Examining the relationship between exercise tolerance and isoproterenol-based cardiac reserve in murine models of heart failure

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Richards DA, Bao W, Rambo MV, Burgert M, Jucker BM, Lenhard SC. Examining the relationship between exercise tolerance and isoproterenol-based cardiac reserve in murine models of heart failure. J Appl Physiol 114: 1202–1210, 2013. First published February 28, 2013; doi:10.1152/japplphysiol.00556.2012.—The loss of cardiac reserve is, in part, responsible for exercise intolerance in late-stage heart failure (HF). Exercise tolerance testing (ETT) has been performed in mouse models of HF; however, treadmill performance and at-rest cardiac indexes determined by magnetic resonance imaging (MRI) rarely correlate. The present study adopted a stress-MRI technique for comparison with ETT in HF models, using isoproterenol (ISO) to evoke cardiac reserve responses. Male C57BL/6J mice were randomly subjected to myocardial infarction (MI), transverse aortic constriction (TAC), or sham surgery under general anesthesia. Mice underwent serial ETT on a graded treadmill with follow-up ISO stress-MRI. TAC mice showed consistent exercise intolerance, with a 16.2% reduction in peak oxygen consumption vs. sham at 15-wk postsurgery (WPS). MI and sham mice had similar peak oxygen consumption from 7 WPS onward. Time to a respiratory exchange ratio of 1.0 correlated with ETT distance (r = 0.64; P < 0.001). The change in ejection fraction under ISO stress was reduced in HF mice at 4 WPS [10.1 ± 3.9% change (Δ) and 8.9 ± 3.5%Δ in MI and TAC, respectively, compared with 32.0 ± 3.5%Δ in sham; P < 0.001]. However, cardiac reserve differences between surgery groups were not observed at 16 WPS in terms of ejection fraction or cardiac output. In addition, ETT did not correlate with cardiac indexes under ISO stress. In conclusion, ISO stress was unable to reflect consistent differences in ETT between HF and healthy mice, suggesting cardiac-specific indexes are not the sole factors in defining exercise intolerance in mouse HF models.

magnetic resonance imaging; myocardial infarction; transverse aortic constriction; treadmill

HEART FAILURE (HF) CONTINUES to be the leading cause of hospital admissions and death in American adults over 55 yr old, coinciding with over 770,000 myocardial infarction (MI) episodes per year (1). Exercise intolerance presents as one of the most debilitating symptoms of HF (29). This intolerance, triggered usually by chest pain and dyspnea, is a growing area of unmet medical need, with patients experiencing reduced independence and quality of life (6).

Mice offer a unique opportunity to study exercise capacity in vivo as they are voluntary runners, needing little encouragement (3). Previous studies have focused on comparing exercise trained mice with a sedentary cohort (33), often using transgenic HF models (13) or surgical HF models (9).

Different exercise tolerance test (ETT) protocols, as well as how murine exhaustion is defined, are two critical sources of variability in murine ETT studies. Studies often use a fixed duration of nonresponse to the shock stimulus to define exhaustion (16, 31). However, some definitions of exhaustion are more qualitative, using end points such as “the inability to continue regular treadmill running,” or simply “exhaustion” (33, 13, 24). Recently, the ability to easily record the peak oxygen consumption (\(\dot{V}_{O_2,max}\)) in mice, known in humans to predict exercise capacity and HF prognosis (14, 20), provides an additional prospective end point for ETT.

To better understand the cardiac index relationship with exercise intolerance in the research setting, cardiac magnetic resonance imaging (MRI) has been used in conjunction with mouse treadmill testing (35). However, since only a weak relationship at best exists between treadmill exercise tolerance and any given at-rest cardiac index (18), the MRI stress test is thought to be of greater relevance in determining cardiovascular function and hence is more relevant to treadmill ETT (35). Inotropic agents, such as isoproterenol (ISO) and dobutamine, activate \(\beta_1\)-adrenoceptors in the heart, evoking acute physiological adaptations, which would normally occur during exercise (35). Here, for the first time, we describe combining regular ETT with MRI stress testing in mouse models of HF. The MRI stress test allows for an estimate of cardiac reserve: the ability of the heart to improve efficiency and function to meet heightened metabolic demands (2). A loss or reduction in cardiac reserve would, therefore, be expected to translate into exercise intolerance.

