Quantification of absolute coronary flow reserve and relative fractional flow reserve in a swine animal model using angiographic image data

Zhang Z, Takarada S, Molloi S. Quantification of absolute coronary flow reserve and relative fractional flow reserve in a swine animal model using angiographic image data. Am J Physiol Heart Circ Physiol 303: H401–H410, 2012. First published June 1, 2012; doi:10.1152/ajpheart.00153.2012.—Coronary flow reserve ( CFR) and fractional flow reserve ( FFR) are important physiological indexes for coronary disease. The purpose of this study was to validate the CFR and FFR measurement techniques using only angiographic image data. Fifteen swine were instrumented with an ultrasound flow probe on the left anterior descending artery (LAD). Microspheres were gradually injected into the LAD to create microvascular disruption. An occluder was used to produce stenosis. Contrast material injections were made into the left coronary artery during image acquisition. Volumetric blood flow from the flow probe ( Qa) was continuously recorded. Angiography-based blood flow ( Qb) was calculated by using a time-density curve based on the first-pass analysis technique. Flow probe-based CFR (CFRb) and angiography-based CFR (CFRg) were calculated as the ratio of hyperemic baseline flow using Qb and Qa, respectively. Relative angiographic CFR (relative FFRg) was calculated as the ratio of the normalized Qb in LAD to the left circumflex artery (LCX) during hyperemia. Flow probe-based FFR (FFRb) was measured from the ratio of hyperemic flow with and without disease. CFRg showed a strong correlation with the gold standard CFR (CFR = 0.91 CFR + 0.30; r = 0.90; P < 0.0001). Relative FFRg correlated linearly with FFRe (relative FFRe = 0.86 FFRe + 0.05; r = 0.90; P < 0.0001). The quantification of CFR and relative FFR using angiographic image data was validated in a swine model. This angiographic technique can potentially be used for coronary physiological assessment during routine cardiac catheterization.

METHODS

Protocol. The study protocol was approved by the University of California, Irvine, Institutional Animal Care and Use Committee. In an open-chest swine model, CFR and FFR measurements were made at various stages of severity of microvascular disruption in the left anterior descending artery (LAD). Microcirculation was disrupted by embolized microspheres. An external vascular occluder was used to produce a moderate epicardial stenosis. The gold standard coronary blood flow from the flow probe was continuously recorded. Coronary angiograms were acquired for each data set.

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ment of the LAD was dissected free. Two transit-time ultrasound flow probes (Transonic Systems, Ithaca, NY) were placed on the proximal segment of the LAD and LCX coronary arteries. An extravascular occluder (IVM In Vivo Metric, Healdsburg, CA) was also placed around the LAD just distal to the flow probe to produce a moderate epicardial stenosis. The approximate arterial lumen diameter and the hyperemic blood flow, using the flow probe, were used to assess the stenosis severity while the vascular occluder was adjusted. Blood flow measurement is important in the case of a severe stenosis since slight changes in diameter will substantially change the blood flow. The position of the flow probe and the occluder was adjusted to ensure that there were no side branches between them. The extravascular occluder was also used to produce a temporary 100% occlusion to create the position of the flow probe and the occluder was adjusted to ensure that there were no side branches between them. The extravascular occluder was also used to produce a temporary 100% occlusion to create the reactive maximum hyperemia to determine the required dose to produce maximum hyperemia. The dose of intravenous adenosine (350–450 µg·kg⁻¹·min⁻¹) was adjusted based on the total occlusion measurement to reach the maximum hyperemia. The left main ostium was cannulated with a 6-F hockey-stick catheter through the left carotid artery under fluoroscopic guidance. For injection of microspheres, the catheter was either repositioned in the proximal segment of the LAD or it was changed to a small catheter depending on size of the LAD. Microcirculation was disrupted by gradual injection 50–100 µm (1.8 × 10⁴ microspheres/ml) microspheres (Polysciences, Warrington, PA; Ref. 3) into the LAD through the catheter. This procedure was repeated for different degrees of severity of microcirculatory embolism. Another 4-F hockey-stick catheter was placed in the right atrium through the right carotid vein to measure the coronary back pressure. Additionally, in eight pigs, an intracoronary pressure wire (Radi Medical System, 0.014 in.) was advanced into the distal segment of the LAD to measure the distal coronary pressure (Pd) to calculate the pressure-derived FFR. Therefore, aortic pressure (Pa), Pd, and right atrium pressure (Pra) were measured continuously with pressure transducers and the pressure wire, respectively.

