means of a standard curve, plotted concentrations vs. time, and used least-squares regression analysis over the initial linear portion of the curve (15–60 min) to determine the theoretical concentration of dye at time 0. The following standard pharmacokinetic formula (25) was used to calculate plasma volume:

\[ V_p = \frac{D_0}{P_C} \]  

(1)

where \( V_p \) = plasma volume, \( D_0 \) = amount of dye injected (mg), and \( P_C \) = plasma concentration of dye (mg/ml). To calculate the volume of blood in each animal, we employed the following equation:

\[ V_B = V_p/(1 - \text{hematocrit}) \]  

(2)

Finally, we expressed \( V_B \) as milliliters of blood per kilogram animal body weight.

EBD is known to leak from fish vasculature even when bound to plasma proteins (20, 21, 29). This raised the possibility that if hemodynamics were different in animals exhibiting ventricular hypertrophy, the loss of dye (and therefore volume estimates) would not be comparable among all animals. Preliminary analysis suggested that \( V_B \) was elevated up to 70% as RVM increased. We questioned the magnitude of this apparent increase, as it seemed likely that the elevated blood pressures observed as ventricles enlarged might have inflated our estimates of \( V_B \). To evaluate this possibility, we used an open two-compartment pharmacokinetic model to calculate the kinetics of EBD in our animals (19, 25). This type of model was appropriate based on the biexponential decay in dye concentration over time, as demonstrated in Fig. 2. We used the following design in our two-compartment model:

\[
\begin{align*}
\text{Compartment 1} & \quad \text{Compartment 2} \\
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\end{align*}
\]

where compartment 1 represents the central compartment or blood volume, and compartment 2 represents the extravascular compartment. The rate constants represent the movement of EBD from compartment 1 to compartment 2 (\( k_{12} \)), from compartment 2 to compartment 1 (\( k_{21} \)), and elimination of drug from the central compartment (\( k_{10} \)). Using this model, we calculated the volume of distribution (\( V_C \)) of EBD in the central compartment as a function of body weight:

\[ V_C = \frac{4\times\text{dose}((A + B)/\text{body wt}) \times 100}{(A + B) \times \text{body wt}} \]  

(3)

where \( A \) and \( B \) are the y intercepts for the distribution and elimination phases, respectively, of the plasma-level time curve (Fig. 2). We then used our calculated \( V_C \) to estimate clearance of EBD from the central compartment:

\[ \text{Clearance} = V_C \times (k_{12} + k_{21}) \]  

(4)

Our analysis indicated that elevated SBP was associated with greater clearance and a larger \( V_C \) for EBD (see RESULTS), suggesting that EBD was being removed or driven out of the vasculature at higher blood pressures. To more accurately estimate \( V_B \) and avoid inflation of our volume estimates caused by extravasation of EBD, we recalculated \( V_B \) based on our earliest sampling point (15 min). Our reasoning was that there would be little loss of EBD from the central compartment at an early sampling time (3).

After the collection of blood samples, animals were killed by a sharp blow to the head. Body weight and fork length were recorded, and the testes and ventricle were isolated, rinsed briefly with 1% NaCl, blotted dry, and weighed. The GSI was employed as an indicator of reproductive maturation (12). We dissected the gill isthmus of several animals to confirm that we had placed the cannula correctly in the VA. Our designation of ventricles as hypertrophied was based on the definition proposed by Clark and Rodnick (4), whereby a ventricle is considered hypertrophied if the RVM exceeds by 30% or more the mean RVM of immature male and female trout in the population from which the study animals were drawn. For the population used in this study, an RVM > 0.11% was considered hypertrophied.

Pressure-Volume Relationships of the Bulbus Arteriosus

Animals. Trout (\( n = 17 \), body wt = 793.5 ± 86.9 g, fork length = 37.6 ± 1.6 cm) were supplied by Clear Springs Foods and housed as indicated previously. All experiments were completed in October of 1998.

