Noninfectious Mitral Annular Disruption
An Unusual Complication of a 25-Meter Fall
Eli V. Gelfand, MD; Kamal Khabbaz, MD

A previously healthy 35-year-old man fell from a height of 25 meters while pruning a tree. His fall was somewhat slowed by the untrimmed tree branches. The ground impact focused on the right thorax and axilla, with resultant closed head injury, right clavicular fracture, and multiple right-sided rib fractures with pneumo- and hemothorax. Emergent tube thoracotomy, tracheotomy, and orthopedic interventions were conducted. The patient’s 10-day hospital course was notable for steady improvement, and, other than for a mild traumatic brain injury, he felt well on discharge. Prior to discharge, a loud holosystolic murmur, consistent with mitral regurgitation, was auscultated. The patient remained afebrile and there was no evidence of heart failure or systemic embolic phenomena. A transthoracic echocardiogram done during the admission was suggestive of severe mitral regurgitation, but images were technically suboptimal. The patient was eventually referred to our facility for transesophageal imaging and a consultation for the question of mitral valve repair. At the time of our evaluation, 6 months after the accident, he reported no cardiopulmonary symptoms and was able to hike 5 to 7 miles without dyspnea or fatigue. A screening transthoracic echocardiogram had been done 5 years before the accident because of a family history of cardiomyopathy, and was normal, without evidence of valvular pathology.

A transesophageal echocardiogram showed intact mitral leaflets, without evidence of endocarditis or paravalvular abscess (Movie I in the online-only Data Supplement). However, there was marked disruption and fistulization of the anterior mitral annulus with a 9 × 7 mm irregularly shaped opening at the base of the A2/A3 mitral valve scallops (Figure A and B, Movies II and III in the online-only Data Supplement). There was systolic left ventricular-to-left atrial flow through the fistula (Figure C, Movie IV in the online-only Data Supplement); its Doppler signal was characteristic of mitral regurgitation (Figure D). Biventricular cavity size, systolic function, and estimated pulmonary artery systolic pressure were within normal limits.

In summary, these findings demonstrated a left ventricular-to-left atrial fistula through the anterior mitral annulus, acquired as a result of rapid deceleration injury from a fall. Because the patient was free of cardiovascular symptoms, and the probability of the need for valve replacement during repair was high, the recommendation was made to delay primary repair of the defect until the onset of symptoms or standard echocardiographic signs of left ventricular decompensation. Frequent clinical and echocardiographic follow-up was scheduled.

Disruption of the mitral annulus is well described as a consequence of (1) infective mitral endocarditis with paravalvular abscess formation and (2) cardiac surgery, usually, mitral or aortic valve replacement. Cardiac trauma as a result of a fall from heights is likewise well described and is frequently fatal, because it typically involves rupture of the atrial or ventricular free wall with rapid exsanguination. In other types of cardiovascular deceleration injury, such as that resulting from motor vehicle accidents, disruption of the most anterior cardiac structures is most frequently seen: avulsion of the tricuspid valve, aortic disruption, and right ventricular contusion. In case of aortic disruption, the ligamentum arteriosum is thought to serve as a rigid anchor, while the surrounding tissues continue in motion on impact, thus shearing the aorta. Likewise, in our patient, the fibrous skeleton of the heart at the confluence of the aortic and mitral valves presumably served as an anchor, while the valve apparatus itself continued motion. This discrepancy resulted in shear injury and fistulization of the anterior mitral annulus. To our knowledge, we describe the first case of survivable traumatic disruption of the mitral annulus.

Disclosures
None.

