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Regional ischemia in hypertrophic Langendorff-perfused rat hearts

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Ashruf, J. F., C. Ince, and H. A. Bruining. Regional ischemia in hypertrophic Langendorff-perfused rat hearts. *Am. J. Physiol.* 277 (*Heart Circ. Physiol.* 46): H1532–H1539, 1999.—Myocardial hypertrophy decreases the muscle mass-to-vascularization ratio, thereby changing myocardial perfusion. The effect of these changes on myocardial oxygenation in hypertrophic Langendorff-perfused rat hearts was measured using epimyocardial NADH videofluorimetry, whereby ischemic myocardium displays a high fluorescence intensity. Hypertrophic hearts, in contrast to control hearts, developed ischemic areas during oxygen-saturated Langendorff perfusion. Reoxygenation of control hearts after a hypoxic episode resulted in a swift decrease of fluorescence in a heterogeneous pattern of small, evenly dispersed, highly fluorescent patches. Identical patterns could be evoked by occluding capillaries with microspheres 5.9 μm in diameter. Ten seconds after reoxygenation there were no more dysoxic areas, whereas reoxygenation in hypertrophic hearts showed larger ischemic areas that took significantly longer to return to normoxic fluorescence intensities. Hypothesizing that the larger areas originate at a vascular level proximal to the capillary network, we induced hypoxic patterns by embolizing control hearts with microspheres 9.8 and 15 μm in diameter. The frequency distribution histograms of these dysoxic surface areas matched those of hypertrophic hearts and differed significantly from those of hearts embolized with 5.9- μm microspheres. These results suggest the existence of areas in hypertrophic Langendorff-perfused hearts with suboptimal vascularization originating at the arteriolar and/or arterial level.

hypertrophy; hypoperfusion; reduced nicotinamide adenine dinucleotide fluorescence; microspheres; microcirculation

HYPERTROPHIC MYOCARDIUM is more susceptible to ischemic damage than normal myocardium because of, among other factors, changes in its vascularization (7, 21). Morphological studies of vasculature in hypertrophic myocardium have demonstrated profound changes in anatomy and function. In hypertrophic myocardium, capillary density and coronary vascular reserve decrease, whereas minimal coronary vascular resistance increases (11, 19, 20). Hypertrophy also increases diffusion distances from capillaries to myocytes (22, 24). These changes are not distributed evenly throughout the myocardium, resulting in a diversion of coronary flow from subendocardial to subepicardial layers (1, 8). Many studies have shown this transmural redistribution of coronary flow in hypertrophy with changes in

the heterogeneity of flow and vascularization between subsequent transmural myocardial layers (1, 22, 23). Less is known, however, of the effect of hypertrophy on the local distribution of oxygen through the myocardial microcirculation. For instance, measurements of local oxygen consumption and supply did not show any difference between normal and hypertrophic *in situ* hearts at rest (25) and during stress (5, 28), whereas a significant functional impairment in hypertrophic hearts was demonstrated. An explanation may be that these hearts were blood-perfused *in situ* hearts with a physiological oxygen supply-to-demand ratio as compared with Langendorff-perfused rat hearts, which are known to have a marginal oxygen supply, resulting in the development of ischemia when the oxygen supply-to-demand ratio decreases slightly (13). In a previous study we found that hypertrophic Langendorff-perfused hearts spontaneously developed hypoxic areas that could be alleviated by factors influencing oxygen free radical concentrations through the addition of fatty acids or superoxide dismutase to the perfusate (12).

The purpose of the present study was to investigate the nature of the spatial distribution of the hypoxic state in myocardial regions and the vascular level at which perfusion is disturbed in myocardial hypertrophy by using epicardial NADH videofluorescence to monitor the mitochondrial energy state (15). Measurement of the mitochondrial autofluorescence of reduced pyridine nucleotide (NADH) of the epimyocardium allows the identification of hypoxic regions because NADH (which accumulates during hypoxia) fluoresces when excited with ultraviolet (UV) light and oxidized NAD^+ does not (6). There is also a fairly linear relationship between the oxygen concentration available to mitochondria and the NADH fluorescence intensity, as has been shown in isolated mitochondria (6). In this way the local mitochondrial energy state of the myocardium can be visualized (2). In normal Langendorff-perfused rat hearts a stepwise transition from anoxic to normoxic perfusion was accompanied by a transition from high epicardial NADH fluorescence intensity to low fluorescence intensity with a reproducible patchy pattern of high-fluorescence anoxic areas lagging behind low-fluorescence normoxic areas (15). These areas are microcirculatory weak units, because in a given heart they were always at the same location and were the first to become hypoxic during tachycardia (2, 15). These weak units were also the first to become hypoxic during inhibition of nitric oxide synthesis in endotoxic rat hearts (4) and could be the cause of shunting pathways during sepsis (17). Hypoxic areas of identical size and pattern could also be elicited by embolization of the capillaries by microspheres whose diameter

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corresponded to the capillary diameter (5.9 μm), whereas embolization of arterioles and arteries by larger microspheres produced larger hypoxic areas not corresponding to the heterogeneous areas seen during recovery from hypoxia (15). We hypothesized that in hypertrophic myocardium this pattern would be altered because of the formerly mentioned circulatory changes, resulting in suboptimally perfused myocardial regions. Furthermore, the size of the hypoxic areas in hypertrophic myocardium could elucidate at which vascular level the impaired perfusion originated. In this study we analyzed the anatomic substrate responsible for the appearance of these dysoxic areas in hypertrophic hearts. Preliminary results were reported elsewhere (3).

