Assessing heterogeneous distribution of blood flow and metabolism in the heart

A. B. Johan Groeneveld
Johannes H. G. M. van Beek
David J. C. Alders

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Abstract

The literature is reviewed on methods to assess heterogeneity of blood flow, substrate uptake and oxidative end energy metabolism in the normal heart, and their interrelations. Even though the factors controlling matching on the regional level remain largely obscure, the evidence that heterogeneous blood flow partially correlates to indicators of metabolism in the normal heart is accumulating, particularly in face of a correlation between acetate metabolism indicative of regional \( O_2 \) consumption to microsphere blood flow. Moreover, the partial matching cannot be explained by vascular anatomical differences from one region to the other, since, although fractal theory can partially describe the branching patterns of the coronaries, vasodilation is similar among regions upon metabolic stimulation of the heart. It is dissimilar among regions, so that blood flow is redistributed, upon maximum vasodilation with adenosine or hypoxia, denoting regionally different maximum vessel diameter and flow reserve. However, regionally differing tissue composition could also contribute somewhat to regional differences in (the need for) blood flow. It is still unknown, because of technical limitations, how the foregoing measures relate to regional work load.

**Key words:** Heterogeneous coronary blood flow – fractal branching patterns – regional substrate uptake – oxidative and energy metabolism – high energy phosphates – regional \( O_2 \) consumption
Introduction

The distribution and control of coronary blood flow has been a challenge to investigators for decades. In the past, authors already noted the dispersion of arteriovenous transit times in bolus indicator dilution assessments of coronary blood flow, suggesting blood flow heterogeneity (22, 44, 46). Various investigators have focused on the characteristics and causes of this blood flow heterogeneity, by relating assessments of regional blood flow to metabolic indicators, among others. This review summarizes the methods to assess heterogeneity of blood flow and metabolism and their interrelations, as reported in the literature, in the normal heart (Table 1).

Table 1 Measuring heterogeneous blood flow and metabolism in the heart

<table>
<thead>
<tr>
<th>Blood flow</th>
<th>Metabolism</th>
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<td>Radioactive, colored or fluorescent microspheres</td>
<td>Radioactive fatty acid or glucose uptake</td>
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<td>“Molecular microspheres”, desmethylimipramine</td>
<td>Positron emission tomography (substrate and O₂ uptake)</td>
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<td>&quot;²⁰¹Thallium or other diffusible tracers</td>
<td>Nuclear magnetic resonance</td>
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<td>Positron emission tomography</td>
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<td>Glycolytic and oxidative enzyme concentrations</td>
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<td>Lactate, inosine, adenosine and S-adenosine-homocysteine concentrations</td>
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<td>Nicotinamide adenine dinucleotide (NADH) fluorescence</td>
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Microspheres
An elegant method to study blood flow heterogeneity in the heart is the use of labeled (generally 15 µm Ø) microspheres, which, upon atrial/ventricular administration, lodge in the arterioles and thereby constitute a fair measure of red cell blood flow (46). Obviously, one of the premises of microsphere flow as a measure of red cell blood flow is that microspheres do not traverse capillaries and that the measurement error of the technique is low enough to allow blood flow measurements in small pieces of tissue in the heart, as assessed after simultaneous injection of multiple labels (1, 4, 11, 22, 46). Interventricular and transmural differences, as commonly described, with higher endo- than epicardial blood flow, are usually less than the piece to piece (of 0.2 – 1.5 g) differences in blood flow, even within one layer of the heart. At extremes, regional blood flow can vary by a factor of 6 or more. Usually, the observed heterogeneity is expressed as the coefficient of variation (CV = standard deviation/mean), amounting to approximately 20 – 30% in 0.5 – 1 g tissue samples (1, 3, 9, 18, 19, 22, 26, 27, 33, 36, 38, 40). The observed heterogeneity can be mathematically broken down into a measurement error (about 5%) on the one hand and (if serial observations are done) into temporal (about 10%) and spatial heterogeneity (about 20%), on the other hand, with the latter greatly predominating over the former two factors in almost all studies, regardless of species (1, 3, 4, 19, 22, 34, 37, 44). This implies that regional blood flows highly correlate with time (1, 4, 7, 8, 14, 22, 23, 27, 36). In fact, spatial heterogeneity of blood flow in the heart has been found in rabbits, dogs, pigs, guinea pigs, sheep and baboons, in a quantitatively similar manner, and in both left and right ventricles and endo-, meso-, and epicardium, while studies to reveal similar heterogeneities in man are hampered by resolution limitations (3, 16, 18, 19, 22, 33). Nevertheless, positron emission tomography (PET) and dynamic parametric polar mapping of \(^{13}\)N ammonia perfusion allows a relatively high resolution, and these studies have revealed human myocardial perfusion heterogeneity of about 18% among 480 segments (10 rings of 48 segments), similar to that in animals (28, 41). Alternatively, rapid computer scanning (CT) may also allow regional blood flow and volume estimations (32).