References

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Figure. Three-dimensional (A through C) and continuous-wave Doppler (D) transesophageal echocardiographic images of the LV-LA fistula. AML indicates anterior mitral leaflet; F, fistula; and LV-LA, left ventricular to left atrial.
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Supplementary Materials for

Autotaxin derived from lipoprotein(a) and valve interstitial cells promotes inflammation and mineralization of the aortic valve

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Methods

Procurement of tissues for analyses

We examined stenotic aortic valves (CAVD) that were explanted from patients at the time of aortic valve replacement. Control non-calcified aortic valves with normal echocardiographic analyses were obtained during heart transplant procedures. Patients with a history of rheumatic disease, endocarditis, and inflammatory diseases were excluded. Valves with moderate to severe aortic valve regurgitation (grade＞2) were excluded. The protocol was approved by the local ethical committee and informed consent was obtained from the subjects.

Remodelling score

The remodeling scores were determined by cardiovascular pathologists (C.C., S.T., S.P.) as previously described.¹

VICs isolation

Human VICs were isolated from control non-mineralized aortic valves obtained from patients undergoing heart transplantation. Aortic leaflets were incubated in 1mg/ml type I collagenase at 37°C for 30 minutes, then washed in HEPES1X, cut into pieces and incubated in 4.5mg/ml type I collagenase at 37°C for 30 minutes. Tissues were then washed in normal medium 3 times and seeded. Cells were used between passages 3 to 7.
ATX activity

Control non-mineralized and CAVD valve tissues were homogenized and harvested in Tris-buffered saline (140 mM NaCl, 5 mM KCl, 1 mM CaCl₂, 1 mM MgCl₂, 50 mM Tris, pH 8.0). Samples were incubated for 15 minutes at 37°C. The reaction was initiated with the addition of FS-3 (Echelon Biosciences, UT, USA) at a final concentration of 3 μM. The reaction was allowed to proceed at 37°C for 1h and the fluorescence was monitored at 520 nm. ATX activity was also measured in human plasma and Lp(a) fraction according to the same protocol. Lp(a) was either purified from human plasma or purchased from (Biomedical Technologies, MA, USA).² Results were normalized to protein contents.

Western blotting

Tissue pieces were mixed with lysis buffer (150mM NaCl, 20mM Tris pH7.5, 10% glycerol, 5mM EGTA, 5mM EDTA, 2mM sodium vanadate, 50mM sodium fluoride, 1% triton X-100, 0.1% SDS, 80mM β-glycerophosphate, 5mM sodium pyrophosphate, 1mM PMSF and protease inhibitor cocktail). Mechanical lysis was performed by using a polytron, following centrifugation, supernatants were harvested and protein loading buffer was added. Extracts were boiled 5 minutes, proteins were loaded onto polyacrylamide gels followed by electrophoresis and transferred onto nitrocellulose membranes. Membranes were blocked with TBS-tween containing 5% non-fat dry milk, incubated with ATX (Fisher ThermoScience, QC, Canada) and β-actin (Sigma-Aldrich, ON, Canada) antibodies overnight at 4°C. Membranes were then washed and incubated with HRP-labeled secondary antibodies (Cell Signaling Technology, MA, USA). Detection was done using clarity western ECL substrate (BioRad, ON, Canada). Images
were acquired and quantification analyses were performed using a ChemiDocMP system (BioRad, ON, Canada).

**Quantification of lysophosphatidylcholine and lysophosphatidic acid by thin layer chromatography**

Aortic cusps were homogenized for 30 seconds in 3mL CHCl₃:CH₃OH (2:1) containing 1% butylated hydroxytoluene. Solids were allowed to settle, then supernatants were decanted and pellets were stored at 4°C. The extraction procedure was repeated twice and samples were evaporated to dryness. Dry extracts were dissolved in 1mL CHCl₃:CH₃OH (10:1) and 20µl was applied to a thin-layer chromatography plate (20cm×20 cm). Samples were first migrated in CHCl₃:CH₃OH:H₂O (65:24:4) and then in hexane: diethyl ether:acetic acid (75:35:1). Visualization was performed using 0.1% Amido Black 10b in 1M NaCl.

