Detection of coronary stenosis with myocardial contrast echocardiography using regadenoson, a selective adenosine A2A receptor agonist

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Aims
Regadenoson is comparable to adenosine in pharmacologic radionuclide stress tests but has not been studied with stress myocardial contrast echocardiography. This study assessed the haemodynamic profile and ability of regadenoson, a novel selective A2A receptor agonist, to detect coronary artery stenosis during myocardial contrast echocardiography.

Methods and results
Myocardial contrast echocardiography was performed to measure myocardial blood volume, myocardial blood flow velocity, and total regional myocardial blood flow before and after administration of regadenoson (5 μg kg⁻¹, 10 s bolus) in 10 open-chest dogs with mild-to-moderate coronary stenosis that was not flow limiting at rest. Regadenoson decreased blood pressure but did not change heart rate. It increased coronary blood flow significantly (P < 0.05) for 30 min, which was attenuated in proportion to coronary stenosis severity. Whereas myocardial blood volume maximally increased by 0.5–0.75-fold in the control region, it did not change in the region supplied by the non-flow limiting stenosis. Perfusion defects were visually and quantitatively detectable for as long as 10 min after administration of regadenoson. No arrhythmias were noted with regadenoson either prior to or during myocardial contrast echocardiography.

Conclusion
Regadenoson can be used as a vasodilator stress agent with myocardial contrast echocardiography to detect the presence of physiologically significant coronary stenosis. The optimum time for image acquisition was 3–10 min after drug administration.

Keywords
Regadenoson • Stress testing • Myocardial contrast echocardiography

Introduction
Pharmacologic stress testing is used routinely to detect coronary artery disease. Adenosine and dipyridamole are used in up to 50% of myocardial perfusion stress tests, but they are often associated with undesirable side effect profiles.¹–³ Both agents act non-selectively to activate all four subtypes of the adenosine receptors (A₁, A₂A, A₂B, and A₃) that can result in chest pain, dyspnoea, hypotension, bronchospasm, and high-grade atrioventricular nodal block. In addition, these agents require weight-based dosing and administration as a continuous infusion,⁴,⁵ which can predispose to preparation error and lengthen the duration of the study.

The ideal vasodilator stress agent would be a potent selective A₂A agonist, have rapid onset of action, adequate duration of maximum action to allow sufficient time for image acquisition, and fewer side effects. In 2008, regadenoson (Lexiscan®; Gilead Sciences Inc, Foster City, CA, USA) was the first selective A₂A agonist approved by the US Food and Drug Administration for use as a vasodilator in conjunction with radionuclide myocardial perfusion imaging. Its counterpart, Rapiscan® was authorized by the European Commission for the same indication in 2010. The

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drug possesses all of the characteristics mentioned above and can be given as a fixed-dose bolus.

Regadenoson and adenosine elicit comparable dose-dependent increases in coronary blood flow via a reduction in coronary vascular resistance. However, the period when the increase in coronary blood flow is at least two-fold higher than baseline is three times longer after bolus administration of regadenoson than after administration of adenosine. Phase 2 and 3 studies of the ADVANCE-MPI trial demonstrated non-inferiority of regadenoson for the detection of reversible myocardial perfusion defect using single-photon emission computed tomography when compared with adenosine regardless of age, gender, body mass index, and diabetes. Due to its selectivity, the side effects of regadenoson were also more tolerable than those of adenosine.

In addition to single-photon emission computed tomography, pharmacological stress testing is also used with echocardiography, where the basis for coronary artery disease detection is an inducible wall motion abnormality. In the USA, dobutamine is the preferred stress agent, whereas in Europe, high-dose dipyridamole in combination with atropine is routinely used since it offers a similar sensitivity, specificity, and accuracy as dobutamine. However, we have previously shown that perfusion abnormalities precede wall motion abnormalities during demand ischaemia and compared with perfusion, wall motion assessment alone can underestimate the extent and severity of coronary artery disease.

