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ORIGINAL ARTICLE
Translational basic science

Development of a small animal model replicating core characteristics of takotsubo syndrome in humans

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Aims

Adequate animal models are necessary to understand human conditions, such as takotsubo syndrome (TS) characterized by the heart's transient regional wall motion abnormalities. This study aims to develop a reproducible, low-mortality TS model that closely mimics the human condition and addresses the limitations of existing models.

Methods and results

We conducted six experiments using 309 Sprague Dawley rats, each approximately 300 g and aged 7–8 weeks. Initially, we replicated an established model using intraperitoneal isoprenaline injections. Subsequent experiments varied the doses and infusion durations of intravenous isoprenaline and assessed the effects of sex, strain, and breeder on the development of reversible akinetic segments. High-resolution echocardiography monitored the regional wall motion over 30 days to correlate with histological changes. Increasing the isoprenaline dose and the infusion time significantly enhanced akinesia ($P < 0.01$), resulting in pronounced apical ballooning observed in three-dimensional imaging. Akinesia peaked at 6 h post-infusion, with recovery observed at 24 h; most rats recovered from akinetic segments within 48–72 h. Optimizing the mode of administration, dose, and duration achieved a TS-like phenotype in 90% of cases, with a 16.7% mortality rate. Histological examinations confirmed that myocardial injury occurred, independent of apical ballooning.

Conclusion

This study presents a refined TS model that reliably replicates the syndrome's key features, including morphological and electrocardiographic changes, demonstrating its transient nature with high fidelity and reduced mortality. The model's reproducibility, evidenced by consistent results across trials, suggests its potential for broader application pending further validation.

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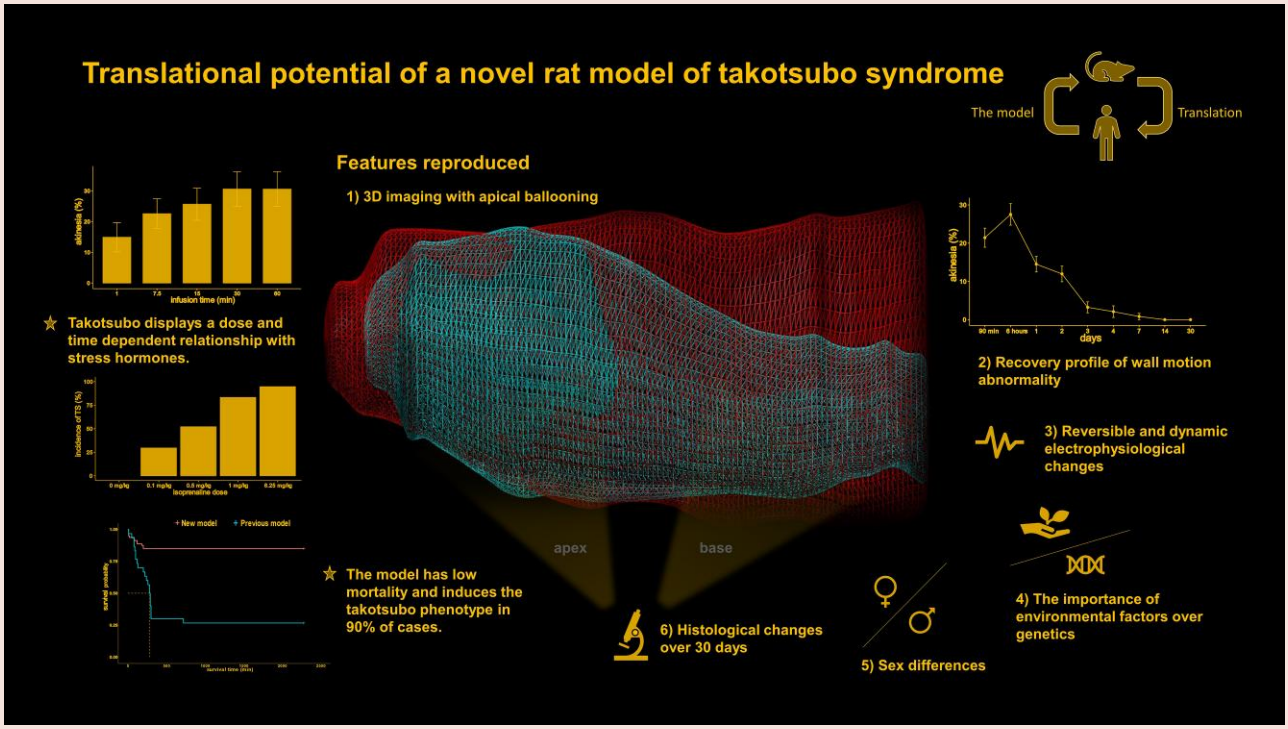
Graphical Abstract



Keywords Takotsubo syndrome • Stress cardiomyopathy • Rat model • Catecholamine

Introduction

Takotsubo syndrome (TS) is an acute form of heart failure with clinical presentation and mortality rates like myocardial infarction.[1] It is characterized by reversible akinesia in the apical segments of the ventricles, often known as 'apical ballooning'.[1] A stressful trigger often precedes TS, and electrocardiographic abnormalities are observed.[2] While the exact mechanisms behind TS are still unclear, there is consensus regarding the involvement of catecholamines.[3]

Modelling human diseases is a crucial aspect of comprehending the pathophysiology of clinical syndromes. Researchers have developed pre-clinical animal models to study TS.[2] However, these models face limited reproducibility, high mortality rates, and an inability to replicate human pathophysiology.[3–9] In our previous work, we established a small animal model of TS that exhibited key clinical features seen in patients.[5] Nonetheless, this model had limitations in reproducibility and high mortality rates.

Building on our previous small animal TS model based on intraperitoneal (IP) administration of isoprenaline in rats, we have developed a novel model using a refined methodology. This new model accurately reproduces the defining characteristics of TS observed in humans. We highlight the reproducibility and low mortality rates observed in several experimental settings, examining multiple factors that impact the development of apical akinesia. Our study investigated the role of catecholamine-induced stress on cardiac function and the development of akinesia. We highlight the variability in TS susceptibility observed in animals like in humans and discuss its implications. Additionally, we outline the natural course of TS using high-resolution echocardiography and histology. Finally, we explore how sex, strain, and breeder differences influence the model.

Methods

Animals and experimental setup

Animal work followed the national guidelines for the use of experimental animals, and the regional Animal Ethics Committee in Sweden approved the study protocol (Dnr 5.8.18-13700/2021). A summary of experimental designs can be seen in *Figure 1*. A total of 309 outbred rats were used, specifically 7- to 8-week-old male nRjHan:SD rats (Sprague Dawley, Janvier Labs). *Table 1* shows the summary statistics for each study group. The rats were housed three to four in open cages in a temperature-controlled (21°C) facility having a 12-h light/dark cycle and food and water *ad libitum*, with an acclimatization period of 1 week. The rats were anaesthetized with ketamine (70 mg/kg) and midazolam (3.5 mg/kg) IP. Isoprenaline (Sigma-Aldrich) was injected IP or intravenously (IV) in the lateral tail vein with an infusion pump (Harvard Bioscience). Oxygen saturation (SpO_2, %) and heart rate were monitored (Kent Scientific Corporation). Core body temperature was measured with a rectal probe and controlled with a heating plate. Data collection and analysis from a lead I electrocardiogram (ECG) were performed using a BIOPAC MP160 ECG data acquisition module (Acknowledge, BIOPAC Systems).

