Minimally Invasive Evaluation of Coronary Microvascular Function by Electron Beam Computed Tomography

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Background—We previously demonstrated that in vivo electron-beam computed tomography (EBCT)–based indicator-dilution methods provide an estimate of intramyocardial blood volume (BV) and perfusion (F), which relate as BV=aF+b/F, where a characterizes the recruitable (exchange) and b the nonrecruitable (conduit) component of the myocardial microcirculation. In the present study, we compared BV and F with intracoronary Doppler ultrasound–based coronary blood flow (CBF) as a method for detecting and quantifying differential responses of these microvascular components to vasoactive drugs in normal (control) and hypercholesterolemic (HC) pigs.

Methods and Results—BV and F values were obtained from contrast-enhanced EBCT studies in 14 HC and 14 control pigs. BV, F, and CBF values were obtained at baseline (intracoronary infusion of saline) and after 5 minutes each of intracoronary infusion of adenosine (100 μg · kg⁻¹ · min⁻¹) and nitroglycerin (40 μg/min). BV and CBF reserves in response to adenosine were attenuated in HC pigs compared with controls (90±36% versus 127±42%, P<0.03, and 485±182% versus 688±160%, P<0.01, respectively). The relationship between BV and F showed consistently lower recruitable BV in HC versus control pigs. Nonrecruitable BV reserve in response to adenosine was attenuated in HC compared with controls (77±20% versus 135±28%, P<0.001). Our findings are consistent with HC-induced impairment of intramyocardial resistance vessel function.

Conclusions—EBCT technology allows minimally invasive evaluation of intramyocardial microcirculatory function and permits assessment of microvascular BV distribution in different functional components. This method may be of value in evaluating the coronary microcirculation in pathophysiological states such as hypercholesterolemia. (Circulation. 2000;102:2411-2416.)

Key Words: myocardium ▪ microcirculation ▪ blood volume ▪ ultrasonics ▪ hypercholesterolemia

The intramyocardial microvasculature plays a major role in regulating myocardial perfusion and flow reserve. Differential responses of different-size microvessels may be of significance in this regulatory process.1 Advances in the ability to assess the coronary microcirculation in the past decade have led to a better understanding of the involvement of intramyocardial microvascular dysfunction in early stages of coronary artery disease.1–3 However, whether particular pathophysiological situations involve selective impairment of specific microvascular components remains to be established.

Quantification of vasodilator reserve with intracoronary Doppler ultrasound is a clinically accepted method to evaluate coronary microvascular function.4 However, measuring coronary blood flow (CBF) in a fairly proximal segment of the coronary artery provides only an index for the integrated physiological response of all vessels downstream of that segment, with limited ability to distinguish functionally distinct microvascular components.

Electron-beam computed tomography (EBCT)–based indicator dilution techniques have been shown to provide minimally invasive quantitative indices of the functional status of the coronary microcirculation.5,6 However, the ability of EBCT methods to discern functional alterations in intramyocardial microcirculatory functional components, ie, exchange vessels and larger intramyocardial conduit vessels, during a chronic pathophysiological condition that targets the coronary microvasculature, has not been evaluated.

Therefore, the 2 aims of this study were to (1) use the discriminatory power of intracoronary Doppler ultrasound studies as the basis for evaluating the feasibility of EBCT to describe normal and altered microcirculatory function and (2) evaluate the degree to which EBCT technology can detect, discriminate, and quantify blood volume (BV) in different components of the porcine coronary microcirculation in normal and hypercholesterolemic (HC) pigs.
Methods

Animal Preparation

The study was reviewed and approved by the Mayo Foundation’s Institutional Animal Care and Use Committee in accordance with the National Institutes of Health guidelines. All animals were male domestic crossbred pigs. The 14 control group pigs (59±4 kg) were fed a standard pig chow, and the 14 HC group animals (56±5 kg) were fed a standardized high-cholesterol diet (2% cholesterol, 15% lard, Harlan Teklad), both over a period of 10 to 12 weeks before the study. Anesthesia was initiated with ketamine 12.5 mg/kg IM and xylazine 2 mg/kg IM and maintained by a titrated infusion of 100 mg ketamine IV in 500 mL normal saline. All animals were intubated, ventilated, and placed supine in a cast for the duration of both EBCT and Doppler studies. A bipolar pacing catheter and a pigtail catheter were inserted via the jugular veins into the coronary sinus and the superior vena cava, respectively, to permit injection of contrast agent and atrial pacing if needed. After injection of 10 000 U heparin IV (followed by infusion of 1000 U heparin/h IV), a left coronary guide catheter was placed in the left main coronary artery through the external carotid artery for coronary angiography and monitoring proximal coronary artery pressure. A 2.2F dual-lumen infusion catheter was placed in the proximal left anterior descending coronary artery (LAD) to selectively infuse drugs and for Doppler ultrasound studies.

