Contrast echocardiography reveals apparently normal coronary perfusion in a rat model of stress-induced (Takotsubo) cardiomyopathy

Bjorn Redfors1,2†*, Yangzhen Shao1†, Johannes Wikström3, Alexander R. Lyon4, Anders Oldfors5, Li-Ming Gan3, and Elmir Omerovic1,2

1Department of Molecular and Clinical Medicine, Sahlgrenska Academy at University of Gothenburg, Bruna sträket 16, SE 413 45 Gothenburg, Sweden; 2Division of Cardiology, Sahlgrenska University Hospital, Gothenburg, Sweden; 3Bioscience, Astra Zeneca Research and Development, Mölndal, Sweden; 4Cardiovascular Biomedical Research Unit, Royal Brompton Hospital, London, UK; and 5Department of Pathology, Sahlgrenska Academy at University of Gothenburg, Gothenburg, Sweden

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Aims
Stress-induced cardiomyopathy (SIC) is an important differential diagnosis to acute myocardial infarction (AMI) that is associated with significant morbidity and mortality. The typical hallmark of SIC is left-ventricular apical akinesia but preserved function in basal segments. Catecholamines are postulated to play an important role in SIC but the precise pathophysiology is incompletely understood. Whether myocardial perfusion of the affected segments is impaired in SIC has been debated and remains unknown.

Methods and results
Myocardial contrast echocardiography (MCE) was used to study regional myocardial perfusion in a rat model of SIC. Twelve rats received 50 mg/kg isoproterenol (ISO) i.p. and were continuously monitored by MCE. Apical and basal perfusion were estimated and expressed as a ratio at baseline, 5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min post-ISO. The rats developed typical apical ballooning after 43 ± 9 min post-ISO injection. The ratio of apical:basal perfusion was close to 1.00 at all time-points and never dropped below 0.89 (95% CI never extended below 0.73). Light and electron microcoscopical investigation revealed no structural damage of myocardial vessels.

Conclusion
Apical perfusion is not impaired in the early phase of SIC in this rat model.

Keywords
Animal models of cardiovascular disease • Catecholamines • Echocardiography • Tako-tsubo syndrome

Introduction
Stress-induced cardiomyopathy (SIC), also known as Takotsubo cardiomyopathy, is an increasingly recognized syndrome and an important differential diagnosis to acute myocardial infarction (AMI). SIC is characterized by severe regional myocardial akinesia, often but not always involving apical segments, in the absence of a coronary lesion that can explain the extent of cardiac dysfunction.1 While the prognosis of SIC is perceived to be excellent, and is indeed favourable when patients with STEMI secondly to coronary occlusion are the comparator, recent data indicate that the syndrome is associated with significant morbidity and mortality during the acute phase.2

The typical morphological presentation of SIC is LV apical dysfunction with characteristic virtual ‘ballooning’ of the apex during systole.3 SIC patients display similar clinical signs and symptoms as patients with AMI and as yet no laboratory or non-invasive diagnostic modality is able to reliably differentiate between the two conditions. Consequently, many SIC patients undergo coronary angiography as a necessary part of the diagnostic work-up in order to exclude acute coronary syndrome. Plasma levels of catecholamines are severely elevated in SIC and are postulated to play an important role in SIC.4 Beyond adrenergic overstimulation, however, the underlying pathophysiology is incompletely understood. Among the proposed mechanisms leading to cardiac dysfunction are direct catecholamine-mediated myocardial toxicity and catecholamine-mediated coronary

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1 Contributed equally.

* Corresponding author. Tel.: +46 31 343 7560; Fax: +46 31 823762; Email: bjorn.redfors@wlab.gu.se

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artery spasm. Some authors have also suggested that the cardiac dysfunction observed in SIC occur due to transient coronary artery occlusion caused by a spontaneously dissolved thrombus, although subsequent studies using intravascular ultrasound in SIC patients have failed to detect atherosclerotic plaque or thrombus. According to the latter two hypotheses, cardiac dysfunction would be caused by impaired myocardial perfusion. Whether or not myocardial perfusion is impaired in SIC has been debated and remains unproved. Although several attempts have been made to study perfusion in SIC patients at different time-points after the development of cardiac dysfunction, no study has addressed the temporal pattern of perfusion in the development of SIC.