High-resolution echocardiography

We evaluated the cardiac function in lightly anaesthetized rats by measuring the fractional area change [FAC (%)] and the extent of akinesia.[5] Akinesia defined as the lack of change in wall thickness over cardiac cycles is also assessed in the wall motion score index—the most widely used echocardiographic tool for quantifying wall motion abnormalities, as described in guidelines.[10,11] Echocardiographic images were acquired in the parasternal long-axis view (LAX) with a 21 MHz transducer (MX250; VEV3100, VisualSonics). Body temperature was maintained (36–38°C), and ECG

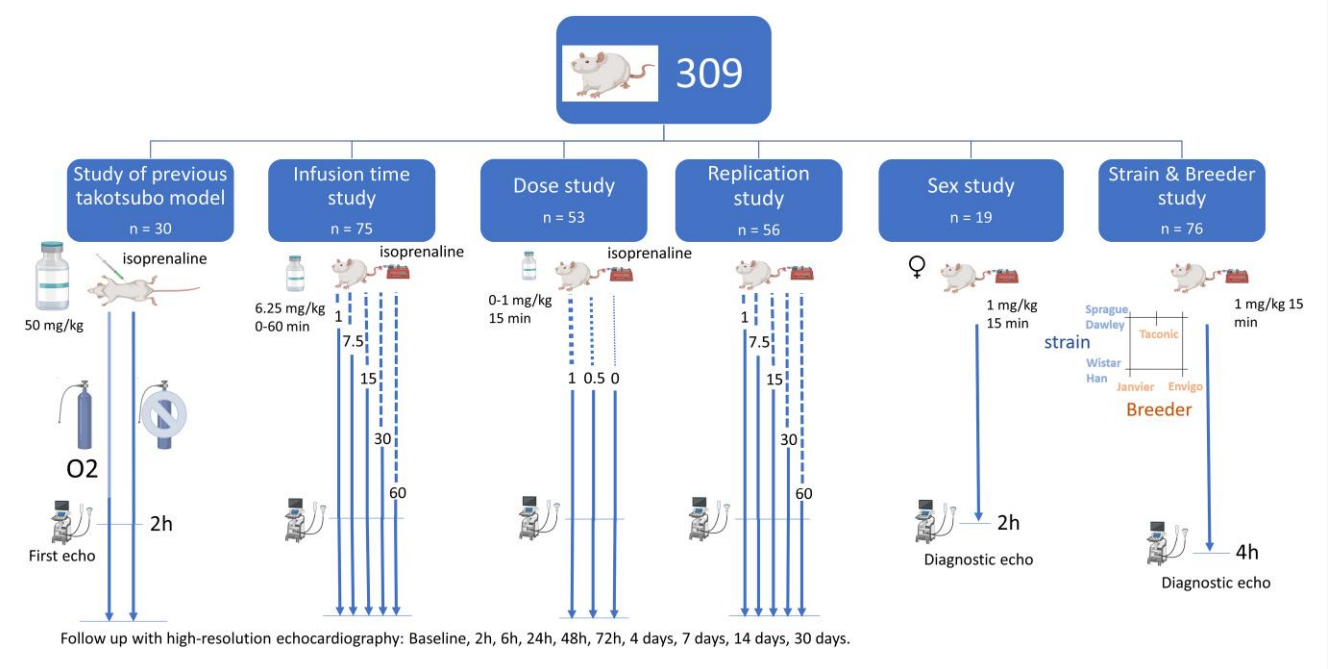


Figure 1 Overview of methodologies employed in six distinct studies. The first study (intraperitoneal study) scrutinized a preceding model of takotsubo syndrome and the impact of oxygen supplementation. The 'infusion time study' assessed varying durations of infusion (ranging from 1 to 60 min). The 'dose study' examined a range of isoprenaline dosages (from 0 to 1 mg/kg). The 'replication study' served as a reiteration of the infusion time study. The 'sex study' investigated the role of female and male sex in the intravenous model. The 'strain study' evaluated the effect of different rat strains and breeders on the newly developed takotsubo syndrome model. High-resolution echocardiography was utilized to assess cardiac function and wall motion abnormalities for a period up to 30 days. Parameters, such as mortality rate, incidence and extent of apical akinesia, and timeframe for recuperation of cardiac function, were investigated across the studies.

was recorded. Baseline imaging was performed under ketamine–midazolam anaesthesia before isoprenaline injection.[12,13] Fractional shortening (%) was assessed with M-mode in the parasternal short-axis view at both the base and the apex. Three consecutive heart cycles were recorded and averaged for each measurement. Post-image analysis was performed by a researcher blinded to the treatment groups and time points using the Vevo Lab 5.6.0 software (FUJIFILM VisualSonics Inc.). Incidence of TS was defined as any visible akinetic segment on echocardiography in LAX extending circumferentially (see Supplementary material online, *Video S1*) and recovery when no akinetic segment was visible. Reconstruction of three-dimensional (3D) images was performed by delineating the endocardium of a serial of images taken in short-axis view with an increment of 150 µm from the aortic root to the apex in diastole and systole, respectively. The resulting diastolic and systolic volumes were overlaid for visualization.

Oxygen supplementation in the intraperitoneal model

We studied the effects of oxygen supplementation in the IP model[5] on survival and development of apical akinesia (*Figure 1*, IP study). Thirty rats received a dose of 50 mg/kg isoprenaline IP and were randomized to oxygen supplementation or air ($n = 15$ per group). The oxygen was administered during the first hour after administration of isoprenaline.

Infusion time and dose finding in the intravenous model

We hypothesized that the IV delivery of isoprenaline would lead to a greater frequency of apical akinesia attributable to reduced pharmacokinetic variability, faster onset of action, and more uniform cardiac stimulation. In addition, avoiding potential complications caused by the local administration of isoprenaline in the abdominal cavity, such as severe inflammation, bleeding, and sepsis, could lower mortality. We performed two separate studies, one with a constant dose and varying infusion times (infusion time study) and another with differing doses and a constant infusion time (dose study). The former study was replicated (replication study). In the infusion time study, 75 rats were randomized to a single dose of isoprenaline 6.25 mg/kg given IV at different infusion times (i.e. immediate administration with a bolus dose, 7.5, 15, 30, and 60 min). The experiment was replicated using an additional cohort of 56 rats. In the dose study, we compared 1 mg/kg with 0.5 mg/kg based on the pilot study in which the dose variation from 6.25 to 50 mg/kg did not produce differences in incidence or extent of akinesia (data not shown). Fifty-three rats were randomized to 0.5 or 1 mg/kg of a single IV dose of isoprenaline over 15 min or saline. We conducted a longitudinal study over 30 days to scrutinize the natural evolution of TS-like phenotype in rats (*Figure 3B*; Supplementary material online, *Figure S3* and *Videos S2* and *S3*).

Effects of sex, strain, and vendor on takotsubo syndrome phenotype

Nineteen female Sprague Dawley rats were administered a single IV dose of 1 mg/kg isoprenaline over 15 min, and echocardiography was performed after 2 h. A two-factor fractional design was employed to assess the influence of different strains and breeders on the TS phenotype. We used two common outbred rat strains, Sprague Dawley and Wistar Han. These strains were crossed with three breeders available to our facility: Janvier Labs (France), Envigo (the Netherlands), and Taconic (Denmark). However, Taconic could not provide Wistar Han rats. Following the induction of TS using the established protocol, echocardiography was performed at 4 h.

