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Original article

# Cardiac phase-targeted dynamic load on left ventricle differentially regulates phase-sensitive gene expressions and pathway activation

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ABSTRACT

The heart has remarkable capacity to adapt to mechanical load and to dramatically change its phenotype. The mechanism underlying such diverse phenotypic adaptations remains unknown. Since systolic overload induces wall thickening, while diastolic overload induces chamber enlargement, we hypothesized that cardiac phase-sensitive mechanisms govern the adaptation. We inserted a balloon into the left ventricle (LV) of a Langendorff perfused rat heart, and controlled LV volume (LVV) using a high performance servo-pump. We created isolated phasic systolic overload (SO) by isovolumic contraction (peak LV pressure >170 mmHg) at unstressed diastolic LVV [end-diastolic pressure (EDP) = 0 mmHg]. We also created pure phasic diastolic overload (DO) by increasing diastolic LVV until EDP >40 mmHg and unloading completely in systole. After 3 hours under each condition, the myocardium was analyzed using DNA microarray. Gene expressions under SO and DO conditions were compared against unloaded control condition using gene ontology and pathway analysis ($n = 4$ each). SO upregulated proliferation-related genes, whereas DO upregulated fibrosis-related genes ($P < 10^{-5}$). Both SO and DO upregulated genes related functionally to cardiac hypertrophy, although the gene profiles were totally different. Upstream regulators confirmed by Western blot indicated that SO activated extracellular signal-regulated kinase 1/2, c-Jun $NH_2$-terminal kinase, and $Ca^{2+}$/calmodulin-dependent protein kinase II (3.2-, 2.0-, and 4.7-fold versus control, $P < 0.05$, $n = 5$), whereas DO activated p38 (2.9-fold, $P < 0.01$), which was consistent with the downstream gene expressions. In conclusion, pure isolated systolic and diastolic overload permits elucidation of cardiac phase-sensitive gene regulation. The genomic responses indicate that mechanisms governing the cardiac phase-sensitive adaptations are different.



## 1. Introduction

There is accumulating evidence that exposure of the heart to various mechanical loads induces diverse myocardial functions, gene expressions, and phenotypic responses [1,2]. In pressure overload such as hypertension or ventricular outflow obstruction, high afterload increases myocardial systolic wall stress. In contrast, volume overload such as valve regurgitation or shunting increases preload and stretches the myocardium in diastole.

Pressure overload-induced concentric hypertrophy, i.e., wall thickening, is an adaptive mechanism to lower systolic wall stress according to the law of Laplace [3]. In contrast, volume overload-induced eccentric hypertrophy cannot lower wall stress if pressure remains unchanged, because of the law of Laplace. However, the enlarged heart eases cardiac filling, increases cardiac output, and in turn lowers cardiac filling pressure. If the reduction in filling pressure overrides the increase in chamber size that determines wall stress, the net effect of chamber enlargement can lower wall stress in diastole. Therefore, in both systole and diastole, normalization of phase-specific wall stress may be the goal of adaptation by the myocardium.

Pressure overload, volume overload [4–6] and stretched myocytes [7] have been reported to induce expression of specific genes and activation of specific signal pathways. However, the mechanism underlying these diverse adaptations remains unclear. Most pathological conditions are associated with mixed loads, both in systole and diastole. In acute *in vivo* experiments, pressure overload inevitably increases systolic and diastolic wall stress, while volume overload increases not only diastolic but also systolic wall stress. In other words, it is never possible to create cardiac phase-targeted isolated load to the heart and evaluate pure responses depending on the cardiac phase in *in vivo* models. To learn more about the mechanisms of these two adaptations, development of an experimental system to impose cardiac phase-targeted load to the heart is indispensable. We hypothesized that systole- and diastole-sensitive mechanisms govern myocardial adaptations. To prove this hypothesis, we overcame the limitations of *in vivo* models by creating pure isolated loading conditions *ex vivo* and investigated the gene expression profiles under systolic and diastolic overload conditions.

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## 2. Materials and methods

### 2.1. Animals and Langendorff-perfused rat heart preparation

The investigation conformed with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996). All surgical and experimental protocols were approved by and performed according to the guidelines for the care and use of animals established by Kyushu University.