Myocardial contrast echocardiography is either superior or equivalent to single photon emission-computed tomography imaging for coronary artery disease detection, and the incremental benefit of having both perfusion and function information can increase the sensitivity of coronary artery disease detection when compared with regional wall motion analysis alone on stress echocardiography. Regadenoson has not been evaluated for the detection of coronary artery disease in conjunction with myocardial contrast echocardiography. In this pre-clinical investigation, we tested the hypothesis that regadenoson is an effective vasodilator stress agent when used with ultrasound contrast and will allow both qualitative and quantitative detection of non-flow limiting coronary artery stenosis.

**Methods**

**Animal preparation**

The study was approved by the Animal Research Committee at Oregon Health and Science University and conformed to the American Heart Association Guidelines for the Use of Animals in Research. Ten adult mongrel dogs (25–35 kg) were studied. They were intubated and ventilated with room air. Sodium pentobarbital was used to induce (40 mg kg⁻¹) and maintain (200 mg h⁻¹) anaesthesia throughout the experiment. Heart rhythm, oxygen saturation, end-tidal CO₂, and temperature were continuously monitored (Advisor® Vital Signs Monitor, SurgiVet, Norwell, MA, USA). Catheters (7F) were placed in both femoral veins for microbubble infusion and administration of fluids and drugs as needed, and in the descending aorta and right atrium to measure pressures.

A left lateral thoracotomy was performed in the fourth intercostal space, and the heart was suspended in a pericardial cradle. A micromanometer-tipped catheter (Millar Instruments, Inc., Houston, TX, USA) was inserted into the left ventricular cavity via the apex to measure dP/dt. The proximal portions of the left anterior descending and left circumflex coronary arteries were dissected free from surrounding tissue. Time-of-flight ultrasonic flow probes (series SC, Transonic, Ithaca, NY, USA) were placed on both arteries and connected to a digital flow metre (model T206, Transonic, Ithaca, NY, USA) to monitor epicardial coronary blood flow. Fluid-filled catheters were inserted into the left atrium and the distal portion of the left anterior descending or the left circumflex coronary artery to measure pressure.

**Haemodynamic measurements**

All catheters and flow metres were interfaced with a multi-channel recorder (PowerLab, AD Instruments, Inc., Colorado Springs, CO, USA). Phasic and mean coronary blood flow and pressures (mean central aorta, right atrial, left atrial, and dP/dt) were digitally acquired continuously and displayed online on a computer system (iMac, Apple, Cupertino, CA, USA). All data were analysed offline using LabChart 6 software.

**Assessment of regional left ventricular function**

Echocardiographic images were acquired, and regional left ventricular function was assessed offline using previously described custom-designed software. Several endocardial and epicardial targets in each frame from end-diastole to end-systole were defined, which were then automatically connected using cubic-spline interpolation to derive epicardial and endocardial contours. To correct for systolic cardiac rotation, the junction of the posterior left ventricular wall and the right ventricular free wall was defined over the epicardium in each frame, and 100 equidistant chords between the two contours were generated starting at this point. Each chord represented the shortest distance between the epicardial and endocardial contours. The chord lengths of the selected myocardial regions were averaged. Plots of wall thickening over the entire systolic contraction sequence were then automatically generated, with time represented in deciles. Maximal wall thickening at any point in systole was taken to represent percent wall thickening.

**Assessment of myocardial perfusion**

Real-time low mechanical index power modulation (IE33, Philips Healthcare, Andover, MA, USA) was used to measure myocardial blood flow during myocardial contrast echocardiography. Depth, focus, compression, persistence, and colour gain were optimized at the beginning of each study and kept constant throughout. Images were acquired after steady state was achieved during a continuous infusion of SonoVue® (Bracco Research, Geneva, Switzerland) at 1 mL min⁻¹ using a proprietary rotation syringe pump (BR-INF 100, Bracco Research, Geneva, Switzerland). The infusate was prepared by mixing 20 mL of normal saline to each vial of SonoVue. A set of 10 pulses of ultrasound at a mechanical index of 0.9–1.0 was transmitted to destroy all microbubbles within the myocardium (flash). The first image acquired immediately after the flash represented the baseline image. At least 15 consecutive end-systolic images were then acquired at the low mechanical index (0.09–0.12). All images were processed for offline analysis. Custom-designed software was used for myocardial contrast echocardiography image analysis. Regions of interest were placed over the myocardium and left ventricular cavity in images acquired at baseline (background) and during subsequent images and included as much
myocardium as possible avoiding artefact. Pulsing interval vs. background-subtracted acoustic density plots were generated offline and were fitted to an exponential function, $y = A (1 - e^{-bt})$, where $y$ is the acoustic density at pulsing interval $t$, $A$ the acoustic density after the ultrasound beam is completely replenished, which represents the capillary cross-sectional area or myocardial blood volume, and $b$ the rate constant that reflects the mean myocardial blood flow velocity. The product, $A \cdot b$, was calculated to derive regional myocardial blood flow.$^{26}$