Using monthly ETT and MRI stress tests, the purpose of this study was to investigate the temporal relationship between treadmill exercise performance and ISO-induced cardiac reserve in healthy and HF mice. Both an ischemic model, MI, and a pressure overload model, transverse aortic constriction (TAC), of HF were used in the present study. These are relatively acute (<3 wk to cardiac decompensation: exercise or MRI) models of HF (28, 35), but little is known of their longer term profiles (>8 wk). It was hypothesized that HF mice would show a reduced exercise capacity vs. sham controls, which would be reflected as a reduction in the cardiac reserve, as assessed by a MRI stress test.

METHODS

All procedures described were carried out following approval by the Institutional Animal Care and Use Committee of GlaxoSmithKline.

Animal study details. Sixty C57BL/6J male mice (Jackson Laboratories), 8 wk of age, were randomized numerically (1–60) and with...
a day of the week (Monday–Friday). Mice underwent all further aspects of this study on the allocated day of the week only, and the study operator was blinded to the surgery group throughout. Acclimation to the metabolic treadmill was performed in week 1. Surgery was performed at week 0: sham (n = 10), MI (n = 34), and TAC (n = 16). Mice underwent ETT during weeks 3, 7, 11, and 15, with a follow-up magnetic resonance imaging (MRI), including isoproterenol (ISO) stress test 1 wk later (Fig. 1). In the final week, 17, mice were euthanized, and left ventricular (LV) tissue was harvested for histological analysis.

**MI and TAC surgical models.** MI was induced via a ligation of the lateral anterior descending (LAD) coronary artery, as described previously (4). Briefly, following a midline sternotomy, an 8–0 silk suture was passed under the LAD artery, 1 mm below the left atrium. The suture was tied off, and correct ligation was confirmed visually by blanching of the apex. The same procedure was used for TAC, except that a 7–0 silk suture was passed under the transverse aorta between the left common carotid and left subclavian branches of the aortic arch, rather than the LAD artery. A 27G needle was placed alongside the transverse aorta, and the suture was tied snugly around the needle. The needle was removed (by sliding out), leaving the suture banding intact. The MI procedure was repeated for sham controls, but the suture was not tied and was removed from the mouse. For all procedures, the incision was closed by layers using 5–0 suture, and the endotracheal tube was removed after spontaneous breathing was regained. Perioperative survival rates (within 24 h following surgery) were high, with death only occurring in two TAC mice.

**Exercise tolerance testing.** All mouse exercise procedures were aimed to be conducted in the morning between 8 AM and 12 PM, with mice running in the same order each time to control for metabolic time-of-day variations. Due to logistics, however, there were five afternoon start times, with the latest being 1:53 PM. Mice were acclimated to the treadmill (1012M-1-E52 Modular Enclosed Metabolic Treadmill, Columbus Instruments, Columbus, OH) on 1 day in week 1, at 10 m/min speed, with a 10° incline for 10 min. An electric shock stimulus was provided by a nonmoving plate located at the rear of the treadmill, operating at an intensity of 1.5 mA (setting 10.0). Within 1–2 min, all mice were found to comply with the protocol and very rarely fell back onto the stimulus plate during the 10-min acclimation period. Before ETTs, treadmill chambers were calibrated in the presence of mice on the treadmill. With the treadmill still inactive, mice were left undisturbed for 30 min, during which mice remained idle or testing under these baseline conditions. Exercise was started after 30 min of baseline, with the acclimation protocol, but, after 5 min, the speed was increased by 2 m/min every 2 min to a 40 m/min maximum, based in part on a previous study (25). A 1-min cycle/sample time was used for the recording of respiratory gases for oxygen consumption (V(02)), VO2max, and time to respiratory exchange ratio (RER) = 1.0, via the Oxymax Equal Flow system (Oxymax Windows V3.23, Columbus Instruments, Columbus, OH). Maximum exercise tolerance and, therefore, exhaustion were determined when the mouse resided on the stimulus plate for 5 s, thereby receiving five consecutive shocks and immediately being removed from the treadmill.

For the measurement of blood lactate concentration ([La]), mice were subjected to a tail snip before an ETT (baseline [La]), and a single drop of blood was analyzed. The resulting blood drop (at least 7 µl) was analyzed with the Lactate Plus Meter (Nova Biomedical). Blood clotting was initiated with Styptic Powder (Kwik Stop) before the animal was returned to its cage. Removal of the clot by hand was used to retest blood lactate following ETTs (postexercise [La]).

