

Effect of coronary perfusion on the basal performance, volume loading and oxygen consumption in the isolated resistance-headed heart of the trout *Oncorhynchus mykiss*

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Summary

Basal performance, volume loading response and oxygen consumption were determined in a resistance-headed preparation of the isolated trout heart. Two groups of hearts were used: the +CF group, in which the coronary vascular tree was perfused with a flow directly related to the pressure generated by the heart, and the –CF group, in which the coronary flow was set to zero. As a criterion for setting basal performance, the atrial input pressure was set in order to induce the ventricle to produce a cardiac output of $15 \text{ ml min}^{-1} \text{ kg}^{-1}$. Once basal conditions were obtained, the preparation was perfused for 30 min, and atrial and aortic pressure, cardiac output, heart rate, coronary pressure and coronary flow were determined at 5 min intervals. At the onset of perfusion, there was no difference in the basal performance between the two groups: the same preload was necessary to get the same cardiac output in both perfusion groups. None of the other performance parameters determined were different. However, after only 5 min of perfusion, the –CF hearts displayed significant adjustments, with increased atrial preload and ventricular preload (mean atrial pressure), and a significant decrease in cardiac output. At the end of the 30 min basal perfusion period, hearts were challenged

with a stepwise increase in preload in order to obtain maximal stroke work (volume loading). The effect of coronary perfusion on the heart's response to volume loading was highly significant: the stroke work–preload relationship was significantly shifted towards higher preload values in the –CF group. Also, the maximal work produced by the heart under the experimental conditions used was lower in the –CF group. Rate of oxygen consumption of the heart increased significantly with volume loading, from a basal value of approximately $20 \mu\text{l O}_2 \text{ min}^{-1} \text{ g}^{-1}$ to approx. $40 \mu\text{l O}_2 \text{ min}^{-1} \text{ g}^{-1}$, but was not significantly affected by the absence of coronary perfusion. Mechanical efficiency under basal conditions was approximately 17%, but was not affected by either volume loading or coronary perfusion. Taken as a whole, these data represent direct evidence of the effect of coronary perfusion on the mechanical performance of the trout heart, but also show that these effects are limited by significant self-adjustments that occur in the heart.

Key words: heart, trout, *Oncorhynchus mykiss*, coronary circulation, volume loading, mechanical efficiency.

Introduction

The majority of teleost fish rely on venous blood for oxygen and nutrient supply to the heart. In these animals the myocardial tissue has a spongy, trabeculated architecture, with specific biochemical and mechanical characteristics (Rodnick and Williams, 1999; Ewart and Driedzic, 1987; Ewart et al., 1988; Tota, 1983; Agnisola and Tota, 1994). However, one third of teleost species, including salmonids, scombrids and anguillids, possess an outer layer of compact myocardium in the ventricle. This layer requires a blood supply via a dedicated vascular tree, the so-called coronary system, which usually originates from the branchial efferent, bringing arterious blood to the heart (Farrell and Jones, 1992).

As only part of the myocardial tissue is supplied by coronary arteries, it is likely that the fish heart does not have an obligatory dependence on its coronary blood flow (Farrell, 2002). Only the skipjack tuna heart, whose ventricle contains up to 60% of compacta, is thought to be obligatorily dependent on its coronary circulation (Farrell et al., 1992). On the other hand, there are several indications that coronary circulation is essential under conditions of both hypoxia and intense swimming activity. In trout, coronary flow can increase during exercise or hypoxia (Gamperl et al., 1994), and coronary ligation resulted in a reduced proportion of compact myocardium together with a decrease in a series of metabolic

enzymes (Farrell et al., 1990; Gamperl et al., 1994). Moreover, the long-term significance of coronary circulation was demonstrated by the fact that arteries can grow around the ablation site in 14 days (Farrell et al., 1990). The modulation of coronary flow under conditions of hypoxia or swimming suggests a neurohumoral and/or local regulation of the coronary resistance and consequently of the perfusion of the myocardium. Although the mechanism for such regulation *in vivo* is not known, a number of studies *in vitro* suggest that a complex interaction between various circulating and paracrine factors is involved (Mustafa et al., 1992; Mustafa and Agnisola, 1994; Agnisola et al., 1996; Mustafa and Agnisola, 1998).