### Experimental protocol

In the closed-chest setting, haemodynamic, regional wall thickening, and myocardial contrast echocardiography data were acquired prior to baseline and at 1 min after intravenous bolus administration of regadenoson (5 $\mu$g kg$^{-1}$) over 10 s to determine to haemodynamic effects of the drug with and without myocardial contrast echocardiography. Haemodynamic data were also recorded at 1 min intervals for the subsequent 30 min, whereas myocardial contrast echocardiography was performed at 1 min intervals for the subsequent 10 min.

Coronary stenoses that did not reduce resting coronary blood flow were then applied on either the left anterior descending or the left circumflex coronary artery in the opened-chest setting in random order using a custom-designed screw occluder. The severity of stenosis was determined by the gradient between the mean central aortic and the distal coronary artery pressures: 1–10 mmHg constituted mild stenosis and 11–20 mmHg constituted moderate stenosis. The non-stenosed artery served as the control region. Similar to the closed-chest protocols, haemodynamic, regional wall thickening, and myocardial contrast echocardiography data were acquired prior to baseline and at 1 min after intravenous bolus administration of regadenoson (5 $\mu$g kg$^{-1}$) over 10 s. Haemodynamic data were also recorded at 1 min intervals for the subsequent 30 min, while myocardial contrast echocardiography was performed at 1 min intervals for the subsequent 10 min.

### Statistical methods

Comparisons between stages were performed using repeated measures analysis of variance. When a difference was found, a paired Student’s t-test was used for inter-stage comparisons. A $P$-value of $<0.05$ (two-sided) was considered statistically significant for all comparisons.

### Results

#### Closed-chest preparation

Heart rate increased 1 min after injection of regadenoson and peaked during 3–5 min at 30% above baseline. It decreased slightly over the next 20 min but still remained elevated at 15% above baseline at 30 min. Immediately after injection of regadenoson, mean aortic pressure decreased significantly (nadir 11% below baseline) and returned to baseline at 5 min (Figure 1A). Continuous
Table 1  Haemodynamic data

<table>
<thead>
<tr>
<th></th>
<th>Min after regadenoson injection</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1</td>
</tr>
<tr>
<td>No stenosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAP (mmHg)</td>
<td>13 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>RAP (mmHg)</td>
<td>11 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>Max dP/dt (mmHg.s⁻¹)</td>
<td>674 ± 44</td>
<td>593 ± 55</td>
</tr>
<tr>
<td>Mild non-flow limiting stenosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAP (mmHg)</td>
<td>13 ± 1</td>
<td>17 ± 1</td>
</tr>
<tr>
<td>RAP (mmHg)</td>
<td>11 ± 1</td>
<td>12 ± 1</td>
</tr>
<tr>
<td>Max dP/dt (mmHg.s⁻¹)</td>
<td>632 ± 126</td>
<td>530 ± 104</td>
</tr>
<tr>
<td>Moderate non-flow limiting stenosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAP (mmHg)</td>
<td>14 ± 2</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>RAP (mmHg)</td>
<td>11 ± 1</td>
<td>13 ± 1</td>
</tr>
<tr>
<td>Max dP/dt (mmHg.s⁻¹)</td>
<td>561 ± 36</td>
<td>476 ± 44</td>
</tr>
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</table>
Figure 2 Effects of regadenoson on heart rate and mean aortic blood pressure (A), left anterior descending coronary blood flow (B), and myocardial blood volume, myocardial blood flow velocity, and myocardial blood flow (C) in the open-chest dog without stenosis. Heart rate, blood pressure, and coronary blood flow values were compared with baseline values. Myocardial contrast echocardiography parameters were represented as the percent change compared with baseline values. Data are mean ± SEM. *P < 0.05 vs. baseline. †P < 0.01 vs. baseline. ‡P < 0.001 vs. baseline.