Image acquisition and processing. All images were acquired using a conventional X-ray tube with a constant potential X-ray generator (Optimus M200; Philips Medical Systems, Shelton, CT). A cesium-iodide-based flat panel detector (FaxScan 4050A; Varian Medical, Palo Alto, CA) was used for image acquisition. The flat panel detector has a 30 cm² field of view and pixel size of 0.194 × 0.194 mm². Images were acquired at 30 frames/s. All images were corrected for X-ray scatter before logarithmic transformation. Publicly available software (ImageJ, NIH, Bethesda, MD) was used for image analysis. Each swine was positioned on its right side under the flat panel detector. The projection angle was optimized for separating the LAD and LCX perfusion beds. Pancuronium (0.1 mg/kg) was administered intravenously before coronary angiograms were acquired. Images of at least one full heart cycle were acquired before contrast injection for selecting a cardiac phase-matched mask image for temporal subtraction. Contrast material (Omnipaque-350, Princeton, NJ) was power injected (Leibel-Flarsheim Angiomat 6000, Cincinnati, OH) at 3 ml/s for 3 s. An image of a calibration phantom positioned over the heart was also acquired to determine the correlation between image gray level and iodine mass (25).

Coronary blood flow measurement based on FPA technique. A previous report (13) has measured the myocardial perfusion reserve using the mean transit time and rise time in a canine model. Molloi et al. (25, 27) have shown that the FPA analysis technique can be used to measure absolute coronary blood flow by analyzing the propagation of a contrast material signal in the coronary system. In the FPA theory, the volume of the vascular bed, which is supplied by a major coronary artery, is modeled as a reservoir with a single input. Coronary blood flow was determined from the change in volume during one cardiac cycle. A regions of interest for flow measurement was drawn around the LAD vascular bed, which encompassed both the visible arteries and the microcirculatory blush (43). Power injection of contrast material was assumed to momentarily replace blood with contrast material. The known iodine concentration in the contrast material and a linear regression analysis between the measured integrated gray levels in the calibration phantom were used to convert the gray level to volume. Therefore, the difference of densitometric signal in the vascular bed can be converted to the volume of contrast bolus entering the vascular bed between successive images using system iodine calibration. The number of image frames and the imaging acquisition rate (30 frames/s) were used to calculate the time interval for one cardiac cycle. The ratio of the measured volume change to the time period of a cardiac cycle yields the volumetric coronary blood flow.

Relative CFR is defined as the ratio of the hyperemic flow in a stenotic artery to another normal artery as shown below:

\[
\text{Relative CFR} = \frac{Q_S}{Q_N} 
\]

In Eq. 1, \(Q_S\) is the hyperemic coronary blood flow of the artery with stenosis while \(Q_N\) is the hyperemic coronary blood flow of another normal artery. One of the main limitations of relative CFR is that it is difficult to always find a reference normal artery (8, 12, 24). Furthermore, the reference normal artery may not have the same normal hyperemic blood flow as the disease artery due to the fact that coronary blood flow changes depending on the arterial perfusion bed size. In addition, congenital variations can also change the normal hyperemic coronary blood flow in the three main coronary arteries (16). Therefore, relative CFR measurement using velocity may not provide an accurate evaluation of the diseased artery (1, 17). However, it is possible to measure the dependent arterial lumen volume using densitometry (25), which can also be related to the myocardial mass (20, 21). Therefore, arterial lumen volume can be used to account for the dependent arterial bed size. Previous reports (26, 27, 43) have shown that a power law relationship exists between the hyperemic blood flow (Q) through a stem and its corresponding crown volume (V);

\[
V_{\text{ref}} (1 \text{ ml}) \text{ is a reference volume to make } V \text{ raised to the power of } 3/4 \text{ unitless. } V^{3/4} \text{ can be used to normalize hyperemic coronary blood flow for different arteries. Therefore, relative FFR can be introduced by combining Eqs. 1 and 2.}
\]

\[
\text{Relative FFR} = \frac{Q_S}{(V_S)^{3/4}} \text{ or } \frac{Q_N}{(V_N)^{3/4}}
\]

Relative FFR is similar to relative CFR except \(Q_S\) and \(Q_N\) are normalized with the corresponding crown arterial volume of the disease artery (\(V_S\)) and the arterial volume of the reference normal artery (\(V_N\), respectively. Therefore, relative angiographic FFR (FFR_a) can be calculated using \(Q\), and their corresponding crown arterial volume from the angiographic images. In our study, LAD was the diseased artery and LCX was selected to be the normal control artery (Fig. 1). The flow-based FFR (FFR_f), which was calculated from the flow probe data before and after producing the disease, was used as a gold standard. Current clinical measurement of FFR is pressure-derived (FFR_P) according to the following expression using \(P_a\), \(P_d\), and \(P_c\):

\[
\text{FFR_P} = (P_a - P_d)/P_c
\]

\[
\text{FFR_f} = (P_a - P_d)/P_c
\]
Diagnosis abilities for microcirculation disruption and epicardial stenosis. The diagnostic abilities of CFRq, CFRa, FFRq, and relative FFRa were tested in three models: 1) the epicardial stenosis model (S model), which included normal conditions and intermediate coronary epicardial stenosis (~75% area stenosis) without any microspheres injection; 2) the microcirculation disruption model (M model), which included normal conditions and different severities of microvascular disruptions with normal epicardial arteries; and 3) the combined S and M model, which included moderate coronary epicardial stenoses and different severities of microvascular disruption with the same moderate epicardial stenoses. The gold standard for detecting microvascular disease is determined by whether microspheres were injected, regardless of the amount of microspheres.