MATERIALS AND METHODS

Experimental setup. Male Wistar rats weighing 200–250 g were operated on, undergoing subdiaphragmatic and suprarenal aortic narrowing to induce left ventricular hypertrophy (18), and were killed 6 wk later when they weighed 250–300 g. For control measurements, rats of similar weight were used. Hearts were removed and perfused according to Langendorff and paced via aortic and right ventricular leads. Mean coronary flow rates (in $\text{ml} \cdot \text{min}^{-1} \cdot \text{g ventricle wet wt}^{-1}$) were measured with an electromagnetic flow probe (Skalar-Medical, Delft, The Netherlands) placed immediately proximal to the aortic cannula. Perfusion pressure was measured with a pressure transducer (Hewlett-Packard 8805B corner amplifier) connected to the aortic cannula. The perfusate was a modified Tyrode solution (128 mM NaCl, 4.7 mM KCl, 1 mM MgCl_2 , 0.4 mM NaH_2PO_4 , 1.2 mM Na_2SO_4 , 20.2 mM NaHCO_3 , and 1.3 mM CaCl_2) containing 11.0 mM glucose. Perfusate temperature was kept at 37°C and equilibrated with either 95% O_2 -5% CO_2 or 95% N_2 -5% CO_2 . Perfusate pH was kept between 7.35 and 7.45. Hearts were paced at 5 Hz. The left ventricle was cannulated and communicated with the atmosphere via its apex to prevent generation of left ventricular pressure (2). In some experiments 10 μM nitroprusside, an endothelium-independent vasodilator, was added to the perfusate. In other experiments microspheres (Polyscience, Warrington, PA) of different diameters were infused into the coronary circulation. When indicated, the oxygen-saturated perfusate contained 10% (vol/vol) fluorocarbon F-43 emulsion (an artificial oxygen carrier) and 1% (wt/vol) fatty acid-free BSA. The stock fluorocarbon emulsion was obtained by sonifying 24 ml of F-43 and 5.6 g of pluronic F-68 in 150 ml of water at 4°C for 1 h under continuous bubbling of CO_2 through the solution (9). Chemicals were obtained from Merck (Darmstadt, Germany).

Fluorescence measurements. The NADH videofluorimeter used has been described previously (2, 15). The UV light from a 100-W mercury arc lamp (Olympus, Tokyo, Japan) was selected by means of a UG-1 barrier filter, centered around 365 nm, to provide the UV light needed for NADH excitation. The NADH fluorescence signal was selected by means of a band-pass filter centered around 470 ± 20 nm and was placed in front of the camera. An image-intensified video camera (MXRi, Adimec, Eindhoven, The Netherlands) with a Micro-Nikkor 105-mm macrolens was used to detect NADH fluorescence images of the left ventricle of the heart. To enable correction of images for changes in the sensitivity of the videofluorimeter and fluctuations in the intensity of the light source, a small piece of uranyl calibration glass was placed next to the heart within the excitation field. Images were

recorded on a video recorder and were computer analyzed off-line with the use of image-processing software (TCLImage, Multihouse, Amsterdam, The Netherlands). Arbitrary units of NADH fluorescence intensities relative to uranyl fluorescence intensity are expressed as percentages, where 100% is taken as the intensity of epicardial NADH fluorescence at the end of a 2-min period of perfusion with nitrogen-saturated medium.

Experimental protocols. Hearts were allowed at least 15 min of stabilization after the start of the Langendorff perfusion at a perfusion pressure of 80 mmHg with oxygen-saturated perfusate before the following experimental protocols were performed.

To examine the fluorescence patterns in hypertrophic left ventricle during oxygen-saturated Langendorff perfusion and posthypoxic recovery, *protocol 1* was carried out. After the stabilization of flow during normoxic perfusion, perfusion was switched to nitrogen-saturated perfusate for 2 min and then restored to normoxic perfusion. Because large ischemic areas already existed during normoxic perfusion at a perfusion pressure of 80 mmHg, perfusion pressure was increased to 100 and then 120 mmHg for 5 min each to increase coronary flow and oxygen transport. This did not cause the ischemic areas to disappear, and perfusion pressure was restored to 80 mmHg. Finally, to ascertain that the ischemic areas already visible at the beginning of perfusion were reversible [and did not represent infarcted (fibrotic) highly fluorescent myocardium (16)], oxygen transport to the myocardium was increased by either adding 10 μM nitroprusside to the oxygen-saturated perfusate or switching perfusion to fluorocarbon-containing oxygen-saturated medium.