**MRI and stress testing.** MRI was conducted 1 wk after each ETT to allow for rest and recovery. Mice were weighed and then anesthetized in a rodent chamber using 2–3% isoflurane-medical air mixture (1 l/min flow rate) for 3–4 min. Mice were placed in the patient cradle (2.8-cm internal diameter) and transferred to the magnet (9.4 T Bruker UltraShield vertical magnet, Bruker, Billerica, MA) when fully anesthetized. During the imaging sequence, respiration was monitored and recorded every 10 min (SA Instruments, Stony Brook, NY). Anesthesia was reduced to 1.5–2.5% isoflurane during imaging, to achieve a respiratory rate of 85 ± 20 breaths/min. A tripilot Intragate FLASH scout image was acquired using the following parameters; (echo time/repetition time = 1.2/267.5 ms, field of view = 40 mm × 40 mm, no. of repetitions = 10, 128 matrix, slice thickness = 1 mm, 312 µm in plane resolution, 10 slices/orientation, total acquisition time = 86 s). A coronal long-axis image was acquired using the following parameters; (echo time/repetition time = 1.8/6.85 ms, field of view = 25 mm × 25 mm, no. of repetitions = 80, 128 matrix, slice thickness 1 mm, 195 µm in plane resolution, total acquisition time = 74 s). A series of 2-mm-thick short-axis (axial) images were acquired through the entire LV of the heart, using the same parameters as long-axis images (19). Following baseline imaging, the probe was removed from the magnet. Mice were given 10 ml/kg body wt (ip) of a 0.1 mg/ml isoproterenol bitartrate (Sigma, St. Louis, MO) stock solution (1 mg/kg dose) while still in the patient cradle. Mice were immediately placed back into the magnet and reimaged as per baseline procedures, such that the between-scan interval (baseline to post-ISO) time was <2 min. This stress imaging protocol was adapted from Wiesmann and colleagues (35). Heart rate (HR) data was manually reconstructed (ParaVision 4.0, Bruker, MA), achieving HRs of 384.4 ± 10.5 and 506.8 ± 8.8 beats/min for baseline and post-ISO, respectively, at week 4. Images of the LV were analyzed using Analyze (AnalyzeDirect, Overland Park, KS) to obtain end-systolic volume (ESV), end-diastolic volume (EDV), and LV volume, from which LV mass, stroke volume (SV), ejection fraction (%EF) and cardiac output (CO; via multiplication with HR) parameters were calculated.

Terminal hemodynamic measurements were conducted using dobutamine. Mice were anesthetized with isoflurane (2% in oxygen), and LV contractile function was determined using Millar Mikro-tip catheter, as our laboratory previously reported (7). After baseline hemodynamic measurement, mice were administered dobutamine at a dose of 1 µg·mL−1·min−1 by intravenous infusion through the right jugular vein for 5 min to determine the cardiac contractile reserve in response to the dobutamine challenge; the difference in maximal change in pressure during a 1-s interval (dP/dt max) between dobutamine challenge and baseline (dP/dt max) was calculated.

**Histology.** In week 17, mice were euthanized by inferior vena cava exsanguination under 3% isoflurane anesthesia. LV tissue was isolated and frozen in liquid nitrogen and stored at −80°C. LV tissues were embedded using OCT cryostat embedding medium (Sakura-Finetek, Dublin, OH), before being cut by a microtome in a cryostat into 6-µm sections and transferred to glass slides. An automated Masson’s Trichrome staining method was used for histological analysis.

**Data analysis.** Kaplan Meier Survival was used to analyze mortality rates following the 24-h perioperative period. Cardiac reserve was assessed as an increase in CO. %EF, HR, and SV responses to ISO were also assessed in the same way. All MRI analysis was completed using Analyze 8.1 software (AnalyzeDirect).

Data are presented as means ± SE, unless otherwise stated. The P values are not adjusted for multiple comparisons. The analysis of the
repeated-measures modeling used SAS version 9.2, and all other analyses and graphing were performed using GraphPad Prism 5.0 (GraphPad Software).