Because observed heterogeneity increases with decreasing tissue sample sizes, authors have also expressed heterogeneity as the slope of the inverse (logarithmic) relation between sample size (x-axis) and (logarithmic) CV (y-axis)
The parameters of this relation may render results among laboratories using varying sizes of tissue samples comparable. Blood flow heterogeneity can indeed be described, quite adequately, in terms of fractal dimensions, suggesting disparate flow distributions at each dichotomous branching in the coronary artery tree, and these dimensions can be calculated from the (logarithmic) CV to sample size relation \((3, 23, 36, 38, 46)\). This implies, for instance, that a difference in blood flow of about 20% between daughter vessels at a branching point gives a CV of about 30% among 1g tissue samples. That the observed heterogeneity can be described in terms of fractals may argue in favor of an anatomic reason for heterogeneity largely based on the branching pattern \((36)\). However, the presence of regional coronary vascular dilatatory potential following an increased workload of the heart with exercise, glucose-insulin-potassium or inotropic drugs argues against an anatomic and in favor of a functional control of blood flow heterogeneity, which results in a fractal pattern, even if heterogeneity decreases or blood flow is slightly redistributed upon 1 stimulation \((9, 13, 19, 22, 27, 46)\). Conversely, the blood flow randomness can be assessed with the help of the autocorrelation between adjacent regional blood flows, with high correlations denoting low randomness \((1, 2, 23, 27, 41)\). Fractal dimensions do not necessarily concord with autocorrelation coefficients \((27)\).

During maximum vasodilation with adenosine/nitrates or hypoxia in the normal heart, blood flow increases in all regions but in some regions more than in others, even if receiving higher or lower than average blood flow at rest, so that blood flow is redistributed regardless whether heterogeneity increases or decreases \((1, 2, 11, 13, 23, 27, 44, 46)\). Arresting the heart does not change the pattern of maximum vasodilation, suggesting that contraction itself does not affect heterogeneity in the normal state \((2)\). Thus, maximum vessel diameter varies among regions and the coronary vascular dilation reserve, as can be evoked during stenosis, also varies among regions, independently of baseline blood flow \((1, 7, 8, 11, 26)\). Therefore, blood flow heterogeneity in the normal heart cannot be explained by regionally exhausted vasodilation \((1, 11, 26)\).

**Diffusible and other flow tracers**

More recently, “molecular” microspheres with very high myocardial extraction and retention have been used and they have the advantage of lacking spherical
hindrance and thereby have preferential flow in high flow regions, as exhibited by the real microspheres. Nevertheless, the distribution of the “molecular microsphere” $^3$H or $^{125/131}$I-desmethylimipramine is very similar to that of 15 µm Ø radiolabeled microspheres, suggesting, among others, that the observed heterogeneity is not determined by the microsphere method itself (3, 4, 27, 31). Hence, microsphere blood flow heterogeneity cannot be merely attributed to preferential red cells flows at branching points into (straight) vessels with the largest diameter. Diffusible flow tracers further include $^{201}$thallium, a diffusible tracer behaving like potassium with relatively high extraction and retention (20, 46). They have also been used to estimate blood flow heterogeneity in animals, but the resolution in humans is still too low to identify and quantify heterogeneity in the absence of ischemic regions in the normal heart. Even when assessed regionally with the help of $H_2$ clearance techniques, blood flow heterogeneity can be demonstrated (24). Recently, Bauer et al. (5) reported on heterogeneity assessed with the help of a high-resolution magnetic resonance technique (NMR), using perfusion-sensitive relaxation times (without contrast agents) and allowing perfusion estimations in 1.5 mm thick and 140 x 140 µm wide tissue areas, at the microscopic level. This will hopefully elicit human studies.

