Journal of Cardiovascular Pharmacology™
24:344–349 © 1994 Raven Press, Ltd., New York

Short Communication

Impact of Isradipine on Contractile Performance, Metabolism, and Coronary Resistance Studied in Isolated Rat Hearts

Daniel Burkhoff and Gary Gerstenblith

Cardiology Division, The Johns Hopkins Hospital, Baltimore, Maryland, U.S.A.

Summary: In anesthetized dogs, isradipine has been reported to induce peripheral vasodilation and increase cardiac output (CO) and myocardial contractility, whereas myocardial oxygen consumption (MVO_2) decreases, suggesting that isradipine may increase overall metabolic efficiency of the ventricle of intact animals. Whether isradipine has any direct myocardial effects that could cause intrinsic increase in metabolic efficiency or whether the observation relates to favorable isradipine-induced changes in hemodynamic loading conditions is not known. Therefore, we determined the direct myocardial effects of isradipine on contractile strength and metabolic efficiency in isolated rat heart. Isolated crystalloid perfused rat hearts were instrumented for measurement of ventricular pressure, volume, and MVO_2. Isradipine decreased developed pressure (DP) and MVO_2 in a concentration-dependent manner; at 32 n*M* irradipine, both quantities were ~70% of their control values. Isradipine caused a downward shift of the end-systolic pressure-volume relation (ESPVR) and in the relation between ventricular work (indexed by pressure-volume area, PVA) and MVO_2, indicating that for any given amount of total mechanical work performed, the rat heart consumed less O_2 during administration of isradipine than under control conditions. However, the magnitude of the downward shift of this relation was nearly identical to that observed in a separate group of hearts in which we decreased contractility by decreasing the perfusate calcium concentration. Thus, isradipine does not appear to have a contractility-independent effect on myocardial efficiency.
Key Words: Coronary resistance—Myocardial oxygen consumption—Myocardial contractility—Metabolic efficiency—Isradipine—Isolated rat heart.

Isradipine (PN 200-110), a calcium channel blocker of the dihydropyridine class, is now being used for treatment of hypertension. In anesthetized dogs, isradipine induced peripheral vasodilation and increased cardiac output (CO) and myocardial contractility, whereas myocardial oxygen consumption (MVO_2) was decreased (1). Therefore, isradipine may increase overall metabolic efficiency of the ventricle of intact animals. Enhancement of overall efficiency could be the result of favorable changes in hemodynamic loading conditions (including secondary effects mediated by autonomic activation owing to the decrease in blood pressure, BP) or of a direct myocardial effect of the drug. However, whether isradipine has any direct effects that could increase myocardial metabolic efficiency is not known. Therefore, we determined the direct myocardial effects of isradipine on contractile strength and metabolic efficiency in isolated rat hearts.

METHODS

Surgical preparation

The methods used to isolate and support rat hearts were identical to those detailed previously (2) and sum-

Received June 9, 1993; revision accepted February 28, 1994.
Address correspondence and reprint requests to Dr. D. Burkhoff at his present address: Division of Circulatory Physiology, Columbia-Presbyterian Hospital, Milstein 5-435, 177 Fort Washington Ave., New York, NY 10032, U.S.A.

marized in Fig. 1. In each experiment, a retired male breeder Sprague-Dawley rat weighing ~500 g was heparinized (1,000 U intraperitoneally, i.p.) and then heavily anesthetized with sodium pentobarbital (140 mg i.p.). Bilateral sternotomy was performed, and the inferior, left superior, and right superior vena cavae were ligated near their insertions into the right atrium. The heart was excised and immediately submerged in oxygenated warmed perfusate (37°C, composition described herein). The severed end of the aorta was fed over a 16-gauge needle connected to a modified Langendorff perfusion system. Perfusate flow was adjusted to provide a perfusion pressure of ~80–90 mm Hg, and flow was then kept constant throughout the experiment.

A thin piece of Tygon tubing with a fenestrated tip was advanced into the right ventricle through the main pulmonary artery and held in place by a suture placed at the base of the pulmonary artery. This tube served to collect all coronary sinus and right ventricular Thebesian perfusate flow.