RESULTS

Survival rates for MI and TAC groups were significantly different from that of the sham group at 11.4 and 23.1%, respectively, compared with 100% (P < 0.001). Following the initial 3 wk postsurgery (3 WPS), mortality only coincided with the ISO MRI stress test in weeks 4, 12, and 16 (Fig. 2).

Serial ETT in MI and TAC mice. Following initial placement into the metabolic chamber, mice were allowed to stabilize, as confirmed by a recorded VO2 of <100 ml·kg⁻¹·min⁻¹ and an RER between 0.70 and 0.90. MI and TAC mice had a significantly reduced running distance at 3 WPS (Fig. 3A), down to 481.8 ± 40.1 m (P < 0.01) and 394.0 ± 46.4 m (and P < 0.001) for MI and TAC, respectively, vs. sham, 630.8 ± 35.9 m. Interestingly, sham mice showed a reduction in exercise capacity at 7 WPS, down to just 415.8 ± 35.9 m (P < 0.05). This reduction resulted in the shams having similar ETT distances to MI mice at 7 WPS, and thereafter there were no significant differences in either VO2 max (Fig. 3B) or time to RER = 1.0 (Fig. 3C). In contrast, TAC mice continued to show exercise intolerance vs. sham (P < 0.05) through weeks 7, 11, and 15, accompanied by a significant 16.2% reduction in VO2 max (98.2 ± 4.3 ml·kg⁻¹·min⁻¹ vs. sham 117.2 ± 3.0 ml·kg⁻¹·min⁻¹ at 15 WPS) (Fig. 3B) and a reduction in time to

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**Fig. 2.** Kaplan Meier survival curves for mice surviving beyond 24 h after surgery. Dotted lines indicate ETT and MRI. MI (11.4%) and TAC (23.1%) survival are significantly less than sham. ***P < 0.001, overall survival vs. sham.

**Fig. 3.** Sham, MI, and TAC mouse exercise capacity for monthly repeated tests. A: maximum running distance (m) determined by exhaustion on the graded ETT. B: maximum recorded oxygen consumption (VO2 max) for any 1 min during the ETT. Due to an air sampling error, VO2 max data for 3 wk postsurgery (WPS) was not included. C: TAC mice show an overall reduction in time to respiratory exchange ratio (RER) = 1.0 vs. sham mice. Data are for sham, MI, and TAC (n = 29, 20, and 15, respectively) combined from weeks 7, 11, and 15. Values are means ± SE. D: running distance showed a positive correlation with time to RER = 1.0. Data are for all groups, with an individual data point per mouse for each of weeks 7, 11, and 15. Multivariate mixed model correlation r = 0.64, P < 0.001, showing that a short time to RER = 1.0 is equivalent to a low running distance. *P < 0.05, **P < 0.01, and ***P < 0.001 vs. sham. #P < 0.05 for sham vs. 3 WPS. #P < 0.05 for MI vs. 3 WPS. E: representative RER traces for sham, MI, and TAC mice at 11 WPS. Averages values for each group are shown at 3-min intervals before and during ETT, up until removal from the treadmill due to exhaustion.
RER = 1.0 (Fig. 3C). Maximum running distance correlated with the time taken to reach an RER of 1.0 when all groups were assessed together (r = 0.64; P < 0.001) (Fig. 3D).

Baseline blood [La] averaged at 1.8–2.2 mmol without significant differences between surgery groups. A wider average range was obtained after exercise (6.6–12.1 mmol; P < 0.001), increasing significantly from baseline in all groups. The change from baseline at 3 WPS was significantly different between sham and MI (P < 0.05) and sham and TAC (P < 0.01), but there were no significant differences between groups at any later time point in the study (Fig. 2G).

MRI of LV function and morphometry. To investigate cardiac reserve, it was necessary to first acquire data for baseline cardiac indexes (Table 1).