There is no direct evidence for the mechanical significance of compacta perfusion. Information on the interplay between coronary physiology and ventricle performance in teleosts is derived from *in situ* and *in vitro* work. One limitation of these studies is that in all the preparations used, the heart was working against a fixed pressure (pressure head) rather than against a resistance. *In vivo*, aortic pressure and flow result from the matching of vascular resistance and the capacity of the heart to produce force and then to move blood. This cannot be simulated under pressure-head *in vitro* conditions, where the heart is constrained by the need to produce a minimal constant pressure in order to get flow. Also, coronary flow can barely be synchronised with heart requirements, something that *in vivo* is self-accommodated *via* changes in the pressure generated by the heart and local, paracrine and/or metabolic mechanisms. The aim of the present study was to define the role played by a well supplied compacta in fish heart performance using a simple protocol (with or without coronary perfusion) in an *in vitro* preparation in which the isolated trout heart was working against a resistance, as *in vivo*, and with the coronary flow related to the ventricular function on a beat-to-beat basis. The results provided evidence for some self-regulating properties of the teleost heart.

Materials and methods

Animals

Rainbow trout *Oncorhynchus mykiss* Walbaum of both sexes were obtained from a local fish farm (Randbøldal, Denmark). Fish were held in 400 liter tanks with running tapwater at $12\pm 0.5^\circ\text{C}$ under an artificial 12 h:12 h L:D photoperiod. They were fed 3 times a week with commercial trout pellets and allowed to acclimatize for 10 days prior to experiments. A batch of 29 animals was used for this study. The mean mass of the animals was 0.375 ± 0.011 kg (\pm S.E.M.). The animals were divided into two groups: +CF, used to obtain an isolated heart preparation with perfusion of the coronary system (mean animal mass 0.362 ± 0.015 kg; mean ventricle mass 0.297 ± 0.011 g), and -CF, used to obtain an isolated heart preparation without perfusion of the coronary system (mean animal mass 0.393 ± 0.018 kg; mean ventricle mass 0.327 ± 0.017 g). There was no significant difference in animal or ventricle mass between the two groups.

Dissection and preparation of the isolated trout heart

Fish were injected with heparin (~ 1 ml kg^{-1} of a 0.9% NaCl solution containing 300 units heparin ml^{-1}) in the caudal vein and killed by a quick blow on the head. The thorax was cut open and after removing the pericardium the entire heart was dissected out. The atrium, ventral aorta and coronary artery were cannulated as previously described (Mustafa et al., 1992; Agnisola et al., 1994). The isolated heart was pre-perfused with ice-cold saline solution during the cannulation procedure to clear the blood from the lumen and coronary arteries. The entire procedure from opening of thorax to coronary cannulation took approximately 15 min. After cannulation the heart was mounted in the perfusion chamber.

The experimental set-up

The perfusion chamber, a double-jacketed chamber similar to that previously described (Houlihan et al., 1988; Agnisola et al., 1994), was connected to a water bath (RM6 Lauda, DRR.WOBSEER GMBH & Co. KG, Lauda-Königshofen, Germany), which maintained a chamber temperature of $10\pm 0.5^\circ\text{C}$. The input and output reservoirs were at the fixed heights of 7 cm and 10 cm from the level of saline in the chamber, respectively, and were connected to each other *via* a small tube as described by Agnisola et al. (1998) (Fig. 1), which enabled perfusion of the isolated heart with 50 ml of recirculating saline. The input and output loadings on the heart were set through resistances according to Agnisola et al. (1998). Briefly, the input loading on the heart was set by a variable resistance (R_i) and the saline level difference between the input reservoir and the heart. Output resistance was set by a fixed resistance (R_o) in the output tube (a teflon tube 17.8 cm long, 0.5 mm i.d.). The input to the coronaries was *via* a side arm on the output tube before R_o , and a constant resistance (R_c , consisting of a nylon tube 10 cm long, 0.3 mm i.d.) allowed the coronary input pressure to be directly related to the pressure generated by the heart. The experimental set-up was placed in a refrigerated cabinet set at 10°C . No detectable temperature changes occurred while opening and closing the glass door of the cabinet.