Histology

Rats with extensive apical ballooning at 6 h were sacrificed at 6 h (peak akinesia), 24 h (recovery phase), and 30 days (recovery) (see Supplementary material online, *Figure S1*). Rats receiving isoprenaline but not developing

Table 1 Summary of animal characteristics

	Dose study ($n = 53$)	IP study ($n = 30$)	IT study ($n = 75$)	Replication study ($n = 56$)	Sex study ($n = 19$)	Strain study ($n = 76$)	Overall ($n = 309$)
Weight (kg)							
Mean (SD)	0.33 (0.022)	0.334 (0.040)	0.32 (0.021)	0.32 (0.022)	0.21 (0.013)	0.29 (0.037)	0.31 (0.041)
Median [min, max]	0.34 [0.28, 0.37]	0.34 [0.27, 0.40]	0.32 [0.28, 0.37]	0.33 [0.28, 0.37]	0.21 [0.20, 0.25]	0.29 [0.21, 0.37]	0.32 [0.20, 0.40]
Age (days)							
Mean (SD)	48.0 (1.18)	48.0 (1.22)	47.2 (1.87)	47.5 (1.63)	43.3 (1.24)	50.6 (3.55)	48.1 (2.83)
Median [min, max]	48.0 [46.0, 50.0]	48.0 [46.0, 50.0]	47.0 [43.0, 51.0]	47.0 [46.0, 55.0]	43.0 [42.0, 46.0]	49.0 [46.0, 56.0]	48.0 [42.0, 56.0]
Sex							
Male	53 (100%)	30 (100%)	75 (100%)	56 (100%)	0 (0%)	76 (100%)	290 (93.9%)
Female	0 (0%)	0 (0%)	0 (0%)	0 (0%)	19 (100%)	0 (0%)	19 (6.1%)
Temperature (°C)							
Mean (SD)	37.5 (0.450)	37.2 (0.634)	37.2 (0.283)	37.3 (0.313)	39.1 (0.259)	37.5 (0.483)	37.4 (0.606)
Median [min, max]	37.4 [36.7, 38.8]	37.2 [35.8, 39.2]	37.1 [36.5, 38.0]	37.3 [36.6, 38.0]	39.1 [38.5, 39.5]	37.5 [36.2, 38.7]	37.3 [35.8, 39.5]
Heart rate (b.p.m.)							
Mean (SD)	399 (49.1)	485 (77.6)	418 (40.4)	452 (54.5)	410 (51.6)	380 (63.0)	419 (63.0)
Median [min, max]	407 [291, 514]	484 [366, 606]	426 [341, 517]	448 [337, 545]	400 [333, 515]	370 [276, 560]	414 [276, 606]
Saturation (%)							
Mean (SD)	96.4 (2.60)	94.0 (7.39)	97.5 (2.93)	93.6 (6.01)	91.6 (7.32)	93.2 (7.22)	95.0 (5.70)
Median [min, max]	96.0 [88.0, 99.0]	98.0 [70.0, 99.0]	99.0 [87.0, 99.0]	95.0 [77.0, 99.0]	93.0 [80.0, 99.0]	97.0 [73.0, 99.0]	98.0 [70.0, 99.0]

Table shows the summary statistics of each study at baseline prior induction of the TS. The dose study evaluated different doses of isoprenaline. The IP study (intraperitoneal study) evaluated a previous animal model of the TS. The IT study (infusion time study) evaluated different infusion times. The replication study was a replication of the IT study. The sex study evaluated the influence of sex on the TS model. The influence of different strains and breeders was evaluated in the strain study.

akinesia at 6 h (no TS) were sacrificed at 6 h and 30 days. Rats receiving saline (control) were sacrificed at 6 h. The hearts were excised, fixed in 4% formalin, and cryo-sectioned for staining and scanning. Apical and basal segments of the individual hearts were stained with haematoxylin and eosin, Mason trichrome (MTC), and Oil Red O (ORO). Image analysis of the histological slides was performed using QuPath.[14] A machine learning algorithm employing a pixel classifier and random forest was utilized to quantify fibrosis, interstitial space, and lipid accumulation. A high resolution of 0.97 µm/px was employed for the classifiers. Two classifiers were trained on MTC- and ORO-stained slides (see Supplementary material online, *Figure S2A and B*). The total percentage of interstitial space, fibrosis, and lipid content was calculated. The apical segments were divided into the right ventricular free wall, sub-endocardium, and sub-epicardium in a subsequent analysis (*Figure 7E and F*). Classifiers were then applied to calculate the different regions' fibrosis and lipid content percentages.

Statistical analysis

Data analyses were conducted using R 4.2.0, IBM SPSS 27, and InVivo Stat 4.2.0. Sample size determination based on a power of 0.8, effect size $\eta^2 = 0.19$, and alpha of 0.05 targeted akinesia as the primary endpoint with an assumed attrition of 0.2. Secondary outcomes included FAC, mortality, and TS incidence. Randomization sheets were blinded and managed via REDCap.[15] Following the intention-to-treat principle, no animal was excluded after randomization. For comprehensive statistical details, see Supplementary material online, *File S2*.

Results

Takotsubo model with higher mortality: the intraperitoneal model

A decrease in SpO_2 (~10%) was observed in both groups. However, oxygen supplementation normalized the SpO_2 decline (*Figure 2A*). All rats developed dynamic ECG changes in ST-T segment morphology, which normalized upon oxygen supplementation (*Figure 2B*). All animals' heart rate and body temperature increased substantially during the first hour ($P < 0.001$). Over the 30-day follow-up, only eight rats survived out of 30, and these recovered fully within 7 days. All other animals died within the first 24 h, and the majority died within the first 5 h (*Figure 2C*). Mortality was usually preceded by gradual decline in SpO_2, an inability to recover from anaesthesia, and poor systolic function (*Figure 2E*). All but one rat developed apical ballooning at 2 h. Oxygen supplementation increased survival but did not affect the extent of akinesia (*Figure 2D*).