**Table 2** Potential contributors to spatial blood flow heterogeneity

<table>
<thead>
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<th>Evidence present</th>
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<tr>
<td>Regional differences in tissue composition, i.e., content of blood volume, water volume, mitochondrial and/or contractile elements</td>
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<tr>
<td>Fractal behavior of vascular tree branching</td>
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<td>Anatomic differences, with respect to maximum diameters and flow reserve</td>
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<td>Differences in substrate uptake and oxidative metabolism</td>
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<table>
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<tr>
<th>No evidence</th>
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<tr>
<td>Differences in arteriovenous diffusional shunting of $O_2$</td>
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<tr>
<td>Differences in hematocrit in branching vessels</td>
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<td>Differences in workload</td>
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<tr>
<td>Differences in “tightness” of metabolic control and metabolic efficiency</td>
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The classifications present and no evidence are the author's interpretation of the literature.
Authors have extensively tried to find explanations for blood flow heterogeneity, when assessed with radioactive microspheres or other flow traces, and have therefore studied relations with regional indicators of metabolism (Table 2).

**Substrate uptakes**
The heterogeneous uptake of radiolabeled fatty acids, the major fuel of heart muscle, and glucose has been studied in relation to regional perfusion (9, 10, 14, 20, 26, 34). Authors have found that regional uptake of substrates was heterogeneous in the dog heart, albeit somewhat less than regional blood flow heterogeneity, and correlated to regional blood flow, even at varying workloads. This provides an argument that regional blood flow is at least partially matched to regional metabolic demand, i.e., $O_2$ uptake in the absence of ischemia, and that heterogeneity of the former can be explained at least in part by heterogeneity of the latter (9, 10, 14, 20–25, 27). Nevertheless, regional substrate uptake may not equal regional $O_2$ consumption, since the relative contributions of the substrate uptakes to the $O_2$ consumption may vary from region to region. Moreover, the transport from blood to tissues is, among others, dependent on the capillary surface (S) area (and permeability P) available for exchange. In fact, regional PS directly relates to regional perfusion, so that a direct relation between uptake and perfusion may be mediated by covariance of regional PS with blood flow, even though not necessarily implying varying metabolism (10, 14, 35). Nevertheless, a relation between regional substrate/$O_2$ consumption and perfusion may be independent of flow-proportional PS (14, 35). Otherwise, proportionality between blood flow and PS may imply minor heterogeneity in substrate extraction, i.e., regional uptake divided by delivery of substrate (10, 20, 35).

**Oxidative and energy metabolism**
The tissue concentrations of glycolytic enzymes and mitochondrial enzymes such as succinate dehydrogenase, indicating oxidative capacity, may be heterogeneous and thereby suggest heterogeneous $O_2$ consumption. Moreover, they may (poorly) relate to regional blood flow, suggesting some coupling of heterogeneous blood flow to metabolic demand (6). Others suggested, however, that the regional contents of enzymes were relatively homogeneous and did not relate to blood flow heterogeneity (34).
That matching of blood flow to metabolic demand may be imperfect and heterogeneous demand cannot fully explain blood flow heterogeneity can be inferred from studies in which regional small arteriolar and venular O$_2$ saturation was assessed by cryomicrospectrophotometry (42, 43). In fact, the small venular saturation varied from 0 to 60 %, at an average coronary sinus hemoglobin O$_2$ saturation of around 30%, while arteriolar saturation generally was above 90 %, suggesting regionally varying O$_2$ extraction (42). Nevertheless, the regional myocardial O$_2$ consumption that can be inferred from these measurements, if combined with microsphere blood flow measurements, may be somewhat heterogeneous (43). Assuming that heterogeneity cannot be explained by measurement error, heterogeneity of the convective O$_2$ supply to demand ratio, diffusional shunting from arterioles to venules, or combinations could have been responsible (37). Diffusional O$_2$ shunting may be negligible, however, and may not account for blood flow heterogeneity, which is unchanged during increased work load and regional coronary blood flow (19, 37). Since hemodilution does not change heterogeneity, regional flow compensations for potentially varying hematocrits at vascular branches may only slightly contribute to (microsphere) blood flow heterogeneity (16, 19, 23).