Once mounted in the perfusion chamber, the heart was wrapped with a transparent plastic film. Although not properly simulating a pericardium (no negative preload was generated), this procedure avoided overstretching of the atrium during volume loading of the preparation.

The saline in the input and output reservoirs was gassed with 99.5% O_2 and 0.5% CO_2 throughout the entire experiment, taking care not to trap any air bubbles in the system. The perfusate was Cortland saline (Wolf, 1963) as modified by Farrell et al. (1986) and Farrell (1987), of composition (in g l^{-1}): NaCl, 7.25; KCl, 0.23, $\text{MgSO}_4\cdot 7\text{H}_2\text{O}$, 0.23; $\text{NaH}_2\text{PO}_4\cdot \text{H}_2\text{O}$, 0.016; $\text{NaHPO}_4\cdot 2\text{H}_2\text{O}$, 0.28; glucose, 1.0; polyvinylpyrrolidone (PVP) 10.0; $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$, 0.37; the pH was adjusted to 7.9 at 10°C with NaHCO_3 (approx. 1 g l^{-1}).

Measurements and calculations

Atrial pressure was measured through a small saline-filled

chamber, and putting an input load on the atrium, the heart started working against the fixed resistance, recirculating the 50 ml saline solution. Any leaks in the heart were discovered by a decrease in the saline level in the input reservoir and these hearts were discarded. The isolated working heart was left for a period of at least 30 min to stabilize, i.e. when the heart had settled into regular beating. Hearts that did not stabilize were discarded. At the end of the stabilization period, the cardiac output was adjusted to approximately $15 \text{ ml min}^{-1} \text{ kg}^{-1}$ (Houlihan et al., 1988; Agnisola et al., 1994) by changing the variable input resistance (R_i). As soon as a basal cardiac output was obtained, automatic recording of pressure at 5 min intervals was begun; seven determinations were made for a total period of 30 min perfusion under basal conditions. At the end of this period, the oxygen consumption of the heart was determined.

Volume loading

At the end of the 30 min period of perfusion under basal conditions, the filling pressure was increased (by varying R_i) in seven steps of 5 min each and the pressure measured at each step. When maximal stroke work was reached, oxygen consumption was measured again.

Statistics

All values are means \pm S.E.M. One-way analysis of variance (ANOVA) was used to test the time course of the effect of different parameters during basal perfusion. Two-way ANOVA was used to analyse the changes in stroke work, ventricular preload and afterload during volume loading. $P < 0.05$ was taken to indicate statistical significance. Statistics were performed with GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA).

Results

Effect of coronary perfusion on basal atrial performance

In the +CF group of hearts, the initial atrial preload necessary to get a cardiac output of about $15 \text{ ml min}^{-1} \text{ kg}^{-1}$ was $0.15 \pm 0.02 \text{ kPa}$ (Fig. 2). Once set, this parameter did not change significantly during the successive 30 min of perfusion. At this load, the atrial performance remained constant with a mean atrial pressure (ventricular preload) that did not change from the initial value of $0.26 \pm 0.02 \text{ kPa}$. Fig. 3 shows that the ventricular performance parameters also remained constant at the initial level throughout the 30 min of basal perfusion.

