Regional myocardial perfusion under exchange transfusion with liposomal hemoglobin: in vivo and in vitro studies using rat hearts

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1Division of Bioengineering, Osaka University Graduate School of Engineering Science, Toyonaka; 2Department of Medical Engineering and Systems Cardiology, Kawasaki Medical School, Kurashiki; and 3Department of Cardiovascular Physiology, Okayama University Graduate School of Medicine and Dentistry, Okayama, Japan

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Matsumoto, T., et al. Regional myocardial perfusion under exchange transfusion with liposomal hemoglobin (LH): in vivo and in vitro studies using rat hearts. Am J Physiol Heart Circ Physiol 288: H1909–H1914, 2005. First published December 2, 2004; doi:10.1152/ajpheart.00976.2004.—The purpose of this study was to test the hypothesis that exchange transfusion with liposomal hemoglobin (LH) reduces the microheterogeneity of regional myocardial flows while sustaining cardiac function. Neo Red Cell mixed with albumin was used as the LH solution, in which the LH volume fraction was 17–18% and hemoglobin density was nearly two-thirds smaller than in rat blood. Regional myocardial flows in left ventricular free walls were measured by tracer digitalangiography (100-μm resolution) in anesthetized rats with or without 50% blood-LH exchange transfusion. Within-layer flow distributions showed lower heterogeneity with (n = 8) than without (n = 8) LH transfusion. No extravasation of hemoglobin was confirmed by 3,3-diaminobenzidin staining (n = 2). Carotid flow increased by 68% due to LH transfusion, whereas arterial pressure and heart rate remained unchanged. On the other hand, cross-circulated rat hearts (n = 7) were used to evaluate the effects of 50% blood-LH exchange on coronary flow and tone preservation under 300-beats/min pacing and 100-mmHg perfusion pressure. Blood-LH exchange caused a 71% increase of coronary flow and 10% decrease of percent flow increase during hyperemia after 30-s flow interruption. Myocardial O₂ supply and consumption increased by 9% and 10%, respectively, whereas myocardial O₂ extraction remained unchanged. The large increases of in vivo carotid flow and coronary flow in cross-circulated hearts due to LH coperfusion could be explained by the reduction of apparent flow viscosity. These results suggest that under LH coperfusion, the microheterogeneity of myocardial flows decreases with increased coronary flow while fairly preserving coronary tone and cardiac function.

It is also notable that LH can easily reach and oxygenate tissue regions with high resistance against RBC without extravasation because of its appropriate particle diameter of 200 nm. Therefore, on the basis of the assumption that LH facilitates regional perfusion, we formulated the hypothesis that blood-LH exchange decreases the heterogeneity of microvascular bed flows in myocardium, where flows are highly heterogeneous in nature (3, 14, 18, 19, 30), with keeping cardiac function. To test this hypothesis, the present study was designed as follows. First, using high-resolution tracer digitalangiography (18–20), we assessed the microheterogeneity of left ventricular (LV) myocardial flows in anesthetized rats with and without 50% blood-LH isovolumic exchange transfusion. In cross-circulated rat hearts under constant heart rate and constant perfusion pressure, we then evaluated the effect of 50% blood-LH replacement on coronary flow and tone preservation (reactive hyperemic flow), which are determinants of myocardial flow heterogeneity. We also measured the distribution of myocardial flows in cross-circulated hearts by digitalangiography and compared its heterogeneity with our previous cross-circulated rat heart study under no LH perfusion (20).

MATERIALS AND METHODS

Experiments were conducted in accordance with the guiding principles of the American Physiological Society (1) and with the approval of the Animal Research Committee of Kawasaki Medical School.

Preparation of LH solution. Neo Red Cell (NRC), polyethylene glycol-modified LH suspended in physiological saline with a volume fraction (Nct) of 25%, was provided by Terumo. The concentration of Hb is 5 g/dl or 0.2 g·Nct⁻¹·dl⁻¹, and the PO₂ required to half-saturate Hb is controlled to 40 Torr. The latter is higher than the PO₂ required to half-saturate Hb of rat RBC (32 Torr), and, accordingly, O₂ unloading to the tissue will be facilitated when an appropriate amount of NRC is mixed with rat blood. In addition, NRC contains a met-Hb reductase system and an allosteric effector (34, 35). NRC was mixed with a human albumin solution (Bayer) and adjusted to pH 7.4 with 1 M NaOH, resulting in a Nct of 17–18% and an albumin concentration of 6 g/dl.