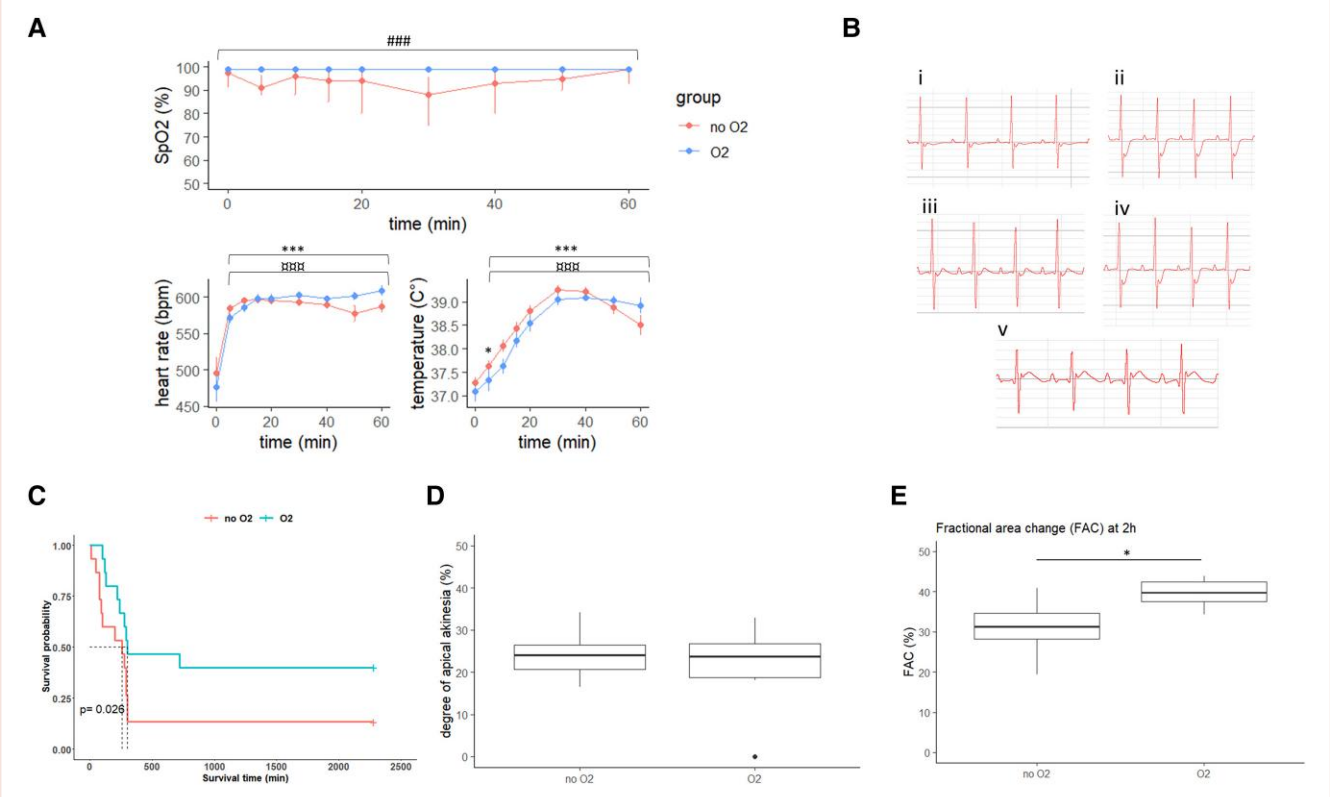


Figure 2 Evaluation of 30-day mortality and physiological parameters in the intraperitoneal model with oxygen supplementation. (*A*) Oxygen saturation and body temperature during first 60 min. Mean ± SEM. Two-way repeated-measures analysis of variance; $n = 18$ per group. $^*P < 0.05$, $^{***}P < 0.001$ vs. baseline (no O_2 supplementation group); ¤¤¤$P < 0.001$ vs. baseline (O_2 supplementation group). ###$P < 0.001$ between groups (no O_2 vs. O_2). (*B*) Electrocardiogram (ECG) changes from baseline (i) to 60 min (v) after induction. ECG changes (ii) occurred in conjunction with increased heart rate and oxygen saturation drop. These changes were dynamic with (iii) and without (iv) oxygen supplementation. (*C*) Although oxygen supplementation increased survival, high 30-day mortality rates were observed. Results presented assuming Weibull distribution; $n = 15$ per group; $P = 0.026$. (*D*) Boxplots depicting the extent of akinesia. Oxygen supplementation did not affect the extent of akinesia. Unpaired two sample *t*-test; $n = 8$ and 12 per group. $P = 0.91$. (*E*) The surviving animals in the oxygen supplemented group had better cardiac function at 2 h. Unpaired two sample *t*-test; $n = 8$ and 6 per group. $P = 0.022$. FAC, fractional area change; SpO_2, oxygen saturation.

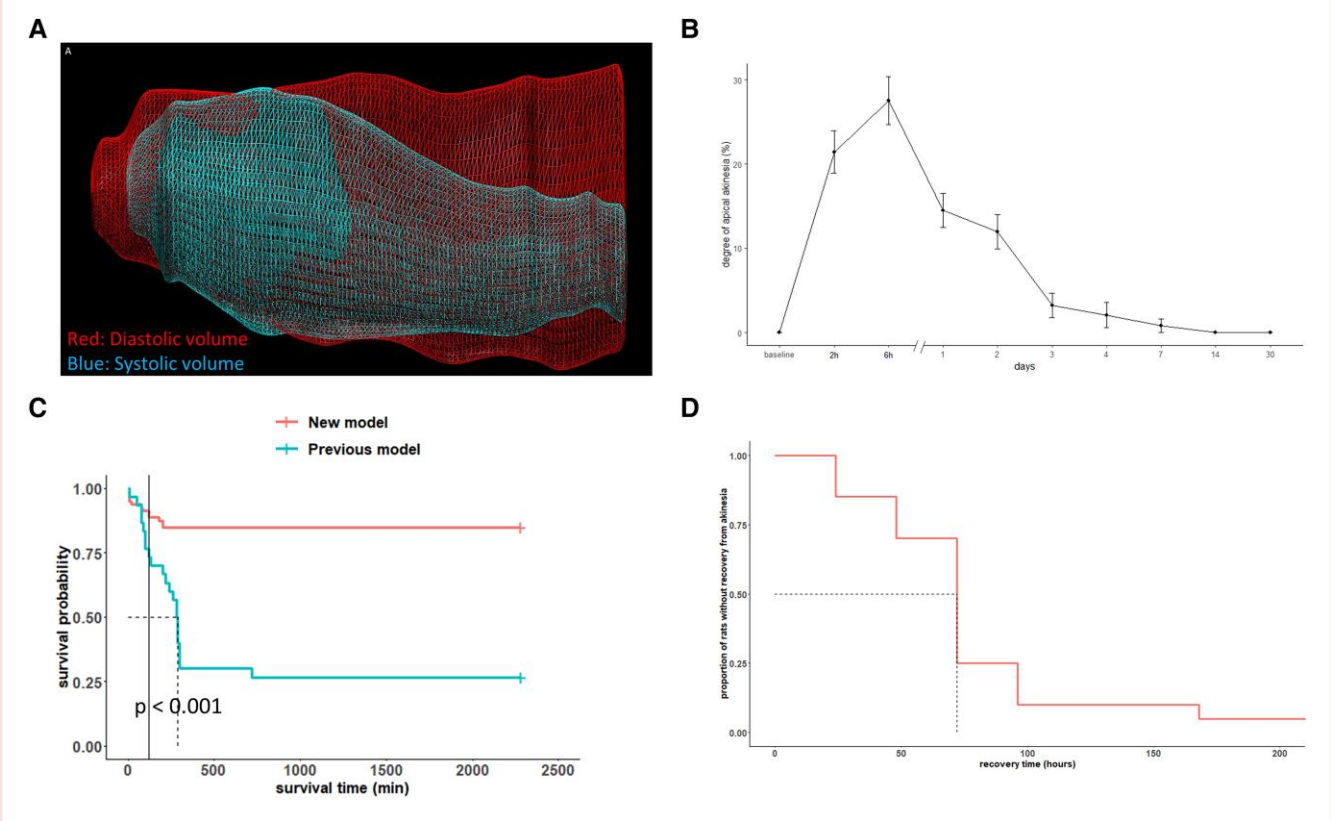


Figure 3 Intravenous model of takotsubo syndrome with low mortality and high reproducibility. (*A*) Representation of the new refined takotsubo syndrome model. Diastolic and systolic three-dimensional volume overlay. (*C*) Survival probability plot comparing the previous model with the new and refined takotsubo syndrome model. Log-rank test; $P < 0.001$. (*B–D*) Natural course of the takotsubo syndrome model over 30 days and time to recovery. Data are from the infusion time study. Mean ± SEM.

Takotsubo model with lower mortality: the intravenous model

We established a TS model with apical akinesia (see Supplementary material online, *Videos S2 and S3*). Approximately 90% of the surviving subjects exhibited apical akinesia, reaching its zenith at the 6-h mark (*Figure 3B*). A recovery phase was observed at 24 h. Left ventricular systolic function and contractility returned to baseline levels between 1 and 14 days, with the mode (45% of all rats, *Figure 3D*) achieving functional restoration within 48 to 72 h. After 30 days, surviving rats demonstrated normal behaviour, electrocardiographic and echocardiographic findings. The infusion study showed that longer infusion times are associated with increased mortality and incidence (see Supplementary material online, *Figure S4A and B*; P for trend < 0.001, P for trend = 0.016). A brief infusion duration of 1 min led to a less pronounced manifestation of apical akinesia, whereas infusion durations exceeding 7.5 min yielded no significant differences (*Figure 4*; Supplementary material online, *Figure S3*). Infusion duration over 30 min produced the highest mortality, reaching ∼50% (see Supplementary material online, *Figure S4*). Our replication study corroborated these observations, albeit with some variability in both incidence and mortality across the experimental groups (see Supplementary material online, *Figure S5*). Despite adhering to an identical experimental protocol, the animals in the replication study exhibited varying degrees of susceptibility to developing TS phenotype and the associated mortality risk. Given that an infusion duration of 15 min demonstrated the most consistent mortality rate at ∼25%, along with an incidence of apical akinesia ranging between 87.5 and 100%, this time frame was chosen as the standardized infusion duration for the subsequent dose–response investigation (see Supplementary material online, *Figure S5*).

In the dose–response study, we noted a significant decline in the incidence of akinesia and mortality with lower doses of isoprenaline (*Figure 4*). Specifically, doses up to 1 mg/kg of isoprenaline influenced the TS incidence, akinesia, and FAC. We observed an elevation in body temperature during the duration of the experimental protocol, which was exacerbated by the isoprenaline infusion (see Supplementary material online, *Figure S6*). The natural trajectory of FAC after isoprenaline is outlined in Supplementary material online, *Figure S7*, showing significant contractile dysfunction at 6 h and Day 1.