LV mass was normalized to body weight to ensure any observed hypertrophy was cardiac specific. LV hypertrophy (LVH) occurred in MI and TAC mice from week 4 and continued to progress for the 16-wk study duration, in the absence of intergroup body weight differences (Table 1). Intermediate data (8 and 12 WPS) show the gradual progression of LVH and indicate that LVH is near maximal after 12 WPS (not shown here). At 4 WPS, there was a significant decrease in LV %EF (−49%, P < 0.001) in the MI group, confirmed further by MRI assessment of the akinetic regions of the LV during the diastole-systole transition. However, this decrease in %EF was accompanied by a significant increase in EDV (P < 0.001), ESV (P < 0.001), and SV (P < 0.05) vs. sham. By contrast, excluding LVH, TAC mice show no significant characteristics of developing HF at 4 WPS. TAC %EF is markedly reduced at 16 WPS vs. sham (P < 0.001). At 16 WPS, cardiac hypertrophy was increased by 47% and 99% for MI and TAC, respectively, vs. sham (Table 1). Examples of LV remodeling progression observed by MRI are shown in Fig. 5. Comparing 4 WPS to 16 WPS, sham mice show expected growth; MI mice show further LV chamber dilation and anterior wall thinning; and TAC mice show continued concentric hypotrophy. The extent of chamber dilation is especially evident for MI mice, who show a tripling of EDV vs. sham (P = 0.001). TAC mice show a doubling vs. sham (P < 0.05).

Fig. 4. Blood lactate concentration ([La]) for mice before and immediately after an ETT (Ex). Note that there are no intergroup differences under either baseline or exercised conditions for sham, MI, and TAC mice. The change from baseline at 3 WPS was significantly different between sham and MI (P < 0.05) and sham and TAC (P < 0.01), but there were no significant differences between groups at any later time point in the study. Values are means ± SE. Exercised blood [La] is elevated for all groups vs. baseline. ***P < 0.001.
Similar HR was recorded for sham, MI, and TAC at 4 WPS, all of which increase significantly at 16 WPS (Table 1). The SV and CO of the TAC mice were reduced compared with sham at 16 WPS. By contrast, the SV and CO of MI mice were elevated compared with sham at 16 WPS.

Cardiac MRI stress test and cardiac reserve assessment. In general, mice responded well to ISO, showing cardiovascular responses predicted to occur in exercise (i.e., increased HR and CO). Congestion was observed in some MI and TAC mice, especially if time under anesthesia was prolonged. A later analysis showed this was most common in mice with more severe LVH and reduced %EF. A total of five mice (2 MI and 3 TAC) were lost during the stress test imaging procedure, deemed to be due to the additional stress resulting from ISO administration.

While all groups showed an increase in cardiac reserve, as reflected by change in CO from baseline following ISO at 4 WPS, there were no significant differences between groups in terms of change in CO or SV from baseline (Table 2). Sham mice again showed a reduction from 4 to 16 WPS for change in CO ($P < 0.01$). However, both MI and TAC mice had a reduced %EF compared with sham in terms of change from baseline following ISO ($P < 0.001$) at 4 WPS. Sham mice underwent a reduction in %EF response to ISO from 4 to 16 WPS, coinciding with a loss of significance between groups at this later time point. The change in HR following ISO was significantly decreased for sham and MI mice, but not for TAC mice from 4 to 16 WPS.

Terminal invasive LV hemodynamics showed that TAC mice had a significantly blunted response to dobutamine (Fig. 6). Maximal average change in dP/dt was $374.0 \pm 265.3$ mmHg/s in TAC compared with sham, $2,917 \pm 819.6$ mmHg/s, $n = 3$ / group, ($P < 0.05$). MI mice showed a trend toward a reduction vs. sham, albeit not significant ($P = 0.26$).

Histology. Fig. 7 shows representative LV free wall histological sections from sham, MI, and TAC mice at 17 WPS. Using Masson’s trichrome staining, TAC mice consistently show the greatest amount of interstitial fibrosis, shown as blue staining indicated by black arrows. MI mice show less fibrosis, but still considerably more than sham mice.

ETT and MRI stress test correlation analysis. To determine the association between cardiac indexes (under ISO stress) and treadmill exercise performance, a correlation analysis was used (Table 3). Total running distance did not correlate with %EF or CO under ISO stress for any surgery group at any point from 3 to 16 WPS. Similarly, $V_{O2\max}$ did not correlate with CO under ISO stress. For MI mice, $V_{O2\max}$ and %EF under ISO stress showed a significant degree of association in both 11–12 WPS and 15–16 WPS ($P < 0.05$, $r = 0.82$, and $P < 0.01$, $r = 0.98$, respectively). Interestingly, this correlation was not seen during 7–8 WPS or in sham or TAC mice.