The left atrium was opened with care to ensure that the interatrial septum was not damaged. A thin latex balloon was attached to the end of a 10–15-cm length of stiff polyethylene tubing with fenestrations on the distal 3 mm of its tip. The fenestrated portion of the tube was advanced into the balloon to facilitate removal of all volume from within the balloon without having the balloon wall occlude the tip of the tube. The balloon was inserted in the left ventricle and held in place by a pursestring suture around the mitral annulus. The balloon and tubing were filled with water and connected to a Statham pressure transducer for measurement of ventricular pressure. Balloon volume was controlled using a calibrated 1-ml syringe (Gilmont). Before each experiment, the PVA relation of the balloon was measured and the balloon was used only if the pressure was zero at an intraballoon volume $\geq$0.4 ml. The volume of the balloon wall plus the tip of the tubing inside the balloon was measured by water displacement after all fluid was withdrawn from within the balloon (range 0.15–0.2 ml); this value was added to the volume infused inside the balloon to obtain total intraventricular volume. A minimum volume of 0.05 ml was used to avoid negative pressures required to collapse the balloon volume to such small volumes.

Pacing electrodes were placed at the right ventricular outflow tract and the remnants of the left atrium. Pacing stimuli were provided by a Grass SD-9 stimulator set at a rate above the native sinus node rate (mean ± SD 188 ± 20 beats/min) and was kept constant throughout each experiment.

The perfusion system consisted of a warmed storage vat for perfusate solutions, a Masterflex adjustable speed rotary pump (model 7523-10), and a condenser. The vat and condenser were warmed by a VWR constant temperature circulator (model 1135) set to heat the solutions to 37°C. Perfusate composition (in m*M*) was glucose 15, NaCl 140, KCl 5, $MgCl_2$ 0.9, $CaCl_2$ 1.5, and HEPES 6. Lidocaine (0.5 μg/ml) was added to suppress ventricular ectopic beats. All chemicals were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). The solution was warmed to 37°C, with pH adjusted to 7.40, and equilibrated with 100% O_2. Perfusate was not recirculated. A 200-n*M* solution of isradipine was prepared (using the oxygenated warmed perfusate as solvent) and infused at desired rates (pump 22, Harvard Apparatus) into the perfusion line. The syringe in which the isradipine solution was stored during the experiments was covered with aluminum foil to minimize its exposure to light. After hearts were attached to the Langendorff perfusion system, they were allowed to stabilize for at least 30 min, during which time left ventricular pressure (LVP) and venous O_2 tension were monitored (as described herein).

MVO_2 measurement

MVO_2 was calculated by multiplying coronary arterial flow by the difference in O_2 content between arterial and venous perfusate. To estimate O_2 contents, we measured O_2 tension using a commercially available platinum O_2 electrode system (Instech, Plymouth Meeting, PA, U.S.A.) which allowed continuous measurements. Sodium dithionate (a compound that extracts O_2 from solution) was used to zero the electrode at the start of each experiment. The gain of the electrode system was calibrated with the perfusate solution equilibrated with 100% O_2. The O_2 content of this solution was 2.3 volume-percents as determined by multiple measurements using a Lex-O_2-CON (model K) machine, which was in good agreement with standard values. Linearity of the O_2 electrode was confirmed by comparison of electrode readings

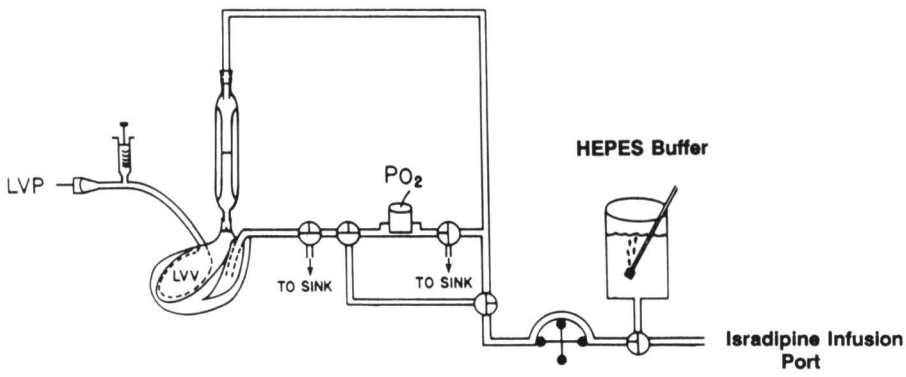

FIG. 1. Experimental setup. Aorta of isolated rat heart is attached to the end of a perfusion apparatus consisting of a perfusate storage vat, a rotary pump, and a condenser. A balloon is placed in the ventricle which in turn is connected to a monometer to measure left ventricular (LV) pressure and a syringe to adjust LV volume. The right ventricle is cannulated through the pulmonary artery for collection of coronary sinus effluent, which passes through a system of stopcocks and tubing for measurement of oxygen tension. The stopcocks can be adjusted to measure O_2 tension of either perfusate or coronary effluent.