In vivo study on regional myocardial flow heterogeneity. In the control (n = 8), a male Wistar rat (400–600 g, 14–16 wk) was anesthetized with pentobarbital sodium (50 mg/kg ip), given heparin (1,000 U/kg iv), and placed supine on a 37°C heated plate. A femoral artery was exposed and cannulated for blood gas sampling and pressure measurement. Ventilation was maintained via tracheotomy and a mixture of O₂ and room air adjusted to keep arterial pH and arterial blood gases within physiological limits (pH 7.35–7.45, PO₂

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35–45 Torr, \( \text{PO}_2 \) 100–200 Torr). Arterial pressure was measured with a manometer catheter and a pressure transducer (Camino Laboratories). Both carotid arteries were exposed by blunt dissection; one was used for the injection of myocardial flow tracer via a cannula advanced into the LV cavity and the other for the measurement of carotid arterial flow with a transit time ultrasonic flow probe and a flowmeter (Transonic Systems). In NRC-transfused rats \((n = 10)\), which mimicked 50% blood-LH isovolumic exchange transfusion, a femoral vein was also exposed and cannulated. Infusion of NRC solution, filtered through a 5-\(\mu\)m pore filter (Millipore), into the femoral vein and simultaneous withdrawal of blood from the femoral artery (together with NRC already circulating) were performed at an exchange rate of 1 ml/min. The total exchange volume for 50% replacement of blood with LH was about 10 ml. Arterial blood was collected immediately before cardiac arrest for the measurements of hematocrit (Hct) and Nct by centrifugation. For eight hearts in each group, regional myocardial perfusion was evaluated by tracer digitalangiography according to the previous studies (18–20). Briefly, after stabilization for 30–60 min, 10 \(\mu\)Ci tritiated desmethylimipramine (HDMI, Life Science Products) was injected into the LV cavity over a period of 4–5 s. Sixty seconds after the tracer injection, when the HDMI retention was still nearly 95% (33), the heart was arrested with a saturated KCl solution. Thus the HDMI deposition in a certain small piece of tissue is virtually proportional to the accumulated flow perfused into that piece. The heart was excised and perfused with saline to wash out residual blood. The LV free wall was then frozen in a plate-like shape and divided into 10-\(\mu\)m-thick slices from subendo- to subepicardium with a cryostat microtome (Zeiss). The slices were carefully put onto a slide glass. Twenty-eight slices without large epicardial vessels and free from the influence of uneven endocardial surface were intended for the digitalangiographic flow measurement; 14 slices in the epicardial side were assigned to the outer layer and the rest of the 14 slices in the endocardial side to the inner layer.

Within-layer HDMI distributions were measured in a 1,024-step gradation with 100-\(\mu\)m resolution using the tritium-sensitive radiactive imaging plate and the imaging analyzer (Fujix). The spatial heterogeneity of HDMI densities (arbitrary units), i.e., relative regional flows, was quantitated by the coefficients of variation (CV) of HDMI densities [CV (%) = \(\text{SD/mean} \times 100\)] in 64 × 64 regions of 100 × 100 \(\mu\)m² and in 16 × 16 coarse-grained regions of 400 × 400 \(\mu\)m². In the latter, HDMI densities were averaged over 4 × 4-pixel aggregates. The coarse-grained regions were comparable in size to the functional length of capillaries connecting an arteriole to an adjacent venule (15).

In another two NRC-transfused rats, the extravasation of NRC was examined by 3,3′-diaminobenzodiazine (DAB) staining (22). The hearts were excised and perfused with saline. LV free walls were then harvested and suspended in 10% neutral buffered formalin for fixation. After being stored in a refrigerator at 4°C, LV walls were embedded in paraffin, and serial sections (6 \(\mu\)m) were made. Slices were stained for Hb using a pseudoperoxidase reaction with DAB and embedded in paraffin, and serial sections (6 \(\mu\)m) were made. Slices were stained for Hb using a pseudoperoxidase reaction with DAB and counterstained with hematoxylin. Standard hematoxylin-eosin (HE) staining was also done for histomorphological evaluation.