Protocol 2 was performed to examine fluorescence patterns in control left ventricle during posthypoxic recovery and progressive hypoperfusion. After the stabilization of flow during normoxic perfusion, perfusion pressure was decreased to 60 mmHg to obtain coronary flows comparable between control hearts and hypertrophic hearts. As in *protocol 1*, control hearts were subjected to a 2-min period of nitrogen-saturated perfusion, after which perfusion was restored to oxygen-saturated perfusate. Hereafter, each heart was subjected to stepwise reduction of perfusion pressure from 60 mmHg to 10 mmHg in steps of 10 mmHg, with each step lasting 3.5 min.

To determine which vascular level determined the appearance of the ischemic areas in hypertrophic hearts, control hearts were embolized with microspheres of different diameters in *protocol 3*. The frequency distributions of ischemic surface areas in embolized control hearts were compared with those of hypertrophic hearts. After the stabilization of flow in control hearts, heterogeneous fluorescence patterns were elicited by switching perfusion from oxygen-saturated perfusate to nitrogen-saturated perfusate (for 2 min) and back. When flow had returned to baseline values, microspheres emulsified in Tyrode solution were infused at a rate of 500–1,000 microspheres/min into the coronary flow (15). Microspheres had fixed diameters of 5.9, 9.8, and 15 μm . Each heart was perfused with microspheres of one diameter, and infusion was stopped when flow was decreased to $\sim 75\%$ of the baseline value. The surfaces of the ischemic areas caused by embolization in control hearts and the surfaces of ischemic areas in hypertrophic hearts during normoxic perfusion were measured in the fluorescence images and expressed in pixels (1 pixel = $670 \mu\text{m}^2$ of myocardial surface). The transition from a normoxic area to an ischemic area is characterized by a small transitional zone (26) where fluorescence intensity increases gradually from normoxic to hypoxic intensity. The surface of an ischemic area was defined as the number of

pixels with a fluorescence intensity larger than or equal to the intensity halfway between the intensity of the normoxic surrounding area and the peak intensity of the ischemic area. Relative frequency distributions were calculated for six intervals of surface areas (see Fig. 7), and the proportion of total ischemic epicardial surface and the number of ischemic areas were measured (see Fig. 8).

Data are presented as means \pm SE. Mean relative frequencies of surface areas per interval were tested for differences between groups using unpaired *t*-tests. Group means of other data were also tested for differences, also using unpaired *t*-tests. The criterion for significance was taken to be $P < 0.05$ for all comparisons.

RESULTS

Five hypertrophic Langendorff-perfused hearts (total ventricle wet wt 1.6 ± 0.2 g) were subjected to *protocol 1*. Hearts instantly developed ischemic areas, indicated by patches of highly fluorescent myocardium (Fig. 1A). These ischemic areas were stable in size and time during the entire period of normoxic perfusion, lasting up to 30 min. After stabilization, the perfusate was switched to nitrogen-saturated medium for 2 min, which caused an increase of coronary flow from 13.8 ± 0.4 (at 2 min in Fig. 2A) to 24.4 ± 0.4 ml \cdot min $^{-1}$ \cdot g $^{-1}$ (at 4 min in Fig. 2A) and an increase of NADH fluorescence intensity of the total epicardium from $32 \pm 3\%$ (Fig. 1A; at 2 min in Fig. 2B) to 100% (Fig. 1B; at 4 min in Fig. 2B). Restoration to oxygen-saturated perfusion decreased coronary flow and epicardial fluorescence intensity (Figs. 1C and 2A). The decrease of NADH fluorescence intensity was spread in uneven patterns across the epimyocardial surface with larger areas having a high fluorescence intensity, persisting even after 20 s of reperfusion (Fig. 1C). Eventually fluorescence intensity decreased to the baseline level at the start of the experiment; this took longer than 5 min. To increase oxygen transport to the myocardium after coronary flow had reached baseline levels, perfusion pressure was increased to 100 and then 120 mmHg. Coronary flow increased from 14.1 ± 0.3 to 17.0 ± 0.5 and then 19.8 ± 0.7 ml \cdot min $^{-1}$ \cdot g total ventricle wet wt $^{-1}$, respectively, but this did not cause ischemic areas to disappear (data not shown). Perfusion pressure was then restored to 80 mmHg. The perfusate was switched to oxygen-saturated medium containing nitroprusside to increase oxygen transport by vasodilatation. Flow increased from 13.5 ± 0.5 to 27.8 ± 1.2 ml \cdot min $^{-1}$ \cdot g total ventricle wet wt $^{-1}$, and ischemic areas completely disappeared (Fig. 1D). To increase oxygen transport in a different manner, in three additional hypertrophic hearts (total ventricle wet wt 1.5 ± 0.1 g) not subjected

to this protocol but which also displayed ischemic areas on commencement of Langendorff perfusion, the perfusate was switched from oxygen-saturated medium to fluorocarbon-containing oxygen-saturated medium. Flow decreased from 14.5 ± 0.7 to 12.1 ± 0.6 ml \cdot min $^{-1}$ \cdot g total ventricle wet wt $^{-1}$ (not significant), and the size of ischemic areas dramatically decreased (Fig. 3).