at various O_2 tensions with measurements using the Lex-O_2-CON machine. A tubing system (Fig. 1) was devised to divert either all the pulmonary effluent or all the oxygenated perfusate flow through the electrode system for on-line sampling.

Protocols

Hearts were allowed to stabilize for 30 min before any measurements were made. Two protocols were used to determine (a) the isradipine concentration dependence of contractility, O_2 consumption, and coronary perfusion pressure at a constant ventricular volume; and (b) the impact of isradipine on the relation between total ventricular work and O_2 consumption at a fixed isradipine concentration. To determine whether the contractile and metabolic changes induced by isradipine can be accounted for simply by the accompanying reduction in ventricular contractility, we compared data with those obtained when contractility was reduced by decreasing the perfusate calcium concentration from the standard 1.5 m*M* to 0.75 m*M*.

Data analysis

To assess ventricular contractile state, end-systolic pressure-volume relation (ESPVR) were constructed by plotting ESP (P_{es}) as a function of LV volume (LVV). These data were subjected to nonlinear regression analysis (3):

$$P_{es} = E'_{es}(LVV - V_o) + \alpha\,(LVV - V_o)^2. \quad [1]$$

V_o is the volume axis-intercept of the nonlinear fit to the ESPVR, E'_{es} is the slope of the extrapolated ESPVR at a volume of V_o, and α is related to the degree of ESPVR curvilinearity. E'_{es} provides one quantitative index of contractile state (3). However, since this measure is somewhat dependent on the shape of the ESPVR and the volume range over which data are collected, $P_{0.4}$ (an alternative index of contractile strength which is independent of these factors) was also quantified. $P_{0.4}$ was defined as the interpolated P_{es} at a fixed volume of 0.4 ml (a volume always within the range of data collected). This parameter was determined by calculating P_{es} from the nonlinear fit to the ESPVR (eq. 1) and setting LVV = 0.4 ml.

Total energy liberated by the ventricle under the isovolumic conditions studied was quantified by the pressure-volume area (PVA), which is defined as the area on the pressure–volume plane circumscribed by the ESPVR (eq. 1), the end-diastolic pressure–volume relation (EDPVR), and the systolic portion of the ventricular pressure–volume trajectory (4). For these calculations, EDPVR was described by a second-order polynomial above the minimum volume at which data were collected and was assumed to be equal to 0 below that volume. In a previous study, we noted very little difference in PVA or the parameter values of the MVO_2–PVA relation when linear or nonlinear equation was used to define the ESPVR (2); since the relation is truly nonlinear in rats, we preferred to continue using the nonlinear form in the present study. Finally, we normalized PVA to LV mass in grams.

MVO_2 of the whole heart was estimated by multiplying perfusion flow by the difference in arterial and venous perfusate O_2 contents. This value represents the MVO_2 of the LV (which will vary with LV workload) plus a small amount of O_2 consumed by the non–work-performing right ventricle, which theoretically would not vary with changes in LV workload. Right ventricular MVO_2 was thus approximated by multiplying total MVO_2 estimated for unloaded contractions by the ratio of right ventricular mass to total heart mass. MVO_2 values were reported as milliliters of O_2/beat/g LV after estimated right ventricular MVO_2 was subtracted. Linear regression analysis was then performed to quantify the parameters of the following relation:

$$MVO_2 = A\ PVA + B. \quad [2]$$

The statistical significance of alterations of this relation noted when contractile state was changed were tested by multiple linear regression analysis. All statistical tests were performed with commercially available software (SYSTAT, IL).