We have recently developed a rat model of catecholamine-induced SIC-like cardiac dysfunction that mimics the most important findings in SIC, including typical LV apical ballooning, electrocardiographic changes, perturbations in myocardial metabolism, and recovery of cardiac function. This small animal model allows for serial assessment of myocardial perfusion from the time of catecholamine administration until SIC-like cardiac dysfunction is fully developed. Myocardial contrast echocardiography (MCE) can reliably quantify myocardial blood flow and detect impairments in perfusion in the rat.

The aim of this study was to decipher whether or not perfusion is impaired in the affected, i.e. akinetic, regions of the myocardium.

**Methods**

All animal work was performed in accordance with the NIH guidelines for the use of experimental animals and the study protocol was approved by the Animal Ethics Committee at the University of Gothenburg. Sprague Dawley rats weighing $300 \pm 12$ g were used in this study. The rats were housed in a temperature-controlled ($25^\circ$C) facility with a 12 h light/dark cycle and given access to food and water. A total of 20 rats were used for MCE and 18 rats were used for assessment of cardiac function. Thirty-two rats received 50 mg/kg isoproterenol (ISO) intraperitoneally, a protocol that induces reproducible Takotsubo-like cardiac dysfunction in two rats received 50 mg/kg isoproterenol (ISO) intraperitoneally, a protocol that induces reproducible Takotsubo-like cardiac dysfunction in two immediately consecutive recordings (Fig. 1).

The remaining six rats were given saline and cardiac function was compared with ISO rats.

The rats were anaesthetized by i.p. administration of ketamine/midazolam ($50$ and $5$ mg/kg, respectively) and placed on a heating pad set to maintain $38^\circ$C. The right jugular vein was dissected free and cannulated. Baseline myocardial perfusion was assessed by contrast echocardiography and ISO ($50$ mg/kg) was then injected i.p. Contrast echocardiography was repeated at $5$, $10$, $20$, $30$, $40$, $50$, $60$, $70$, $80$, and 90 min post-ISO administration.

**Myocardial contrast imaging**

MCE was performed using a $15$ MHz linear transducer (15L8) connected to an echocardiographic system (Acuson Sequoia C512, Siemens Medical Solutions, Mountain View, CA, USA). All recordings were stored and analysed offline using a dedicated software analysis program (Research Arena 1.2.2.50, TomTec Imaging Systems, Unterschleibheim, Germany).

The chest was shaved and hair removal gel was applied to prepare for echocardiography. The paws were connected to an ECG. An optimal parasternal long-axis view, i.e. visualization of the aortic and mitral valves and maximal LV area, was achieved. The probe was then fixed in the optimal position for the remainder of the echocardiographic study. ECG-gated intermittent triggered imaging was performed. Appropriate triggering, i.e. identical position in the cardiac cycle in each image, was ensured. Before contrast was injected, the gain was adjusted to obtain images in which the myocardium was without signal. Contrast agent (SonoVue, Bracco International, 8 $\mu$L/mL, $10$ mL/min) was then infused. At the start of each recording, the contrast bubbles were cleared from the myocardial blood pool by briefly switching to high-energy power Doppler mode (mechanical index $>1.5$). Reappearance of the bubble contrast was recorded and analysed as the measure of perfusion for each myocardial segment. At least two recordings were made at each time point in each rat. Between each contrast imaging time-point, real-time B-mode echocardiography was carefully studied to detect the time of development of LV apical akinesia.

Images were analysed by TOMTEC software (Research-Arena 1.2.2.50). Two myocardial regions of interest (ROIs), one apical and one basal, were selected in the anterior wall. Real-time cine loops were carefully studied before selection of the ROIs in order to ensure placement of the apical ROI in a myocardial region that would develop akinesia and placement of the basal ROI in a region that remained unaffected. Myocardial blood flow (MBF) in the apex (akinetic part) relative to the base (normokinetic part) was expressed as a ratio. The formula used for calculation is given below.

$$A_{\text{apex}} \times \beta_{\text{apex}} / A_{\text{base}} \times \beta_{\text{base}}$$

where $\beta$ is the blood exchange frequency and $A$ is the myocardial plateau intensity for the respective ROI (Fig. 1). The parameters $A$ and $\beta$ were obtained from the refill curve signal intensity $y$ by the previously validated and widely used function: $y(t) = A 	imes (1 - e^{-t/\beta})$.

