Abnormalities of capillary microarchitecture in a rat model of coronary ischemic congestive heart failure

Jiqiu Chen, Elisa Yaniz-Galende, Heather J. Kagan, Lifan Liang, Saboor Hekmaty, Chiara Giannarelli and Roger Hajjar*

Cardiovascular Research Center. Mount Sinai School of Medicine. New York, NY 10029

* Corresponding author:
Roger J. Hajjar, MD (Roger.hajjar@mssm.edu)
Cardiovascular Research Institute
Mount Sinai School of Medicine
1470 Madison Ave. 7th Floor
New York, NY 10029
Tel: 212-824-8901
Fax: 212-241-4080

Words: 6,220
ABSTRACT

Background: The aim of the present study is to explore the role of capillary disorder in coronary ischemic congestive heart failure (CHF). Methods: CHF was induced in rats by aortic banding plus ischemia/reperfusion followed by aortic de-banding. Coronary arteries were perfused with plastic polymer containing fluorescent dye. Multiple fluorescent images of casted heart sections and scanning electric microscope of coronary vessels were obtained to characterize changes in the heart. Cardiac function was assessed by echocardiography and in vivo hemodynamics. Results: Stenosis was found in all levels of the coronary arteries in CHF. Coronary vasculature volume and capillary density in remote myocardium were significantly increased in CHF compared with control. This occurred largely in micro-vessels with diameter $\leq 3$ μm. Capillaries in CHF had a tortuous structure, while normal capillaries were linear. Capillaries in CHF had inconsistent diameters, with assortments of narrowed and bulged segments. Their surfaces appeared rough, potentially indicating endothelial dysfunction in CHF. Segments of main capillaries between bifurcations were significantly shorter in length in CHF than in control. Transiently increasing preload by injecting 50 μl of 30% NaCl demonstrated that the CHF heart had lower functional reserve; this may be associated with congestion in coronary microcirculation. Conclusions: Ischemic coronary vascular disorder is not limited to the main coronary arteries, as it occurs in arterioles and capillaries. Capillary disorder in CHF included stenosis, deformed structure, proliferation and
roughened surfaces. This disorder in the coronary artery architecture may contribute to the reduction in myocyte contractility in the setting of heart failure.

**Key words**: coronary artery, capillary, congestive heart failure, hypertension, ischemia/reperfusion.

**Abbreviations**: Congestive heart failure = CHF, left ventricle = LV, left coronary artery = LCA; myocardial infarction = MI; ischemia/reperfusion = I/R; aortic banding = Ab, aortic de-banding = DeAb; ejection fraction = EF; fractional shortening = FS.

**INTRODUCTION**

It is widely accepted that a decrease in intrinsic contractility of the myocardium is the primary defect in congestive heart failure (CHF)(5). CHF can be caused by many conditions which reduce the efficiency of the myocardium through damage or overload, including myocardial infarction, hypertension, valve defects, diabetes, arrhythmia and genetic mutations(9, 28, 30). Coronary artery disease is the most common cause of CHF, comprising approximately two-thirds of patients with severe class IV heart failure(21, 25). However, the detailed mechanisms of reduced mechanical function remain unclear. Distinct defects of excitation–contraction coupling compartments and organelles have been
identified in CHF cardiac samples, and include changes in Ca$^{2+}$ cycling proteins(4, 32, 37).

In this study we wanted to address the specific question of what happens to coronary architecture in rodent models in the setting of congestive heart failure. Transient (< 30 minutes) arterial ligation, moderate occlusion or blockage of a small vessel leads to sub-myocardial infarction (MI) and minor alternation or no change in cardiac function(11). Severe ischemia (>30 minutes) of a major coronary artery induces a large MI and serious heart dysfunction, usually due to pathological physical geometry—large MI reduce left ventricular (LV) ejection fraction (EF) and increase LV volume as myocardial mass is replaced by the non-contractile scar tissue(9). However, the remote myocardium is not ischemic.