DISCUSSION

In this present investigation, we have shown ETT on mouse treadmill exercise test can be used to detect exercise intolerance in HF models most clearly demonstrated by the accompanying reduction in $V_{O2\max}$ in TAC mice. Importantly, due to the marked increase in blood [La] postexercise, we were confident that mice achieved their maximal exercise capacity. Our models reflect the HF condition achieved, indicated by a reduction in %EF and an increase in resting HR, known to be an early sign of HF, in MI and TAC mice vs. sham. Use of ISO stress testing further indicated HF, with reductions in cardiac reserve for MI and TAC mice compared with sham at 4 WPS. Interestingly, this relationship is lost at 16 WPS.

Exercise tolerance testing. It has been suggested repeated treadmill exercise capacity tests in rodents are not reproducible within each mouse with regard to total running time or running distance (22). Aside from uncontrolled differences between mice (e.g., physical differences, motivation to perform, feeding, or fatigue state), variability would usually arise from study operator differences. The use of the rodent ETT, however, is ongoing, with the addition of varying protocol intensities, mouse strains, and determinants of exhaustion (10, 33). Here, having acknowledged the need for the utilization of a metabolic end point as verification of exercise exhaustion, we decided on two such measures: RER = 1.0 and $V_{O2\max}$, both of which are objective in nature with a real-time readout. Due to the correlation observed between RER = 1.0 and total running distance, we believe that RER = 1.0 has the potential to be used as an objective physiological end point in future ETT. The use of a physiological end point may help to reduce the variability of observer-based subjective determination of
Table 2. ISO-evoked change in cardiac reserve for sham, MI, and TAC at 4, 8, 12, and 16 WPS

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>MI</th>
<th>TAC</th>
</tr>
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<tbody>
<tr>
<td><strong>EF, %</strong></td>
<td>32.0</td>
<td>11.0</td>
<td>10.2</td>
</tr>
<tr>
<td><strong>SV, l</strong></td>
<td>6.65</td>
<td>6.60</td>
<td>6.64</td>
</tr>
<tr>
<td><strong>HR, beats/min</strong></td>
<td>633</td>
<td>606</td>
<td>627</td>
</tr>
<tr>
<td><strong>ACO, ml/min</strong></td>
<td>8.26</td>
<td>7.81</td>
<td>7.58</td>
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</table>

Values are mean changes (Δ) from baseline MRI parameters SE on isoproterenol (ISO) administration; n, no. of mice. SV, stroke volume.

Fig. 6. LV contractility as assessed by invasive hemodynamics. TAC mice show a significantly blunted response to dobutamine, as determined by change in maximal change in pressure during a 1-s interval \( \Delta \text{dP/dt}_{\text{max}} \) during an invasive terminal procedure. \( \text{dP/dt}_{\text{max}} \) was initially determined at rest, before repeating with dobutamine infusion (1 mg/kg iv). The change in maximal response between rest and dobutamine-infused was then calculated for each mouse \( (n = 3 \text{ per group}) \). Values are means ± SE. TAC mice show a low response to dobutamine, \( *P < 0.05 \) vs. sham.

exhaustion. In the present study, most, but not all, mice achieve RER = 1.0 during the ETT. For the mice that did not achieve RER = 1.0, we believe that the ETT was stopped prematurely, and true exhaustion may not have been achieved. This may be expected when using an end point, which is influenced by animal compliance and motivation to complete the task. Figure 3E suggests that all mice are capable of achieving RER = 1.0, regardless of HF condition. Implementation of RER = 1.0 as a primary ETT end point may reduce inter- and intraoperator variability and contribute to a more robust method of assessment; however, a separate study would first need to be conducted to specifically test this hypothesis.

The significant reduction in exercise capacity observed in sham mice between 3 and 7 WPS was initially thought to be due to ETT variability. Aside from individual motivation to perform (which would be expected to average out among the number of mice), dosing with ISO was identified as the only explanation for a reduced exercise capacity, since the exhaustion criteria was always met (see METHODS), accompanied by an explanation for a reduced exercise capacity. How- ever, from 8 WPS onwards, which shows further progressive deterioration of exercise capacity, baseline HR reduces cardiac reserve and appears to be at least a contributing factor to the decreased exercise capacity. However, from 8 WPS onwards, which shows further progressive loss of cardiac reserve, sham ETT appears unchanged, indicating that ISO may have an initial, but not ongoing, effect on exercise capacity.

