Spatial Heterogeneity in the Heart: Recent Insights and Open Questions

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Within the left ventricular myocardium and despite its rather homogeneous structure, local myocardial perfusion varies substantially. Areas of low and high local flow differ with regard to substrate uptake, energy turnover, and demand. This spatial heterogeneity is related to distinct differences in local protein expression, forming the basis of a novel homeostatic mechanism.

In recent years, it has become increasingly clear that the regulation of myocardial energy metabolism and perfusion cannot be fully understood without taking into account the substantial spatial heterogeneity that can be found within the heart on a regional, local, and subcellular level. Functional differences between atria and cardiac chambers as well as between left and right ventricle have long been recognized. This review will therefore focus on the emerging evidence that, even within the apparently homogeneous left ventricular free wall, in each transmural layer, the coordinated functions of flow, energy metabolism, and possibly contraction display substantial spatial heterogeneity when studied at high resolution. In fact, this heterogeneity even challenges the idea of the myocardium as a functional syncytium.

Spatial heterogeneity of flow

The initial evidence for spatial heterogeneity within the left ventricular myocardium was obtained when analyzing local myocardial blood flow by the microsphere technique (see below). At a resolution of 300 µl (mg), in the awake dog a patchy pattern of local microsphere deposition is observed as shown in Fig. 1. This pattern is present not only in the subepicardial but also in all myocardial layers. It is commonly described by a normalized frequency distribution (Fig. 1), which is characterized by the following features:

- Although the majority of tissue samples receives an average number of microspheres and thus of flow, ~6% received <0.5 and another 11% received >1.5 of the mean, with individual samples ranging from <0.2 to >2 (14).
- The coefficient of variation (CV) observed (0.34) by far exceeds the inherent error of the microsphere technique (0.07) and is therefore a valid index of biological variability. It increases with higher spatial resolution.
- This substantial spatial heterogeneity is stable for weeks (Fig. 1C; Ref. 14) and possibly months. In other words, the patchy pattern of local perfusion will persist for extended periods of time.

Similar data have been obtained by several groups in awake baboons and in dogs, sheep, rabbits, and rats (for review, see Ref. 11).

Considering the apparently homogeneous structure and function of the myocardial wall, the fundamental importance of oxidative phosphorylation for cardiac function, and the nearly complete oxygen extraction, the finding of a considerable spatial heterogeneity of local perfusion may appear somewhat disconcerting. The question arises as to whether the spatial heterogeneity of microsphere deposition is indeed predominantly a function of local myocardial blood flow. Microspheres with a diameter of, for example, 15 µm are given to the left atrium, resulting ideally in a homogeneous distribution in the cardiac output. Following transport into all arteries supplied vascular beds in proportion to the respective volume flow, microspheres are logged in precapillary arterioles by virtue of their size. Following tissue excision, the deposition density in an organ or tissue region is determined and related to that of a known reference organ. For a 10% precision at a 95% confidence level, ~400 microspheres per sample are required. Since the number of microspheres conventionally applied is designed to block <1/1,000 of myocardial capillaries, the spatial resolution of this technique is limited to 1 mg, with most studies being performed in a range from mg to 1 g. Whereas conventionally microspheres are regarded as the gold standard for tissue perfusion (for review, see Ref. 16), it is very conceivable that the probability of an individual microsphere entering a distinct portion of the arteriolar tree is not only influenced by erythrocyte or plasma flow but also by the geometry at the individual branching points. To address this concern, Bassingthwaighte et al. (3) assessed local plasma flow by iododesmethylinipramine (IDMI) uptake. IDMI plays a nearly complete extraction during a single capillary passage, and its uptake is therefore solely flow limited. IDMI uptake correlated very well with microsphere deposition (r = 0.93). However, samples with a high IDMI uptake frequently demonstrated an even higher microsphere deposition, indicating a small systematic bias of the microspheres (3). It is currently unclear whether the somewhat greater heterogeneity of myocardial microsphere deposition represents the true heterogeneity of erythrocyte flow compared with plasma flow in the heart or whether it exaggerates the extent of myocardial blood flow heterogeneity to a minor degree.

It is worth noting that the concept of a spatial heterogeneity...
of myocardial blood flow under resting conditions is not only based on measurements of tissue deposition of molecular or particulate markers. It is also supported by model analysis of indicator dilution curves in the canine circulation (17) and most recently by high-resolution MRI in the saline-perfused rat heart (4).