Statistical analysis. Linear regression analyses were performed between 1) \( Q_{q} \) and \( Q_{a} \) at baseline, 2) \( Q_{q} \) and \( Q_{a} \) at maximum hyperemia, 3) CFRq and CFRa, and 4) FFRq and relative FFRa to determine the coefficients in the regression equations. The correlation coefficient (r) and standard error of estimate (SEE) were determined by a linear regression analysis. The degree of agreement between different methods was assessed using the Bland-Altman analysis. Receiver operating characteristic (ROC) curves were made for CFRq, CFRa, FFRq, and relative FFRa measurements in both S and M models. The gold standard for detecting microvascular disease was determined by whether microspheres were injected, regardless of the amount of microspheres. Also, the gold standard for detecting microvascular disease is determined by whether microspheres were injected, regardless of the amount of microspheres.

Table 1. Summary list of the all flows and flow-derived variables and the relationship between the different \( Q \) and different types of CFR and FFR

<table>
<thead>
<tr>
<th>Target Indexes</th>
<th>Abbreviations</th>
<th>Needed Parameters for Calculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gold standard flow probe-based absolute CFR</td>
<td>CFRq</td>
<td>( Q_{q} ) of the diseased artery at maximum hyperemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( Q_{q} ) of the diseased artery at baseline</td>
</tr>
<tr>
<td>Angiography-based absolute CFR</td>
<td>CFRa</td>
<td>( Q_{a} ) of the diseased artery at maximum hyperemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( Q_{a} ) of the diseased artery at baseline</td>
</tr>
<tr>
<td>Gold standard flow probe-based FFR</td>
<td>FFRq</td>
<td>( Q_{q} ) of the diseased artery at maximum hyperemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( Q_{q} ) of the same artery without diseases at maximum hyperemia</td>
</tr>
<tr>
<td>Pressure derived FFR</td>
<td>FFRp</td>
<td>Coronary distal pressure (( P_{d} ))</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aortic pressure (( P_{a} ))</td>
</tr>
<tr>
<td>Angiography-based relative FFR</td>
<td>Relative FFRa</td>
<td>( Q_{a} ), the diseased LAD artery at maximum hyperemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>( Q_{a} ), the normal LCX artery at maximum hyperemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>V of the diseased LAD artery perfusion bed at maximum hyperemia</td>
</tr>
<tr>
<td>Gold standard flow probe-based relative CFR</td>
<td>Relative CFRq</td>
<td>V of the normal LCX artery perfusion bed at maximum hyperemia</td>
</tr>
<tr>
<td>(not used in the current study)</td>
<td></td>
<td>( Q_{q} ), another normal similar size artery at maximum hyperemia</td>
</tr>
<tr>
<td>Angiographic directly measured FFR</td>
<td>FFRa</td>
<td>( Q_{a} ), the diseased artery at maximum hyperemia</td>
</tr>
<tr>
<td>(not used in the current study)</td>
<td></td>
<td>V of the diseased artery perfusion bed at maximum hyperemia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Aortic pressure (( P_{a} ))</td>
</tr>
</tbody>
</table>

CFR, coronary flow reserve; FFR, fractional flow reserve; \( Q_{q} \) (in ml/min), gold standard coronary blood flow from the flow probe; \( Q_{a} \) (in ml/min), angiographic coronary blood flow based on the first-pass distribution analysis technique; V (in ml), corresponding crown volume based on angiography; LAD, Left anterior descending artery; LCX, left circumflex artery.
Table 2. Hemodynamic data for individual animals