Electron Beam Computed Tomography

All animals were positioned in the scanner with the heart centered in the imaging field. The field of view was 26 or 30 cm (pixel size 0.52 to 0.69 mm², voxel size 3.64 to 4.83 mm³, 7-mm slice thickness, acquisition time 50 ms). Short-axis images were obtained at a mid left ventricular level at 80% of the RR interval. Initially, contrast agent was injected selectively into the LAD (5 mL over 1.3 seconds) to highlight the cross-sectional LAD perfusion territories (Figure 1), which were of similar sizes in HC and control pigs (435±65 versus 417±118 mm², respectively, P>0.1). The volume of myocardium encompassed by these regions of interest averaged ~3000 mm³. The initial scan sequence was followed by a series of flow studies, each performed with a rapid bolus injection of the contrast agent iodipol (0.33 mL/kg over 2 seconds) into the superior vena cava. The same region of interest as in the initial scan sequence was used for all these consecutive scanning sequences, which were each followed by 20-minute recovery and washout periods with intracoronary injection of saline (infusion rate 1 mL/min). Each sequence included 40 scans obtained every 1 to 2 heartbeats. Hemodynamic data were recorded just before each EBCT study. The first image in each sequence was obtained before injection of contrast agent to obtain background image intensity. The first scanning sequence was a baseline study (selective intracoronary infusion of normal saline at 1 mL/min), followed by scanning sequences after 5 minutes of intracoronary infusion of adenosine (100 μg · kg⁻¹ · min⁻¹) and then after 5 minutes of intracoronary infusion of nitroglycerin (NTG, 40 μg/min). Image data were evaluated with the Analyze software package (Biomedical Imaging Resource) to obtain indices of intramyocardial perfusion (F [mL · g⁻¹ · min⁻¹]) and intramyocardial BV (mL/g) as previously described.⁶

Calculation of Intramyocardial Microvascular BV in Functional Components

We modeled the intramyocardial microcirculation as 2 sets of vessels arranged in series as previously described.⁶ In brief, 1 set consists of functionally nonrecruitable conducting vessels, which are assumed to be always patent but can change their diameter in response to various stimuli. Vascular resistance in these vessels is assumed to follow Poiseuille’s law, which relates flow to the fourth power of the vessel radius, ie, the square of the cross sectional area (CSA), and thus F=CSA². Because the length of these vessels does not change much relative to the change in CSA, the intraluminal BV of these nonrecruitable vessels (BVnr) should be proportional to CSA. Hence, it should follow that F=ΣBVnr², or BVnr=ΣF/F. The other microvascular component consists of functionally recruitable vessels (presumably mostly capillaries, small arterioles, and venules), which are assumed not to change appreciably in diameter but in the number of vessels perfused. Because the BV within recruitable vessels (BVr) should be proportional to the number of recruited vessels, it should follow that Fr=ΣBVr. Because the 2 sets of vessels are arranged in series, the relationship BV=BVr+BVnr=ΣFr+BVnr/F should hold, where a and b are coefficients to be established experimentally.

Intracoronary Doppler Ultrasound and Coronary Angiography

Average peak velocities (cm/s) and selective coronary artery diameters were measured to calculate CBF (mL/min) as previously described.⁷,⁸ As in the EBCT study, 100 μg · kg⁻¹ · min⁻¹ of adenosine and 40 μg/min of NTG were infused into the LAD continuously over 5 minutes before Doppler and hemodynamic measurements were recorded.

Statistical Analysis

The values for each group are reported as mean±SD. Paired and unpaired Student’s t tests were used to evaluate the significance of differences between the reported variables. An ANCOVA model was used to evaluate differences in BV between HC and control pigs after...
adjustment for changes in perfusion. Initially, we assessed whether the slopes of the lines summarizing the group-specific relationship between BV and perfusion were significantly different between the groups. When this was not the case, we fit a model under the assumption of equal slopes and tested for differences between groups. Differences were considered significant at a value of  P < 0.05.