Initial attempts to explain the observed spatial heterogeneity of blood flow focused on structural features. A close analysis of the individual neighbor areas of low- and high-flow samples revealed a higher probability for a similar flow in the local environment. This can already be seen in the example given in Fig. 1 and was true at different levels of spatial resolution. Both the flow distribution (2) and the branching pattern of the vascular tree show a self-similar fractal pattern, extending to small spatial scales. In fact, the structure of the vascular tree was taken to predict the observed flow heterogeneity (20). However, several studies demonstrated that local perfusion in both low- and high-flow areas can be substantially increased (e.g., Ref. 1). It is thus highly unlikely that simply the vessel architecture dictates the heterogeneity of local flow.

**Spatial heterogeneity of energy metabolism**

As long as only flow and structural features were investigated, the relation between local myocardial flow and local energy turnover was dominated by speculation. Given the more than threefold difference in local perfusion between those areas receiving <50% or >150% of the average flow (low- and high-flow areas, respectively), substantial differences in local oxygen consumption may have been expected. Alternatively, low-flow areas might have been close to hypoxia, whereas high-flow areas were receiving a luxury perfusion. However, low- and high-flow areas showed only minor differences in their local ATP content. More importantly, the free cytosolic concentration of adenosine (Fig. 2A), a sensitive indicator of tissue hypoxia, as well as the local lactate content were not enhanced in low-flow regions under basal conditions (15). This virtually ruled out tissue hypoxia in low-flow regions and indicated a lower oxygen consumption and energy turnover.

Since the direct measurement of local oxygen consumption or energy turnover at high spatial resolution has hitherto not been achieved, indirect measures had to be developed to test the hypothesis that low- and high-flow areas differed by a factor of 3 both in flow and energy turnover. Initial studies related substrate uptake to local flow. Long-chain fatty acid uptake correlated closely with local flow, indicating enhanced fatty acid membrane permeability in high-flow regions in a model analysis (7). Similarly, glucose uptake increased with local flow (19), a finding that was attributed to an enhanced glucose metabolism in high-flow areas (10). Whereas the enhanced substrate uptake in high-flow areas clearly suggested an enhanced metabolic activity, the in vitro analysis of key enzymes involved in energy metabolism did not unravel major differences, in line with the structural homogeneity of the left ventricular myocardium in terms of capillary and mitochondrial density (19). It was therefore important to test whether the enhanced substrate uptake in high-flow areas translated into increased metabolic activity, i.e., an enhanced turnover of the citric acid cycle.

Measuring the metabolism of $^{13}$C-labeled pyruvate in the citric acid cycle using $^{13}$C-NMR spectroscopy, Decking et al. (9) demonstrated in the dog heart in situ that high-flow areas were characterized by a higher turnover of the TCA cycle than low-flow areas. In fact, the threefold difference in local flow was associated with a 3.4-fold difference in local TCA cycle turnover (9). Since the generation of reducing equivalents in the TCA cycle feeds almost directly into the respiratory chain and oxidative phosphorylation, the data established both a link between local energy turnover and local flow and a substantial spatial heterogeneity of local energy turnover within the heart.

**FIGURE 1.** Spatial heterogeneity of myocardial blood flow. A: spatial distribution of local flow in a representative left ventricular free wall. Data are from Ref. 14. B: frequency distribution of local myocardial blood flow, as assessed by the deposition density of radioactive microspheres in a total of 1,385 samples (297 ± 97 mg wet weight) from the left ventricular free wall in 7 dogs, and normalized to the mean flow in each individual heart. Data are from Ref. 14. C: temporal stability of local myocardial blood flow. Reproduced from Ref. 14.
the left ventricular free wall. This conclusion is further supported by data obtained in the saline-perfused rabbit heart. Here, the local H$_2$O residue following perfusion with 18O-saturated medium was consistent with a close correlation between local flow and local oxygen consumption, as indicated by model analysis (18). Thus in the past few years, substantial evidence was obtained demonstrating within the left ventricular free wall not only a spatial heterogeneity of flow but also of local substrate uptake and energy turnover, with local differences exceeding a factor of 3.