In the -CF group, although the initial set of atrial preload was not significantly different from that of the +CF group, the atrium adjusted within 5 min to a significantly higher preload value (Fig. 2), which remained constant during the remaining perfusion period at the basal setting. As a consequence, mean atrial pressure also increased up to about 0.3 kPa. The increased preload in the -CF group of hearts was associated with a significant reduction in cardiac output and power output (Fig. 3A). Stroke volume and stroke work were consistently lower in the -CF hearts (Fig. 3B), although the difference was

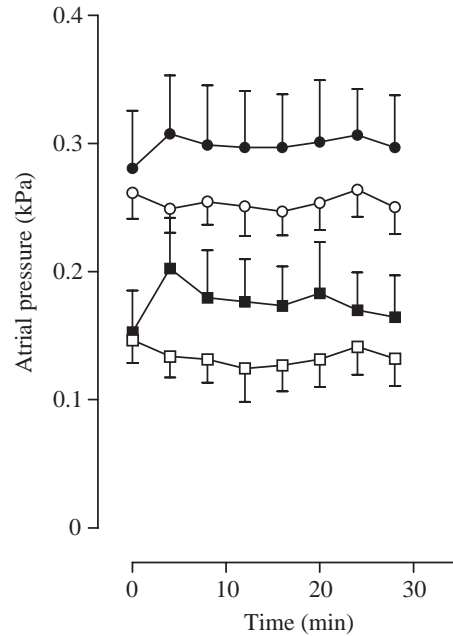


Fig. 2. Atrial (squares) and ventricular (circles) preload values during 30 min perfusion of the isolated working trout heart under basal conditions, with (+CF, open symbols) and without (-CF, closed symbols) coronary perfusion. At time 0 the atrial preload was set in order to get a cardiac output of about $15 \text{ ml min}^{-1} \text{ kg}^{-1}$. Any successive change indicates a self-adjustment of the heart. Two-way ANOVA indicated a significant difference between +CF and -CF data ($P < 0.01$) in both parameters. Repeated-measures one-way ANOVA and Dunnett *post-hoc* test applied to -CF data indicated a significant increase in both atrial and ventricular preload 5 min after the beginning of perfusion ($P < 0.05$).

not statistically significant. There was no difference in afterload and heart rate between the two groups (Fig. 3C).

In the +CF group, coronary performance, as evaluated by coronary pressure, flow and resistance values, was constant throughout the 30 min perfusion period under basal conditions (Fig. 4).

Effect of coronary perfusion on the heart's response to volume loading

At the end of the 30 min period of basal perfusion, hearts were challenged with a stepwise increase in input pressure, with consequent increases in atrial and ventricular preloads (volume loading). The stroke work-preload curves determined in the two experimental groups are shown in Fig. 5. Volume loading induced a doubling of stroke work in both groups, although the overall response was significantly affected by the perfusion of coronaries. In the +CF hearts the curve was significantly shifted left: higher stroke work values were obtained at lower preloads. Also, the maximal stroke work value observed was lower in the -CF hearts ($3.36 \pm 0.35 \text{ mJ g}^{-1}$) than in the +CF hearts ($3.77 \pm 0.28 \text{ mJ g}^{-1}$). The coronary flow in the +CF group of hearts remained constant during volume loading (Fig. 5, inset A), while there was a significant