**Immunostaining analyses**

Immunostaining analyses were performed with the following antibodies: ATX (Fisher ThermoScience, QC, Canada), ox-LDL (Accurate chemical, ON, Canada), E06 (Avanti Polar Lipids, AL, USA), Lp(a) (Novus Biologicals, ON, Canada) and Lp-PLA2 (Abgent, CA, USA). Slides were fixed in acetone: methanol (60:40) 10 minutes at -20°C, washed in TBS1X and incubated in dual enzyme block for 10 minutes (Dako, CA, USA). Slides were incubated with primary antibodies overnight at 4°C in 1% BSA in TBS1X and then washed with TBS1X. The EnVision Dual Link System-HRP and the AEC substrate (Dako, CA, USA) were used to detect signal. To assess the numbers of CD45 (Abcam, Toronto, ON, Canada) positive cells, representative regions, rich in cells, were detected in each section and the number of cells was counted by two observers blinded to clinical
results. Cells were counted at 400X in triplicates, and a mean value was attributed to each valve. Data were reported as the average number of cells per 400X field. To document the amount of ox-LDL in the aortic valve, a semi-quantitative score was used; score 1, 2 or 3 were given respectively when less than 25%, 25-50%, and more than 50% of the valve area was specifically immunostained.

**Immunofluorescence of human aortic leaflets**

Tissues were fixed in a solution of acetone-methanol (60:40), quenched in 50mM NH₄Cl for 30 minutes and blocked with 1% BSA in TBS1X for 30 minutes. Slides were then incubated with ATX (Fisher ThermoScience, QC, Canada) and Lp(a) (4H1, Fisher ThermoScience, QC, Canada) or vimentin antibodies (Sigma-Aldrich, ON, Canada) in 1% BSA in TBS1X overnight at 4°C. Slides were then incubated with alexa568-conjugated anti-rabbit and alexa488-conjugated anti-mouse secondary antibodies (Molecular Probes/Thermo Fisher Scientific, ON, Canada). Images were acquired using a confocal microscope system (FV1000, Olympus, ON, Canada, objective 60X oil, NA1.42) driven by Fluoview software (FV-10 ASW 3.1, Olympus). Merges were performed with ImageJ 1.47g (NIH, USA).

**Proximity ligation assay**

Six μm slices were cut from OCT embedded human mineralized aortic valve leaflets. Tissues were fixed with acetone:methanol (60:40) 10 minutes at -20°C, followed by quenching in 50mM NH₄Cl for 30 minutes at room temperature. Permeabilization was then performed in 0.2% triton (in PBS1X) for 10 minutes with constant agitation. Following permeabilization, the proximity ligation assay (Olink Bioscience, Sweden)
was performed according to the manufacturer’s instructions using monoclonal antibody 4H1 (Fisher ThermoScience, QC, Canada) that recognizes apolipoprotein (a) (fragment 4330-4521) and polyclonal antibody against ATX (Fisher ThermoScience, QC, Canada). Samples were analysed using a confocal microscope system (FV1000, Olympus, ON, Canada, objective 60X oil, NA1.42) driven by Fluoview software (FV-10 ASW 3.1, Olympus). Merges were performed with ImageJ 1.47g (NIH, USA).

Immunofluorescence of cells

Human VICs were seeded on poly-L-lysine coated glass coverslips. The following day, cells were washed once with PBS1X and fixed in 3.7% formaldehyde for 30 minutes at 37°C. Cells were treated 15 minutes with 50mM NH₄Cl in PBS1X. Cells were incubated in PBS1X containing 5% milk for one hour at room temperature with constant agitation. Incubation with anti-ATX (Fisher ThermoScience, QC, Canada) was performed in PBS1X containing 1% milk overnight at 4°C. Cells were incubated one hour with FITC-conjugated anti-rabbit secondary antibodies (Molecular Probes/Thermo Fisher Scientific, ON, Canada). Confocal images were acquired using a Zeiss microscope driven by the Zen software (Objective 40X oil, 1.4 NA, Zeiss, ON, Canada). Image processing and quantification were performed with ImageJ 1.47g (NIH, USA).