Fibrosis and lipid accumulation

Observations at the 30-day mark post-isoprenaline revealed the presence of fibrosis, while lipid accumulation was evident as early as 6 h (*Figures 5–7*). This lipid accumulation was subsequently normalized after 24 h. Notably, fibrosis and lipid accumulation were more pronounced in the apical compared with the basal segments. These apical changes were manifest, irrespective of the presence or absence of akinesia at the 6-h stage. Further compartmental analysis within the apical region indicated that the sub-endocardial zone was susceptible to elevated lipid accumulation and fibrosis levels (*Figure 7*). Moreover, a significant expansion of the interstitial space—a measure of oedema—was observed in the apical region during the acute phase (see Supplementary material online, *Figure S8A*). Summary statistics of rats can be found in Supplementary material online, *Tables S1 and S2*.

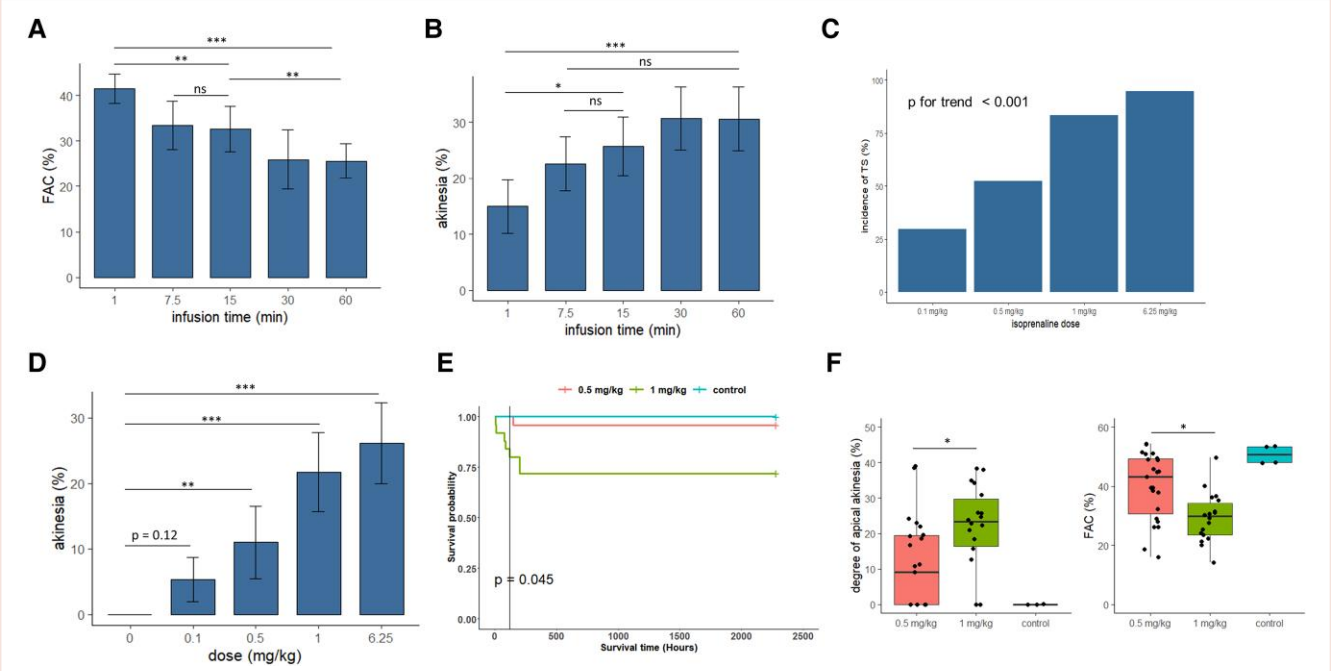


Figure 4 Dose- and time-dependent relationship with akinesia, mortality, and cardiac function. (*A–B*) Mean fractional area change (%) and akinesia (%) from pooled infusion time and replication study data with a 95% confidence interval. The data were analysed using one-way analysis of variance. Study period was used as a blocking factor; $n = 20$ per group. Results are presented as pairwise comparisons using Benjamini–Hochberg's procedure. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. (*C–D*) Pooled data from the dose, infusion, and a low-dose pilot study. Dose-dependent relationship in incidence and extent of akinesia. $n = 32$, 23, 18, and 23 per group. Extended Mantel–Haenszel χ^2 P for trend < 0.001. Mean ± SEM. (*E*) Mortality results are presented using a log-rank test; $P = 0.045$. (*F*) Akinesia (%) and fractional area change (%) results are presented using the Kruskal–Wallis test; $n = 23$, 18, and 4 per group. ns ≥ 0.05, $^{*}P < 0.05$. Data from the dose study. Median + interquartile range. FAC, fractional area change; ns, not significant.

Effect of sex

The TS model successfully reproduced the TS phenotype in female rats. No sex differences in the incidence or extent of akinesia were observed (*Figure 8A and B*; Supplementary material online, *Table S3*). The development of TS in the model was significantly affected by the type of breeder ($P = 0.006$). However, the choice of strain did not significantly impact the development of TS ($P = 0.604$; *Figure 8C and D* and Supplementary material online, *Table S4*). A significant interaction between strain and breeder was observed ($P = 0.032$).

Discussion

Our aim was to create a pharmacologically induced TS-like phenotype in rats with low mortality and high reproducibility. We examined dose-dependent effects, infusion time, and sex differences. The most important results show that isoprenaline dosage, duration, and way of administration impact the TS incidence, severity, and survival. Higher dosages and more extended infusion periods enhanced apical akinesia incidence and extent. Sex did not affect these results. The model demonstrated heart function recovery within 2 days, establishing TS' temporary nature, like in humans. These findings form the basis for the model's translational relevance.

Two-thirds of patients with TS have an identifiable trigger preceding the condition.[16] Our model can serve as a suitable representation of TS triggered by both emotional and physical factors because the catecholamine overstimulation is the common denominator. The IP model established in 2012 by our group and histologically validated with patient samples[5] is the most reproduced model by others.[17,18] However, the IP model has a mortality rate of 73% at 30 days, much higher than what is reported in patients.[19,20] Most of the deceased animals from the IP model displayed an inability to maintain proper oxygenation and regain consciousness after anaesthesia and a rapid decline in cardiac function with a notable spasm-like dysfunction.[5] The mortality in the IP model is substantially reduced by oxygen supplementation. These findings suggest isoprenaline toxicity as the cause of death. These excessive deaths were seen in only a minority of cases in the IV model. Our refined TS model had a 30-day mortality across the studies at 16%. In addition to a lower dose of isoprenaline, we adjusted the infusion duration to maximize the incidence of akinesia and minimize deaths. The study found that both the death rate and the presence and extent of akinesia increased with longer infusion times, indicating that not just a rapid rise in stress but also its sustained presence is crucial for the development. This finding suggests that a similar mechanism may exist in humans, where the pharmacokinetics of stress hormones could play a crucial role in the onset and severity of TS. However, further research is needed to confirm this hypothesis in clinical settings. This paper reports doses up to 500 times lower than the one used in the IP model, which makes the model much more relevant from the translational point. However, as the dose of isoprenaline decreases, so does the representation of the syndrome. The incidence and extent of apical akinesia were dose dependent. This was most obvious at lower doses of up to 1 mg/kg when the effects of isoprenaline toxicity were lower. We also reproduced ECG changes in the ST–T segment morphology during the induction of TS, like in humans.