Fig. 1 Model of $^{13}$C-acetate enrichment of glutamate in the tricarboxylic acid cycle (after ref 39, 40).
Schwanke et al. (35) estimated regional tissue \( \text{O}_2 \) consumption from regional \( \text{H}_2^{18} \text{O} \) residues after addition of \( ^{18} \text{O} \) to perfusate of isolated rabbit hearts. The residues correlated with regional microsphere blood flow, suggesting that the latter was at least in part determined by regional differences in metabolic demand. Van Beek et al. (39, 40) recently modified a \(^{13}\text{C}\)-acetate NMR method for regional assessment of the turnover rate of the tricarboxylic acid (TCA) cycle as a measure of regional \( \text{O}_2 \) consumption. Indeed, for the total heart, the estimated \( \text{O}_2 \) consumption from the TCA turnover rate with the \(^{13}\text{C}\)-acetate method correlated with directly estimated \( \text{O}_2 \) consumption from coronary flow and in- and outflow \( \text{O}_2 \) contents, over a wide range of workloads (40). The principle of the method is as follows (Fig. 1). Infusion of \(^{13}\text{C}\)-enriched substrate for the TCA cycle leads to \(^{13}\text{C}\) labeling of glutamate. The fractional enrichment of glutamate after start of \(^{13}\text{C}\)-acetate infusion, as measured by NMR, follows fixed routes, so that at higher turnover rates the total \(^{13}\text{C}\) label at the 4, 3 and 2 carbon positions in glutamate increase faster. Progressively more complicated multiplet patterns appear after the second and third turn of the cycle. NMR analysis of the multiplet patterns therefore allows one to estimate TCA turnover rate using a computer model, during a certain interval of \(^{13}\text{C}\)-acetate infusion. In most recent studies, the relation of regional microsphere blood flow to \(^{13}\text{C}\)-acetate turnover and calculated \( \text{O}_2 \) consumption was studied, and it suggested a fair correlation between the two in the left ventricle of the anesthetized pig (Fig. 2). The preliminary observations suggest that blood flow heterogeneity may be explained for about 50% by heterogeneity of \( \text{O}_2 \) uptake, so that there is some, albeit incomplete, coupling. That regional blood flow may relate to regionally varying metabolic demand can also be inferred from other studies showing that low flow regions in the normal heart do not exhibit increased concentrations of lactate or adenosine triphosphate (ATP) breakdown products including adenosine and inosine, so that there is no \( \text{O}_2 \) deficit in low-flow regions of the normal heart (7, 8, 26, 34). Conversely, low or high flow regions exhibit similar increases in concentrations of these substances, if fractional flow decreases are similar during coronary artery obstruction (7, 26). A similar technique of regional \( \text{O}_2 \) consumption assessed by the TCA flux rate following \( ^{3}\text{C}\)-pyruvate infusion has been recently reported by Decking et al., but these authors did not report a correlation between regional blood flows and \( \text{O}_2 \) uptakes (12).
Fig. 2 Pooled data (n = 36) for 7 pigs (3–8, 43 to 252 mg dry weight left ventricular tissue samples per pig) of microsphere blood flow (left atrial injection; normalized for individual means) on the x-axis and O₂ uptake (normalized for individual means) on y-axis, showing that at least 50% (linear correlation = 0.7) of the heterogeneity of the former can be explained by heterogeneity of O₂ consumption (unpublished observations). The local O₂ consumption was determined using the double label ¹³C-acetate NMR technique, after a 30 min saturation of metabolism by unlabeled acetate (40).