Some authors have argued that inclusion of LV luminal ROIs in the estimation of myocardial perfusion improves accuracy and compensates for regional ultrasonic beam inhomogeneities and contrast shadowing. Two additional ROIs were therefore placed in the LV lumen immediately below the respective myocardial ROIs and an alternative estimation of apical to basal MBF was calculated as $A_{\text{apex}} / (A_{\text{apex}} + A_{\text{base}}) \times \beta_{\text{apex}} / \beta_{\text{base}}$, where $A_{\text{apex}}$ and $A_{\text{base}}$ are the plateau intensity of the luminal ROIs (Figure 1).

**Reproducibility**

Relative error of measurement was calculated for $A$, $\beta$, and MBF. The same recording was evaluated at two separate time-points ($n = 8$) and difference between the measurements was expressed as the percentage of the mean of the two measurements.

Reproducibility of the MBF estimate was also estimated by calculation of apical to basal flow ratio in two immediately consecutive recordings ($n = 4$). Again, the difference between the measurements was expressed as the percentage of the mean of the two measurements.

**Assessment of cardiac function**

Echocardiography was performed 90 min post-ISO using a VisualSonics 770 VEVO imaging station, which includes an integrated rail system for consistent positioning of the ultrasound probe. The hair from the chest was removed with an electrical clipper and a hair removal gel prior to the examination. The animals were placed on a heating pad and connected to an ECG while rectal temperature was monitored. A 35 MHz linear transducer (RMV 707) was used for imaging. An optimal parasternal long-axis (LAX) cine loop (i.e. visualization of both the mitral and aortic valves, and maximum distance between the aortic valve and the cardiac apex) was acquired. The extent of akinesia was traced in the long axis and expressed as the percentage of total LV endocardial length. Stroke volume (SV) and cardiac output (CO) was calculated according to conventional formulas.
Histology
Morphological investigations of base and apex of hearts were performed in four rats 1, 5, or 4 h after intraperitoneal isoproterenol injection and one untreated control. Specimens were either snap frozen in liquid nitrogen and subjected to cryostat sectioning followed by staining with haematoxylin and eosin or fixed in buffered glutaraldehyde and processed for electron microscopy using standard methods.

Statistics
STATA software was used for statistical analyses. 95% confidence intervals for the ratio of apical to basal myocardial perfusion at each time-point were calculated as log(MBFapex/MBFbase). Normal plot and Kolmogorov–Smirnov test was used to confirm the appropriateness of assuming normal distribution of the log10 transformed data. Student’s t-test was used to compare indices of cardiac function between control rats and rats that received ISO. Data are expressed as mean ± SD.

Results
All rats developed sustained tachycardia (>500 bpm), hair erection, and mucous hypersalivation. Out of the 20 rats that underwent CME, four rats died within 90 min post-ISO. Three of these rats showed progressive worsening of cardiac contractile function with bradycardia and death, whereas one rat developed fatal ventricular fibrillation. Fourteen rats developed typical LV apical ballooning (Figure 2, Supplementary data online, Video S1) with preserved basal function. Of these, two rats died before 90 min. Complete echocardiography studies from the surviving 12 rats were analysed.

Among the 12 rats that were given ISO and in which more detailed assessment of cardiac function was performed, two rats died. No rat died in the group that received saline (n = 6).

Cardiac function
All rats developed varying extent of akinesia involving the left-ventricular apical myocardium with typical LV apical ballooning during systole. Ejection fraction and stroke volume were decreased compared with control rats but cardiac output was maintained near normal (Table 1).

Apparently normal myocardial perfusion post-isoprenaline
Akinesia could first be detected at 43 ± 9 min following intraperitoneal ISO administration and had reached its full extent at 86 ± 12 min post-ISO.

Goodness of fit for the function $y(t) = A \times (1 - e^{-\beta t})$ was always $> 0.8$ (mean 0.94). Apical to basal MBF ratio was similar at all time-points and 95% confidence intervals included 1.00 at all times (Figure 3, Supplementary data online, Video S1). When LV luminal ROIs were included in the calculation, MBF ratio increased but still remained similar at each time-point.