Experimental procedures. Animals were anesthetized with 0.02% MS-222 and killed by a sharp blow to the head. We recorded body weight and fork length and then exposed the heart and bulbus arteriosus with a single ventral incision. After determining the in vivo length of the bulbus, we excised the intact ventricle and bulbus, being careful to include at least 0.5 cm of the VA. The ventricle was cut away, leaving a small portion still attached to the bulbus, and the ventricle and bulbus were rinsed with 1% NaCl at 15°C, blotted dry, and weighed. A 14-gauge needle was inserted through the

![Fig. 2. Representative semilog plot showing concentration of Evans blue dye (EBD) in trout plasma for each sampling time. Regression lines highlight biexponential nature of EBD disappearance in trout blood, indicating that an open, 2-compartment pharmacokinetic model is appropriate. Dotted line represents exchange of EBD between compartments (distribution phase), and solid line represents slow elimination of EBD from animal (elimination phase). A and B represent y-intercepts for each regression line and are used to calculate clearance of dye.](http://ajpregu.physiology.org/DownloadedFrom)
ventricular-bulbar valve and secured with 3-0 surgical silk. The needle hub was attached to a three-way plastic stopcock, which was connected to a calibrated pressure transducer (Digimed BPA 200a; Micro-Med) and a calibrated syringe pump (ISCO, Lincoln, NE). Air was evacuated from the preparation by flushing the bulbus lumen with 1% NaCl followed by tying the ventral aorta with 3-0 silk to prevent leakage. The bulbus surface was kept moist throughout the procedure by frequent external applications of 1% NaCl. The bulbus was inflated with 1% NaCl (1 ml/min) to a maximum lumen pressure of 80 mmHg then deflated. Tracings were captured on a Model L45 2-channel flatbed recorder (IITC, Woodland Hills, CA) set to record at a rate of 1 mm/s. Each bulbus was subjected to three preliminary inflation-deflation cycles, followed by four cycles that were recorded for analysis. At the end of the experiment, the bulbus minus the VA and ventricle was weighed.

Data analysis. Pressure tracings were transcribed with a digital caliper. Volumes were calculated by using the time component on the chart recorder and factoring the syringe pump rate (1 ml/min). Plots of bulbus volume vs. pressure were used subsequently to describe the hemodynamic characteristics of the bulbus. Based on our tracings, we identified the range of greatest compliance (i.e., the portion with minimal slope) for the pressure-volume curve for each animal and calculated compliance and distensibility over this range by means of the following formulas:

\[
\text{Distensibility} = 100 \left( \frac{\Delta V}{\Delta P \times V} \right) \tag{5}
\]

\[
\text{Compliance} = \frac{\Delta V}{\Delta P} \tag{6}
\]

where \(\Delta V\) and \(\Delta P\) refer to the change in volume and pressure, respectively, and \(V\) refers to the initial volume at the beginning of this compliant range. We used this range to determine the average pressure at which bulbi were most compliant, as well as the scope of the range. Finally, we estimated maximal lumen volume as the volume of the bulbus arteriosus at a lumen pressure of 80 mmHg, which represented the point at which \(\Delta P/\Delta V\) rose exponentially for each animal.

Statistical Analyses

We used simple correlation analyses to describe relationships between RVM and both hemodynamic and pharmacokinetic variables and between blood pressure (peak systolic and mean) and the volume-pressure relationship of the bulbus. A modified Bonferroni correction was employed in all cases to avoid the effects of familywise error (18). Thus a \(P\) value > 0.01 was considered nonsignificant. The predicted linear relationships between RVM (or SBP) and hemodynamic variables (e.g., MAP, \(V_p\), clearance, and compliance) were determined by the method of least squares.

RESULTS

Reproductive Maturation Induces Ventricular Hypertrophy

Our data demonstrate that an increase in the gonadosomatic index was positively and strongly \((y = 0.027x + 0.091; r^2 = 0.91; P < 0.001)\) associated with enlargement of the ventricle (RVM; Fig. 3). Indeed, increasing mass of the testes was associated with up to a 2.5-fold increase in RVM. Because body weight and fork lengths were similar among all animals, this increased RVM reflects an absolute hypertrophy of the ventricle.

Ventricular Hypertrophy is Associated with Altered Hemodynamics

Our data also demonstrate that hemodynamics differ as the magnitude of hypertrophy (i.e., RVM) increases from normal to mature trout. RVM was positively associated with increased SBP \((y = 172.4x + 24.6; r^2 = 0.59; P < 0.01; \text{Fig. 4A})\), MAP \((y = 102.1x + 23.9; r^2 = 0.47; P < 0.01; \text{Fig. 4C})\), and PP \((y = 98.1x + 2.6; r^2 = 0.70; P < 0.01; \text{Fig. 4D})\), but DBP was similar among all animals (mean \(= 32.7 \pm 1.5 \text{ mmHg}; \text{Fig. 4B})\). Ventricular hypertrophy also correlated positively and strongly \((P < 0.001)\) with \(+dP/dt\) \((y = 444.4x - 2.9; r^2 = 0.75; \text{Fig. 5A})\) and \(-dP/dt\) \((y = 371.5x - 23.3; r^2 = 0.80; \text{Fig. 5B})\), but HR decreased by as much as 34% \((y = -117.9x + 60.8; r^2 = 0.60; P < 0.01; \text{Fig. 5C})\) with increasing RVM. The absolute duration of arterial systole was similar among all animals \((0.32 \pm 0.01 \text{ s}; \text{Fig. 5D})\), but the relative duration of arterial systole decreased by as much as 33% \((y = -58.0x + 31.3; r^2 = 0.62; P < 0.01; \text{Fig. 5E})\) as RVM increased.