Results

Cholesterol Levels and Hemodynamic Parameters

Serum cholesterol levels were 326 ± 99 mg/dL in HC pigs versus 82 ± 15 mg/dL in control pigs (  P < 0.0001). In the Doppler ultrasound study, heart rates of control pigs increased in response to adenosine (from 59 ± 17 to 63 ± 17 bpm,  P < 0.05) and NTG (to 66 ± 15 bpm,  P < 0.05). Mean blood pressure decreased in response to adenosine (from 108 ± 15 to 103 ± 17 mm Hg,  P < 0.05) and NTG (to 104 ± 12 mm Hg,  P = NS). Mean blood pressure during NTG administration in the Doppler study was significantly lower in HC versus control pigs, with no difference between groups in changes from baseline values (−5.9 ± 13% versus −2.6 ± 4%,  P = NS, for HC and control pigs, respectively). All other parameters were indistinguishable between HC and control pigs and between EBCT and Doppler studies.

Doppler CBF

Baseline CBF was similar in HC and control pigs (23.2 ± 8.1 versus 27.1 ± 13.0 mL/min,  P = NS). CBF increased significantly in both groups in response to adenosine, but the increase was attenuated in HC animals (Figure 2A). CBF also increased significantly in both groups (  P < 0.05) in response to NTG, by 33 ± 38% versus 38 ± 28%, respectively, with no differences between groups.

EBCT-Based Intramyocardial Perfusion

F was similar in HC and control animals at baseline (0.91 ± 0.2 and 0.79 ± 0.1 mL · g⁻¹ · min⁻¹,  P = NS). F increased significantly in both groups in response to adenosine, by 200 ± 70% in HC pigs (  P < 0.001) and by 262 ± 99% in control pigs (  P < 0.001). The increase in F in response to adenosine tended to be lower in HC than in control pigs (  P = 0.086). F increased in both groups in response to NTG, by 10 ± 29% and 16 ± 17%, respectively (  P = NS for the difference between groups), which reached statistical significance only in the control group (  P < 0.05).

CBF Versus Myocardial Perfusion

We compared Doppler-based CBF with EBCT measurements of F for all animals at baseline and after administration of drugs and found a good linear correlation between these 2 variables (Figure 3).

EBCT-Based Intramyocardial BV

Total intramyocardial BV was similar between control and HC pigs at baseline (0.13 ± 0.02 versus 0.14 ± 0.02 mL/g,  P = NS). BV increased significantly in both groups in response to adenosine, but the increase was blunted in HC pigs (Figure 2B). Total BV increased in response to NTG by 0.002 ± 0.02 mL/g in HC pigs (  P = NS) and by 0.01 ± 0.03 mL/g in the control group (  P = NS), with no difference between the groups.

Myocardial BV in Relation to Myocardial Perfusion

Figure 4 shows the BV-to-F relationship for baseline and adenosine values in control and HC pigs. In both groups, experimental data were fitted with the function $BV = aF + b\sqrt{F}$. The figure demonstrates an attenuated in-
crease in BV per increase in F in HC pigs during adenosine administration. ANCOVA confirmed that during adenosine infusion, HC pigs reached a significantly lower BV (by 0.02 mL/g) than control pigs after control for perfusion ($P_{0.05}$).

In response to NTG, there was no difference between groups in changes in BV per change in perfusion.

**BV in the Recrutable Component of the Microvasculature**

In the recruitable component (Figure 5), BV, at baseline was lower in HC than in control pigs (0.018±0.004 versus 0.039±0.005 mL/g, $P<0.0001$). In response to adenosine, these values increased in both groups ($P<0.001$, Figure 5) by a similar amount (0.030±0.018 mL/g in HC and 0.031±0.011 mL/g in control pigs, $P=NS$ for differences between groups). Relative to baseline, these changes were higher in HC than in control pigs (179±67% versus 80±50%, $P<0.001$). After NTG, BV increased in HC pigs by 9±28% ($P=NS$) and in control pigs by 16±17% ($P<0.02$), with no differences between groups.

**BV in the Nonrecrutable Component of the Microvasculature**

In the nonrecrutable component (Figure 6), BV in baseline was higher in HC than in control pigs (0.119±0.013 versus 0.090±0.006 mL/g, $P<0.05$). In response to adenosine, these values increased in both groups ($P<0.001$, Figure 6). The increase in absolute BV in HC versus control pigs (0.09±0.02 versus 0.12±0.02 mL/g, $P<0.01$), as was the change relative to baseline (77±20% versus 135±28%, $P<0.001$). After NTG, BV in HC pigs increased by 3.6±13% ($P=NS$) in HC pigs and by 7.1±9% ($P<0.01$) in controls, with no difference between groups.