RESULTS

Isradipine dose-dependent impact on function, MVO_2, and coronary perfusion pressure

The impact of isradipine on peak isovolumic pressure, MVO_2 and coronary perfusion pressure were determined at a constant ventricular volume over a range of isradipine concentrations of 4–32 n*M* (n = 5), with coronary perfusion flow held constant. Under baseline conditions, developed pressure (DP) averaged (±SD) 104 ± 12 mm Hg, MVO_2 was 0.18 ± 0.04 ml O_2/min, and coronary perfusion pressure was 85 ± 7 mm Hg. Mean (±SD) results are summarized in Fig. 2, in which the value of each parameter is normalized to its control value. Ventricular contractile strength decreased in a concentration-dependent manner and was paralleled by a proportionate decrease in MVO_2. Both DP (defined as the difference between peak and minimum ventricular pressure on a beat) and MVO_2 decreased to ~65% of control values at an isradipine concentration of 32 n*M*. Coronary perfusion pressure decreased by ~20% with 4 n*M* isradipine but did not change further with increased isradipine concentration. Thus, when the effect of isradipine on the coronary vasculature was maximized (i.e., at 4 n*M*), the effect on DP and MVO_2 was minimal.

Impact of isradipine on ESPVR and MVO_2–PVA relation

P_{es}, P_{ed}, and MVO_2 were measured at several volumes under control conditions and with a fixed concentration of isradipine (16 n*M*). A typical result is shown in Fig. 3. As compared with control, ESPVR was shifted downward with 16 n*M* isradipine, indicating a decrease in contractile state at this dose. Isradipine had no impact on EDPVR.

To assess the impact of isradipine on overall ventricular efficiency, we examined the relation between total mechanical energy liberation and MVO_2. For this purpose, we indexed total mechanical energy liberated for each beat by the PVA,

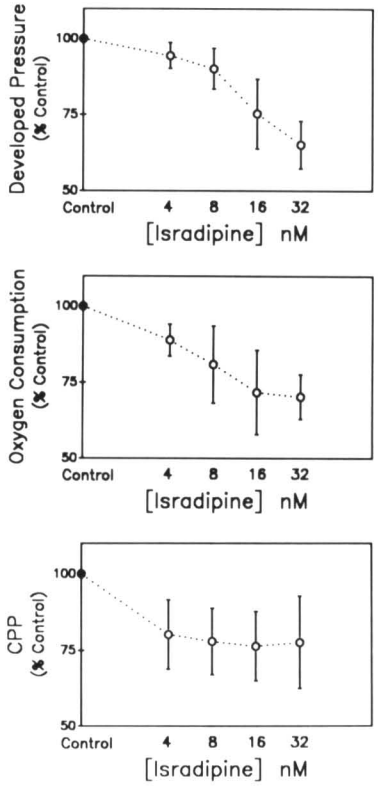

FIG. 2. Dose–response curves of developed pressure, MVO_2, and coronary perfusion pressure at a constant coronary flow for four different doses of isradipine.

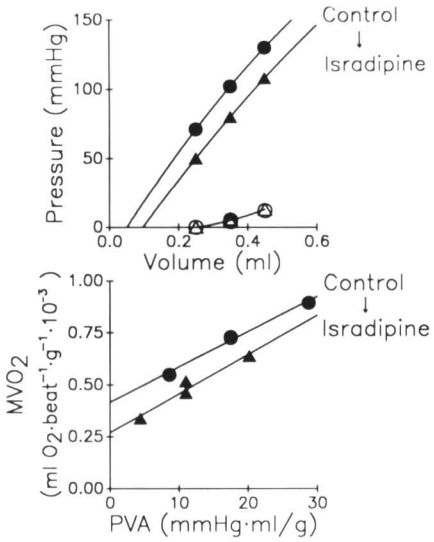

FIG. 3. Effect of 16 n*M* isradipine on contractile state as assessed by the end-systolic pressure–volume relation (ESPVR) **(top)** (solid symbols) and end-diastolic pressure–volume relation (EDPVR) **(top)** (open symbols) and on the relation between total energy liberation (indexed by pressure volume area, PVA) and O_2 consumption. As with other negative inotropic agents, both ESPVR and MVO_2–PVA relations shift downward and EDPVR is unaffected. Control data (circles), isradipine (triangles). Described in detail in text.