Blood Volume and Clearance of EBD are Increased with Ventricular Hypertrophy

Our preliminary analysis (using standard indicator dilution methods and back-calculating to time 0) indicated that plasma volume increased by up to 68% with ventricular enlargement (data not shown). Hematocrit was similar in all animals \((26.8 \pm 0.9\%), \text{ which resulted in up to a 70% expansion in } V_p \text{ (data not shown) as RVM increased. Our concerns about the loss of EBD from the vascular compartment were supported by our pharmacokinetic analysis, which indicated that elevated SBP was associated with up to a threefold greater clearance of EBD (range: 0.80–3.35 ml·kg\(^{-1}\)·h\(^{-1}\); \(y = 0.08x - 1.91; r^2 = 0.77; \text{Fig. 6A})\). The apparent \(V_c\) in the vascular compartment, expressed as a percentage of body weight, increased by as much as 75% with greater RVM (range = 1.95–3.43%; \(y = 0.04x + 0.89; r^2 = 0.60; \text{Fig. 6B})\). This agrees with the apparent increase in \(V_b\).
determined by standard indicator dilution methods. However, using only the earliest sampling time (15 min) to calculate plasma and blood volumes, we observed up to a 27% increase in plasma volume ($r^2 = 0.61$; $P < 0.01$; data not shown) and as much as a 34% elevation in $V_B$ ($r^2 = 0.64$; $P < 0.01$; Fig. 7) as ventricles enlarged. These increases in $V_B$ are proportionally smaller compared with results obtained with standard techniques or with pharmacokinetic analysis, perhaps as a result of the reduced loss of EBD at the earlier time point.

**Bulbus Pressure-Volume Characteristics are Unchanged with Ventricular Hypertrophy**

The pressure-volume relationship for each bulbus had a characteristic sigmoidal shape (Fig. 8). Bulbus weight (0.20 ± 0.01 g) and maximum lumen volume (0.84 ± 0.03 ml) were similar among all animals despite the wide range of RVMs (0.067–0.183%). We observed no changes in bulbus compliance (0.024 ± 0.002 ml/mmHg) or distensibility (5.38 ± 0.29%) over the range of physiological pressures as ventricles enlarged. Likewise, we noted no differences among animals in the average pressure at maximal compliance (45.8 ± 3.0 mmHg) or the scope of maximal compliance (9.9 ± 0.8 mmHg).

**DISCUSSION**

Despite the essential role hemodynamic load plays in inducing cardiac growth in mammals (30), few studies have examined whether increasing workload promotes growth of the fish heart. The aim of our study was to investigate whether the well-documented ventricular enlargement of mature rainbow trout is associated with elevated blood pressure and/or blood volume. Our approach was to measure hemodynamic variables in reproductively immature and mature male rainbow trout and subsequently describe the hemodynamic load on the heart.

**Trout Exhibiting Ventricular Hypertrophy are Hypertensive and Hypervolemic**

Our study demonstrates for the first time that the ventricular hypertrophy observed as trout become reproductively mature is correlated with increased blood pressure and elevated $V_B$. The importance of elevated blood pressure as a promoter of cardiac hypertrophy in mammals is well known (26, 30), and our results suggest that elevated blood pressure may be important to cardiac growth in trout as well. More specifically, the positive linear relationship between PP and RVM that we noted for trout (Fig. 4D) agrees closely with hemodynamic studies of human cardiac hypertrophy (7, 23). Based on our indicator dilution analysis of the earliest (15 min) blood samples, the degree of ventricular hypertrophy was also associated with up to a 34% increase in $V_B$ (Fig. 7), suggesting that the hearts of mature animals are faced with a volume overload. Our determination of apparent $V_B$ is dependent on the distribution kinetics of EBD, which may be altered by hypertension (discussed in Blood Volume Estimates and Evans Blue Distribution Kinetics are Dependent on Blood Flow). The idea that a sustained increase in $V_B$ can increase cardiac preload is supported by the observation that acute hypervolemia in trout elevates central venous pressure, which can result in a greater end diastolic volume (31). It has been demonstrated that exercise training can induce hypervolemia in humans.
via a similar sequence of events (6), leading to the implication of hypervolemia in the development of cardiac hypertrophy in these individuals.