We found that the extent of apical akinesia—considered a hallmark of TS[21]—peaked at 6 h, while the recovery phase commenced at 24 h. The majority of akinetic segments regained normal contractility within

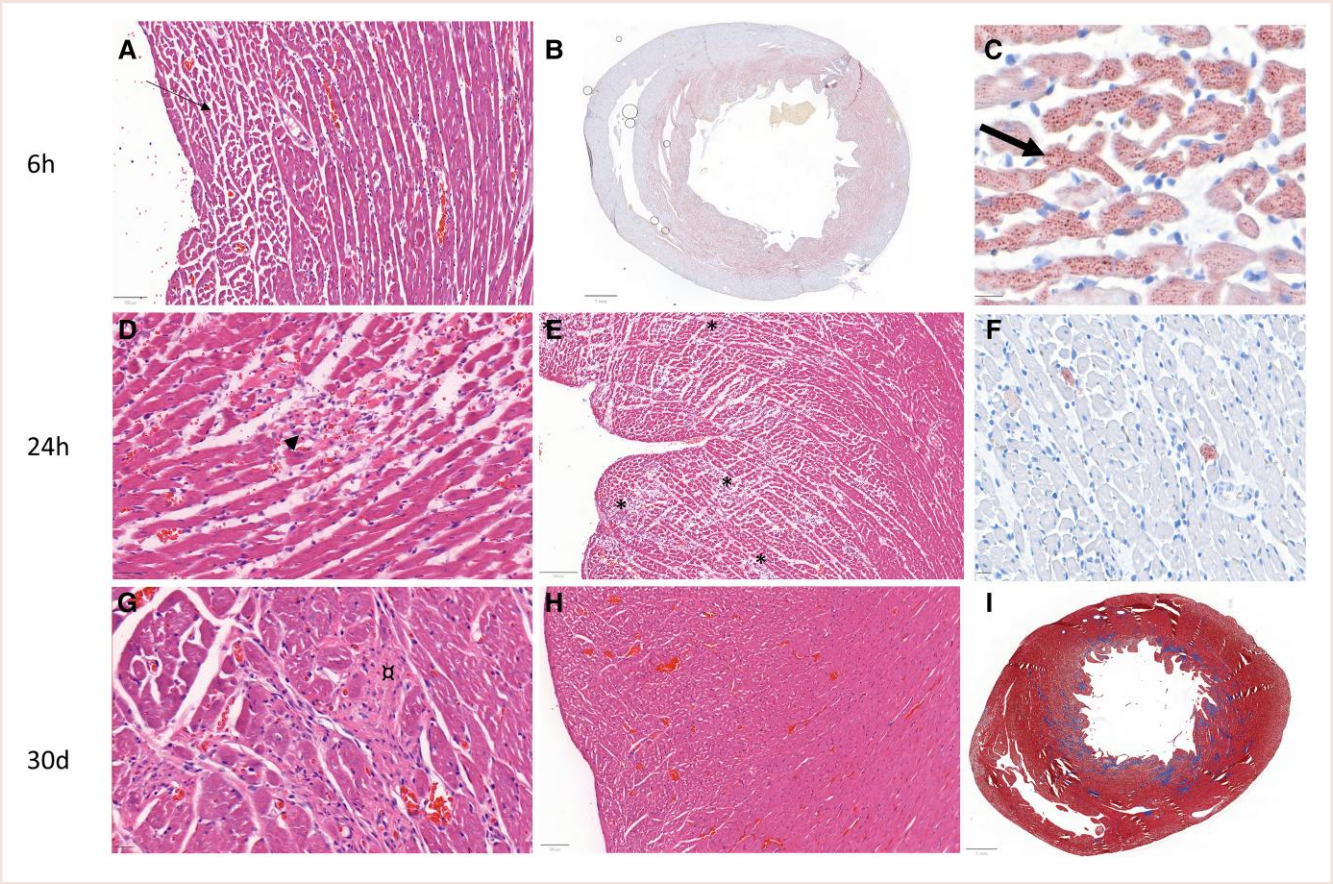


Figure 5 Visualization of histological changes over 30 days after isoprenaline infusion. At 6 h, oedema (*A*, thin arrow) and sub-endocardial deposition of lipid droplets (*B–C*, thick arrow) were observed. Foci of necrosis at 24 h (*D*, arrow head) were primarily located as patches in the sub-endocardium (*E*, *). Lipid accumulation was subsequently normalized after 24 h (*F*). Fibrosis was present at 30 days (*G*, ¤) distributed sub-endocardially (*I*, blue) and without the presence of oedema (*H*). Staining (scale bar, magnification): haematoxylin and eosin (100 μm, ×6), Oil Red O (1 mm, ×0.3), Oil Red O (20 μm, ×22), haematoxylin and eosin (50 μm, ×10), haematoxylin and eosin (200 μm, ×2.5), Oil Red O (20 μm, ×15), haematoxylin and eosin (20 μm, ×15), haematoxylin and eosin (100 μm, ×4), and Mason trichrome (1 mm, ×0.3).

48–72 h. A similar time-lapse was observed for the global cardiac function. The normalization of ST–T segment changes was observed between Days 14 and 30, indicating that the recovery timeline in rats' parallels that seen in humans.[16,22] No clinical nor experimental studies so far have performed evaluations of cardiac function and morphology in TS longitudinally as detailed as we did.

Patients with typical TS demonstrate a spectrum of wall motion abnormalities, ranging from isolated apical to extensive mid-segment involvement. This variability is mirrored in the model discussed herein. To systematically assess this variability, we quantified the extent of akinesia, an established metric in both pre-clinical and clinical studies of TS.[6,23] The response to catecholamines is pivotal and warrants deeper exploration to elucidate why certain individuals exhibit apical ballooning, while others do not under analogous conditions. Further, it is imperative to differentiate the transient loss of cardiac function in specific regions following stress, a phenomenon well-documented in TS patients. This element is incorporated into the current diagnostic guidelines by the European Society of Cardiology[22] and elaborated upon in the International Expert Consensus Document on TS.[2,24] Our study aligns with these diagnostic criteria by focusing on akinesia to maintain translational relevance. Nonetheless, the definition of TS varies across different animal models. It is essential to acknowledge these differences when comparing models. Some define TS as a decrease in contractility relative to baseline[25,26] and others as an overall reduction in global systolic function,[27,28] regional wall motion abnormality,[6,29–31] or by catecholamine induction.[18]

In the IV model, fibrosis was observed at 30 days. However, the presence of fibrosis did not impact echocardiographic measures of cardiac function at that time. The development of fibrosis after catecholamine administration is a known dose-dependent phenomenon.[32,33] This fibrosis primarily occurred within the sub-endocardial region. Several mechanisms contribute to this process, including activation of cardiac fibroblast through adrenergic receptors,[34–36] oxidative stress,[37] calcium overload,[38] and myocardial ischaemia.[39] In our study, all rats that received a 1 mg/kg infusion of isoprenaline over 15 min developed fibrosis within the sub-endocardial region at the apex by Day 30. However, the extent of fibrotic response did not correlate with the development and extent of segmental akinesia. These findings suggest that apical akinesia—the most characteristic expression of TS syndrome—does not follow the severe tissue injury pattern, but rather that catecholamines are the common cause of reversible and irreversible tissue injury and function. Additionally, lipid accumulation was predominantly observed within the sub-endocardial region at the apical segment. Lipid accumulation has been attributed to direct catecholamine stimulation, hypoxia, and ischaemia.[8,40,41] The extent of lipid accumulation peaked at 6 h and recovered at 24 h. Like fibrosis, lipid accumulation was