which is defined as the area on the PV plane bounded by the ESPVR and the EDPVR between V_o (the volume axis-intercept of the ESPVR) and the actual volume in the heart (4). Accompanying the decrease in contractile state, the relation between MVO_2 and PVA was shifted downward in a parallel manner (Fig. 3), indicating that less O_2 was consumed for any given amount of total mechanical work performed by the heart during isradipine administration. Figure 4 summarizes the way in which isradipine impacted on contractile strength and the slope and intercept of the MVO_2–PVA relation in 5 rat hearts (*$p < 0.05$ vs. control as determined by multiple linear regression analysis). Contractile strength is indexed by $P_{0.4}$, which is P_{es} at a volume of 0.4 ml (2). $P_{0.4}$ decreased from 136 ± 17 to 99.6 ± 15 mm Hg ($p < 0.001$). No isradipine-mediated change occurred in *A*, the slope of the MVO_2–PVA relation (1.37 ± 0.18 vs. 1.24 ± 0.47 ml $O_2 \cdot$ mm $Hg^{-1} \cdot ml^{-1} \cdot 10^{-5}$). The MVO_2–PVA intercept, *B*, which is the MVO_2 of mechanically unloaded contractions, decreased from 0.54 ± 0.13 to 0.33 ± 0.07 ml $O_2 \cdot beat^{-1} \cdot g^{-1} \cdot 10^{-3}$ ($p < 0.05$).

Comparing effects of isradipine to changes in perfusate calcium concentration.

The effects of isradipine already described were qualitatively similar to those observed in rat heart

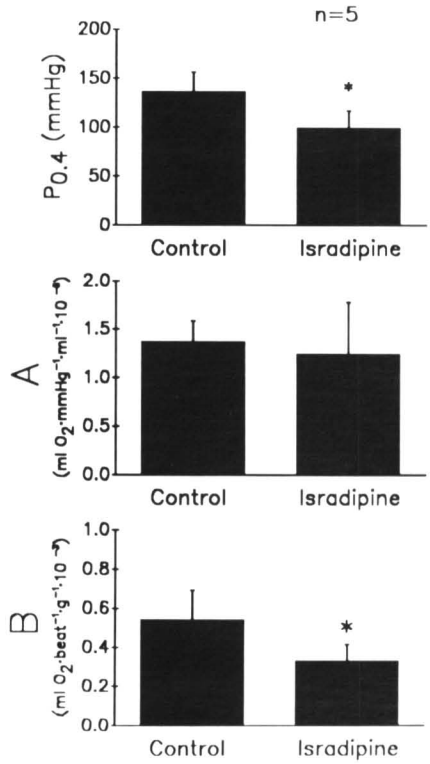

FIG. 4. Summary of effect of isradipine 16 n*M* on contractile strength indexed by $P_{0.4}$, and the slope **A,** and intercept, **B,** of the MVO_2–pressure/volume area (PVA) relation. Isradipine significantly $P_{0.4}$ and B, but A was not affected.

when contractile strength was decreased by a reduction in perfusate calcium concentration (n = 6). When calcium was reduced from 1.5 to 0.75 m*M*, $P_{0.4}$ decreased significantly (144 ± 41 vs. 94 ± 32 mm Hg, $p < 0.001$), MVO_2–PVA slope did not change (1.57 ± .65 vs. 1.33 ± .47 ml $O_2 \cdot$ mm Hg/ml$^{-1} \cdot 10^{-5}$), and MVO_2–PVA intercept was significantly reduced (0.51 ± .26 vs. 0.34 ± 0.25 ml $O_2 \cdot beat^{-1} \cdot g^{-1} \cdot 10^{-3}$, $p < 0.01$). To determine whether the reduction in the MVO_2–PVA intercept (*B*) observed during isradipine administration was comparable to that observed when perfusate calcium concentration was decreased, *B* was plotted as a function of $P_{0.4}$ (5) for both the calcium and isradipine protocols. The results (Fig. 5) indicate that the reductions in both parameters were comparable for both protocols, suggesting that the decrease in *B* observed with isradipine can be accounted for simply by the accompanying decrease in myocardial contractility.