In contrast to studies in mammals, only two studies in fish have suggested a correlation between increased cardiac workload and ventricle growth. First, Houlihan et al. (16) demonstrated that increased workload and power output in an in vitro trout heart preparation induced greater fractional rates of protein synthesis in the ventricle. An increase in protein synthesis within hours of hemodynamic overload is considered a marker of cardiac growth in mammals (30). Second, a recent report demonstrated that parasitic infestations of the bulbus arteriosus in Sheepshead minnows (Cyprinodon mariegatus) resulted in fibrosis and narrowing of the lumen diameter of the bulbus, leading to ventricular hypertrophy in infected animals (5). This observation stresses how the functional status of the bulbus can influence ventricular function and growth. Additional support for the effects of increased workload on cardiac growth comes from morphometric studies. In mammals, increased end diastolic volume and wall stress induces myocyte elongation and thickening, whereas increased afterload and strain results predominantly in increased myocyte diameter (15). We reported recently (4) that ventricular hypertrophy in reproductively mature trout results from cardiomyocyte hypertrophy, specifically increased cross-sectional area (183%) and length (131%). The fact that myocytes grew predominantly by thickening suggests that increased afterload may promote ventricular hypertrophy in mature trout, although it does not exclude increased preload as a factor influencing ventricle growth.

We acknowledge that factors other than hemodynamics may be important in the development of ventricular hypertrophy in trout. Blood concentrations of androgens are higher in reproductively mature male trout (24), and androgen supplementation in young male and
female trout is associated with ventricular hypertrophy (8, 27). Despite the anabolic effect androgens exert on numerous tissues, a direct link between elevated androgens and cardiomyocyte hypertrophy has not been established in fish. We suggest that elevated androgens in mature male trout may influence ventricular growth indirectly through volume expansion or increasing blood pressure.

### Hemodynamics and the Role of the Bulbus Arteriosus in Ventricular Hypertrophy

Although elevated blood pressure may facilitate ventricular growth in trout, it is important to identify what factor(s) are responsible for the increase in blood pressure. The gill vasculature functionally separates the heart from the systemic vessels. This suggests that the elevated VA pressures observed in this study were dependent on changes in either ventricle-bulbus dynamics or increases in systemic vascular resistance. Our results indicate that a lack of change in ventricle-bulbus dynamics might promote ventricular hypertrophy in mature trout and further highlight the important role the bulbus plays in influencing ventricular function (5, 9, 17). Franklin and Davie (12) demonstrated that ventricular hypertrophy in trout was associated with increased maximal stroke volume. A greater stroke volume would place increased demands on the bulbus, which functions to depulsate blood flow (9). To maintain normal VA pressures, this alteration in ventricular function should be matched by morphofunctional adaptations in the bulbus, such that the bulbus exhibits increased distensibility (i.e., elasticity) or compliance (which is influenced by changes in maximal lumen volume). We did not observe such compensatory changes in bulbus function, nor did the bulbus mass increase with ventricle growth. Indeed, the fact that arterial +dP/dt was greater (Fig. 5B) despite an unchanged duration of arterial systole (Fig. 5D) as RVM increased strongly suggests that ventricular and bulbar functions were disjoined. Using trout of similar size to those used in our investigation, Forster and Farrell (11) described values for bulbus compliance (0.019 ml/mmHg, estimated from graphs) and a shape of the bulbus pressure-volume curve that are similar to what we observed, but they cited a lower average VA pressure at maximal compliance (37.5 mmHg). Notably, our study shows that both the pressure at maximal...
compliance (45.8 ± 3.0 mmHg) and the scope of maximal compliance (9.9 ± 0.8 mmHg) of the bulbus agree reasonably with the mean SBP of trout with normal ventricles, but the mean SBP of trout with hypertrophied ventricles appears to be higher than bulbus compliance. Regardless of the potential role the bulbus may play in ventricular hypertrophy, the combined observations of 1) increased -dP/dt and 2) unchanged DBP and bulbus hemodynamics indicate that the bulbus continues to function as an effective Windkessel (9) in mature male trout.