P65 translocation

Cells were treated with lysophosphatidylcholine for 30 minutes at 37°C, fixed in 3.7% formaldehyde for 30 minutes at 37°C and treated 15 minutes with a 50 mM NH₄Cl solution. Cells were then permeabilized 10 minutes with 0.2% triton in PBS1X, blocked for 1 hour in 5% milk PBS1X and incubated with mouse monoclonal NF-κB p65 antibody (Clone 2A12A7) (BioVision, CA, USA) at 4°C overnight. Cells were incubated
with an alexa488 conjugated anti-mouse secondary antibody (Molecular Probes/Thermo Fisher Scientific, ON, Canada). Cells were mounted in DAPI-containing mounting medium and samples were analysed using an epifluorescence microscope system BX51 (Olympus, ON, Canada), mounted with an Evolution QEi camera (Media Cybernetics, MD, USA) driven by Image-Pro Plus 7.0 (Olympus, ON, Canada). Merges were performed with ImageJ 1.47g (NIH, USA).

**Real-time PCR**

RNA was extracted from valves explanted from patients, mice and from cells during in vitro experiments. Total RNA was isolated with RNeasy micro kit from Qiagen (ON, Canada). The RNA extraction protocol was performed according to manufacturer’s instructions. The quality of total RNA was monitored by capillary electrophoresis (Experion, Biorad, ON, Canada). One μg of RNA was reverse transcribed using the Quantitec Reverse Transcription Kit from Qiagen. Quantitative real-time PCR (qPCR) was performed with Quantitec SYBR Green PCR kit from Qiagen on the Rotor-Gene 6000 system (Corbett Robotics Inc, CA, USA). Primers for Lp-PLA2, IL6, TNF-α, ATX (human and mouse), BMP2 (human and mouse), Runx2, Osteonectin, ALP and COL1A1 were obtained from Qiagen (ON, Canada). The expression of the hypoxanthine-guanine phosphoribosyltransferase (HPRT) gene (human and mouse) (Life technologies/ Thermo Fisher Scientific, ON, Canada) was used as a reference gene to normalize the results.
**In vitro analyses of calcification**

Cells were incubated for 7 days with a pro-calcifying medium containing: DMEM + 5% FBS, 10^{-7} \text{ M insulin}, 50 \mu g /\text{ml ascorbic acid} and \text{NaH}_{2}\text{PO}_{4} \text{ at 2 mM. The medium was supplemented with lysophosphatidylcholine (100 nM) (Sigma-Aldrich, ON, Canada), lysophosphatidic acid (10 \mu M) (Sigma-Aldrich, ON, Canada), BAY11-7085 (20\mu M) (inhibitor of IкB phosphorylation; Calbiochem Gibbstown, NJ, USA), noggin (2.5\mu M) (inhibitor of BMP2 and 4, Calbiochem, Gibbstown, NJ, USA), Ki16425 (10 \mu M) (antagonist of lysophosphatidic receptors 1-3 (LPAR1) and (LPAR3), Tocris Bioscience, MI, USA).}

**Determination of calcium concentrations**

Calcium content in cell cultures was determined by the Arsenazo III method (Synermed, Monterey Park, CA, USA), which relies on the specific reaction of Arsenazo III with calcium to produce a blue complex. Results are measured at 650 nm on the Modular P800 Elecsys of Roche Diagnostics apparatus (Roche Diagnostics, QC, Canada). This reaction is specific for calcium. Magnesium is prevented from forming a complex with the reactive. Results were normalized to protein contents.