DISCUSSION

Isradipine, a calcium antagonist, decreases ventricular contractility and MVO_2 in isolated rat heart in a concentration-dependent manner. At an isradipine concentration of 32 n*M*, both these quantities were decreased to between 65 and 70% of their control values. Coronary perfusion pressure measured at a constant coronary flow decreases, but the concentration dependence of this phenomenon is not strong, possibly owing to baseline vasodilatation in this crystalloid perfused preparation. Isradipine caused a parallel downward shift of the relation between ventricular work (indexed by PVA) and MVO_2, similar to all previously tested negative inotropic agents (4). This indicates that rat hearts, for any given amount of total mechanical work performed, consumed less O_2 during administration of isradipine than in control conditions. However, the magnitude of the downward shift of this relation was almost identical to that observed in a separate group of hearts in which contractility was decreased by reducing the perfusate calcium concentration. Thus, because isradipine has no impact on the MVO_2–PVA slope, and the decrease in MVO_2–PVA intercept is that which is expected based simply on the change in contractility, isradipine does not appear to have a contractility-independent effect on myocardial efficiency.

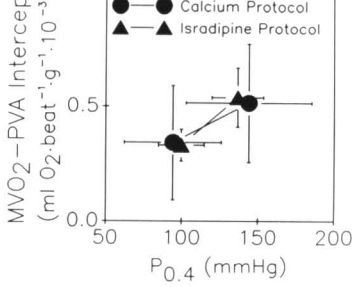

FIG. 5. Comparison of metabolic and contractile effects of isradipine 16 n*M* to with observed when contractile strength was decreased by decreasing perfusate calcium concentration from 1.5 to 0.75 m*M*. Graph shows relation between $P_{0.4}$ (contractility index) versus intercept of the MVO_2–pressure/volume area (PVA) relation, **B.** Data point **(right)** corresponds to control conditions, data point **(left)** shows values obtained with either isradipine administration (triangle) or decreased perfusate calcium (circle). Data for isradipine and decreased perfusate calcium superimpose on each other, suggesting that isradipine-induced changes in O_2 utilization can be accounted for simply by its negative inotropy mediated by calcium antagonism.

In studying the effects on the MVO_2–PVA relation, we tested only one dose of isradipine (16 n*M*), raising the question of whether the results would be the same at other doses. As shown in Fig. 2, isradipine had a monotonic impact on both ventricular function and MVO_2: the more isradipine given, the greater the reduction in both these parameters, as occurs when calcium concentration is reduced. Therefore, the impact on ESPVR and MVO_2–PVA relations probably would also follow a similar monotonic relation with isradipine dosing. Thus, in terms of answering the specific question posed (i.e., does isradipine exert a direct, metabolic efficiency enhancing action on the myocardium?), investigation of one intermediate dose does not pose a major limitation. Furthermore, 16 n*M* isradipine is a clinically relevant dose. Blood levels in patients peak ~1.5 h after ingestion of the drug and reach levels of 1 ng/ml for a 1-mg dose (molecular weight 381 g/mol). A 5-mg dose (a typical clinical dose) would correspond with a peak blood level of 13 n*M*. Therefore, the 16-n*M* concentration of isradipine tested in this portion of the study is in the clinically relevant range.

Our results therefore suggest that the previously observed increase in contractility with isradipine administration in anesthetized dogs (1) probably relates to reflex-induced changes associated with the decrease in BP. Furthermore, the concomitantly improved overall ventricular efficiency observed after isradipine administration in that same preparation most likely relates to peripheral and coronary vasodilatory actions of this agent rather than a direct energetically favorable impact.

Acknowledgment: This work was supported in part by a grant from Glaxo, North Carolina. D.B. was supported by a fellowship grant from the American Heart Association, Maryland Affiliate.

REFERENCES

1. Hof RP, Hof A, Scholtysik G, Menninger K. Effects of the new calcium antagonist PN 200-110 on the myocardium and

the regional peripheral circulation in anesthetized cats and dogs. *J Cardiovasc Pharmacol* 1984;6:407–16.
2. Wannenburg T, Schulman SP, Burkhoff D. End-systolic pressure-volume and MVO2-pressure-volume area relations of isolated rat hearts. *Am J Physiol* (*Heart Circ Physiol 31*) 1993;262:H1287–93.
3. Burkhoff D, Sugiura S, Yue DT, Sagawa K. Contractility-dependent curvilinearity of end-systolic pressure-volume relations. *Am J Physiol* (*Heart Circ Physiol 21*) 1987;252: H1218–27.
4. Suga H. Ventricular energetics. *Phys Rev* 1990;70:247–77.
5. Burkhoff D, Yue DT, Oikawa RY, Franz MR, Schaefer J, Sagawa K. Influence of ventricular contractility on non-work-related myocardial oxygen consumption. *Heart Vessels* 1987;3:66–72.