Table 2 Regional function data

<table>
<thead>
<tr>
<th>Control region</th>
<th>Baseline WT (%)</th>
<th>Regadenoson WT (%)</th>
<th>P-value vs. baseline</th>
<th>Stenosed region</th>
<th>Baseline WT (%)</th>
<th>Regadenoson WT (%)</th>
<th>P-value vs. baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open-chest no stenosis</td>
<td>36 ± 3</td>
<td>44 ± 7</td>
<td>&lt; 0.01</td>
<td>35 ± 4</td>
<td>43 ± 5</td>
<td>&lt; 0.001</td>
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<tr>
<td>Mild Non-flow limiting stenosis</td>
<td>36 ± 1</td>
<td>41 ± 3</td>
<td>&lt; 0.01</td>
<td>37 ± 1</td>
<td>42 ± 4</td>
<td>&lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>Moderate non-flow limiting stenosis</td>
<td>36 ± 4</td>
<td>41 ± 4</td>
<td>&lt; 0.01</td>
<td>37 ± 2</td>
<td>39 ± 8</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>
myocardial blood flow after regadenoson exhibited a similar pattern, and generally, the magnitude of change in the control region exceeded that in the stenosed region (Table 3).

Heart rate and aortic pressure responses to regadenoson in the presence of a moderate stenosis were also similar to the changes observed in the other open-chest stages (Figure 4A). Coronary blood flow in the control region increased by three-fold 5 min after injection and remained significantly elevated at 30 min. On the contrary, coronary blood flow in the stenosed region increased only slightly (Figure 4B). Regadenoson resulted in a significant increase in wall thickening in the control region but not in the moderately stenosed region (Table 2). However, wall thickening was not significantly different when compared with the mild stenosis and no stenosis groups. The change in the myocardial blood volume in the stenosed region was negligible, while that in the control group reached a maximum level of approximately 25% higher between 4 and 7 min after regadenoson injection (Table 3). Similar to the mild stenosis group, there was a trend showing a greater increase in myocardial blood flow velocity and myocardial blood flow in the control region compared with the stenosed region (Table 3).

Figure 3 Changes in heart rate and mean aortic blood pressure (A), coronary blood flow in the control and stenosed regions (B) in the presence of a mild non-flow limiting stenosis. All variables were compared with baseline values. Data are mean ± SEM. *P < 0.05 vs. baseline. †P < 0.01 vs. baseline. ‡P < 0.001 vs. baseline.

**Discussion**

In this experimental study, we report for the first time that regadenoson (5 μg kg⁻¹ bolus over 10 s) can be used as vasodilator stress agent with SonoVue ultrasound contrast microbubbles to...
<table>
<thead>
<tr>
<th>% Change from baseline</th>
<th>Minutes after regadenoson injection</th>
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<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td><strong>Myocardial blood volume</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20 ± 13</td>
</tr>
<tr>
<td>Mild non-flow limiting stenosis</td>
<td>−5 ± 12</td>
</tr>
<tr>
<td>Control</td>
<td>−13 ± 14</td>
</tr>
<tr>
<td>Moderate non-flow limiting stenosis</td>
<td>−37 ± 14</td>
</tr>
<tr>
<td><strong>Myocardial blood flow velocity</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>80 ± 24</td>
</tr>
<tr>
<td>Mild non-flow limiting stenosis</td>
<td>21 ± 40</td>
</tr>
<tr>
<td>Control</td>
<td>36 ± 50</td>
</tr>
<tr>
<td>Moderate non-flow limiting stenosis</td>
<td>20 ± 20</td>
</tr>
<tr>
<td><strong>Myocardial blood flow</strong></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>118 ± 35</td>
</tr>
<tr>
<td>Mild non-flow limiting stenosis</td>
<td>14 ± 46</td>
</tr>
<tr>
<td>Control</td>
<td>7 ± 24</td>
</tr>
<tr>
<td>Moderate non-flow limiting stenosis</td>
<td>0 ± 36</td>
</tr>
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</table>

