**Appendix**

***In Silico* Parameter Validation**

Simulations were performed to test the performance of the systemic-pulmonary model and to validate the parameters used to mimic rat cardiac physiology. The *in silico* model was implemented in MATLAB (Mathworks, US).

The *in silico* model architecture was similar to that described in Figure 1, with some necessary changes made to the active compartments. In the *in silico* model, as there was no physical muscle to generate a force twitch, the pressure (*P*) developed in each ventricle was instead modeled by Equation S1 using the time-varying elastance theory (Suga *et al.*, 1973; Suga & Sagawa, 1974)

*Equation S1*

where *e*(*t*) is the activation function (a normalized isometric force twitch measured from an isolated rat trabecula; Figure S1, DOI: 10.17608/k6.auckland.24116526), *V* is the current ventricle volume*, Vo* isthe volume of the ventricle when ventricular pressure is zero*,* and *EES* is the ventricle-specific end-systolic elastance, defined as the slope of the end-systolic pressure-volume relation. This relation is usually approximated to be linear within low volume ranges (Suga *et al.*, 1986; Wannenburg *et al.*, 1992), although experimentally, it is curvilinear over greater volume ranges (Kass *et al.*, 1989; Sato *et al.*, 1998). For these simulations, the equation was simplified by assuming *V0* to be zero, which constrained the end-systolic relation to intersect the origin of the pressure-volume (PV) axis.

The use of a mathematically-prescribed ventricular PV relation during *in vitro* experiments was unnecessary since the trabecula determined the stress-length relation. Hence, the simplified end-systolic PV relation was only used for model development and simulation purposes. There was also no need to explicitly model the end-diastolic PV relation because the passive behavior is inherent to the isolated muscle sample during *in vitro* experiments.

For the simulation, the force-twitch was modelled at a rate of 2 Hz (Figure S1). The *in silico* model was run until the volume distribution between compartments reached a steady state. Figure S2 (DOI: 10.17608/k6.auckland.24116526) illustrates an example of the simulation output, adopting the parameters (Table 1) for pressure (Panel A) and flow rates (Panel B) relevant for determining the dynamics of left-ventricular PV loops. Aortic pressure (*PAO*) represents afterload, defined as the pressure immediately downstream from the left ventricle that must be overcome by the left ventricle to initiate ejection. The rate and dynamics of this ejection (*QAV*) were therefore determined by the pressure gradient formed across the aortic valve between *PLV* and *P­AO*. The venous return pressure determined the preload via the flow rate through the mitral valve (*QMT*), which arose from the pressure difference between pulmonary venous pressure (*PPU*) and *PLV* during diastole. Therefore, this venous return pressure determined the end-diastolic volume before the next contraction.

Figure S3A (DOI: 10.17608/k6.auckland.24116526) displays simulated PV loops for *Rp* = 175 GPa·s·m-3 (solid) and *Rp* = 115 GPa·s·m-3 (dashed line), produced by the systemic-pulmonary model using the parameters in Table 1. Each loop exhibits a different initial volume and afterload. Panel B shows the loops for a range of *RSYS* values, with the arrow indicating an increase of systemic resistance from low values (50 GPa·s·m-3) to high values (500 GPa·s·m-3). The resultant loops exhibit simultaneous increases in both preload and afterload, as would be expected and have been observed previously experimentally (Suga *et al.*, 1973; Cingolani & Kass, 2011).

These *in silico* results provided confidence in the derived parameters (Table 1) and enabled us to commence *in vitro* experiments on isolated trabeculae.

**References**

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**Figure Captions**

Figure S1: Normalized isometric force twitch used as the driver of the model that serves as the activation function (*e(t)*) in simulating the systemic-pulmonary hemodynamic model. This force twitch was measured from an isolated rat cardiac trabecula contracting in vitro (Taberner et al., 2011).

Figure S2: Pressures (A) and flow rates (B) of the left ventricle computed from simulation of the six-compartment systemic-pulmonary model.

Figure S3: Simulated PV loops for the six-compartment systemic-pulmonary model. Panel A displays PV loops with systemic resistance (*RSYS*) values of 115 GPa·s·m-3 (dashed) and 175 GPa·s·m-3 (solid) used to tune the model, coinciding with muscle length change from 0.95 *Lo* to *Lo*. Panel B displays PV loops for *RSYS* values ranging from low (50 GPa·s·m-3) to high (500 GPa·s·m-3), as indicated by the arrow.