**Alizarin red staining of cultured cells**

Cells were stained with 2% Alizarin red solution. Alizarin red solution was prepared by dissolving 2 g of Alizarin red (Sigma, Oakville, ON, Canada) in 100 ml distilled water, mixed well and pH was adjusted to 4.2 with 10\% \text{NH}_{4}\text{OH}. The solution was filtered before use. Cells were washed one time with PBS and fixed with 3.7\% formaldehyde (Sigma, Oakville, ON, Canada) for 30 minutes, and then washed once with distilled
water. Filtered 2% alizarin red solution was added to the cells for 2-3 minutes and washed with distilled water and acetone.

**siRNA transfection**

Cells were plated at a density of $6 \times 10^4$ cells / well (12-well plates) or $1 \times 10^5$ cells / well (6-well plates). The following day, cells were transfected by using HiPerfect reagent (Qiagen, ON, Canada) with 300-600 ng siRNA against IL6 or ATX (Qiagen, ON, Canada). Reduction of target genes was measured by qPCR, ELISA assay or enzymatic activity assay.

**Quantification of IKKα and pIKKα**

VICs were collected in cell lysis buffer. CAVD tissues were homogenized in lysis buffer. Lysates were used directly in ELISA kit (PathScan®IKKa/Phospho-IKKα (Ser176/180), Cell Signaling Technology, Inc., USA). The quantification of IKKα and phospho-IKKα was determined in accordance with the manufacturer's instructions and normalized with protein content.

**Measurement of IL-6 in supernatants**

IL-6 was measured in supernatants of human VICs exposed to lysophosphatidylcholine, Ki6425 or lysophosphatic acid for 48 hours. IL-6 was measured by ELISA (R&D Systems, MN, USA) according to manufacturer's instructions.

**Measurement of ATX in supernatants**

ATX was measured in supernatants of human VICs using ELISA (Echelon Biosciences, UT, USA) according to manufacturer's instructions and normalized with protein content.
Animals

All animal protocols were conducted according to guidelines set out by the Laval University Animal Care and Handling Committee and are conform with the NIH guidelines for the care and use of laboratory animals. LDLR<sup>−/−</sup>/ApoB<sup>100/100</sup>/IGF2 (on C57Bl/6J background) were generated from an established colony at the Quebec Heart and Lung Institute of Laval University from original founders kindly provided by Dr. Seppo Ylä-Herttuala (University of Eastern Finland, Finland). C57BL/6 mice were purchased from Jackson Laboratories (MA, USA). Male mice were housed in a pathogen-free, temperature-controlled environment under a 12:12 hour light-dark cycle and fed ad libitum of a high fat, high sucrose, cholesterol diet (55% calories from fat, 28% from sucrose, 0.2% cholesterol) for 6 months starting at 12 weeks of age. At the end of protocol, mice were sacrificed by anesthesia under isoflurane (2-3%, inhalation) and cardiac puncture, which was performed by a qualified animal care technician.

Echocardiography in mice

The investigator performing echocardiography analyses was blinded to the group allocation. Transthoracic echocardiography was performed under 2.5%-isofluorane anaesthesia, with a L15-7io (5-12 Megaherz) and S12-4 (4-12 Megaherz) probes connected to a Philips HD11XE ultrasound system (Philips Healthcare Ultrasound, Netherlands). Left ventricular (LV) M-mode imaging was obtained in parasternal short-axis view at the level of the papillary muscles. LV dimensions were measured at end-diastole (LVDd) and end-systole (LVDs), LV fractional shortening (LVFS) was calculated as (LVDd – LVDs)/LVDd× 100%. LV volumes and ejection fractions (EF) were calculated using the Teicholz formula. The diameter of the LV outflow-tract
LVOTD was measured in a zoomed parasternal long-axis view. LVOT cross sectional area (LVOTCSA) was calculated as $\pi (D/2)^2$. LVOT flow velocity was obtained by pulsed-wave Doppler (PW) in the apical 5-chamber view. The LV stroke volume (SV) was calculated as LVOTVTI x LVOTCSA, where LVOTVTI is the velocity-time integral measured in the LVOT. Aortic valve area (AVA) was calculated using the formula: (LVOT area x LVOT peak velocity)/ peak jet velocity across the aortic valve. Cardiac output (CO) was measured as: HR x SV and indexed for weight (cardiac index [CI]). Pulsed-wave Doppler was used to record transmitial flow in the apical 4-chamber view and from this signal we measured peak velocity of E- and A- waves. Mitral annulus motion velocity was recorded by tissue Doppler imaging, velocity during early filling E’ was measured, and E/E’ ratio was calculated. Continuous-wave Doppler was also used to record aortic jet flow velocity in the apical 5-chamber view and peak aortic jet velocity and velocity-time integral were measured. The average of 3 consecutive cardiac cycles was used for each measurement. Special care was taken to get similar imaging planes at follow-up studies.