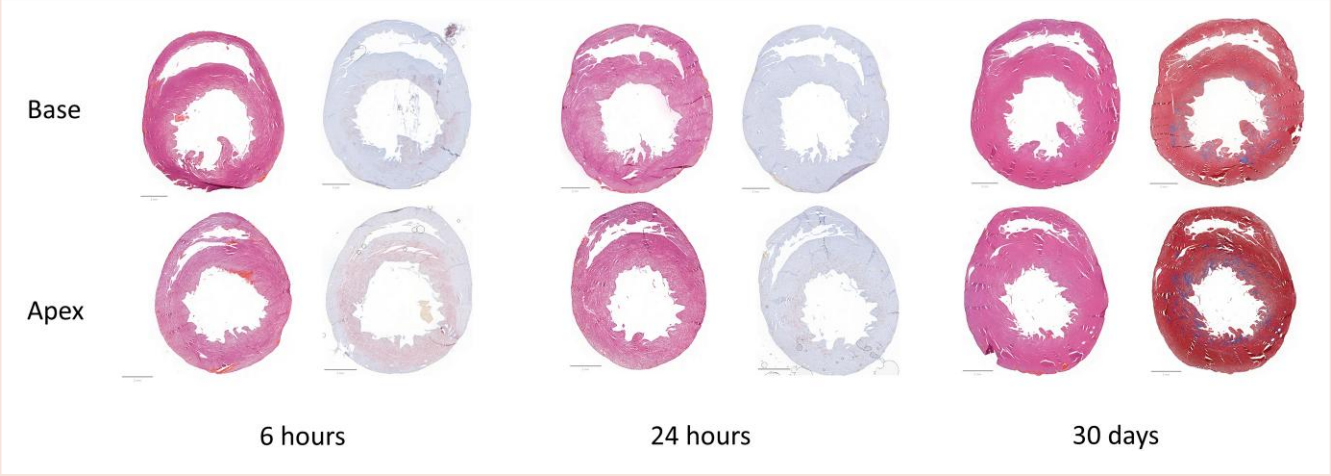


Figure 6 Histological overview of the apical and basal sections. Scale bar: 1 mm. Magnification: ×0.3. Staining: haematoxylin and eosin, Oil Red O, and Mason trichrome.

observed in all rats receiving isoprenaline, irrespective of akinesia. Given their similar distribution, fibrosis, lipid accumulation, and segmental akinesia share a common pathogenesis but are not necessarily causally related. The current study using the IV model did not find a significant association between akinesia and lipid accumulation, unlike previous models.[5,6,8] These differences may be due to variations in lipid estimation methods, timing of histological analysis, dosage, or mode of administration. The future studies should evaluate how perturbations in lipid metabolism and formation of lipid droplets may influence the development of the TS phenotype.

The TS model was initially predicated on young adult male rats. Subsequent experiments revealed no significant differences between the sexes in the incidence or severity of akinesia, thereby validating clinical findings that show a lack of sex-based differences in the younger population.[16] The translational validity is thus sustained, as both young male and female rats are appropriate models for studying TS features. Although TS occurs more frequently in older men than in pre-menopausal women, it predominantly affects women in post-menopausal age.[16] Such findings prompt further inquiry into how factors beyond sex, notably age and hormonal status, may influence TS pathophysiology. Consequently, this model serves as a robust framework for exploring the multifaceted roles of sex, age, hormonal changes, and other elements in TS.

Strain did not have a significant effect on the development of akinesia in our study. However, we observed a significant effect of breeder type and an interaction effect between strain and breeder. Outbred rat stocks exhibited genetic diversity within the population, and differences can exist between individuals, although specific genetic comparisons between rat strains have not been conducted. The results suggest that environmental factors, rather than genetic predisposition, may play a more significant role in TS development after acute stress. This is further supported by the relatively late onset of the disease and few familial cases reported in humans. The notion that environmental factors can facilitate the development of TS has been reported elsewhere.[42,43] The observed differences between breeders could be attributed to variations in stress response and transportation-related stress. When using our model, consideration should be given to both breeder type and strain. Further investigation is needed to better understand the individual propensity for developing TS. This characteristic of the TS model makes it more clinically relevant. It offers the possibility to study the individual propensity to develop TS, which is characteristic of the human condition.

There are limitations to the model that must be considered. First, while the TS model presented in this work effectively replicates several defining features of the syndrome, further validation of other features reported in TS patients is necessary. If a model exhibits high construct validity, it is reasonable to assume that the pathophysiology is accurately represented, providing a robust platform for translational research. Second, using outbred rats to model TS introduces species differences that may impact the relevance of human TS. Nevertheless, the observation that stress can induce similar characteristic apical ballooning in various species in experimental setups suggests potential shared features across different species. Furthermore, small animals have long been the primary choice for cardiovascular research, with findings successfully translating to humans.

Third, anaesthesia is used during induction and imaging, potentially affecting development and natural course. However, considering its necessity for animal welfare and quality imaging, and given the successful induction of the TS phenotype with and without various anaesthetic approaches in prior research, we advocate its use while carefully minimizing its duration and depth. Lastly, our model administers catecholamines IV to enable control of its kinetics. However, stress induces wide-ranging physiological adaptions, including nervous, inflammatory, and endocrine responses, which are influenced by variabilities in both triggers and individuals. For example, despite the constant dose and infusion time of isoprenaline, there is variability in tissue injury, cardiac function, and the extent of akinesia observed in the animals. While this translational aspect of variability is maintained, controlling for the catecholamine-driven response could limit the representation of adaptive responses observed across the full spectrum of human TS cases, which are heterogeneous. However, this limitation does not necessarily reduce the model's utility for understanding or drawing pathophysiological conclusions. Strain analysis was not included in this study due to its scale and focus and to maintain alignment with methodologies from the ongoing clinical trial (trial ID: NCT04448639). Evaluating different aspects of wall motion abnormalities may be valuable for understanding the pathophysiology of TS, and future pre-clinical research should investigate various assessment methods for these abnormalities post-stress.