Control hearts ($n = 5$, total ventricle wet wt 1.1 ± 0.1 g) were subjected to *protocol 1*, which differed from *protocol 2* (hypertrophic hearts) in that, at the start of the protocol, perfusion pressure was decreased to 60 mmHg to obtain coronary flows comparable to those of the hypertrophic hearts. Coronary flows of control hearts 2 min before the start of nitrogen-saturated perfusion did not differ significantly from those of hypertrophic hearts (Fig. 2A). At the start of perfusion there were no ischemic areas (Fig. 4A). When the perfusate was switched from oxygen- to nitrogen-saturated medium, NADH fluorescence homogeneously increased over the entire epicardial surface (Figs. 2B and 4B). Also, coronary flow increased from 14.3 ± 0.8 (at 2 min in Fig. 2A) to 28.3 ± 2.0 ml \cdot min $^{-1}$ \cdot g $^{-1}$ (at 4 min in Fig. 2A). During reperfusion with oxygen-saturated medium, NADH fluorescence rapidly decreased in a heterogeneous pattern (Fig. 4C) of small areas with fluorescence intensities ranging from a low (normoxic) to a high (hypoxic) fluorescence intensity, as described previously (15). Ten seconds after reperfusion, control hearts no longer displayed ischemic areas (data not shown), whereas in hypertrophic hearts postreperfusion ischemia persists much longer (compare Fig. 1, A and C; 20 s after reperfusion the hypertrophic hearts have ischemic areas that are still larger than they were at the start of perfusion). This is also shown in Fig. 2B; after reperfusion, mean NADH fluorescence intensity decreases in both control and hypertrophic hearts but remains significantly higher in hypertrophic hearts for at least 2 min. Figure 2A also shows that after reperfusion, coronary flow decreases faster in hypertrophic hearts than in control hearts (for instance, 1 min after reperfusion, coronary flow in the hypertrophic hearts is 15.2 ± 0.7 ml \cdot min $^{-1}$ \cdot g $^{-1}$, whereas in control hearts it is 24.1 ± 1.0 ml \cdot min $^{-1}$ \cdot g $^{-1}$).

These results suggest that Langendorff-perfused rat hearts that are hypertrophic are hypoperfused, causing ischemic areas to appear. To investigate whether hypoperfusion of control hearts causes similar ischemic areas, perfusion pressure was decreased in a stepwise manner from 60 to 10 mmHg, with each step lasting 3.5 min (*protocol 2* continued) after flow returned to baseline levels after the reperfusion experiment. Flow con-

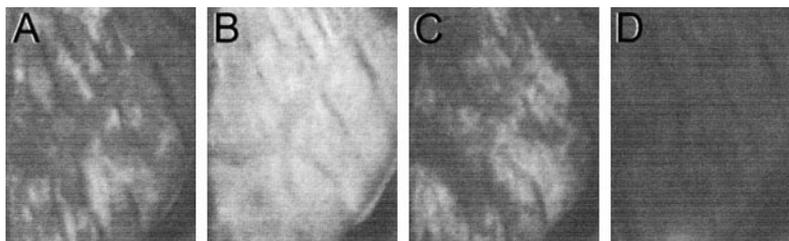


Fig. 1. NADH fluorescence images of a hypertrophic heart at the start of oxygen-saturated perfusion (A), at the end of nitrogen-saturated perfusion (B), 20 s after reoxygenation (C), and during perfusion with oxygen-saturated nitroprusside-containing medium (D).