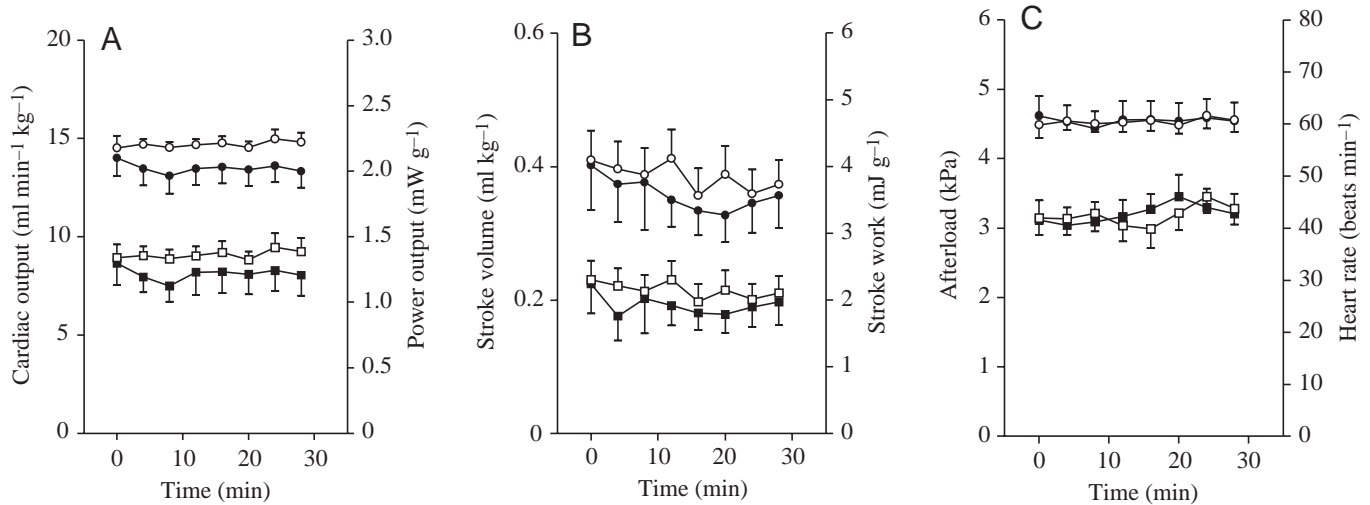


Fig. 3. Ventricular performance during 30 min perfusion of the isolated working trout heart under basal conditions, with (+CF, open symbols) and without (-CF, closed symbols) coronary perfusion. (A) Cardiac output (circles) and power output (squares); (B) stroke volume (circles) and stroke work (squares); (C) afterload (circles) and heart rate (squares). Two-way ANOVA indicated a significant difference between +CF and -CF data ($P < 0.01$) for cardiac output and power output only.

enlargement in coronary resistance that increased from a basal value of $0.30 \pm 0.09 \text{ TPa s m}^{-3}$ up to $0.91 \pm 0.30 \text{ TPa s m}^{-3}$ at maximal loading (significant change; repeated-measures one-way ANOVA, $P < 0.05$).

It is worth noting that in our resistance-headed preparation, volume loading induced increases in both stroke volume and aortic pressure. As can be seen in Fig. 5, inset B, coronary perfusion did not significantly affect pressure generation. In both the +CF and -CF hearts, there was a significant increase in afterload (about 40%). This increase was associated with a significant increase in both diastolic and systolic aortic pressure, with no significant differences between +CF and -CF hearts. In fact, in the +CF group, diastolic pressure increased from the basal level of $2.98 \pm 0.19 \text{ kPa}$ up to $4.45 \pm 0.22 \text{ kPa}$ at the maximal volume loading. The corresponding systolic

pressure values were $6.14 \pm 0.34 \text{ kPa}$ and $9.94 \pm 0.55 \text{ kPa}$, respectively. In the -CF group, diastolic pressure augmented from the basal level of $3.28 \pm 0.43 \text{ kPa}$ up to $4.68 \pm 0.40 \text{ kPa}$ at the maximal volume loading. The corresponding systolic pressure values were $6.25 \pm 0.59 \text{ kPa}$ and $9.78 \pm 0.98 \text{ kPa}$, respectively. So, there was an approximately 70% increase in pulse pressure in both groups, associated with a corresponding significant increase in stroke volume, which then accounted for most of the increase in stroke work during volume loading. Pulse pressure and stroke volume were consistently lower in the -CF hearts (data not shown).

Effect of coronary perfusion on oxygen consumption and mechanical efficiency of the trout heart.

The total oxygen consumption and the mechanical efficiency of the heart measured under basal perfusion conditions and at maximal volume loading are shown in Fig. 6. In both +CF and -CF hearts, volume loading induced a significant increase in rate of oxygen consumption, from a basal level of approximately $20 \mu\text{l O}_2 \text{ min}^{-1} \text{ g}^{-1}$ to approx. $40 \mu\text{l O}_2 \text{ min}^{-1} \text{ g}^{-1}$ at maximal volume loading. In both groups, basal mechanical efficiency was about 17% and was not affected by volume loading. Neither rate of oxygen consumption nor efficiency were affected by coronary perfusion.