Cross-circulated heart study on coronary flow and tone. A cross-circulation model \((n = 7)\) similar to that described in detail in a previous study (20) was used for measurements of coronary flow and cardiac function under constant heart rate and constant perfusion pressure before and after blood-NRC replacement. In brief, a support male Wistar rat (\(>500\) g, 14–18 wk) was anesthetized with pento-barbital sodium (50 mg/kg ip) and given heparin (1,000 U/kg iv). Ventilation was maintained via a tracheotomy and a mixture of \(\text{O}_2\) and room air. The rat was placed supine on a 37°C heated plate. A femoral vein and a carotid artery were exposed by blunt dissection and cannulated. Blood from the carotid artery was pumped into a 37°C water-jacketed reservoir and then perfused to the isolated heart of another male Wistar rat \((<500\) g, 10–14 wk) prepared according to the Langendorff technique. The isolated heart was put in the 37°C mist of warmed saline and paced at 300 beats/min by a pacemaker (Medtronic) through platinum wires sutured to the right atrium and right ventricular apex. A drain was created in the LV apex by puncture with an 18-gauge needle to allow egression of blood from Thebesian vessels. Myocardial effluent was collected manually with a syringe and transferred to another water-jacketed reservoir connected to the femoral vein of the support rat. Perfusion head pressure was maintained at 100 mmHg through continuous overflow to the venous side reservoir.

Arterial and venous blood were collected for blood gas measurements from the aortic line just above the aortic cannula and through a catheter placed in the pulmonary artery of the isolated heart, respectively. Arterial pH and blood gases were kept within physiological limits through the experiment. Arterial blood was also used for measurements of Hct and Nct by centrifugation. A cannulated, saline-filled balloon was placed in the LV via a left atriotomy for isovolumic LV pressure measurement. The balloon was connected to a pressure transducer (Camino Laboratories) and inflated to adjust the end-diastolic pressure at 5–10 mmHg. The perfusion rate was measured in the aortic inflow line with a transit time ultrasonic flow probe and a flowmeter (Transonic Systems). Reactive hyperemia was elicited by interrupting coronary perfusion for 30 s, and the percentage of maximal flow increase to basal flow was assessed.

After basal data were obtained, filtered NRC solution was poured into the venous side reservoir drop by drop, and myocardial effluent was removed until Hct decreased by nearly half (50% blood-NRC exchange). Hearts were allowed to stabilize for 30–60 min, and hemodynamic data were obtained again. Finally, 2 \(\mu\)Ci HDMI was injected into the aortic inflow line, and the heart was arrested 20 s later. Within-layer HDMI distributions were quantitated and evaluated as described before.

Statistics. All measured signals were recorded with a sampling rate of 500 Hz using a personal computer (Macintosh G3) with LabView (National Instruments). Between-group differences in hemodynamic variables and in CV were assessed with the Mann-Whitney U-test. Hemodynamic differences between cross-circulated hearts before and after NRC coperfusion were assessed with Wilcoxon matched-pairs signed rank test. A value of \(P < 0.05\) was considered statistically significant. Data are represented as means ± SD.

RESULTS

Microheterogeneity of myocardial flows (in vivo hearts). In NRC-transfused rats, Nct amounted to 8 ± 1%. Hct and carotid arterial flow were 23 ± 4% and 5.8 ± 2.3 ml/min, respectively, which were different significantly from baseline values (42 ± 2% and 3.4 ± 0.9 ml/min). Mean arterial pressure (113 ± 8 mmHg) and heart rate (459 ± 39 beats/min) remained unchanged from baseline values (122 ± 9 mmHg and 467 ± 35 beats/min). In control rats, Hct, mean arterial pressure, carotid arterial flow, and heart rate were 43 ± 1%, 123 ± 8 mmHg, 3.4 ± 1.2 ml/min, and 450 ± 29 beats/min, respectively. Carotid arterial flow was higher significantly in NRC-transfused rats than in control rats.