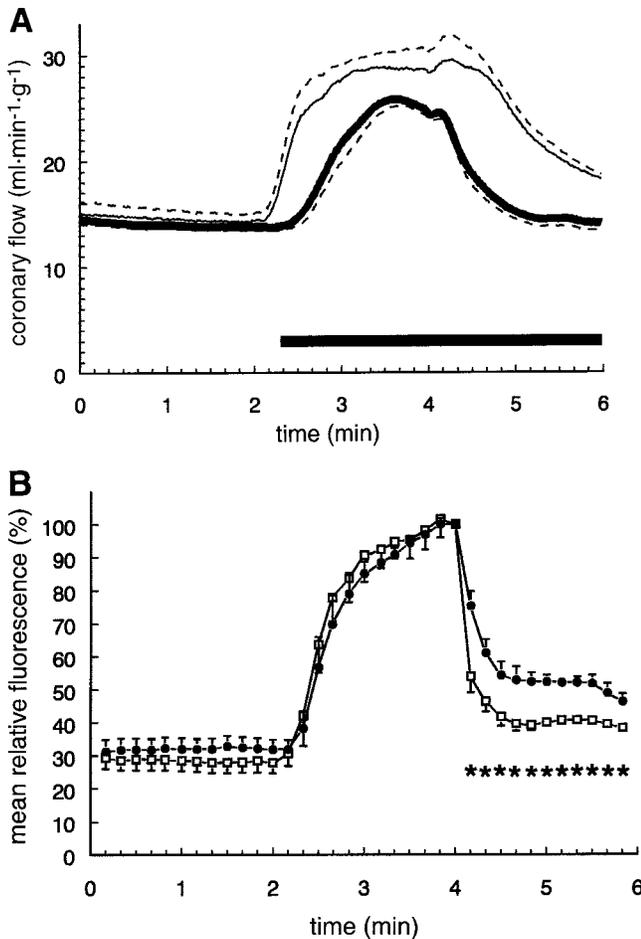


Fig. 2. A: coronary flow changes in rat hearts during stepwise changes in oxygenation. Thick line, mean coronary flow of control heart during *protocol 2* ($n = 5$); thin line, mean coronary flow of hypertrophic heart during *protocol 1* ($n = 5$); dashed lines, \pm SE. Horizontal bar indicates period of statistically significant ($P < 0.05$) difference in coronary flow between control and hypertrophic hearts. B: mean relative NADH fluorescence in rat hearts during stepwise changes in oxygenation. □, NADH fluorescence of control heart during *protocol 2*; ●, NADH fluorescence of hypertrophic heart during *protocol 1*. Values are means \pm SE. Steps: 0–2 min, perfusion with oxygen-saturated medium; 2–4 min, perfusion with nitrogen-saturated medium; 4–6 min, reoxygenation with oxygen-saturated medium. *Statistically significant ($P < 0.05$) difference in mean relative fluorescence between control and hypertrophic hearts.

comitantly decreased (Fig. 5E) and stabilized in < 2 min after each drop in perfusion pressure. Ischemic areas grew progressively larger during perfusion pressures of ≤ 30 mmHg (Fig. 5, B–D). The highly fluorescent ischemic areas were identical in pattern to those seen after switching from nitrogen- to oxygen-saturated perfusion and were evenly distributed over the epicardium (compare Figs. 4C and Fig. 5, B–D). In these experiments, however, patterns with relatively large ischemic areas coexisting with large normoxic areas as seen in hypertrophic hearts did not appear.

To identify at which level of arteriolar and/or arterial vasculature the ischemic areas originate in hypertrophic myocardium during oxygen-saturated perfusion, control hearts were embolized with microspheres of different diameters according to *protocol 3*; five hearts

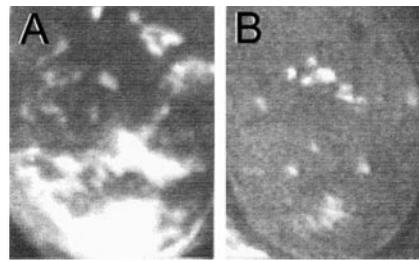


Fig. 3. NADH fluorescence images of a hypertrophic heart during perfusion with oxygen-saturated medium containing no fluorocarbons (A) and 3 min after perfusion with oxygen-saturated medium containing fluorocarbons (B).

were embolized with 5.9- μ m microspheres, three with 9.8- μ m microspheres, and three with 15- μ m microspheres. The larger the diameter, the larger the ischemic areas, as shown in Figs. 6 and 7. Figure 7 shows the frequency distribution of ischemic surface areas of hearts embolized with 5.9-, 9.8-, and 15- μ m microspheres and of the hypertrophic hearts of *protocol 1*. The mean relative frequency distribution of ischemic surface areas in hypertrophic hearts did not differ significantly from those in control hearts embolized with 9.8- and 15- μ m microspheres (except for the interval from 600 to 800 pixels, where there was a significant difference between hypertrophic hearts and control hearts embolized with 9.8- μ m microspheres), whereas it did differ significantly from the frequency distribution of hearts embolized with 5.9- μ m microspheres (Fig. 7). The proportion of ischemic epicardium and number of ischemic areas of hearts embolized with the different microspheres and of hypertrophic hearts are shown in Fig. 8.

DISCUSSION

Earlier work identified hypoperfused ischemic areas in hypertrophic Langendorff-perfused hearts (12). The present study was performed to locate the vascular level at which these hypoperfused areas originate. The main findings of this study were that the ischemic areas in hypertrophic Langendorff-perfused rat hearts were significantly larger than ischemic areas evoked by capillary embolization or by normoxic recovery from nitrogen-saturated perfusion. The ischemic surface areas had a frequency distribution closely resembling that of normal hearts embolized at the arteriolar and/or

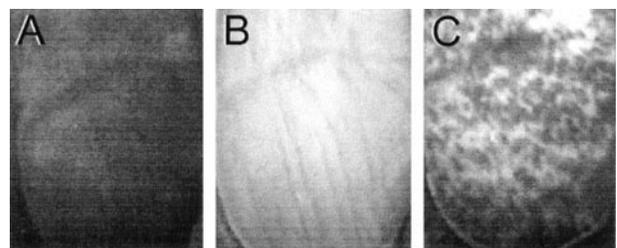


Fig. 4. NADH fluorescence images of a control heart (*protocol 2*) at the start of oxygen-saturated perfusion (A), at the end of nitrogen-saturated perfusion (B), and 5 s after reoxygenation with oxygen-saturated medium (C).