The similarity in exercise capacity between the sham and MI mice from 7 WPS onward is confounding. First, this finding
was unexpected and occurs in contrast to the human condition; a fair assumption is that most humans are incapable of full exercise capacity recovery following a cardiac ischemic insult, especially with reduced %EF. In addition, the recovery of exercise tolerance occurs despite the progressive, elevated LV EDV and ESV, thought to be key predictors of developing HF and, ultimately, patient death (26). A logical explanation for this could be that the amount of healthy cardiac tissue can compensate for the noncontractile ischemic tissue. This can be seen with MRI, in which basal regions of MI hearts (regions superior to the ligation site) maintain a higher %EF (≈65–80%) under basal conditions, compensating for the ischemic apical tissue. The initial sudden onset of lung congestion following MI surgery may also impede pulmonary function (4). However, lung congestion generally resolves within a few weeks, as shown by shamlike levels of \( \dot{V}\text{O}_2 \text{max} \) from 7 WPS. Natural revascularization post-MI occurs in mice, possibly mediated by cathepsin L and may have contributed to maintained exercise capacity (32). The potential role of repeated ETT may have contributed to a reduction in oxidative stress of the MI group (8). It has also been demonstrated that repeated exercise training in MI patients has improved the anti-inflammatory profile by enhancing the anti-inflammatory cytokine interleukin-10 (30). Regardless of the mechanisms associated with this return to normal exercise capacity, it can be concluded the MI model of HF is only suitable for short-term exercise assessment, perhaps in the context of reducing lung congestion.

In TAC mice, the concentric hypertrophy-mediated reduction in LV luminal diameter could be responsible for exercise incapacity. Despite shamlike CO in the first 12 WPS by MRI analysis, the actual blood flow through the stenosis in the aortic arch may be limited and impede exercise ability. In addition, suspected decreased LV compliance caused by marked interstitial fibrosis (Fig. 7) could contribute to diastolic dysfunction and inadequate LV filling (21). This would lead to a decreased CO, which, when combined with pulmonary function, is considered of high importance in adequate tissue perfusion during exercise (34). LV compliance is reported to worsen with age, supporting the progressive loss of %EF for TAC mice (5) and the reduction in contractility responses to dobutamine in the terminal hemodynamic assessment. It is important to consider also that observations could be attributed equally to repeated ISO injections, which are known to reduce LV compliance (12) and seem to have done so in sham mice.

**Development of the cardiac MRI stress test and correlation to ETT.** The MRI stress test uncovered initial differences (4 WPS) in %EF reserve between sham and MI or TAC, consistent with the literature (35). By investigating later time points, we found significant intergroup differences were not maintained. Both the ETT and MRI stress test may lead to mortality, but, in the present study, only the latter occurred. As such, we may assume that the MRI stress test, at ≥1 mg/kg ISO, is more severe than the ETT with respect to evoked cardiac reserve. This raises the question regarding whether or not the MRI stress test provides an accurate representation of the exercised condition. The lack of correlation between MRI end points with ISO-induced cardiac reserve and exercise capacity reiterates this concern (Table 3). It was anticipated that ETT components would correlate with that of cardiac MRI stress test indexes; however, data in this study indicate that parameters other than cardiac reserve dictate exercise tolerance in mice. First, the end point during ETT is determined somewhat by the individual mouse, similar to that of the cardiopulmonary exercise test in humans. In contrast, the MRI stress test will push the hearts of mice to a near maximum capacity, limited only by the efficacy of ISO. The lack of correlation between ETT and

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**Table 3. Correlation analysis between ETT parameters and MRI-derived cardiac performance indices under ISO stress**