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Blood Pressure, mmHg</th>
<th>Heart Rate, beats/min</th>
<th>(Q_{\text{baseline}}), ml/min</th>
<th>(Q_{\text{hyperemia}}), ml/min</th>
<th>CFR</th>
<th>Relative FFR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>48.41 ± 19.74 (10)</td>
<td>126.34 ± 15.44 (10)</td>
<td>19.09 ± 3.27 (3)</td>
<td>48.22 ± 31.19 (7)</td>
<td>1.90 ± 1.41 (7)</td>
<td>0.52 ± 0.40 (4)</td>
</tr>
<tr>
<td>2</td>
<td>67.96 ± 11.69 (19)</td>
<td>107.22 ± 10.88 (19)</td>
<td>31.44 ± 4.81 (6)</td>
<td>75.20 ± 20.05 (13)</td>
<td>2.39 ± 1.04 (13)</td>
<td>0.47 ± 0.19 (10)</td>
</tr>
<tr>
<td>3</td>
<td>64.47 ± 10.02 (9)</td>
<td>89.07 ± 4.28 (9)</td>
<td>19.32 ± 5.24 (3)</td>
<td>58.08 ± 33.13 (6)</td>
<td>1.69 ± 1.35 (6)</td>
<td>0.46 ± 0.32 (5)</td>
</tr>
<tr>
<td>4</td>
<td>55.89 ± 4.24 (13)</td>
<td>96.99 ± 11.71 (13)</td>
<td>26.45 ± 10.15 (5)</td>
<td>59.89 ± 31.49 (8)</td>
<td>2.16 ± 1.48 (8)</td>
<td>0.59 ± 0.23 (8)</td>
</tr>
<tr>
<td>5</td>
<td>67.84 ± 13.70 (16)</td>
<td>110.07 ± 22.15 (16)</td>
<td>20.91 ± 1.68 (5)</td>
<td>39.13 ± 15.82 (11)</td>
<td>2.19 ± 0.68 (11)</td>
<td>0.52 ± 0.26 (10)</td>
</tr>
<tr>
<td>6</td>
<td>64.62 ± 7.44 (12)</td>
<td>105.23 ± 14.52 (12)</td>
<td>19.07 ± 14.66 (4)</td>
<td>40.01 ± 12.25 (8)</td>
<td>3.25 ± 1.59 (8)</td>
<td>0.38 ± 0.18 (8)</td>
</tr>
<tr>
<td>7</td>
<td>66.77 ± 10.69 (27)</td>
<td>99.06 ± 3.88 (27)</td>
<td>26.59 ± 3.69 (7)</td>
<td>41.88 ± 27.42 (20)</td>
<td>1.64 ± 1.11 (20)</td>
<td>0.56 ± 0.30 (15)</td>
</tr>
<tr>
<td>8</td>
<td>72.28 ± 7.12 (25)</td>
<td>89.03 ± 15.32 (25)</td>
<td>15.56 ± 7.80 (7)</td>
<td>40.27 ± 8.35 (18)</td>
<td>2.62 ± 1.21 (18)</td>
<td>0.61 ± 0.29 (17)</td>
</tr>
<tr>
<td>9</td>
<td>76.10 ± 2.25 (31)</td>
<td>86.81 ± 9.47 (31)</td>
<td>19.28 ± 9.92 (5)</td>
<td>41.94 ± 28.18 (26)</td>
<td>2.49 ± 1.12 (26)</td>
<td>0.63 ± 0.23 (15)</td>
</tr>
<tr>
<td>10</td>
<td>66.53 ± 8.83 (24)</td>
<td>88.50 ± 4.15 (24)</td>
<td>27.80 ± 2.64 (5)</td>
<td>58.40 ± 32.16 (19)</td>
<td>1.72 ± 0.93 (19)</td>
<td>0.43 ± 0.22 (14)</td>
</tr>
<tr>
<td>11</td>
<td>62.04 ± 6.91 (33)</td>
<td>89.60 ± 4.75 (33)</td>
<td>22.95 ± 4.13 (8)</td>
<td>64.27 ± 44.74 (25)</td>
<td>2.97 ± 2.32 (25)</td>
<td>0.42 ± 0.26 (13)</td>
</tr>
<tr>
<td>12</td>
<td>65.60 ± 11.55 (18)</td>
<td>88.86 ± 8.89 (18)</td>
<td>33.23 ± 5.86 (4)</td>
<td>75.12 ± 42.97 (14)</td>
<td>2.57 ± 1.63 (14)</td>
<td>0.44 ± 0.28 (11)</td>
</tr>
<tr>
<td>13</td>
<td>48.51 ± 10.10 (28)</td>
<td>81.33 ± 2.82 (28)</td>
<td>14.03 ± 3.06 (6)</td>
<td>47.09 ± 8.83 (22)</td>
<td>1.93 ± 0.43 (22)</td>
<td>0.47 ± 0.13 (18)</td>
</tr>
<tr>
<td>14</td>
<td>57.50 ± 5.92 (45)</td>
<td>87.35 ± 5.66 (45)</td>
<td>29.73 ± 7.47 (8)</td>
<td>69.75 ± 23.15 (37)</td>
<td>3.27 ± 2.30 (37)</td>
<td>0.54 ± 0.27 (28)</td>
</tr>
<tr>
<td>15</td>
<td>64.90 ± 4.44 (28)</td>
<td>97.66 ± 2.96 (28)</td>
<td>23.71 ± 7.99 (4)</td>
<td>41.81 ± 21.96 (24)</td>
<td>3.04 ± 0.66 (24)</td>
<td>0.43 ± 0.28 (17)</td>
</tr>
</tbody>
</table>