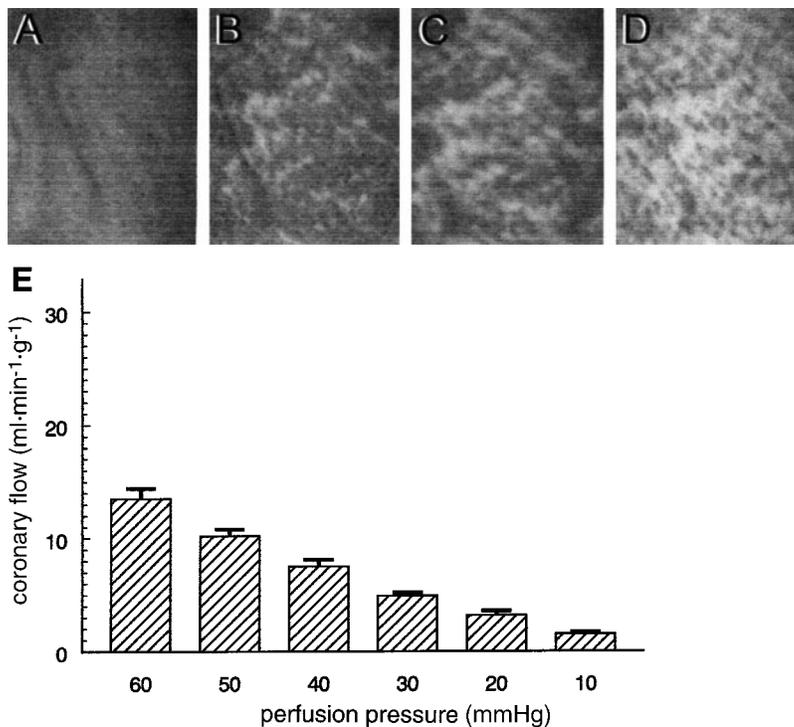


Fig. 5. NADH fluorescence images of a control heart during oxygen-saturated perfusion (protocol 2) at a perfusion pressure of 40 (A), 30 (B), 20 (C), and 10 mmHg (D). E: coronary flow in control hearts ($n = 5$) during stepwise decreases of perfusion pressure.

arterial level, suggesting the existence of hypoperfusion originating at the arteriolar and/or arterial level.

In this study we used epicardial NADH fluorescence to measure epicardial ischemia. The definition of ischemia in terms of fluorescence intensity requires further elaboration. Anoxia induced in Langendorff-perfused rat hearts leads to a four- to fivefold increase in NADH fluorescence intensity relative to the normoxic NADH fluorescence intensity (15). In this study we measured the surface areas of ischemic epicardial zones with a NADH fluorescence intensity greater than the intensity halfway between the intensities of the normoxic surrounding area and those of the ischemic epicardial zones. The NADH fluorescence intensities of the ischemic zones were at least threefold greater than the NADH fluorescence intensities of normoxic epicardium. Because fluorescence measurements were made in beating hearts, we minimized motion artifacts by selecting images from the hearts in identical positions in the cardiac cycle (2).

The NADH fluorescence intensity is also dependent on the amount of work output and the mitochondrial state (2, 6). High work output induces a decrease of the basal normoxic fluorescence intensity. The left ventricle was cannulated and communicated with the atmo-

sphere in all experiments to prevent the development of left ventricular pressure buildup, resulting in similar mitochondrial states in both hypertrophic and control hearts. This prevented confounding of the fluorescence measurements by variations in work output (2). Still, differences in ADP substrate levels between control hearts and hypertrophic hearts could cause differences in NADH fluorescence intensities. However, as can be deduced from Refs. 2 and 6, differences in fluorescence induced by ADP substrate-level variation are not as large as differences induced by ischemia.

Another factor influencing NADH fluorescence intensity is substrate availability. It was shown earlier that the normoxic NADH fluorescence intensity is dependent on the substrate: pyruvate increases normoxic fluorescence the most, oleate less, and glucose even less (2, 12). In this study, however, all experiments were performed with 5.5 mM glucose. Normoxic fluorescence intensity did not differ between hypertrophic and control hearts (data not shown), and highly fluorescent areas in hypertrophic hearts disappeared on improvement of myocardial oxygenation (Figs. 1D and 3B), thereby excluding the confounding of NADH fluorescence measurements by differences in substrate availability between control and hypertrophic hearts.

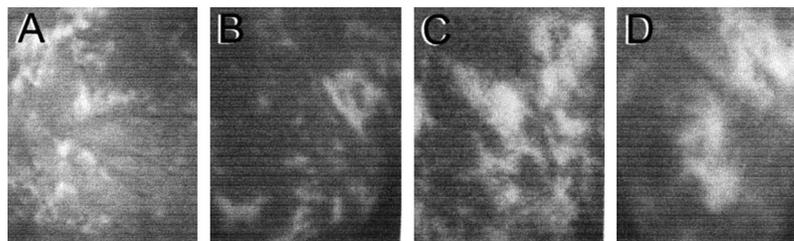


Fig. 6. NADH fluorescence images of control hearts during embolization of vasculature with microspheres of different diameters: 5.9 μm (A), 9.8 μm (B and C), and 15 μm (D).