Figure 1 shows a typical example of within-layer flow distributions in control and NRC-transfused hearts represented at the original (100 \(\mu\)m) and coarse-grained (400 \(\mu\)m) resolution. These are the distributions of normalized HDMI density (mean = 1), i.e., the flow distributions on the relative basis. Shading in each pixel is proportional to flow; a darker region received higher flow. Both distributions show heterogeneous patterns; however, the degree of heterogeneity looks lower in the NRC-transfused heart. The CV values of these distributions are 46.8% (control) and 33.9% (NRC transfused) at 100-\(\mu\)m...
resolution and 20.6% (control) and 13.4% (NRC transfused) at 400-μm resolution. In NRC-transfused hearts, the CV was 38.2 ± 5.7% at 100-μm resolution and 13.7 ± 3.2% at 400-μm resolution, which were lower than 50.4 ± 11.4% and 21.3 ± 5.6%, respectively, in control hearts. Thus the myocardial flow heterogeneity was lower in NRC-transfused hearts. Figure 2 shows the CV of outer and inner layer flows. Irrespective of myocardial depth or resolution, the CV was lower in NRC-transfused than control hearts. No CV difference was found between outer and inner layers in both control and NRC-transfused hearts.

Figure 3 shows a DAB-hematoxylin-stained myocardial slice. Microscopic examination revealed no brown cytoplasm (Hb) in the extravascular space, indicating no extravasated Hb. RBC alone was positive for DAB staining. No morphological abnormality was also found in HE-stained slices (data not shown).

**Coronary flow and tone (cross-circulated hearts).** Through blood-NRC exchange, Hct decreased from 41 ± 3% to 22 ± 4% and Nct amounted to 7 ± 2%. Coronary afferent and efferent pH, PO2, and PCO2 remained unchanged (7.41 ± 0.03 vs. 7.39 ± 0.04, 194 ± 29 vs. 187 ± 32 Torr, and 39 ± 2 vs. 38 ± 6 Torr for aortic inflow; 7.40 ± 0.03 vs. 7.37 ± 0.06, 57 ± 8 vs. 57 ± 9 Torr, and 41 ± 5 vs. 40 ± 5 Torr for pulmonary arterial flow).

Figure 4 shows the coronary perfusion rate, percent flow increase during reactive hyperemia, and LV developed pressure before and after blood-NRC exchange. Because of NRC coperation, the coronary perfusion rate increased largely and, to a lesser extent, the percent flow increase during hyperemia decreased and LV developed pressure increased.

In cross-circulated hearts after NRC coperation, the CV was 54.8 ± 6.3% at 100-μm resolution and 36.5 ± 5.9% at 400-μm resolution, respectively. These were significantly lower than 73.5 ± 11.0% and 56.2 ± 12.1%, respectively, in control hearts.

Fig. 1. Myocardial flow images of control and Neo Red Cell (NRC)-transfused hearts (in vivo) resolved into 64 × 64 pixels of 100 × 100 μm² (original resolution) and into 16 × 16 pixels of 400 × 400 μm² (coarse-grained resolution) shaded proportionately with flows normalized by respective means.

Fig. 2. Coefficient of variations of flows (CV) within outer and inner layers in control and NRC-transfused hearts (in vivo). The CV values were calculated for flow distributions resolved into 64 × 64 pixels of 100 × 100 μm² (original resolution) and into 16 × 16 pixels of 400 × 400 μm² (coarse-grained resolution). *P < 0.05 vs. control.

Fig. 3. Myocardial slice of an NRC-transfused heart (in vivo) stained with 3,3-diaminobenzidin-hematoxylin showing Hb containing cytoplasm (brown) and nuclei (blue). Brown cytoplasm was observed in red blood cells but not in the extravascular space, indicating no evidence of NRC extravasation.

Fig. 4. Coronary perfusion rates at baseline and at the peak during reactive hyperemia after 30-s flow interruption (top), percent flow increase during reactive hyperemia (bottom left), and left ventricular (LV) developed pressure before (control) and after NRC coperation (bottom right) in cross-circulated hearts. *P < 0.05 vs. control.
cross-circulated hearts under no NRC coperfusion, which were obtained in our previous study using a similar technique (20).

DISCUSSION

The present study provided the first detailed evaluation of LH (NRC) coperfusion effects on regional myocardial flows and coronary hemodynamics. Flow heterogeneity at a microvascular bed level (CV) decreased both in vivo and in cross-circulated hearts due to blood-NRC exchange. In vivo exchange transfusion increased carotid flow but had little effect on arterial pressure and heart rate. The histological evaluation showed no evidence of Hb extravasation and tissue edema. In cross-circulated hearts, NRC coperfusion increased coronary flow largely while having only a modest impact on reactive hyperemia and LV developed pressure.