Values are average ± SD for each parameter, and the number of the measurements in parenthesis is for the 15 swine experiments. Values include measurements in both normal and different stages of disease conditions.

standard for detecting epicardial disease was whether a stenosis was created by the occluder, regardless of the severity. The areas under each curve (AUC) were calculated to compare the diagnostic abilities of the different indexes. A \( P < 0.05 \) was considered to be statistically significant for all statistical analyses.

**RESULTS**

Coronary blood flow and CFR measurements. From a total of 338 coronary blood flow measurements of LAD, there were 80 measurements made at baseline and 258 measurements at maximum hyperemia in the normal and disease conditions (Table 2). \( Q_a \) showed a strong correlation with the reference standard \( Q_b \) at both baseline and hyperemia (baseline: \( r = 0.92 \), S.E.E. = 3.32 ml/min; hyperemia: \( r = 0.96 \), S.E.E. = 7.63 ml/min). The regression line was determined to be \( Q_a = 0.89 Q_b + 3.79 \) ml/min \((P < 0.001)\) at baseline and \( Q_a = 0.90 Q_b + 6.57 \) ml/min \((P < 0.001)\) at hyperemia (Fig. 2). Additionally, in the Bland-Altman plot, the mean differences between the two coronary blood flow measurements were 1.4 ± 0.67 ml/min at baseline and 2.5 ± 1.59 ml/min at hyperemia.

From 258 LAD measurements, CFR\(_b\) correlated linearly with CFR\(_q\) as CFR\(_b\) = 0.91 CFR\(_q\) + 0.30 \((P < 0.001)\) with a good correlation coefficient \((r = 0.90; \text{SEE} = 0.54; \text{Fig. 3})\). In the Bland-Altman plot, the mean differences between the two measurements were 0.12 ± 1.07 for CFR. There was no statistically significant difference from zero, implying a lack of bias between the two techniques. Figure 4 shows the changes in Q and CFR while microsources were gradually injected for one of the animals. The measured normal values (no stenosis and microsources) for the CFR\(_b\) and CFR\(_q\) were 3.92 ± 0.74 \((n = 50)\) and 3.99 ± 0.79 \((n = 50)\), respectively.

Relative FFR and FFR\(_q\) measurements. Normalized angiographic blood flow was calculated as \( Q_a \) divided by the crown volume to the power of 3/4. In one animal, LAD, LCx, and six artery branches were selected to calculate \( Q_a \) and normalized \( Q_a \) (see Fig. 5). The arteries’ diameters ranged from 1.8 to ∼4.0 mm. The mean values and SD of \( Q_a \) and normalized \( Q_a \) were 51.43 ± 33.11 and 87.09 ± 9.99 ml/min, respectively. The results indicated that the measured normalized \( Q_a \) decreased the flow variance between different arteries.
A total of 193 FFR\(_q\) and relative FFR\(_a\) measurements were made for LADs (Table 2) of 15 swine in the normal and disease conditions. A linear regression analysis of FFR\(_q\) and relative FFR\(_a\) measurements (Fig. 6A) showed a good correlation (relative FFR\(_a\)/11005 \(= 0.86\) FFR\(_q\)/11001 \(= 0.05; r^2 = 0.86;\) SEE = 0.54; \(n = 193; P < 0.0001\)). Also, the Bland-Altman analysis (Fig. 6B) showed that the mean differences between these two indexes were \(-0.01 \pm 0.23\). Figure 6C shows the correlation between relative FFR\(_a\) and the pressure-derived FFR (FFR\(_p\); relative FFR\(_a\)/11005 \(= 1.11\) FFR\(_p\)/11002 \(= 0.18;\) \(r^2 = 0.766;\) SEE = 0.12; \(n = 54; P < 0.0001\)). The measured normal values (no stenosis and microspheres) for the FFR\(_q\), relative FFR\(_a\), and FFR\(_p\) were \(0.97 \pm 0.06\) (\(n = 18\)), \(0.91 \pm 0.08\) (\(n = 18\)), and \(0.94 \pm 0.06\) (\(n = 13\)), respectively.