Blood Volume Estimates and Evans Blue Distribution

Kinetics are Dependent on Blood Pressure

Compared with previous studies (20), our initial determination of $V_B$, using standard indicator dilution calculations, yielded estimates of $V_B$ that were clearly inflated for animals with hypertrophied ventricles. Subsequent pharmacokinetic analysis established that the distribution of EBD was dissimilar among animals and in fact was strongly dependent on blood pressure. The increased clearance (Fig. 6A) and greater apparent volumes of distribution (Fig. 6B) of EBD strongly suggest that the elevated blood pressures in mature animals enhanced extravasation of dye. EBD binds to circulating albumin, and it is well known that albumin is not confined to the vasculature of teleosts (20). Furthermore, studies in mammals have demonstrated that elevated hemodynamic loads can increase extravasation of albumin and EBD (21, 29). Thus the elevated blood pressure in mature trout appears to increase extravasation of EBD, and presumably albumin, thereby causing inflated estimates of $V_B$. We attempted to circumvent this by using our 15-min sampling point to estimate $V_B$ rather than extrapolating back to a hypothetical concentration at time 0, as the slope of the EBD disappearance curve would influence the latter. These 15-min volume estimates ranged from 38.5 to 58.4 ml/kg body wt (Fig. 7), suggesting that $V_B$ is indeed elevated in trout as ventricles enlarge. Notably, $V_B$s for our normal (nonhypertrophied) animals (~40 ml/kg) are similar to values reported for trout in the literature (~35 ml/kg; see Ref. 20). Loss of EBD from the vasculature (both normal extravasation and loss resulting from elevated SBP) is probably responsible for some inflation in our estimates of $V_B$ even at 15 min postinjection, particularly as RVM (and SBP) are increased. Nevertheless, we believe that the loss of EBD by 15 min was minimal, and that our estimates of $V_B$ are justifiable. We therefore conclude that there is an increase in $V_B$ with RVM, but there is also evidence for greater capillary leakage in animals with elevated blood pressure. We agree with Olson (20) in urging caution when using EBD to estimate $V_B$ in fish, particularly when there is reason to suspect changes in vascular pressure.

Perspectives

Our study demonstrates that increased hemodynamic loads are associated with ventricular hypertrophy in mature trout. Based on numerous studies of cardiac hypertrophy in mammals, we suggest that sustained elevated workloads may be a primary determinant of ventricular growth in mature trout. We emphasize, however, that this hypothesis was not tested empirically in the present investigation, and we are unaware of any definitive studies in fish. As mentioned earlier, previous studies in trout have implicated elevated androgens as a factor promoting ventricular hypertrophy (8, 27). We propose two scenarios that integrate the possible roles of androgens and elevated hemodynamic loads in influencing cardiac growth in mature male trout. First, androgens may influence ventricle growth indirectly through increases in blood volume and pressure. In this scenario, an androgen-dependent expansion of blood volume leads to increased venous pressure, which enhances stroke volume. A greater stroke volume could promote higher arterial pressure, especially if the morphophysiology of the bulbus remains unchanged. It is also possible that androgens may influence arterial tone and resistance, which would further increase blood pressure. The elevated blood pressure in these animals would then promote growth of the cardiac ventricle. As an alternative scenario, androgens may interact with androgen receptors on the heart (22) and directly induce cardiac growth, leading to an increased stroke volume. Because the morphophysiology of the bulbus does not change, this elevated stroke volume would lead to increased arterial pressure, pulse pressure, and $+/-dP/dt$. In both scenarios, the elevated workload of the heart is compensated for by the enhanced pumping ability of the enlarged heart. This hypothesis is supported by the results from the present study, particularly the maintenance of the duration of arterial systole despite elevated arterial pressure, PP, and $+/-dP/dt$. Further support comes from the observations by Franklin and Davie (12), who demonstrated that hypertrophied ventricles of mature trout had a greater maximum cardiac power output and could better oppose elevated output pressure compared with normal hearts. Clearly, additional work needs to be done on the proximate causes and functional consequences of ventricular hypertrophy in mature male trout. Ultimately, we believe that additional studies of ventricular hypertrophy in mature male trout will lead to new insights about cardiovascular plasticity and function in fish.

We acknowledge the technical assistance of Steven J. Merrill in measuring bulbus compliance. Dr. Anna Ratka’s contribution to our pharmacokinetic analysis was essential and much appreciated. Methodological advice on surgery and blood pressure measurements in fish were generously provided by Drs. A. Kurt Gamperl and Joseph J. Cech, Jr., respectively. We are grateful for the critical insights of Dr. Kenneth R. Olson concerning our analysis of blood volume. The support and assistance of the staff at Clear Springs Hatchery, particularly Scott R. Williams, was invaluable during this project. This research was funded through grants from the Idaho State Board of Education (to K. J. Rodnick) and the Graduate Student Research and Scholarship Committee at Idaho State University (to R. J. Clark).

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Received 7 JJuly 1998; accepted in final form 24 May 1999.
REFERENCES