Effects of decreased viscosity. Blood-NRC exchange increased both carotid flow in vivo and coronary flow in cross-circulated hearts by as much as 70%. This flow increase will be attributed largely to decreased apparent viscosity accompanying hemodilution. Actually, viscosity measurements with the cone plate viscometer (Toki Sangyo) at 10 and 50 rpm, which correspond to 75 and 375 s⁻¹, respectively, showed that a mixture of blood (Hct = 40%) and NRC solution in equal proportions had a 33–46% smaller viscosity than blood. The decrease of apparent viscosity of this magnitude could account for considerable parts of carotid and coronary flow increases, implying only little involvement of vascular resistance in these flow increases. A slight decrease of arterial pressure in vivo was also probably due to afterload reduction accompanied by a decrease of apparent viscosity.

Heterogeneity of regional myocardial flows. Decreased myocardial flow heterogeneity under NRC coperfusion will be explained by RBC flow properties. RBC aggregates, causing momentary block of microvessels (21, 37), occur with higher frequency in lower flow regions. Accordingly, this RBC contribution for increasing apparent viscosity will be higher in lower-flow regions and enhance the relative flow differences between high- and low-flow regions consequently. Thus through decreasing apparent viscosity and increasing flow to a higher degree in originally lower flow regions, blood-NRC exchange would lead to a decrease of flow heterogeneity.

The capillary endothelial glyocalyx may also be involved in determining flow heterogeneity under NRC coperfusion because its layer thickness may change according to flow shear rate and Hct (10, 12), especially in capillaries exposed to low flow (24). That is, the redistribution of capillary resistances possibly occurs through the thickness change of glyocalyx layer, affecting microheterogeneity of flows.

Myocardial flow heterogeneity was higher in cross-circulated hearts than in hearts in vivo. Tracer injection procedure, isovolemic contraction, right ventricular pacing, nonautologous blood perfusion, and nonrestriction of pericardium will all be influential more or less in shaping higher heterogeneous flow distributions in cross-circulated hearts. Blood-NRC exchange, however, decreased myocardial flow heterogeneity significantly either in vivo or in cross-circulated hearts. It will be worth exploring the therapeutic value of NRC in microvascular diseased hearts, which are in a state of high myocardial flow heterogeneity.

Blood-NRC exchange increased coronary flow largely in cross-circulated hearts and would also increase coronary flow in vivo presumably to a similar degree. Besides, after NRC coperfusion, arterial pressure was lowered in vivo and LV developed pressure increased in cross-circulated hearts slightly. These changes raise the possibility of altering flow heterogeneity through flow-dependent or autoregulatory coronary tone changes (2). However, arterial pressure was still within the effective range of autoregulation, and the increase of flow shear rate would be counterbalanced by decreased apparent viscosity. Therefore, coronary tone would be fairly preserved and would not be involved in lowering myocardial flow heterogeneity. Coronary tone preservation under NRC coperfusion is also conjectured from the measurement of reactive hyperemic flow in cross-circulated hearts. The effects of NRC transfusion on coronary tone in vivo need to be further investigated, though.

Phase separation at microvascular bifurcations (13, 23) will make RBC fluxes more heterogeneous, and, accordingly, regional O₂ supplies will be more heterogeneous than regional flows. Under NRC coperfusion, however, O₂ supply heterogeneity due to biased RBC fluxes will be largely compensated with O₂ deliveries by NRC particles circulating together with plasma. That is, the reduction of O₂ supply heterogeneity through NRC perfusion will be more pronounced than the reduction of flow heterogeneity.

Coronary hemodynamics in cross-circulated hearts. The increased coronary perfusion rate and lowered flow heterogeneity under NRC coperfusion would increase LV developed pressure through facilitating a wash out of metabolic waste products and probably elevating myocardial O₂ utilization.

Blood-NRC exchange caused a small reduction of percent flow increase during reactive hyperemia, in which apparent viscosity as well as coronary vasodilation would be involved. The reduction of apparent viscosity with increased flow (non-Newtonian effect) during coronary vasodilation increases flow further in an additive manner, and this non-Newtonian effect emerges more distinguishably at higher Hct (9). That is, the observed attenuation of hyperemic flow under NRC coperfusion could be partly due to Hct reduction, implying that the difference between hyperemic vasodilation before and after blood-NRC exchange was still smaller than expected only from flow measurements. Thus coronary tone would be fairly preserved under NRC coperfusion. Metabolic vasodilatory response to NRC coperfusion was also unlikely to occur because of sustained O₂ supply and extraction as mentioned below.