These data suggested a close coupling of local oxygen consumption and local flow not only globally in the heart but also in each individual flow region. In such a scenario, any moderate impairment of local oxygen delivery would compromise oxygen consumption and decrease local oxygenation, both in high- and low-flow areas. Indeed, as shown in Fig. 2, when an occluder reduced coronary blood flow to ~½ of its previous value, a significant rise in local adenosine was observed in all of those regions where local flow fell by <50%. In fact, local adenosine rose to similar levels in former (i.e., basal) low- and high-flow areas when local flow was reduced by the same percentage (15). These data underline the notion that high-flow areas do not receive a luxury perfusion but are adequately supplied for the local tissue requirements. The same conclusion was reached when assessing the rise in local lactate, both in canine and porcine hearts (6, 15). Thus it is not only the local flow and energy turnover but also the local energy demand that appears to vary within the left ventricular myocardium. Assuming high-flow areas to be areas of substantially higher local energy turnover and demand, these areas may be the first to suffer during a complete cessation of myocardial perfusion. Consistent with this assumption, in conscious baboons the local flow before ischemia-reperfusion predicted the risk of local necrosis (13). In addition, partial coronary occlusion in the rat resulted in a spatially heterogeneous impairment of myocyte viability (5), indicating again that local energy demand is inhomogeneous in the myocardial wall.

**Spatial heterogeneity of protein expression**

What is the molecular and functional basis of this spatial variation in local energy demand? Given the high temporal stability of the spatial heterogeneity of local flow (Fig. 1C; Ref. 14) and, presumably, local energy turnover and demand, substantial differences in local myocardial structure and protein expression may be expected. Surprisingly, an initial assessment of mitochondrial, myofibril, and capillary density revealed no difference between low- and high-flow areas (19). Consistent with this finding, in a recent proteome analysis, marker proteins for mitochondria, the myofibril apparatus, and the intravascular space showed identical expression levels in low- and high-flow samples (Table 1), as did most of the 400 protein species quantified (14) and the ischemia-sensitive heat shock protein 70.

In contrast, the phosphorylated myosin regulatory light chain (RLC) showed a patchy fiber-to-fiber distribution within a spatial gradient from low (endocardial) to high (epicardial) levels of phosphorylation. This was shown to result in enhanced stretch activation and to be associated with a spatial gradient of a myosin light-chain kinase from apex to base (8). This heterogeneity may not only have profound functional implications but also affect the cellular architecture of the heart. The spatial distribution of protein expression in the myocardial wall is likely determined by regional differences in gene expression. This is supported by the finding that in the canine and porcine heart, no differences in the expression of critical proteins were observed between low- and high-flow samples (Table 1). This suggests that the spatial heterogeneity of protein expression is determined by regional differences in gene expression and is not simply a consequence of regional differences in flow or energy turnover.

**TABLE 1. Differentially expressed proteins in low- and high-flow areas**

<table>
<thead>
<tr>
<th>Enhanced Expression in Low-Flow Areas</th>
<th>Expression Ratio</th>
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<tbody>
<tr>
<td>Dimethylarginine dimethylaminohydrolase</td>
<td>4.8/1</td>
</tr>
<tr>
<td>Glyceraldehyde-3-phosphate dehydrogenase</td>
<td>1.9/1</td>
</tr>
<tr>
<td>Phosphoglucose kinase 2</td>
<td>1.4/1</td>
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<tr>
<th>Enhanced Expression in High-Flow Areas</th>
<th>Expression Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron transfer flavoprotein β-subunit</td>
<td>1/1.3</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>1/1.3</td>
</tr>
<tr>
<td>Desmin</td>
<td>1/1.4</td>
</tr>
<tr>
<td>Short chain 3-hydroxyacyl CoA dehydrogenase</td>
<td>1/1.4</td>
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</tbody>
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<tr>
<th>Similar expression in high and low flow areas</th>
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<tr>
<td>Cytochrome c oxidase polypeptide Va; ATP synthase β-chain; isocitrate dehydrogenase subunit-a; fatty acid binding protein</td>
</tr>
<tr>
<td>Myosin light chain 1, ventricular isoform; myosin regulatory light chain 2, ventricular isoform; tropomyosin α-chain, skeletal muscle</td>
</tr>
<tr>
<td>Haptoglobin; hemoglobin β-chain</td>
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*Data are from Ref. 14.*
consequences (8) but may also contribute to the spatial heterogeneity of flow and energy turnover. To analyze the local proteome in relation to local flow, Laussmann et al. (14) determined local myocardial perfusion in the awake dog, selected low- and high-flow samples, and employed two-dimensional gel electrophoresis for protein separation. This approach revealed significant differences in nitric oxide (NO) metabolism and substrate utilization. In low-flow areas, the enzyme dimethylarginine dimethylaminohydrolase (DDAH1) was almost fivefold increased both on the protein and mRNA levels (Fig. 3). This resulted in a reduction of its substrate asymmetric dimethylarginine (ADMA) down to 25% (Fig. 3). Although ADMA is a potent endogenous inhibitor of NO synthase, local NO synthase expression showed no difference, pointing to an enhanced NO formation in low-flow areas. This effect will be augmented by a lower expression of myoglobin (Table 1), which has recently been shown to be a potent NO scavenger in the heart (12). Furthermore, although low-flow samples displayed a higher protein expression of glycolytic enzymes, proteins closely related to fatty acid metabolism were more prevalent in high-flow areas (Table 1). These findings strongly indicate a homeostatic mechanism by which areas of low local flow, and thus limited O₂ supply, are protected (Fig. 4). In these areas, the local concentration of NO will be elevated due to increased NO formation as well as decreased NO transport and metabolism, resulting in a greater contribution of NO to the maintenance of vascular tone. A higher NO concentration may even contribute to lower O₂ consumption in low-flow areas. In addition, the higher capacity for glycolysis in low-flow areas will reduce the O₂ demand and consumption for a given ATP turnover. These factors together improve the balance between O₂ supply and demand in areas receiving <50% of the mean myocardial perfusion. This homeostatic mechanism may also explain the low adenosine concentration measured in basal low-flow regions (Fig. 2).