**Discussion**

In this study, we evaluated intramyocardial microvascular function in normal and HC pigs by virtue of intramyocardial microvascular BV and perfusion quantification with EBCT. We found that (1) intramyocardial microvascular BV and perfusion quantification identified alterations in microvascular function in HC pigs consistent with findings in intracoronary Doppler ultrasound studies, (2) the EBCT-based indices also provided BV quantities in the intramyocardial microvascular functional components, and (3) changes in recruitable and nonrecrutable microvessels showed appreciable differences in HC versus control pigs consistent with HC-induced impairment of resistance vessel function.

These findings suggest that minimally invasive EBCT-based indicator dilution methods may be of value for the detection and quantification of altered coronary microvascular function as the basis for the evaluation of normal and pathological states such as hypercholesterolemia.

**Intramyocardial BV Quantification at Baseline**

Total intramyocardial vascular BV in control pigs at baseline was similar to values previously reported by Lerman et al., who used similar methodology. Moreover, Kassab et al. quantified BVs in porcine coronary arteries, capillaries, and veins in isolated hearts using a capillary silicone cast and reported BV of magnitudes comparable to our findings in the recruitable and nonrecrutable components at resting conditions in control pigs. The similarity of their data to ours supports the ability of our model to estimate BV distribution in functional components of the porcine microvasculature.
BV in the recruitable component was significantly and consistently lower in HC than in control pigs, whereas BV in the nonrecruitable component was higher in HC than in control pigs. Lerman et al. recently demonstrated that intracoronary infusion of N\textsuperscript{G}-monomethyl-L-arginine (L-NMMA), a competitive inhibitor of nitric oxide (NO) synthase, led to a significant decrease in BV\textsubscript{r}, whereas BV\textsubscript{nr} increased. Because experimental hypercholesterolemia is associated with a decrease in endogenous NO bioavailability,\textsuperscript{11,12} it probably induces physiological effects similar to those with L-NMMA. The NO pathway is involved in modulating basal vasomotion,\textsuperscript{13,14} and it may be speculated that our observation at baseline is related to “derecruitment,” ie, a functional shift of BV from recruitable to nonrecruitable microvessels.\textsuperscript{6} Impaired resistance vessel function may be responsible for this decrease in capillary recruitment distal to the resistance component, whereas proximal vessels dilate with an associated increase in nonrecruitable BV. Another possible mechanism is an HC-induced decrease in the number of recruitable vessels with an increased number of less responsive nonrecruitable vessels or a combination of both. However, the previous observation that a similar trend was elicited in normal pigs in response to L-NMMA infusion\textsuperscript{6} favors the hypothesis of derecruitment being a functional phenomenon.

**Effect of Drugs on Intramyocardial BV and BV Distribution**

In control and also in HC pigs, adenosine increased BV in both the recruitable (BV\textsubscript{r}) and nonrecruitable (BV\textsubscript{nr}) components. It has previously been shown that the microcirculatory effects of adenosine are mediated through both capillary recruitment\textsuperscript{6,15} and vasodilation of resistance and conducting microvessels.\textsuperscript{1,16} In the HC group, however, the increase in BV\textsubscript{nr} was significantly attenuated, conceivably as a result of a combination of a higher BV\textsubscript{nr} at baseline and an impaired responsiveness of these conducting and resistance vessels. This is consistent with findings by Stepp et al.,\textsuperscript{17} who recently suggested that flow-related vasodilation (as induced by adenosine), particularly in arteries with diameters \(>160\) \(\mu\)m, is regulated partly by endothelial NO release, which is impaired in experimental hypercholesterolemia.\textsuperscript{11,12} Interestingly, we observed that BV\textsubscript{r} increased by similar absolute amounts in HC and control pigs, suggesting a similar number of recruited exchange vessels. Given effectively similar levels of BV\textsubscript{nr} after adenosine, it is conceivable that recruitment of capillaries and the functional status of the governing conducting and resistance vessels are interrelated. However, the underlying mechanism(s) and their interactions in this regulatory process still remain to be defined.