We used male Sprague Dawley rats (Japan SLC, Hamamatsu, Japan) weighing 448 ± 11 g (21–23 week of age; $n = 36$) for the experiments. A rat was anesthetized with a mixture of medetomidine (0.3 mg/kg), midazolam (4 mg/kg) and butorphanol tartrate (5 mg/kg) according to our institutional recommendation, intubated for artificial ventilation, and heparinized (1,000 units i.v.). A median sternotomy was performed and the heart was rapidly excised into ice-cold perfusion fluid. The aorta was cannulated with a shortened and blunted 14-gauge needle and perfusion was initiated at 80 mmHg using the apparatus illustrated in Fig. 1. We minimized ischemia by quick isolation and perfusion (completed within 20 s). In all experiments, a modified Krebs–Henseleit perfusion fluid was used, which contained (mM): NaCl, 118; glucose, 11; $NaHCO_3$, 25; KCl, 4.7; $MgSO_4$, 1.2; $KH_2PO_4$, 1.2; $CaCl_2$, 2.5. The perfusate was pumped by a roller pump upward to a reservoir above the heart, where the fluid was bubbled with a mix of 95% $O_2$ and 5% $CO_2$ at 34 °C to give a pH of 7.4 and $pO_2$ of 500 mmHg, and was filtered through an in-line 0.45 μm Sterivex-HV filter (Merck Millipore, Billerica, MA) before delivery to the heart. The heart was perfused by gravity only. Thebesian fluid accumulated in the left ventricle (LV) was vented via a polyethylene drain through the apex of the heart. A thin latex balloon with an unstressed volume of 290 μl connected with a stainless-steel tube was inserted into the LV through an incision in the left atrial appendage. The ventricular balloon was connected to a high-performance servo-controlled piston pump (Labworks Inc., Costa Mesa, CA) to regulate the intra-balloon volume. The intra-balloon volume was measured by a linear variable differential transformer. The volume of the balloon material (90 μl) was added to the intra-balloon volume to estimate the real LV volume (LVV). A 2F micromanometer catheter (Millar instruments, Houston, TX) was inserted into the balloon to measure LV pressure (LVP). The LV electrocardiogram was recorded, and the heart rate (HR) was maintained at 270 beats/min by right atrial pacing. The average time from initial perfusion to completion of the setup was 5 min. We started the experiments after stabilization for 15 min under a perfusion pressure of 150 mmHg. The temperature was maintained at 34 °C.

### 2.2. Experimental protocols

Each heart was assigned to one of the following three conditions: phasic systolic overload (SO), phasic diastolic overload (DO), and no loading throughout the cardiac cycle (CN; control in which both end-systolic pressure and end-diastolic pressure equal 0 mmHg). We created SO by isovolumic contraction (peak systolic LVP >170 mmHg) at a constant diastolic volume with end-diastolic pressure (EDP) equals 0 mmHg. For DO, we increased LVV in diastole so that EDP reached 40 mmHg, and unloaded quickly in systole to minimize LVP generation. At the end of 3 hours, the myocardium from each heart was collected for subsequent analyses.

### 2.3. Lactate dehydrogenase (LDH) assay

LDH concentration in the collected efflux of perfused heart was measured using LDH-Cytotoxicity Assay Kit (Wako, Osaka, Japan) according to the manufacturer's protocol. Briefly, the fluid was reacted with reagent for 30 min at room temperature. Then the absorbance at 560 nm was measured with a microplate reader (TECAN, Männedorf, Switzerland). Total LDH release was estimated from the product of concentration and efflux volume.

### 2.4. Microarray analysis

Total RNA was isolated from the free wall of LV using an RNeasy Mini Kit (Qiagen, Hilden, Germany). The RNA was purified by ethanol precipitation and dissolved in RNase-free water. This preparation was electrophoresed on an Experion system (Bio-Rad, Hercules, CA), and RNA quality was judged by measuring the ratio of band intensity between 28S and 18S rRNA. The RNA samples passing the quality check were subjected to a DNA microarray analysis. Total RNA (200 ng) was converted to its biotinylated-cRNA according to the manufacturer's procedures (Illumina TotalPrep RNA Amplification Kit, Ambion, Austin, TX). Briefly, reverse transcription of extracted mRNA to the first-strand cDNA was performed for 2 hours at 42 °C, using reverse transcriptase (ArrayScript, Ambion) and an oligo (dT) primer bearing a T7 promoter.

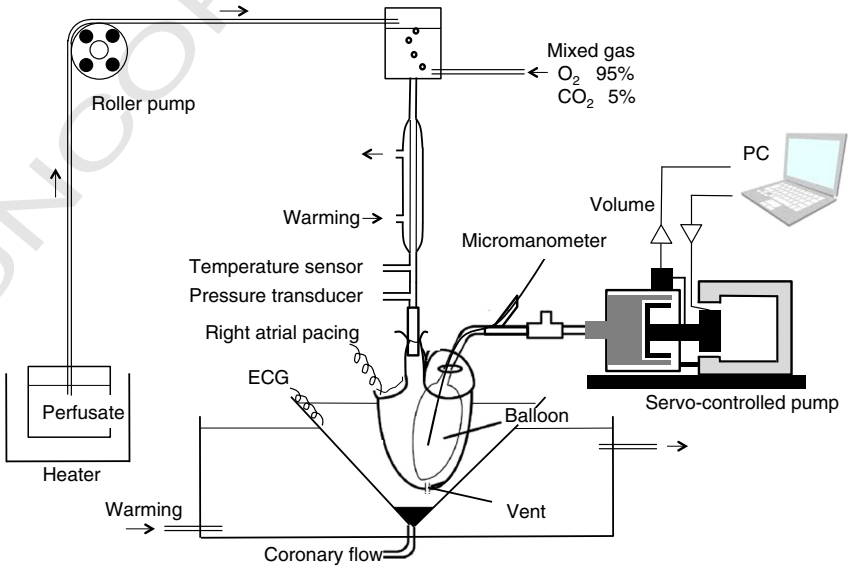


**Fig. 1.** Schematic diagram of the perfusion apparatus. Perfusate is pumped upward to the reservoir by a roller pump with an overflow fixed at 150 mmHg above the heart. The perfusate is maintained at a temperature of 34 °C by a heating coil, and at $pO_2$ of 500 mmHg and pH 7.4 by bubbling a gas mixture containing 95% $O_2$ and 5% $CO_2$. The latex balloon is inserted into the left ventricle through the left atrium. The intra-balloon volume is regulated by a servo-pump under right atrial pacing.



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