**Implications and open questions**

To fully understand the spatial heterogeneity within the heart, several questions need to be addressed. First, what are the factors ultimately governing the spatial heterogeneity of protein expression and metabolism? As indicated in Fig. 4, locally decreased mechanical strain and work may translate into reduced energy demand and turnover, O₂ demand, and consumption as well as coronary flow, and these factors in turn may modulate local gene and protein expression. In such a scenario, the individual myocyte could be the primary control unit. However, this regulatory cascade is not yet verified and the signal transduction mechanisms (e.g., AMP kinase? O₂ or redox-dependent?) and transcription factors involved require elucidation. Also, the size of the smallest unit defining the spatial heterogeneity of energy demand and flow is currently unknown.

Second, to what extent is the spatial heterogeneity of energy turnover reflected in local contractile function, and what is its functional role? The currently available MRI maps of local myocardial fiber strain and activation time (tagging and DENSE) do not reveal a substantial heterogeneity. However, these techniques rely on displacement measurements of local volume elements and do not measure local force or work. Thus a better understanding of local stress-strain relations is required.

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**FIGURE 3.** Dimethylarginine dimethylaminohydrolase (DDAH1), asymmetric dimethylarginine (ADMA), and endothelial nitric oxide (NO) synthase (eNOS) levels in low- and high-flow areas. Top: DDAH1 protein and mRNA levels. Middle: metabolic pathway linking DDAH1, ADMA, and NO synthesis. Bottom: ADMA concentration and eNOS mRNA expression level in low- and high-flow areas. Data from Ref. 14.

**FIGURE 4.** Proposed homeostatic feedback control of supply and demand by increased NO formation and enhanced glycolysis in myocardial areas of low local flow and energy turnover. PGK, phosphoglycerate kinase; ETF, electron transfer flavoprotein; HADH, hydroxyacyl CoA-dehydrogenase. Modified from Ref. 14.
Finally, can the spatial variability, e.g., of local perfusion, be visualized by noninvasive techniques? So far, clinically applied techniques for flow measurements such as PET and MRI gadolinium-DPTA perfusion show a rather homogeneous pattern. This may be due to the low spatial resolution of PET and the still unresolved difficulties in quantitative flow measurements by MRI. Enhancing the temporal and spatial resolution would provide multiple opportunities not only to study the spatial heterogeneity of myocardial perfusion but, in conjunction with functional and metabolic measurements, would also allow us to further our understanding of the regulation of coronary flow and oxidative phosphorylation in the heart.

The surprising extent of spatial heterogeneity of protein expression, energy turnover, and flow contrasts with the apparent uniformity of myocardial morphology and gross mechanical function. It indicates that our understanding of myocardial contractile function is not yet complete, especially with regard to local contractility and work. Since myocardial oxygenation and perfusion are regulated at the local level, the spatial heterogeneity of energy turnover and flow clearly has to be taken into account in our efforts to mechanistically understand the close match between myocardial work, oxygen consumption, and myocardial perfusion. Finally, since the local energy demand dictates the vulnerability to ischemic insults, the spatial heterogeneity within the heart may explain the spatial development of myocardial infarction and its frequently patchy pattern.

I thank Drs. Balaban, Laussmann, Han, and Schrader as well as Alexander Janosi for stimulating discussions.

References