In response to NTG, we found an increase in total microvascular BV in the control group due to an increase in BV in both the nonrecruitable and the recruitable components. Lerman et al.\textsuperscript{6} also found a significant increase in total microvascular BV in response to NTG administration in normal pigs, which, however, was attributable to an increased nonrecruitable BV only. NTG elicits its effect primarily in the nonrecruitable component of the coronary microvasculature, ie, microvessels with diameters \(>200\) \(\mu\)m,\textsuperscript{18,19} which most likely explains the increase in BV\textsubscript{nr}. Whereas Lerman et al obtained their data after a bolus injection of NTG, our measurements were obtained at steady state after 5 minutes of continuous NTG infusion. Capillary recruitment, ie, the increase in BV\textsubscript{r} in control pigs, may have occurred secondary to prolonged changes in upstream vasomotion. In our HC pigs, however, this increase in BV\textsubscript{nr} was not observed, suggesting an impaired vasodilatory response in resistance and upstream conducting vessels, similar to our findings after adenosine infusion. BV, also remained unchanged in HC pigs, which, again, is consistent with a modulating effect of upstream resistance vessel function on capillary recruitment.

**Methodological Considerations**

In this study, EBCT-based indices of microvascular function were directly compared with intracoronary Doppler–based CBF. Although these 2 methods provide different measures of myocardial blood supply, regional myocardial perfusion measurements correlated well with Doppler measurements of CBF. CBF measurements showed an impaired microvascular response to adenosine compared with control pigs. Whereas the trend toward an attenuated increase in intramyocardial perfusion in response to adenosine in HC pigs reached no statistical significance, changes in total intramyocardial BV and also the relationship between intramyocardial BV and perfusion allowed us to distinguish HC from control pigs. This is consistent with previous studies that recognized that intramyocardial BV quantification may be a highly sensitive indicator of microvascular function and appears to provide an independent evaluation of microvascular sequelae of diseases such as epicardial coronary artery stenosis\textsuperscript{20,21} or, as in this study, chronic hypercholesterolemia. The physiological significance of a reduced maximal BV in chronic hypercholesterolemia, however, remains to be defined.

In addition to showing that quantification of intramyocardial BV alone allowed us to distinguish HC from normal pigs, we demonstrated how the relation of intramyocardial BV to perfusion can be used to distinguish recruitable and nonrecruitable microvessels. These microvessels, however, are not visually resolvable by EBCT technology. Other investigators have used more invasive techniques to directly visualize microvessels on myocardial surfaces in vivo,\textsuperscript{22,23} which could potentially be used as an independent reference technique to quantify intramyocardial BV distribution in chronic hypercholesterolemia in response to vasoactive drugs. To the best of our knowledge, however, such studies have not yet been performed. Nonetheless, EBCT-based quantification of BV distribution in the 2 components corresponded to measurements obtained with other methods,\textsuperscript{10} and the functional behavioral trend of these components, as inferred from the relationship between myocardial BV and perfusion, is consistent with previously described hypercholesterolemia-induced impaired resistance vessel function.\textsuperscript{24}

It has been shown that myocardial perfusion is spatially heterogeneous.\textsuperscript{25} Consequently, our evaluation, which focused on 1 midventricular cross-sectional level, could conceivably not be representative of the entire LAD perfusion territory. However, heterogeneity of myocardial BV and perfusion can be quantified by evaluating relative dispersion, ie, the SD of measurements in all subregions divided by their
mean value, of these estimates. Relative dispersion decreases with increasing areas of regions of interest by integrating the spatially heterogeneous functional phenotype. BV and perfusion values obtained in an area of the region of interest >1000 mm$^2$ is adequately representative for the entire perfusion territory. In addition, even though both intramyocardial perfusion and BV are spatially heterogeneous, the BV-to-flow relationship is spatially homogeneous and hence is representative of the entire myocardium.

Conclusions

This study demonstrates that EBCT imaging provides information similar to that of intracoronary Doppler ultrasound by virtue of quantifying intramyocardial BV and perfusion. Moreover, the relationship of intramyocardial BV to perfusion also allowed characterization of differential functional behavior of microvascular components. This approach might be applicable to any technique providing simultaneous quantitative measurements of intramyocardial BV and perfusion and may provide useful insight into microvascular function in normal and pathophysiological states, especially presymptomatic stages of cardiac diseases such as cardiomyopathies and of major systemic diseases such as atherosclerosis, hypercholesterolemia, arterial hypertension, and diabetes mellitus.

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