*P < 0.05 control vs. stenosis.
detect the presence of a non-critical coronary stenosis. The duration of haemodynamic changes associated with regadenoson was 10 min, which should allow sufficient time to acquire myocardial contrast echocardiography images. Dysrhythmias were not observed at any time during low and high mechanical index ultrasound pulse exposure in the presence of regadenoson and SonoVue. Mild and moderate coronary stenoses were both visually and quantitatively detected when myocardial contrast echocardiography was performed with regadenoson. The 5 mg kg\(^{-1}\) dose of regadenoson used in this study is comparable with the human fixed dose of 400 mg, and the 10 s bolus injection used in this study is also the recommended means of administration during myocardial perfusion imaging in clinical studies.

Haemodynamic profile of regadenoson and its effects in the presence of microbubble contrast

Regadenoson injection was followed by an increase in heart rate in the closed-chest but not the open-chest dogs. Previous studies have also noted a period of regadenoson-induced tachycardia in closed-chest preparations, but these studies were conducted in a chronic setting where the animals were allowed to recover for up to 10 to 14 days after surgery and studied without sedation or anaesthesia.\(^6,27\) This tachycardia response was also observed in patients\(^8,28\) and is believed to be due to both a direct sympathomimetic effect of the A\(_{2A}\) receptor activation and baroreflex response following peripheral dilation. The latter pathway has also previously been reported with intravenous administration of adenosine and dipyridamole.\(^28,29\)

In open-chest dogs, we did not observe an increase in heart rate even though the same anaesthesia was used and the same relative reduction in blood pressure was noted immediately after injection of regadenoson. This paradox was previously reported where in conscious closed-chest dogs volume loading increased heart rate and not stroke volume, but in the anaesthetized open-chest dogs, the opposite occurred.\(^30\) We believe that in our study, a lack of chronotropic response in the open-chest model was probably associated with an absent pericardial constraint and release of intra-thoracic pressure.

Coronary blood flow increased immediately after regadenoson injection and remained elevated during the subsequent 30 min. This time course is consistent with previous experimental\(^6,27\) and clinical observations.\(^8,31\) The magnitude of increase, however,
was variable and coronary artery-dependent. In addition, it was also attenuated proportional to stenosis severity. As expected, regadenoson did not increase either right or left atrial pressure significantly. These two parameters have not been measured previously. We also did not find any significant change in maximum left ventricular dP/dt, contrary to a previous report, which was probably due to the absence of a sympathetic reflex response to the decrease of blood pressure in our open-chest dogs.

During the entire experimental protocol, no arrhythmias of any kind were noted. This is contrary to the findings of previous studies in rodents and humans where premature ventricular contractions were noted with the use of microbubbles during exposure to ultrasound.22–24 In all of these previous studies, however, high mechanical index and microbubbles whose composition is different than SonoVue were used. Lack of arrhythmia in our study further supports the lower adverse effects profile of the selective A2A-adenosine agonist regadenoson relative to adenosine, and specifically the reduced risk of atrioventricular nodal block mediated by the A1 receptor. Furthermore, regadenoson has no effect on the QT interval, which virtually eliminates the potential for development of torsades de pointe.25 To the best of our knowledge, the clinical studies have not noted any arrhythmias, although a very small number of patients in the ADVANCE phase 3 multicentre international trial did report palpitations.9

The effect of regadenoson on normal myocardium

In the absence of a stenosis, regadenoson increased epicardial coronary blood flow and corresponding myocardial blood flow by approximately 2.5-fold. The increase in myocardial blood flow was mediated predominantly by an increase in myocardial blood flow.
velocity and, to a smaller extent, myocardial blood volume. These changes were visually observed in real-time and were confirmed with quantitative analysis. Wall thickening also increased significantly immediately in the presence of regadenoson, which has also been reported with adenosine and dipyridamole.\(^8,29\) Changes in myocardial blood volume were greater in the open chest compared with closed chest despite an absence of positive chronotropic response and is most likely due to soft-tissue attenuation when imaging a closed-chest animal. It is unlikely to be due to a difference in wall thickening or myocardial demand because the improved wall thickening was similar in both experimental conditions.