**Alizarin red staining of mouse tissues**

Hearts were perfused with HEPES, and embedded in OCT. Hearts were cut exposing leaflets and 6 µm slices were made. Sections were fixed with acetone-methanol (60:40), rinsed 2 minutes in water and stained with an alizarin red (Sigma-Aldrich, ON, Canada) solution (2% alizarin red in water, pH 4.2 adjusted by using 10% ammonium hydroxide in water) for 3 minutes. Sections were incubated in acetone for 30 seconds and then rinsed with water 30 seconds. Finally, sections were mounted in cytoseal (Dako, ON, Canada). Pictures were acquired using a Zeiss Axio Observer microscope using the Zen
software (Zeiss, ON, Canada), with a LD A-Plan 10x/0.25 Ph1 objective (Zeiss) in polarised light. Images were processed and quantifications were performed using Image J1.47g (NIH, USA).

**Osteosense 690EX calcium quantification**

Tissues were fixed in a solution of acetone-methanol (60:40), washed 3 times with PBS1X, tissue were then stained for 1 hour with OsteoSense 680EX (Perkin Elmer, MS, USA) diluted to 1:10 in PBS 1X. Slides were then washed 3 times with PBS1X and mounted. Images were acquired with Nikon Eclipse TE 2000 epifluorescence microscope (NY, USA) equipped with a MicroMax 512BFT CCD camera (Princeton Instruments, NJ, USA). Quantifications were performed using Image J1.47g (NIH, USA).

**Immunofluorescence of mouse aortic leaflets**

Tissues were fixed in a solution of acetone-methanol (60:40), washed 3 times with TBS1X, quenched in 50mM NH₄Cl for 30 minutes and blocked with 1% BSA in TBS1X for 30 minutes. Slides were then incubated with ATX (Fisher ThermoScience, QC, Canada) or BMP-2 (Novus Biologicals, ON, Canada) antibodies in 1%BSA in TBS1X overnight at 4°C. Slides were washed with TBS1X and incubated with an alexa568-conjugated anti-rabbit secondary antibody (Molecular Probes/Thermo Fisher Scientific, On, Canada). Images were acquired using an Olympus BX51 microscope (Olympus, ON, Canada), mounted with an Evolution QEi camera (Media Cybernetics, MD, USA) driven by Image-Pro Plus 7.0 (Olympus, ON, Canada). Quantifications were performed using Image J1.47g (NIH, USA).
Cholesterol and triglycerides measurements in mice

Plasma cholesterol and triglycerides assay was done according to the protocol Company (Randox, Crumlin, United Kingdom).