**Diagnosis abilities for microcirculation disruption and epicardial stenosis.** The gold standards for detecting microvascular disease and epicardial disease were determined by whether microspheres or stenosis were induced, respectively. From 85 measurements made using the S model, the AUCs for CFR\(_q\), CFR\(_a\), FFR\(_q\), and relative FFR\(_a\) were 0.803, 0.806, 0.844, and 0.835, respectively (Fig. 7A). From 131 measurements made using the M model, the AUCs were 0.836, 0.835, 0.712, and 0.718, respectively (Fig. 7B). FFR\(_q\) and relative FFR\(_a\) had slightly higher AUCs than CFR measurements \((P < 0.05)\) in the S model while the AUCs for CFR\(_q\) and CFR\(_a\) were significantly higher than that for FFR\(_q\) and relative FFR\(_a\) \((P > 0.05)\) in the M model. Table 3 also shows the sensitivity and specificity of the best cutoff value for each index. Additionally, in the combined S and M model, the AUCs for CFR\(_q\), CFR\(_a\), FFR\(_q\), and relative FFR\(_a\) were 0.737, 0.700, 0.671, and 0.712, respectively (Fig. 7C).

**DISCUSSION**

This study demonstrated that CFR\(_a\) based on the FPA technique strongly correlates with the gold standard CFR\(_q\) from the transit time flow probe. Relative FFR\(_a\) and FFR\(_q\) measurements correlated linearly with a good correlation coefficient. The Bland-Altman analysis also showed that there was no significant bias between these two methods. Additionally, each pair...
of indexes (CFRq and CFRa; FFRq and relative FFRa) had similar diagnostic abilities with microcirculation disruption and epicardial stenosis conditions.

Comparison of CFR and relative FFR. The growing awareness that coronary microcirculatory dysfunction is an important pathophysiological component in many cardiac conditions has previously led some studies (19) to use CFR as the theoretical framework to study microcirculation (19). Absolute CFR is considered as a marker for the integrity of epicardial coronary circulation and microcirculation (38). The clinical values of absolute CFR are twofold: 1) a normal CFR (>2.5) implies low microvascular and epicardial resistance and thus adequate myocardial perfusion, and 2) CFR can be used to assess microvascular function in individuals who have an entirely normal epicardial vessel. If there are any abnormalities in the flow, CFR is secondary to microvascular dysfunction (11, 24).

Fig. 5. Normalized Qa measurements in one animal. An example of normalized angiographic coronary blood flow (normalized Qa) by corresponding arterial volume comparison for the LAD, LCx, and their branches was shown in one animal (A). Normalized Qa could decrease the variance between the different size arteries (B).

Fig. 6. Relative FFRa and FFRq measurements. A linear regression analysis (A) and the Bland-Altman analysis (B) of relative angiographic FFR (relative FFRa) and the flow probe-based FFR (FFRq) measurements. (relative FFRa = 0.86 FFRq + 0.05; r² = 0.81; SEE = 0.11; n = 193; P < 0.0001). C: correlation between relative FFRa and the pressure-derived FFR (FFRq) (relative FFRa = 1.11 FFRq − 0.18; r² = 0.766; SEE = 0.12; n = 54; P < 0.0001).
However, the cutoff value for CFR is very sensitive to hemodynamic changes (29). Also, CFR cannot differentiate between the relative contributions of the epicardial or microvascular compartments toward changes in blood flow (47). From the current ROC analysis, the diagnostic abilities of CFRa and CFRq were similar. Additionally, AUCs for CFR measurements, in both S and M models, were always higher than 0.80, which may be considered as a verification of the CFR two-compartment limitation.

Relative CFR was developed to overcome the two-compartment limitation of CFR (36, 41). It was meant to reduce the influence of confounders on CFR by indexing flow reserve in the interrogated vessel to an adjacent reference normal vessel (24). While relative FFR is similar to relative CFR, it differs because the latter is determined as the maximum hyperemic blood flow ratio of the disease artery to the normal artery. Furthermore, relative FFR overcomes some of the limitations presented by relative CFR. First, in the previous relative CFR measurement, the reference artery was always chosen by the stem diameter estimation; this method neglected to account for the perfusion bed size. However, relative FFR can theoretically eliminate the normal hyperemic flow difference between the diseased and normal arteries. Even though the arterial perfusion bed sizes were different, the arterial volume can be used to normalize the hyperemic blood flow to the same level as shown in Fig. 5. Second, relative CFR has very limited applicability in patients with multivessel lesions for which there may be no suitable reference vessel (23). Relative FFR measurement increases the number of potential choices for reference artery. Even small branches (diameter $>1.50$ mm) could be selected as normal control. From the current swine study, relative FFRa had a good correlation with the gold standard FFRq. In the ROC curves, the AUCs of relative FFRa were close to the AUCs of FFRq in both models. However, the ROC shapes of relative FFRa and FFRq in the M model were different. One explanation for the difference could be that the big range of microspheres (Fig. 4) caused variance between the two indexes. Another reason may be that FFR, which is specific to the epicardial vessel, is not significantly influenced by microcirculation. The AUCs from the FFR measurements may further decrease if the sample size of the M model is increased.