<table>
<thead>
<tr>
<th>WPS</th>
<th>3–4</th>
<th>7–8</th>
<th>11–12</th>
<th>15–16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance vs. %EF</td>
<td>0.775</td>
<td>0.057</td>
<td>0.541</td>
<td>0.520</td>
</tr>
<tr>
<td>Sham</td>
<td>0.954</td>
<td>0.198</td>
<td>0.318</td>
<td>0.136</td>
</tr>
<tr>
<td>MI</td>
<td>0.517</td>
<td>0.975</td>
<td>0.282</td>
<td>0.215</td>
</tr>
<tr>
<td>TAC</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Distance vs. CO</td>
<td>0.396</td>
<td>0.707</td>
<td>0.960</td>
<td>0.995</td>
</tr>
<tr>
<td>Sham</td>
<td>0.990</td>
<td>0.553</td>
<td>0.521</td>
<td>0.265</td>
</tr>
<tr>
<td>MI</td>
<td>0.104</td>
<td>0.341</td>
<td>0.757</td>
<td>0.362</td>
</tr>
<tr>
<td>TAC</td>
<td></td>
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<td></td>
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<tr>
<td>( V_\text{O}_2 \text{max} ) vs. %EF</td>
<td>0.335</td>
<td>0.757</td>
<td>0.239</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0.331</td>
<td>0.025*</td>
<td>0.004*</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>0.415</td>
<td>0.306</td>
<td>0.379</td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( V_\text{O}_2 \text{max} ) vs. CO</td>
<td>0.240</td>
<td>0.506</td>
<td>0.186</td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>0.206</td>
<td>0.176</td>
<td>0.884</td>
<td></td>
</tr>
<tr>
<td>MI</td>
<td>0.942</td>
<td>0.773</td>
<td>0.231</td>
<td></td>
</tr>
<tr>
<td>TAC</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Comparison is between exercise performance (distance run during exercise tolerance testing (ETT) or peak oxygen consumption \( V_\text{O}_2 \text{max} \)) and the stated MRI component under ISO during the following week. Regression P values are given. *P < 0.05 for statistical significance. Distance, total distance run during ETT. Note that some values are missing due to recording equipment failure at 3 WPS.
MRE-derived cardiac reserve might, therefore, be explained, in part, by the fact that the ability to perform does not always lead to the desire to, exemplified by mice with an elevated VO2 max, but no increase in exercise capacity (23). Last, the ETT is influenced by other factors, such as pulmonary and muscular adaptations, which will influence the ability to exercise. Therefore, comparing a test (ETT) with multiple sources of influence (cardiac, pulmonary, and muscle) to a cardiac-specific stress test (ISO) may not allow for an adequate correlation, given different external sources of influence between the two tests.

Contractility (determined by %EF) in TAC mice is shown to increase over time in response to ISO. However, contractility as determined by invasive terminal hemodynamic pressure changes, ΔdP/dt max, implies that TAC mice are still significantly decompensated compared with sham (Fig. 6). The ΔdP/dt max describes contractile ability of the LV in terms of pressure changes (7). ΔdP/dt max compares resting conditions with changes elicited by dobutamine or other inotropic agent.

Shifting baseline MRI properties. Here, the primary aim was to characterize our HF model in the absence of therapeutic agents. MI mice mimic a similar long-term profile to sham mice, in that they show elevations in HR, SV, and CO from 4 to 16 WPS. This may further explain why MI mice share a high exercise capacity, indicating this shift could be beneficial to mice. This change is possibly brought about due to β-adrenergic stimulation from ISO, which may sensitize mice through the upregulation of receptors. Maturity and senescence are also shown to increase responsiveness to ISO in mice (36). With an increased receptor density, naturally circulating catecholamines would have a greater inotropic and chronotropic effect on the heart, as indicated by higher baseline HR, SV, %EF, and CO. Conversely, an increase in these indexes, tethered to a decrease in cardiac reserve (shown by sham %EF, HR, and CO, as well as MI HR), could indicate a compensatory response to myocardial damage. ISO is shown to mediate a reduction in fatty acid content in the mouse LV (17). A reduction in cardiac fatty acid content is indicative of a pathological switch to glucose metabolism in the early stages of HF. If this is the case, then ISO can be deemed responsible for the increased cardiac indexes observed over time.

Conclusions. In conclusion, the present study indicates that the TAC HF model is best suited for ETT assessment due to permanent intolerance shown for an extended period. In contrast, ISO was unable to show consistent cardiac reserve differences between HF and healthy mice, indicating cardiac-specific indexes and their reserves are not the sole components in defining exercise intolerance. This study also suggests that repeat administrations of ISO may cause myocardial damage, therefore compromising its evaluable ability, despite any desired augmentations in cardiac performance.

DISCLOSURES
All authors were employed by GlaxoSmithKline at the time of this work. D. A. Richards was on a yearlong research internship through Bristol University in the UK. The subject matter of this paper poses no financial conflicts of interest as it relates to characterizing a preclinical HF animal model with imaging and ETT.

AUTHOR CONTRIBUTIONS

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