Statistical analyses

Results were expressed as means ±SEM. Continuous data were tested for normality of distribution with the Shapiro-Wilk test and compared with Student t-test. For continuous data with a non-normal distribution or with a n ≤ 10 the values were compared between groups with nonparametric Wilcoxon-Mann-Whitney or Kruskal-Wallis test when two or more than two groups were compared respectively. Post-hoc Steel-Dwass multiple comparisons test were performed when the p value of the Kruskal-Wallis test was <0.05. Categorical data were expressed as a percentage and compared with Fisher exact test. A p value <0.05 was considered as statistically significant. Statistical analyses except for the Wilcoxon-Mann-Whitney and Kruskal-Wallis tests were performed with commercially available software package JMP 10.0. Wilcoxon-Mann-Whitney and Kruskal-Wallis tests were performed with Prism 6.0.
Supplementary figure 1: Relationships between ATX level (dichotomized at the median value) and (a) ox-LDL score (b) CD45 positive cells (c) Lp-PLA2 mRNA levels (d) TNF-α mRNA levels and (e) weight of aortic valve. p values: Wilcoxon-Mann-Whitney
Supplementary table 1: Assessment of biological parameters in IGFII mice.

<table>
<thead>
<tr>
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<th>IGFII</th>
<th>IGFII LPA</th>
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</thead>
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<tr>
<td>Cholesterol (mmol/l)</td>
<td>16.2±2.0</td>
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<td>Triglycerides (mmol/l)</td>
<td>1.2±0.3</td>
<td>1.4±0.4</td>
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<td>Glucose (mmol/l)</td>
<td>8.5±0.8</td>
<td>8.8±1.2</td>
<td>0.60</td>
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**Supplementary table 2:** Echocardiographic parameters. LV: left ventricle; FS: fractional shortening, EF: ejection fraction, SV: stroke volume, CO: cardiac output, E/E’: mitral inflow, LVOT: left ventricular outflow tract, and AVA: aortic valve area.

<table>
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<tr>
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<th>IGFII Baseline</th>
<th>6 months</th>
<th>p</th>
<th>IGFII LPA Baseline</th>
<th>6 months</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Mice weight (g)</td>
<td>24±3</td>
<td>39±2</td>
<td>0.001</td>
<td>24±5</td>
<td>35±5</td>
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<tr>
<td>LV systolic function</td>
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<tr>
<td>FS (%)</td>
<td>39±4</td>
<td>34±4</td>
<td>0.005</td>
<td>39±3</td>
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<tr>
<td>EF (%)</td>
<td>77±4</td>
<td>69±7</td>
<td>0.021</td>
<td>77±4</td>
<td>69±7</td>
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<tr>
<td>SV (ml)</td>
<td>0.06±0.01</td>
<td>0.06±0.01</td>
<td>0.080</td>
<td>0.06±0.01</td>
<td>0.06±0.01</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>337±32</td>
<td>412±91</td>
<td>0.062</td>
<td>349±79</td>
<td>379±93</td>
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<td>CO (ml)</td>
<td>19±3</td>
<td>28±8</td>
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<td>19±6</td>
<td>21±6</td>
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<td>LV diastolic function</td>
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<td>E/E’</td>
<td>26.6±3.2</td>
<td>24.7±3.4</td>
<td>0.30</td>
<td>23.2±1.8</td>
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<td>Aortic Valve Hemodynamics</td>
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<td>Peak aortic jet velocity (cm/s)</td>
<td>96.1±18.0</td>
<td>138.2±8.6</td>
<td>0.003</td>
<td>89.8±10.8</td>
<td>171.0±22.3</td>
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<td>Peak LVOT velocity (cm/s)</td>
<td>90.7±14.5</td>
<td>100.0±17.6</td>
<td>0.12</td>
<td>82.4±6.7</td>
<td>88.2±13.5</td>
<td>0.18</td>
</tr>
<tr>
<td>LVOT area (mm$^2$)</td>
<td>1.42±0.11</td>
<td>1.58±0.09</td>
<td>0.54</td>
<td>1.43±0.16</td>
<td>1.46±0.11</td>
<td>0.34</td>
</tr>
<tr>
<td>AVA (mm$^2$)</td>
<td>1.31±0.14</td>
<td>1.18±0.20</td>
<td>0.43</td>
<td>1.32±0.12</td>
<td>0.77±0.14</td>
<td>0.030</td>
</tr>
</tbody>
</table>