Additionally, another method to directly measure FFR using angiographic images was also validated recently (26, 45). The key point of this direct FFR measurement technique is to predict the expected maximum blood flow by using arterial volume. Compared with the current relative FFR, the direct angiographic FFR measurement does not require a normal artery as the reference and it can be easily applied to the multivessel coronary disease. However, the absolute angiographic FFR would underestimate the real myocardial flow ratio FFR when severe stenosis occurs (36), because of the collapse of arterioles.

Table 3. Diagnosis abilities for epicardial stenosis and microcirculation disruption

<table>
<thead>
<tr>
<th></th>
<th>S Model $(n = 85)$</th>
<th></th>
<th>M Model $(n = 131)$</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC</td>
<td>Best Cutoff Value</td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>CFRq</td>
<td>0.803</td>
<td>3.033</td>
<td>0.677</td>
<td>0.889</td>
</tr>
<tr>
<td>CFRa</td>
<td>0.806</td>
<td>3.272</td>
<td>0.677</td>
<td>0.833</td>
</tr>
<tr>
<td>FFRq</td>
<td>0.844</td>
<td>0.777</td>
<td>0.694</td>
<td>0.833</td>
</tr>
<tr>
<td>Relative FFRa</td>
<td>0.835</td>
<td>0.787</td>
<td>0.742</td>
<td>0.833</td>
</tr>
</tbody>
</table>

Areas under the receiver operating characteristic curves (AUC) and the best cutoff values of the CFR and FFR measurements in both epicardial stenosis model (S) and microcirculation disruption (M) models.
Advantages of the angiographic measurement based on FPA technique. There are a number of different methods to estimate CFR and FFR. Currently, the average peak velocity-derived CFR and pressure-derived FFR are the most widely used methods in the clinical settings (5, 31, 33). Several noninvasive and less invasive methods such as PET and transthoracic Doppler echocardiography have been developed. Angiographic blood flow measurement based on the FPA technique has important advantages over the previously reported methods. The angiographic CFR and FFR methods, which require no wire, reduce the cost and procedure time compared with the other techniques. All procedures can be completed during a routine diagnostic cardiac catheterization (46). The arterial lumen volume can be used to 1) predict the expected maximum blood flow in an arterial tree (21, 26), and 2) normalize the maximum hyperemic blood flow for different size arteries (46, 47). Lumen volume can be used to calculate CFR using only image data (45). The current clinical decisionmaking remains challenging in patients with intermediate lesions. Standard coronary angiography is still a poor predictor of coronary artery events. The current technique can provide both anatomical and physiological information from the same angiographic images.

Normalized blood flow using corresponding arterial volume. We have previously developed techniques to angiographically measure coronary blood flow (28) and luminal volume (25). However, the measured blood flow and lumen volume are dependent on the artery size and myocardial mass that it perfuses. Flow measured with PET is normalized with the myocardial mass (ml·min⁻¹·g⁻¹), making it possible to establish a normal perfusion at maximum hyperemia. While it is not possible to directly measure regional myocardial mass with angiography, it is possible to use densitometry to measure the dependent arterial lumen volume (25), which is linearly related to myocardial mass (20, 21). Therefore, arterial lumen volume can be used to account for the dependent arterial bed size. Previous reports (26, 27, 43) have shown that a power law relationship exists between the hyperemic blood flow through a stem and its corresponding crown volume. There were two main approaches to validate the flow normalization with the perfusion territory. One method is to use tracer microspheres. Another method is to delineate the perfusion territory with a postmortem dye injection. We have previously validated the relationship between the distal arterial lumen volume and its corresponding regional myocardial mass in the postmortem hearts of swine by injecting colored polymer materials in the coronary arteries (20).

In the current study, arterial lumen volume was used to normalize the maximum hyperemic blood flows of LAD and LCx for relative FFR measurements. Moreover, by using the relationship between flow and crown volumes, normalized regional flow for small branches can be calculated (see Fig. 5). The normalized flow is expected to be the same for different normal coronary arteries and their branches. Furthermore, the regional CFR and FFR of different coronary arteries and their branches may be calculated based on the normalized regional blood flow by using angiographic images.

Coronary microembolization. Coronary microembolization has become a focus of attention with the awareness that coronary microembolization and its sequelae are a frequent iatrogenic complication of percutaneous coronary interven-tions (PCIs; Ref. 15). The area of no reflow is then confined to the area of infarcted myocardium and is characterized by obstructive capillary damage; however, it is a consequence and not a cause of infarction. Thus no or slow reflow and coronary microembolization should be viewed as distinct phenomena. A previous report (34) has shown that coronary microembolization reduced coronary and inotropic reserves in dogs’ LCx. Also, this finding supported the notion that patients who have increased baseline flow and reduced CFR after PCIs together with marker release that reflects microinfarction may indeed have experienced microembolization (34). Limitations of the coronary microembolization in the current study included the injection of microspheres, which may cause heterogeneous microinfarcts, which are not the same pathological changes as normal myocardial infarction or diffuse microvascular disease (3). Microspheres are chemically inert and not chemoattractant and thus are very different from microemboli as identified in patients at autopsy or when retrieved during PCI (15).