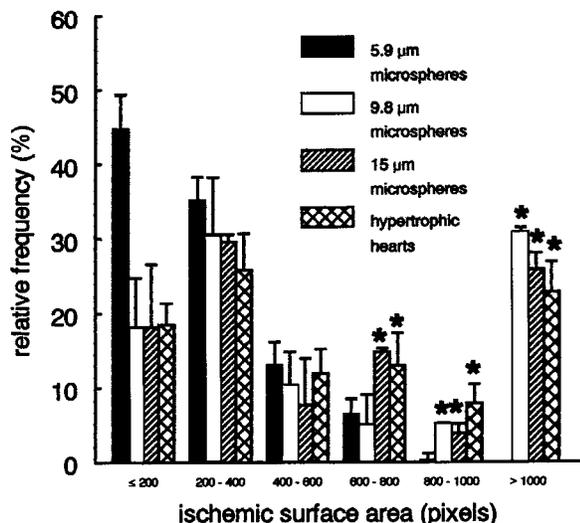


Fig. 7. Frequency distribution histogram of ischemic surface area (1 pixel = $670 \mu\text{m}^2$) in hearts embolized with microspheres $5.9 \mu\text{m}$ ($n = 5$), $9.8 \mu\text{m}$ ($n = 3$), and $15 \mu\text{m}$ ($n = 3$) in diameter and in hypertrophic hearts ($n = 5$). Columns represent mean frequency and bars represent SE. *Statistically significant ($P < 0.05$) different from $5.9\text{-}\mu\text{m}$ microspheres.

In this study we decreased perfusion pressure in control hearts to obtain comparable coronary flow rates between control and hypertrophic hearts. This did not result in qualitatively different NADH fluorescence patterns in control hearts (data not shown). We did not increase perfusion pressure in hypertrophic hearts to this end, because this would have resulted in nonphysiologically high perfusion pressures in hypertrophic hearts. In all experiments in this study the left ventricular cavity communicated with the atmosphere via a cannula inserted through the apex of the left ventricle to ensure the lowest possible cardiac work output in all hearts, thereby reducing the confounding of NADH

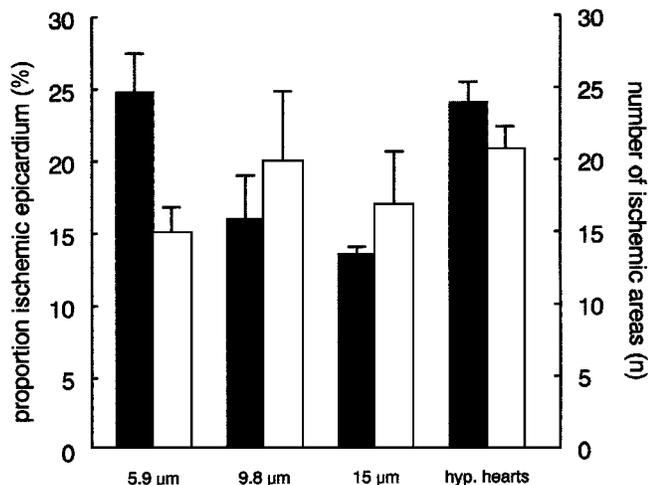


Fig. 8. Proportion of ischemic epicardium (open bars) and number of ischemic areas (filled bars) of hearts embolized with microspheres $5.9 \mu\text{m}$ ($n = 5$), $9.8 \mu\text{m}$ ($n = 3$), and $15 \mu\text{m}$ ($n = 3$) in diameter and in hypertrophic (hyp) hearts ($n = 5$). Columns represent mean values and bars represent SE.

fluorescence measurements by differences in oxygen consumption (2).

Normal rat hearts Langendorff perfused with oxygen-saturated medium continuously release lactate in their effluent, indicating that they are borderline aerobic (13). However, epicardial NADH fluorescence measurements under these circumstances do not reveal dysoxic areas (Fig. 4A) (15). Hypertrophic hearts are known to have a decreased myocardium-to-vascularization ratio (8, 22). Because of this less optimal vascularization, we expected hypertrophic Langendorff-perfused hearts to develop highly NADH-fluorescent ischemic areas during normoxic perfusion, as shown earlier (Fig. 1A) (3, 12). That study had suggested that the development of ischemia was related to the production of oxygen free radicals and acidosis in hypoperfused areas of hypertrophic hearts because of the finding that these ischemic areas could be relieved by perfusion with superoxide dismutase, a scavenger of oxygen free radicals, or by perfusion with fatty acids, resulting in a protection from acidosis-initiated loss of capillary flow. Steenbergen et al. (26) also observed the development of relatively large ischemic areas induced by acidosis in normal hearts and suggested that acidosis-induced coronary (arteriolar and/or arterial) changes were responsible.

Ischemic areas in hypertrophic hearts were elicited at flow rates that did not cause local ischemia in control hearts, suggesting the existence of local hypoperfused areas in hypertrophic Langendorff-perfused rat hearts. To ascertain that these highly fluorescent areas were still viable areas of myocardium and were dysoxic because of impaired oxygenation, oxygen transport was increased in several ways. Adding fluorocarbons or nitroprusside to the perfusate resulted in either complete disappearance (nitroprusside, Fig. 1A) or significant reduction of ischemia (fluorocarbons, Fig. 3). Furthermore, histological analysis in formaldehyde-fixed hypertrophic hearts stained with hematoxylin-eosin revealed no evidence of infarction (data not shown). Increasing coronary flow by increasing perfusion pressure, however, did not produce a significant decrease in ischemic areas (data not shown) in hypertrophic hearts. An explanation for this observation could be that because of the increase in coronary perfusion pressure, oxygen consumption increases, thereby more or less keeping the ratio between oxygen supply and demand unchanged (10). Alternatively, enhancing flow by increasing perfusion pressure could only affect shunting flow, thereby leaving the hypoperfused areas still dysoxic.