On the basis of PO₂ and flow measurements in the cross-circulated heart experiment, the percent increase of myocardial O₂ supply due to blood-NRC exchange (ΔO₂up) could be estimated according to the following equation:

\[ΔO₂up(%) = \left[ \frac{Q_{\text{post}} \times (AO₆^{R \text{post}} \times 0.3Hct_{\text{post}} + AO₆^N \times 0.2\text{Net})/ (Q_{\text{pre}} \times AO₆^{R \text{pre}} \times 0.3Hct_{\text{pre}} - 1)}{100} \right] \]

where Q is the coronary perfusion rate and AO₆⁰ and AO₆¹ are arterial O₂ saturation of RBC and NRC, respectively. Here, AO₆⁰ and AO₆¹ were calculated on the assumption that Hill’s coefficient, P₅₀, and Hb concentration were 2.3, 32 Torr, and 0.3 g·Hct⁻¹·dl⁻¹ for rat blood and 2.1, 40 Torr, and 0.2 g·Nct⁻¹·dl⁻¹ for NRC, respectively (data provided by R&D Center, Terumo). Pre and post denote values before and after...
blood-NRC exchange. The myocardial O₂ supply increased after blood-NRC exchange (ΔO₂sup = 8.8 ± 8.7%, P < 0.05) despite hemodilution decreasing Hb density by >30%. Increased coronary flow is likely to compensate for hemodilution.

Myocardial O₂ extraction (Oext) could be also estimated by the following equation:

\[
O_{\text{ext}}(\%) = \frac{(A VO^R \times 0.3Hct + A VO^N \times 0.2Nct)}{(A O^R \times 0.3Hct + A O^N \times 0.2Nct)} \times 100
\]

where AVO^R and AVO^N are the differences of coronary arteriovenous O₂ saturation of RBC and NRC, respectively. No difference was found in Oext before and after blood-NRC exchange (20.4 ± 6.0% vs. 20.3 ± 4.9%). Under NRC coperfusion, however, Oext from NRC (AVO^N/AVO^R × 100, 28.0 ± 5.5%) was significantly higher than Oext from RBC (AVO^N/AVO^R × 100, 18.7 ± 4.6%), attributed to the higher P50 of NRC. Increased Oext with elevating P50 of O₂ carriers was also reported in earlier studies (6, 29). The myocardial O₂ consumption calculated in a similar manner increased by 10.3 ± 9.8% (P < 0.05) after blood-NRC exchange, which is consistent with the small increase of LV developed pressure after NRC coperfusion.

**Methodological limitations.** Tracer digitalradiography using HDMI is an ideal technique for quantitating myocardial flow heterogeneity, especially in small animal hearts, resolving flow distribution down to a capillary bed level because of its high solubility. The weakness of digitalradiography for flow measurement is, however, that repeated measurement of regional perfusion is not allowed. No diffusible flow tracer provides adequate myocardial extraction and retention. Although a two-time measurement of flow distribution is possible by using HDMI with iodine-labeled desmethylinipramine, for example, their myocardial retention decreases rapidly with time (33) and the possible time interval after the first injection is a few minutes at most.

In this regard, the microsphere method is superior to tracer digitalradiography because of its tight intravascular deposition and the availability of various labeled spheres as well. However, the microsphere deposition at the present high-resolution level is under the influence of not only flows but also the anatomic features of arteriolar trees (11). Furthermore, microspheres tend to be deposited preferentially in high-flow regions (4, 5), and, accordingly, their deposition is likely to follow RBC fluxes more closely than flows, especially at a high-resolution level. Thus the microsphere method is not suitable for evaluating the NRC effect on microvascular bed perfusion.

In conclusion, microheterogeneity of regional myocardial flows decreased with increased coronary flow through blood-LH (NRC) exchange, whereas coronary tone and cardiac contractility were highly sustained at least within the short period of 30–60 min examined here. This LH effect of decreasing flow heterogeneity may be beneficial against microvascular flow abnormalities, e.g., no-reflow phenomenon (16, 17), through reducing capillary derecruitment and increasing functional capillaries. The therapeutic and the long-term effects of LH perfusion on myocardial perfusion are of future interest.

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**REFERENCES**