Discussion

The heart is an organ with complex self-regulating properties, in which the interaction between myocytes, coronary vasculature and neurohumoral factors play a central role (Kresh and Armour, 1997). The self-regulating mechanisms of the heart are tuned to provide a cardiac output that is constantly adequate for the demand, and are constrained

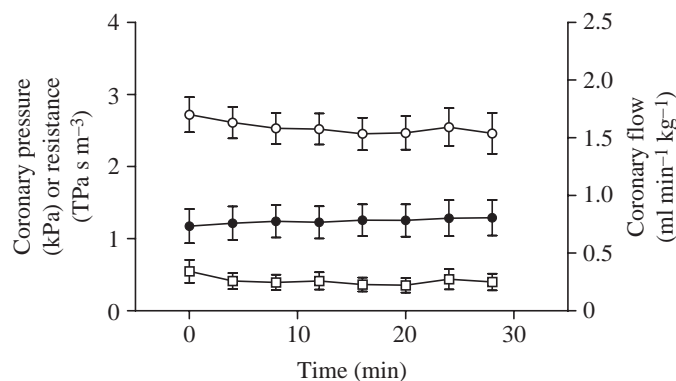


Fig. 4. Coronary flow (filled circles), pressure (open circles) and resistance (open squares) during 30 min perfusion of the isolated working trout heart under basal conditions in the +CF group of hearts. One-way ANOVA indicated that these three parameters were not dependent on perfusion time.

by the continuous need to match heart function with the compliance and resistance properties of arterial vessels (Berne and Levy, 1992).

A main feature of the present study is the use of a resistance-headed isolated heart preparation. In this preparation, the actual aortic pressure and flow result from matching a fixed resistance (R_o), simulating the peripheral resistance *in vivo*, and the intrinsic capacity of the heart to produce force and then to move the perfusion saline (Agnisola et al., 1998). The resistance-headed condition, as occurs *in vivo*, was hereby reproduced. More importantly, in this set-up the coronary flow was continuously and automatically linked to the pressure generated by the heart. *In vivo*, ventral aorta pressure and gill resistance determine the driving force for coronary perfusion (the coronary pressure), a situation that was reproduced in the perfusion set-up we used thanks to the fixed resistance R_c . This allowed maintenance of the intrinsic and reciprocal interactions between ventricle and coronaries on a beat-to-beat basis, as occurs *in vivo*. While lacking the potential to dissect the role of single specific features on heart performance (e.g. the effect of pressure loading), the resistance-headed preparation that we used did allow the isolated heart preparation to express some of the intrinsic self-regulating mechanisms that determine the heart's global pump function *in vivo* (Kresh and Armour, 1997). Thus, this preparation is well suited for the study of the relative significance of a well-perfused compacta on trout heart performance.

The basal performance of the resistance-headed heart with the perfused coronary vascular tree (+CF group) was close to that of a similar preparation previously reported (Agnisola et al., 1998), and similar to that of *in situ* or *in vitro* pressure-headed preparations (Farrell et al., 1986; Houlihan et al., 1988; Davie and Farrell, 1991; Davie et al., 1992). As no attempt was made to simulate the role of the pericardium in determining absolute values of atrial preload (Farrell et al., 1988), the input pressure necessary to get basal cardiac output was higher than the ambient pressure (approx. 0.1 kPa), and similar to that reported previously for both pressure-headed (Houlihan et al., 1988) and resistance-headed (Agnisola et al., 1998) isolated trout heart preparations. The pressure generated by the heart was similar to that reported *in vivo* in the trout ventral aorta (Kiceniuk and Jones, 1977; Wood and Shelton, 1980), while the coronary pressure was somewhat lower than the *in vivo* dorsal aorta pressure (Kiceniuk and Jones, 1977; Gamperl et al., 1995). This was probably a consequence of the lower coronary resistance *in vitro* with respect to the value *in vivo*, due to the lower viscosity of saline with respect to blood (by a factor of 2.5–3.5; Farrell, 1987). In agreement with previously reported data *in vitro* (Agnisola et al., 1998; Houlihan et al., 1988), coronary flow was 2–5% of cardiac