Radiographic anatomy and physiological assessment. Different imaging modalities such as coronary angiography, computed tomography angiography, and magnetic resonance angiography can make the visualization of the coronary artery anatomy possible. However, visual assessment of stenosis correlates poorly with its physiological significance. This is especially true in the case of an intermediate severity stenosis (18, 40). The presence of inducible ischemia related to a coronary artery stenosis is important when deciding whether to revascularize the stenotic lesion (6, 10, 32, 39, 42). The present study proposes a simple angiographic method to assess the coronary physiology as a potential solution to the well-known limitations of the current visual assessment of coronary anatomy. However, given the variability of angiographic anatomically based FFRp in our ideal controlled experimental model, any angiographic anatomically based estimate of FFRp may lack the precision of directly pressure derived FFRp in the critical range of 0.6 to 0.9 necessary for clinical decisions.

Study limitations. The current experimental animal model only introduced moderate epicardial stenosis. More severe stenosis should be included in the future studies. Additionally, the current angiographic method cannot distinguish flow changes due to serial stenoses along a vascular tree (15). The current concept of CFR and FFR measurements also needs to be validated in a clinical setting. The impact of other disease conditions such as ventricular hypertrophy, hypercholesterolemia, diabetes mellitus, diffuse coronary artery disease, and previous myocardial infarction requires additional studies.

The current study measured CFR and FFR in a large range of normal and diseased states and showed a good correlation between the proposed method and the gold standard. However, in the clinical settings, FFR values in the critical range of 0.6 to 0.9 with a cut-off value of ~0.8 are very important for the therapeutic decisionmaking. The current results (see Fig. 6) show a relatively large variability in this clinically important range. This variability is also present in the flow (see Fig. 2) and CFR (see Fig. 3) results. Therefore, further optimization of the proposed angiographic technique is necessary before clinical implementation of the technique. Motion misregistration is another limitation of background subtraction in angiography. The current technique minimizes motion misregistration artifacts by only requiring a short time interval for image acquisition (2–3 s). Nonetheless, misregistration of artifacts could
still occur. A possible solution would be to use unsubtracted images for blood flow measurements (44). Additionally, the overlapping of vascular beds is also a potential limitation, which can easily affect the measured arterial lumen volume. This can increase the error in the measured CFR and FFR using angiographic images in humans.

A recent report (14) has suggested that adenosine, which does not eliminate all active coronary vasomotor tone, is not the predominant mediator of physiological regulation of coronary blood flow. In the present study, the level of hyperemia produced by intravenous adenosine was evaluated by using a vascular occluder to produce a maximum reactive hyperemia.

The existence of collateral flow might lead to assessment errors because myocardial perfusion combines coronary and collateral flows (30). This study’s primary interest is in intermediate severity coronary stenoses. Furthermore, since pigs have no significant collaterals (7), the collateral flow is not expected to be significant in the swine studies. The proposed technique measures the total contributions of the epicardial (segmental stenosis or diffuse disease) and microvascular compartments toward changes in blood flow. Therefore, to distinguish the relative contributions of epicardial and microvascular disease conditions in the clinical settings, measurement of relative CFR\textsubscript{A} or CFR\textsubscript{p} can be used. This assumes that there is at least one artery or arterial branch without epicardial disease and the arteries of interest share the same level of microvascular disease. In the cases where there are no arterial branches without epicardial disease (three vessel disease) or microvascular disease is not uniform for different territories, it might be necessary to use FFR\textsubscript{A} in conjunction with FFR\textsubscript{p} (47).

Conclusions. Angiographic flow measurements based on the FPA technique can be used to calculate CFR for assessing epicardial stenosis and microvascular disruption. This angiographic method is quantitative and easy to perform, requiring only angiographic image data. The technique can easily be automated. Since it uses the corresponding arterial volume to normalize coronary blood flow, relative CFR could potentially be a simpler and less expensive method to measure FFR to assess a specific coronary condition in patients with stable chest pain during routine coronary arteriography.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: Z.Z. and S.T. performed experiments; Z.Z. analyzed data; Z.Z. prepared figures; Z.Z. drafted manuscript; S.T. and S.M. interpreted results of experiments; S.T. and S.M. edited and revised manuscript; S.M. conception and design of research; S.M. approved final version of manuscript.

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