PET can also be used in humans to assess regional cardiac oxidative metabolism using ¹¹C-acetate clearance, and such studies indeed reveal some heterogeneity, together with heterogeneous substrate uptake (17, 21). The technique will likely improve in resolution, thereby allowing further characterization of heterogeneous blood flow and metabolism in man (17, 21).
Regional differences in tissue shortening and composition

A rise or fall in workload imposed on the heart and thereby in cardiac work is associated with similar changes in both global and regional blood flows, suggesting regional blood flow to match demand (9, 13, 19, 22, 27, 46). In fact, during increased myocardial workloads, heterogeneity of microsphere blood flow may be unchanged, so that the fractional increase in blood flow is essentially similar among regions, irrespective of baseline flow differences (13, 19, 27). This suggests that a rise in workload is equivalent among regions and matched by appropriate rises in blood flow, and that baseline blood flow heterogeneity is caused at least in part by heterogeneous metabolic demand (13, 19, 27).

There is some evidence, using ultrasonic gauges or NMR tagging, that regional myocardial strain and shortening varies from region to region in the canine heart, and that the strain changes upon changes in excitation induced by regional pacing (25, 29–31, 45). Moreover, an altered distribution of regional fiber strain during pacing is accompanied by a concordant redistribution of regional blood flow (29, 45). It is unknown, however, if and how these differences relate to regionally varying O₂ consumption and metabolic efficiency. The shortening and thickening of muscle segments can be assessed using NMR by applying a grid of magnetic tags noninvasively and then following the deformation of this grid (30, 31, 45). Strains can be translated into local work, but many assumptions are necessary (30, 45). Nevertheless, this technique will hopefully result in the future in a resolution high enough for assessment of the relation between local perfusion and work load in humans.

Regional differences in glycolytic or mitochondrial enzyme contents, such as succinate dehydrogenase, and in substrate and high-energy phosphate concentrations (with ATP primarily located in mitochondria and creatine compounds mainly in cytosol) could indicate regional differences in tissue composition (6, 15). Moreover, ATP contents better relate to blood flow than creatine kinase, lactate dehydrogenase and glycogen contents (15). This would imply varying amounts in energy-producing mitochondria and energy-consuming contractile elements from one region to the other. The finding may explain the (weak but significant) correlations of the regional content of these high-energy compounds to microsphere blood flow, as reported by some...
investigators, in the absence of metabolic indicators of ischemia (lactate) in areas with low flow and low ATP contents (Fig. 3; ref 7, 8, 15, 24, 26). There is some direct relation between ATP and blood flow, when both are normalized for total creatine content in the tissue (Fig. 3), suggesting that the mitochondrial/extramitochondrial content ratio is a determinant of regional blood flow. On the other hand, microscopic morphometric evaluation, done by others, of the content of structural elements including myofibrils, mitochondria and capillary density may not reveal large heterogeneity related to blood flow heterogeneity (34).

There are small regional differences in blood (red cell and plasma) volume and interstitial spaces, which could imply regional differences in cellular spaces per g tissue (6, 8, 16, 32). In fact, blood flow weakly and inversely relates to blood volume in the rabbit, suggesting higher blood flow in tissue samples with less blood volume and interstitial space and more cardiomyocytes (16). Others, however, described a direct relation between blood flow and volume in the pig heart (32). Moreover, the dry/wet weight fraction may be somewhat heterogeneous and regional blood flow may partially and directly correlate to dry weight, per g tissue wet weight, even though the heterogeneity of water volumes may be much less than the heterogeneity of blood flow (6, 8, 16).

Fig. 3 Pooled data for 6 open-chest pig experiments, where the left ventricle was cut into ±100 pieces of ±1 g each, wherein adenosine triphosphate (ATP) content, total creatine content (phosphocreatine PCr and creatine Cr) and microsphere blood flow were determined. A fair relationship is shown between heterogeneous blood flow on the one hand and tissue content of both ATP and PCr+Cr on the other (after ref 8).
Conclusion

While many measures show heterogeneity in perfusion and metabolic indicators of cardiac muscle, there is no single optimal measure to prove control of regional blood flow by demand and to identify the mechanisms involved. However, the evidence is accumulating that blood flow heterogeneity in the heart relates at least in part to heterogeneity of metabolic indicators. The latter may involve regional differences in content of $O_2$- and energy consuming mitochondria and contractile elements, and differences in the metabolic rate and requirements of these elements. Nevertheless, it is still unknown how these indices relate to regional work load.
References