Reproducibility
Indices of reproducibility were as follows. The relative errors of measurement were 1.82% for A, 9.45% for $\beta$, and 8.33% for MBF ($A \times \beta$). The apical-to-basal MBF ratio measurement error was
5.47% when measured in the same recording and 7.93% when measured in two immediately consecutive recordings.

**Histology**

Light and electron microscopical investigation revealed no structural damage of myocardial vessels (Figure 4).

**Discussion**

The main finding of this study is that myocardial perfusion defects do not precede development of SIC-like apical akinesia and that myocardial perfusion initially remains normal in akinetic segments.

**Table 1.**  Echocardiographic indices of cardiac function in isoproterenol vs. control rats

<table>
<thead>
<tr>
<th>Echocardiographic parameters of cardiac function in rats that developed SIC-like cardiac dysfunction</th>
<th>SIC (n = 10)</th>
<th>Control (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>328 ± 17</td>
<td>329 ± 9</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>605 ± 10</td>
<td>413 ± 19</td>
</tr>
<tr>
<td>Akiniesia (% of left ventricle)</td>
<td>30.58 ± 2.94</td>
<td>—</td>
</tr>
<tr>
<td>End-diastolic volume (µL)</td>
<td>288.92 ± 48.77</td>
<td>287.58 ± 28.75</td>
</tr>
<tr>
<td>End-systolic volume (µL)</td>
<td>133.89 ± 49.22*</td>
<td>42.91 ± 22.22</td>
</tr>
<tr>
<td>Stroke volume (µL)</td>
<td>155.03 ± 45.94*</td>
<td>244.67 ± 16.58</td>
</tr>
<tr>
<td>Cardiac output (mL)</td>
<td>93.44 ± 26.73</td>
<td>101.28 ± 9.30</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>53.77 ± 14*</td>
<td>85.49 ± 7.14</td>
</tr>
</tbody>
</table>

Rats that received isoproterenol developed tachycardia and left-ventricular apical akinesia that involved one-third of the left ventricle. Estimates of stroke volume and ejection fraction were significantly decreased in these rats, whereas estimated cardiac output was similar.

*P < 0.05 vs. control.

SIC is an acute cardiac syndrome characterized by extensive regional LV dysfunction that frequently extends beyond the territory of a single coronary artery and that may lead to acute heart failure, cardiogenic shock, malignant arrhythmias, LV rupture, and death. The absence of culprit obstructive coronary lesions in SIC patients has led many researchers to abandon the idea that SIC is an occlusive coronary syndrome, whereas others propose that a spontaneously dissolving coronary thrombus causes widespread but transient diffuse myocardial ischemia (DAMI) and the classical term "Takotsubo cardiomyopathy" is not impaired in Takotsubo cardiomyopathy.

**Figure 2** End-systolic images of SIC-like left-ventricular ballooning in the rat. Representative B-mode images without (A) and with (B) contrast from two different rats obtained in the parasternal long axis at end-systole. Typical apical ballooning with preserved basal function. AA, anterior apex; AB, anterior base; Ao, aorta; PA, posterior apex; PB, posterior base; LA, left atrium.

**Figure 3** Ratio of apical-to-basal myocardial blood flow (MBF) after 50 mg/kg i.p. isoprenaline (ISO). Myocardial contrast echocardiography (MCE)-derived indices of apical and basal perfusion were obtained before ISO (time 0) and 5, 10, 20, 30, 40, 50, 60, 70, 80, and 90 min post-ISO. Development of regional LV apical akinesia was observed at 43 ± 9 min post-ISO (arrow). 95% confidence intervals include 1, i.e. equal perfusion in the dysfunctional apical region and in the normally functioning basal region, at all time-points and never decreased below 0.73.
regional ischaemia. Involvement of apical regions that extend beyond the territory that is typically supplied by the left-anterior coronary artery (LAD) has been explained by the presence of a variant coronary pattern in SIC, a so-called ‘wrap around LAD’ that supplies also the inferior part of the cardiac apex. However, recent data suggest that ‘wrap around LAD’ is no more common in SIC patients than it is in the general population and is therefore an unlikely explanation.16