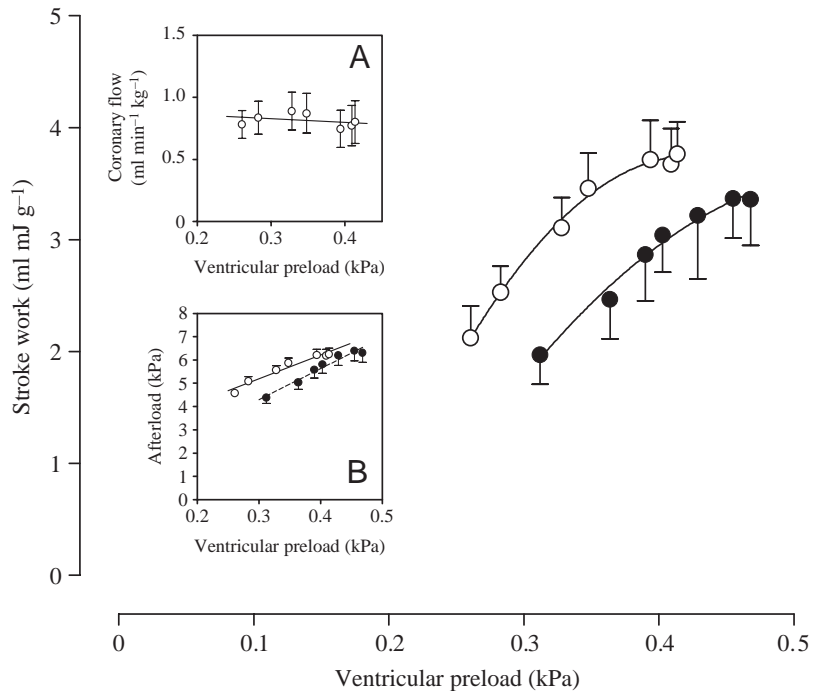


Fig. 5. Volume loading of the isolated working trout heart with coronary perfusion included (+CF, open circles) or excluded (–CF, filled circles). Two-way ANOVA indicated a significant effect of both coronary perfusion ($P < 0.01$) and preload ($P < 0.001$) on stroke work. (Inset A) Relationship between coronary flow and preload in the +CF group. One-way ANOVA indicated no-significant effect of preload on coronary flow. (Inset B) Effect of ventricular preload on afterload of the isolated working trout heart with coronary perfusion included (+CF, open circles) or excluded (–CF, filled circles). Repeated-measures one-way ANOVA indicated a significant effect of preload on afterload in both groups. Two-way ANOVA indicated no significant effect of coronary perfusion on afterload.

output, and higher than the *in vivo* values (1–2%, Axelsson and Farrell, 1993; Gamperl et al., 1994), probably because both the viscosity and oxygen content of saline were low compared with blood.

The absence of a coronary supply did not affect the heart rate and pressure generated by the heart, but significantly did affect the heart's capacity to maintain basal cardiac output, initially set at $15 \text{ ml min}^{-1} \text{ kg}^{-1}$, resulting in a significant reduction in the heart's power output. This result suggests that coronary perfusion was significantly affecting cardiac contractility. However, the consequences of this effect were apparently limited by self-regulating mechanisms, which led to a significant increase in preload which, *via* the Starling mechanism, would oppose the stroke volume reduction. This may explain the non-significant, although consistent, reduction in stroke volume and stroke work.