In a previous study (15) we had shown with NADH fluorescence measurements in normal hearts that occlusion of vessels of increasing diameter induced patchy ischemic areas of increasing surface area. Furthermore, normoxic recovery in control hearts from perfusion with nitrogen-saturated medium was accompanied by a heterogeneous NADH fluorescence pattern with small, highly fluorescent patches lagging behind areas with a much faster decrease of fluorescence. These ischemic areas were shown to be microcircula-

tory units originating at the capillary level, which were the last to be reoxygenated during reperfusion and could also be elicited by occluding capillaries with microspheres 5.9 μm in diameter (Fig. 6A) (15). A recent histological study by Vetterlein et al. (27) in normal rat hearts in vivo found that tissue located within the capillary bed in proximity to the draining venule is more prone to the development of hypoxia in critical oxygen supply conditions. Hypoxia develops during hypoperfusion because of a combination of disturbances of perfusion in feeding areas of arterioles and the loss of oxygen lengthwise along capillaries. Apparently the microcirculatory units described by Ince et al. (15) are located in capillary beds close to venules. Ischemic areas in hypertrophic hearts at the start of perfusion are significantly larger than those elicited by capillary occlusion in normal hearts (compare Figs. 1A and 6A), suggesting that they originate at a vascular level proximal to the capillaries. This is also supported by the finding that the embolization of increasingly larger vessels (protocol 3) produces increasingly larger ischemic areas that resemble those of hypertrophic hearts (compare Figs. 6, B–D, and 1A). One could argue, taking into account the study of Vetterlein et al. (27), that in hypertrophy the disturbances of perfusion of certain individual arterioles predominate over the loss of oxygen lengthwise along capillaries, thereby inducing larger ischemic areas. Another argument in favor of this hypothesis is the close resemblance of the frequency distribution histogram of ischemic surface areas of hypertrophic hearts to that of control hearts with embolized arterioles and/or arteries (Fig. 7).

Reperfusion with oxygen-saturated medium after nitrogen-saturated perfusion in hypertrophic hearts revealed a significantly different fluorescence pattern from that of control hearts. Mean fluorescence intensity decreased significantly slower with, 1 min after reperfusion, a decline in mean fluorescence intensity of $52 \pm 2\%$ in hypertrophic hearts compared with $40 \pm 2\%$ in control hearts (Fig. 2B). This was accompanied by a significantly faster decrease of flow and thus less oxygen transport after reperfusion in hypertrophic hearts ($15.2 \pm 0.7 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ 1 min after reperfusion) compared with control hearts ($24.1 \pm 1.0 \text{ ml} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ 1 min after reperfusion) (Fig. 2B). Peak flow during nitrogen-saturated perfusion was significantly higher in control hearts compared with that in hypertrophic hearts (Fig. 2B). These findings indicate a decreased coronary flow reserve in hypertrophic hearts, as was found in earlier studies (11, 19, 20). The accompanying fluorescence images during the reperfusion phase show the persistence of larger ischemic areas (Fig. 1C) with high fluorescence even 20 s after reperfusion, whereas in control hearts fluorescence images reveal no ischemia even before that time. The ischemic areas persisting in hypertrophic hearts after reperfusion are also larger than those in control hearts (compare Figs. 1C and 4C) and resemble those after occlusion of arterioles and/or arteries with microspheres (Fig. 6). This finding suggests the existence of hypoperfused areas of myocardium in hypertrophy,

originating at the arteriolar and/or arterial level. To examine the effect of hypoperfusion on control hearts, perfusion pressure was progressively decreased (protocol 2). This induced ischemic areas that match the pattern elicited by occluding capillaries (15) and are much smaller in size than the dysoxic areas seen in hypertrophic hearts. We were never able to induce patterns of ischemia in control hearts resembling those seen in the hypertrophic hearts. Apparently hypertrophy produced by the method used in this study induces changes in arteriolar and/or arterial vascular control that result in ischemia of myocardial areas during suboptimal coronary perfusion. Earlier work (12, 14) has shown that a possible mechanism accounting for such an effect could be scavenging of nitric oxide (endothelium-derived relaxing factor) by increased oxygen free radical production in ischemic myocardial areas in hypertrophic hearts.

In conclusion, in this study we found that the pattern of oxygen transport to the epimyocardium is profoundly altered in hypertrophy. These changes are the result of dysregulation of flow at the arteriolar and/or arterial level. The areas with the slowest reoxygenation after ischemia in hypertrophy are larger than the microcirculatory units described by Ince et al. (15) and have a surface area distribution matching that of the ischemic areas evoked by arteriolar and/or arterial embolization.

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