Some researchers believe that catecholamine-mediated single- or multivessel spasm and/or microvascular dysfunction causes impaired perfusion and ischaemia in SIC.5 Contrast echocardiography and single photon emission computed tomography (SPECT) have been used to assess myocardial perfusion in the clinical setting. Some studies have shown various degrees of impaired perfusion in the apical myocardium when studied within the first week after presentation, whereas others have reported normal MBF.9,17–22 However, these studies also show that metabolic activity is decreased in the akinetic areas to a much greater extent than is perfusion.20,21 One could therefore speculate that the lower perfusion observed in this setting is secondary to reduced metabolic demand of the tissue, and/or reduced myocardial relaxation velocity critical for diastolic coronary flow. Several magnetic resonance imaging (MRI) studies have shown that SIC can be associated with reversible swelling and oedema formation in the affected regions.10,23 Cellular damage and swelling could cause various degrees of microvascular obstruction.24 Also in this scenario, any detectable perfusion defects could occur secondary to cardiac dysfunction and/or damage with subsequent cellular swelling, oedema, and microvascular obstruction. Attempts to address the question of whether or not impaired perfusion is an important factor in the development of SIC in the clinical setting are limited by the issue that perfusion is assessed after cardiac dysfunction has already developed. It is thus difficult, if not impossible, to infer causality from such observations.

In the present study, the ratio of apical to basal perfusion was stable near 1.00 with 95% confidence intervals never extending below 0.73 at any time-point. Reproducibility was good with acceptable intra-observer variability and small differences in MBF ratios derived from two consecutive recordings. Previous studies have shown good correlation between MCE and MBF derived by fluorescent microsphere injection. The correlation between MCE and microsphere-derived MBF was moderate for absolute values of MBF but was particularly strong for MBF ratios between two ROIs.12 In a rat model with partial occlusion of the left-anterior descending coronary artery MCE-derived MBF ratios between the area at risk, i.e. the myocardium perfused by the partially occluded artery, and the remote area was significantly decreased, whereas no differences could be observed in conventional echocardiographic parameters.12 In their model, the authors reported no wall motion abnormalities or significant functional impairments despite an average area-at-risk to remote area MBF ratio of 0.64.12 In our model, we observe an akinetic apex but an MBF ratio between 0.89 and 1.02 (95% confidence interval never extended below 0.73). Taken together, these observations indicate that apical MBF, as assessed by MCE, is not sufficiently impaired in our model to explain the degree of regional dysfunction.

The mean time to development of cardiac dysfunction was 43 ± 9 min, and we believe the 90 min time interval would have been sufficient to detect any potential causative perfusion defects. We therefore interpret our data to support the hypothesis that catecholamines cause regional cardiodepression in the setting of SIC through mechanisms that do not primarily involve microvascular impairment or coronary artery spasm.

Taken together, the results obtained in this study suggest that absolute or relative perfusion deficiency, i.e. ischaemia, is not the underlying cause of SIC. An alternative hypothesis is that catecholamines...
act directly on the cardiomyocyte. Among the proposed mechanisms by which catecholamine depress cardiomyocyte function are calcium overload, β2-adrenoreceptor-mediated inhibition of the contractile apparatus, and oxidative stress caused by reactive catecholamine-metabolites.\(^{25,26}\) We (A.R.L.) have previously proposed a primary mechanism based upon β2-adrenoreceptor stimulus trafficking to the Gi protein signalling pathway in response to high epinephrine levels, and the findings of this study would be in keeping with this hypothesis.\(^{6,27}\) We have also recently shown that lipid metabolism is altered in the dysfunctional myocardium but not in normally contracting regions of the heart in both SIC patients and rats.\(^{17}\) Further studies should address whether catecholamine-induced metabolic alterations are the cause or the consequence of contractile dysfunction in SIC.

In conclusion, no detectable perfusion defects precede development of cardiac SIC-like dysfunction in the rat. These data indicate that myocardial ischaemia is not the primary pathological substrate causing SIC, and the mildly to moderately impaired perfusion that is sometimes observed in SIC patients is the consequence rather than the cause of cardiac damage and/or dysfunction.

**Supplementary data**

Supplementary data are available at *European Journal of Echocardiography* online.

**Conflict of interest:** None declared.

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**References**