The dependence of cardiac contractility on a well-supplied compacta was confirmed by the response of hearts to the volume-loading protocol. This was accomplished by a stepwise increase in input pressure, with no attempt to control other parameters. In particular, as the heart was working against a fixed resistance, the increase in atrial preload caused a

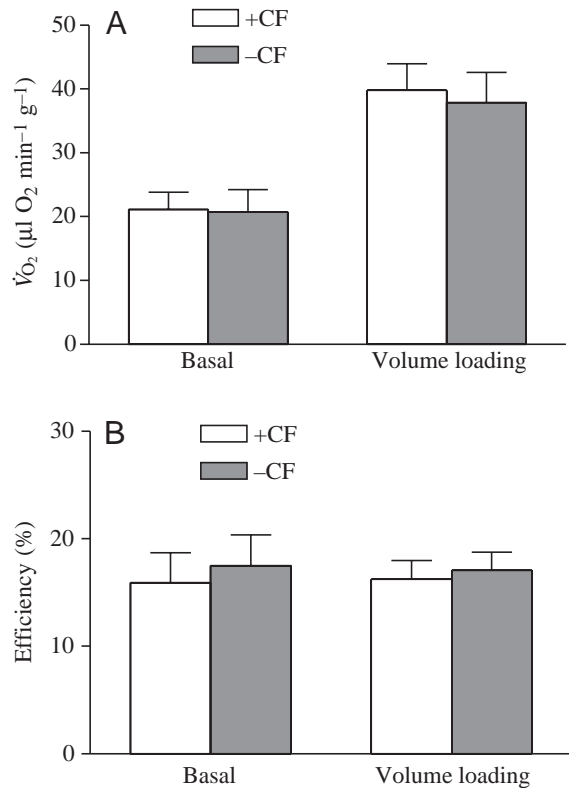


Fig. 6. Effect of coronary perfusion on (A) oxygen consumption and (B) mechanical efficiency of the isolated working trout heart under basal perfusion conditions and at maximal volume loading. Two-way ANOVA with Bonferroni *post-hoc* test indicated a significant effect of preload on $\dot{V}O_2$, but no significant difference was observed between +CF and -CF. Efficiency was not affected by either the perfusion conditions or the presence of coronary perfusion.

significant increase in both stroke volume and afterload (Berne and Levy, 1992). Thus the maximal stroke work thereby obtained was the combination of an increase in both pressure and volume work. In the +CF hearts, this effect was probably part of intrinsic adjustments that resulted in a constant coronary flow despite the increased coronary resistance due to the increased extravascular compression produced by the myocardium (systolic strangulation of coronary perfusion; Gamperl et al., 1995). In the absence of coronary perfusion, the shift of the stroke work–preload curve towards the right, shown in Fig. 5, confirmed that a main effect of coronary perfusion and a main role for the compacta were the increase in ventricular contractility, allowing the heart to produce a greater stroke work at the same preload.

Several theoretical models based on the mammalian left ventricle have suggested that the vascular and ventricular properties are matched to achieve maximal transfer of mechanical energy or maximal ventricular metabolic efficiency (Sunagawa et al., 1983; Burkhoff and Sagawa, 1986; Van den Horn et al., 1985; Toorop et al., 1988). De Tombe et al. (1993) have suggested that, when confronted with variable vascular loading, these parameters are both kept nearly

maximal, although not precisely optimised. An interesting result of the present study is the apparent independence of mechanical efficiency from volume loading. This contrasts with previous data, obtained using pressure-headed fish heart preparations (Farrell and Steffensen, 1987; Davie and Franklin, 1992), showing lower efficiency at lower loads. It is possible that the combined effects of the increased stroke volume (which should increase efficiency; Shipke, 1994) and afterload (which may decrease efficiency; Shipke, 1994) would help to maintain efficiency at a constant, nearly optimal level. On the other hand, neither oxygen consumption nor efficiency were affected by coronary perfusion. This result, which contrasts with the reduction observed by Houlihan et al., (1988) on a pressure-headed preparation, may in part reflect the fact that coronary perfusion did not affect afterload and heart rate, two major determinants of oxygen consumption in fish (Farrell and Jones, 1992). However, it may also indicate that the interplay and integration of the different homeodynamic mechanisms operating in the heart, help control its energetics under a wide range of variations of extrinsic and intrinsic factors, including coronary perfusion.

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