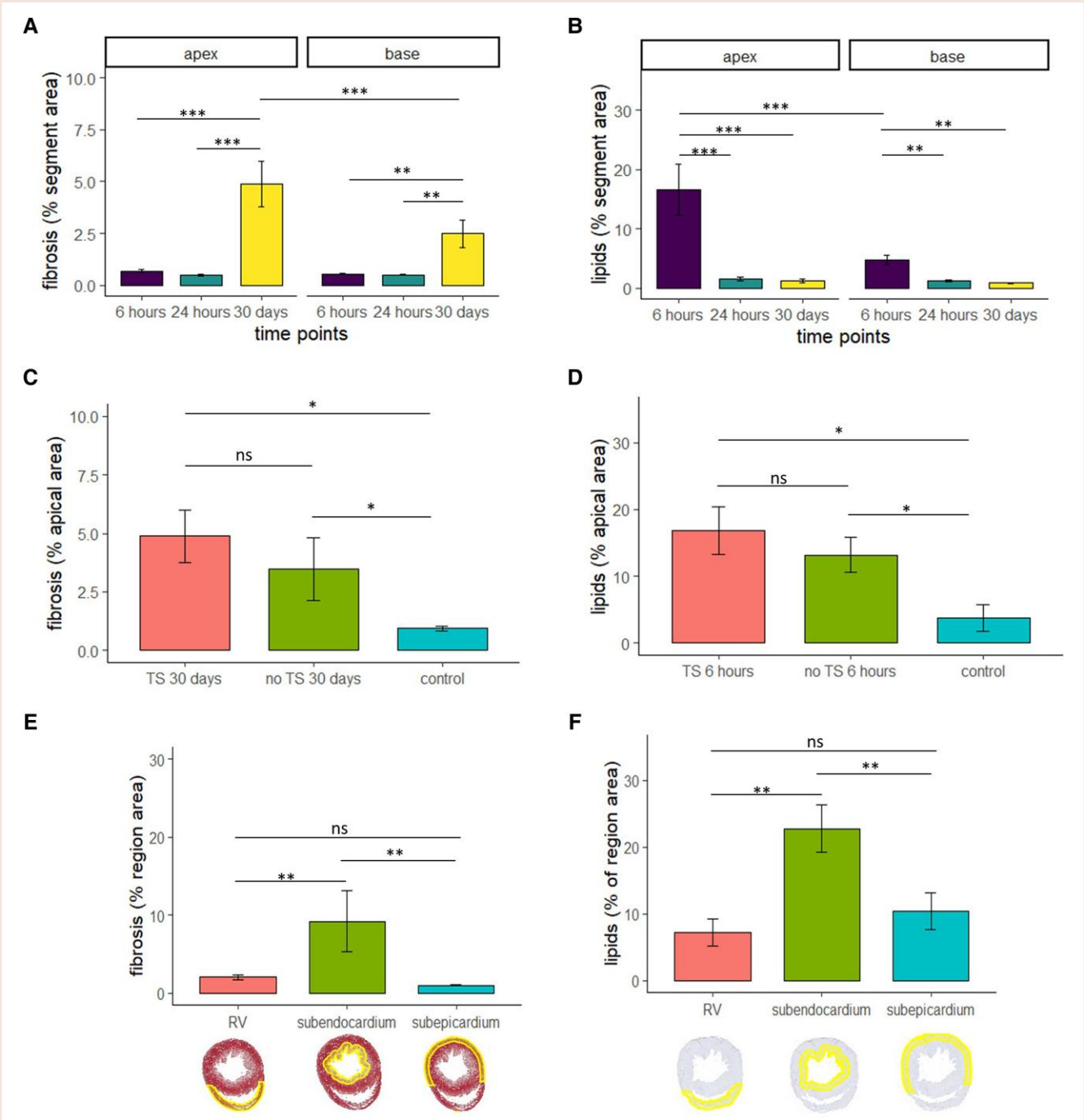


Figure 7 Apical akinesia does not follow tissue injury. Cardiac fibrosis (*A*) and lipid accumulation (*B*) change over time were analysed in takotsubo hearts using a two-way repeated-measures mixed-model approach. (*C–D*) Cardiac fibrosis and lipid accumulation in the apex of different cardiac states were analysed using a one-way analysis of variance approach. The response was log10 transformed before analysis to stabilize the variance. *P*-values were adjusted using Benjamini–Hochberg's multiple comparison procedure. $n = 5$/group and time point. ns; $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. Mean ± SE. no takotsubo syndrome: rats receiving stress and without development of takotsubo syndrome. Control: receiving saline only. Fibrosis and lipid accumulation were predominantly observed in the sub-endocardial region of apex. Fibrosis (*E*) and lipid accumulation (*F*) at 30 days respectively 6 hours after catecholamine challenge. Analysed using one-way repeated-measures analysis of variance, with region as the repeated factor. *P*-values were adjusted using the Benjamini–Hochberg multiple comparison procedure. Takotsubo and non-takotsubo rats presented. $n = 10$/region. Greenhouse–Geisser < 0.001. ns; $P > 0.05$; **$P < 0.01$. Mean ± SE. RV: right ventricular free wall. ns, not significant.

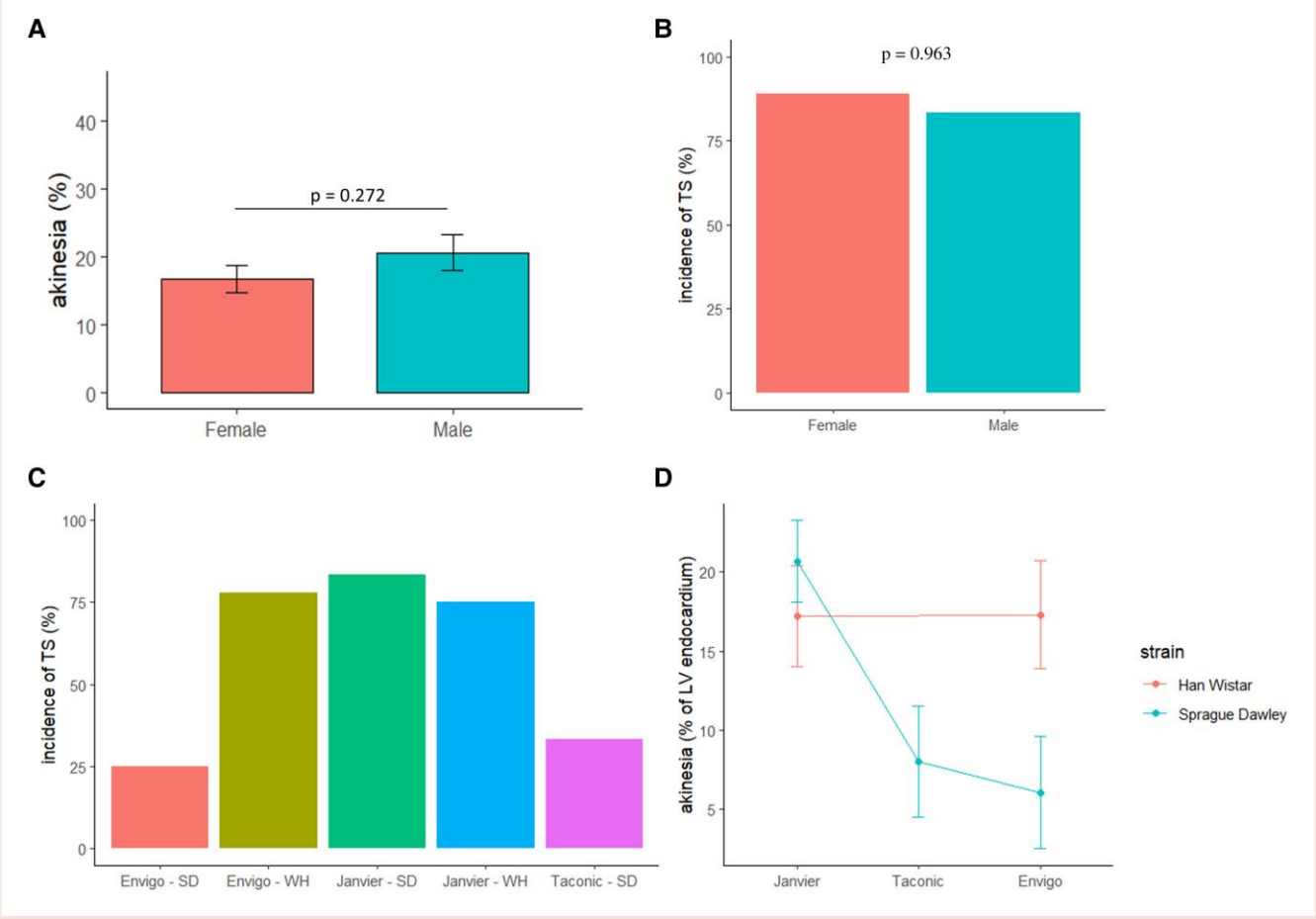


Figure 8 Takotsubo syndrome induced in both sexes and affected by type of breeder and not type of strain. (*A*) Female rats had a similar extent of akinesia as male rats (unpaired *t*-test. $n = 19$ and 25. $P = 0.272$). Mean ± SE. (*B*) Incidence of takotsubo syndrome in female rats compared with male rats. Analysed using the Fisher exact test. $P = 0.963$. The incidence of takotsubo syndrome (*C*) and extent of akinesia (*D*) by different strains and breeder. Analysed using a two-way analysis of variance approach, with group and strain as factors. Mean ± SE. Strain, $P = 0.604$; breeder, $P = 0.006$; strain*breeder, $P = 0.032$. LV, left ventricle; TS, takotsubo syndrome.

Conclusion

In the present article, we describe the development and characteristics of a refined and novel model of TS in rats. It reproduces several critical human features, including the following: (i) the acute onset of the characteristic morphological change, 'apical ballooning', represented here with two-dimensional cine loops and 3D imaging; we further show that this phenotype occurs in a dose- and time-dependent manner with stress hormones, and an optimal administration can be achieved by providing high incidence and low mortality; (ii) the natural course of reversible wall motion abnormalities within days; and (iii) the characteristic dynamic and reversible ECG changes. Other features, such as the importance of environmental factors over genetics, sex differences in young adults, and histological changes, were also examined and successfully reproduced. We present evidence indicating that akinesia in the apical segments is not caused by myocardial damage induced by isoprenaline but rather by the fact that catecholamines are the common factor behind reversible and irreversible cardiac damage and function. This model can enable us to explore the mechanisms, evaluate therapeutic alternatives, or identify diagnostic indicators in TS.

Lead author biography

Ermir Zulfaj, MD, graduated from Lund University, Sweden, and is currently engaged in translational research within Cardiology at the Department of Molecular and Clinical Medicine at Sahlgrenska Academy, University of Gothenburg.

Data availability

The data underlying this article are available in the article and in its online Supplementary material.

Supplementary material

Supplementary material is available at *European Heart Journal Open* online.

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