**Detection of coronary artery stenosis**

In this study, we have shown that regadenoson could be used to easily and quickly detect the presence of a non-flow-limiting coronary artery stenosis both qualitatively and quantitatively. Clinically, we anticipate that images from all views could be acquired within 10 min of injection of regadenoson by an experienced sonographer, after which, the drug action could be terminated with an injection of aminophylline only if the patient develops intolerable side effects. Since regadenoson is associated with fewer side effects,\(^3,8–10\) it is expected that symptom-guided reversal with aminophylline would be used even less often than 17% of the time, which is the rate associated with dipyridamole.\(^3\)

During the first 10 min, changes in coronary blood flow were sustained at the maximum level and thus provided the highest sensitivity for perfusion defects detection. The relative percent change in myocardial blood volume from baseline averaged 0.5–0.75-fold higher in the control region, whereas it was negligible in both the mild and moderately stenosed regions. This differential change in myocardial blood volume in the presence of the vasodilator accounts for the ability to detect the perfusion defect in the stenosed region and can be easily appreciated both on the end-systolic still frames depicted in Figure 5A and in real-time on the accompanying videos. Of course, the more severe the stenosis, the more obvious the perfusion defect will be and the earlier it will appear after the flash frame. In the presence of mild stenosis, this stress modality will increase the sensitivity of detection by displaying a perfusion defect in the absence of regional wall motion abnormality.\(^16,36\) In the presence of a more severe stenosis, interpretation of both function and perfusion data simultaneously will increase the specificity since during stress abnormal wall motion does not occur without abnormal perfusion.

**Clinical implication**

The current study was performed in dogs and similar studies need to be performed in humans to test the value of regadenoson myocardial contrast echocardiography. Furthermore, the sensitivity and specificity of the test in humans will unlikely be as good as in open chest dogs because of limitations such as artefacts, patient breathing, obesity, and performance skills of the echocardiographer.

*In vitro* studies indicate that despite possessing relatively low affinity for the A\(_{2A}\) adenosine receptor, regadenoson produced coronary vasodilation that is equivalent in magnitude to high-affinity agonists and has the advantage of more rapid termination of effects.\(^37\) Clinical studies have confirmed this finding.\(^8,9\) Similar to single photon emission computed tomography imaging, we anticipate that regadenoson will be equivalent to adenosine or dipyridamole when used with myocardial contrast echocardiography to evaluate patients for reversible perfusion defects when they present with chest pain that is suspicious for obstructive coronary artery disease. In our study, there were no regional wall motion abnormalities despite obvious perfusion defects in mild non-flow limiting stenosis. The additional benefit of using regadenoson with real-time myocardial contrast echocardiography is that it may permit detection of less severe but clinically relevant coronary artery disease because it provides myocardial perfusion data. Furthermore, the combination of both perfusion and function data has been shown to be superior for providing prognostic information when compared with single photon emission computed tomography imaging\(^38\) and supine bicycle stress echocardiography alone.\(^39\) Other major advantages of using regadenoson-stress myocardial contrast echocardiography over single photon emission computed tomography imaging are that it eliminates ionizing radiation exposure, is portable, and can potentially decrease downstream personnel and resource costs. Regadenoson like adenosine and dipyridamole may also offer an advantage over dobutamine because of less cardiac translation and respiratory artefacts.

Finally, even though we used Sonovue\(^46\) in our study, we anticipate that our findings will be equally applicable when used with other commercially available ultrasound contrast microbubbles. The ultrasound machines are now equipped with standard low-mechanical index contrast software packages, and full assessment can be obtained without further post-processing. If desired, available commercial quantification software can also be used for quantitative analysis.

**Supplementary data**

Supplementary data are available at *European Heart Journal - Cardiovascular Imaging* online.

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**Conflict of interest:** none declared.

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