So, how does contractile depression occur? In response to pressure overload or myocardial injury, the LV progressively remodels — remote non-injured myocardium hypertrophy and collagen fibrosis develop(20, 34). Hypertrophic myocytes usually have enhanced muscle contractility as long as blood supply is sufficient(26, 38). There is no enough evidence to show that deficient blood supply in remote myocardium post-MI or in pressure-overloaded hypertrophic myocardium leads to decompensation and CHF. In many cases, CHF has appeared in hearts with no history of MI, or hearts which have endured a small MI. Use of inotropic agents in patients with CHF has been limited by adverse effects on outcomes because positive inotropic agents that increase intracellular concentration of cytoplasmic [Ca$^{2+}$] and enhance contractility are accompanied
by increase of myocardial oxygen demands, leading to development, exacerbation or intensification of ischemia and/or life-threatening dysrhythmias (2, 24). This phenomenon indicates that: (i) myocytes in CHF are reasonably responsive to beta adrenergic drugs, and; (ii) the primary reason for energy starvation and decrease of myocyte contractility could be the reduction of blood supply reservation (14). Many studies in CHF patients have demonstrated impairment of coronary flow reserve and microvascular perfusion via PET, SPECT or contrast echo, and histological abnormalities of the microvasculature (23, 42). However, the systematic feature and role of abnormalities of coronary vasculature from main artery to capillary have not yet been elucidated, especially in rodent model of congestive heart failure.

In the present study, we presume that arterial stenosis or neointimal narrowing is one of compensatory mechanisms which attempt to reduce massive myocyte loss and improve general pumping performance, by restricting injury to existing ischemic areas while increasing blood supply to remote but relevant vital myocardium. Compensatory overshoot results in capillary disorder which contributes to the reduction of vascular reservation and ultimately leads to impaired intrinsic contractility of the myocardium in ischemic CHF. The primary goal of current study is to explore a new approach in order to enhance understanding of the initial cause of CHF.

METHODS
All procedures were followed by the recommendations of the Guide for the Care and Use of Laboratory Animals (Department of Health and Human Services publication number NIH 78-23, 1996) and were approved by the Mount Sinai School of Medicine Animal Care and Use Committee.

Animal Protocol:

Congestive heart failure was induced by consecutively performing aortic banding for two months, ischemia/reperfusion (I/R) for one month and aortic debanding (Ab + I/R + DeAb) for one month as previously described (9). Briefly, aortic banding (Ab) was performed in male Sprague-Dawley rats (150 – 180 g) by constricting the ascending aorta with a 4-0 suture against a PE-50 tube through right thoracotomy at the second intercostal space. Two months following Ab, rats underwent left coronary artery (LCA) ligation for 30 minutes followed by reperfusion to induce I/R injury. One month post I/R, the rats underwent a third thoracotomy at the upper-right side of the sternum. The roots of 2 ~ 4 ribs were cut along the right side of sternum to expose the aorta. The aortic banding suture was cut with micro dissecting scissors and separated with micro dissecting forceps. For all surgical procedures, anesthesia was induced by intraperitoneal administration of ketamine (65 mg/kg) + xylazine (13 mg/kg) + acepromazine (1 mg/kg). Animals underwent intratracheal intubation and mechanical ventilation.

Echocardiography. The animals were sedated by intraperitoneal injection of ketamine (40 mg/kg). Echocardiograms were performed as previously
described(9). Left ventricle ejection fraction (EF) and fractional shortening (FS) data were obtained by using M-mode.

In vivo hemodynamics. Left ventricular pressure was acquired and analyzed as previously described(9). Briefly, rats were anesthetized and maintained on isoflurane (1 - 2%). Thoracotomy was performed to expose the heart and provide access for an apical approach for PV conductance catheter placement (1.9Fr, Scisense, Ontario, Canada). Preload response was induced by injecting 50 µl of 30% saline into the jugular vein. This hypertonic saline bolus injection caused a transient increase of left ventricle preload.

Coronary artery resin casting. After all other methods were completed, hearts were perfused with resin polymer using Batson’s No. 17 Plastic Replica and Corrosion Kit (cat. # 07349, Polysciences, Inc., www.polysciences.com) as previously described(11). Proper fluorescent dye (www.blacklightworld.com) was mixed with red pigment (1:4 in weight) and added into casting polymer mixture. Briefly, under Ketamine anesthesia (90 mg/kg ip), the chest was opened, the right atrium was cut, and red latex (30 ml) was infused into the left ventricle after blood was flushed out with 60 ml of PBS (pH 7.4, RT) containing heparin (0.2 ml, 1.000 USP u/ml) with a 14 G catheter. Fifteen minutes later, the heart was removed, washed in PBS and photographed. Next, heart samples for section were cut into 5 ~ 6 pieces (each 2 mm thick), frozen in OCT and stored at - 80°C. Samples for scanning electric microscope imaging, heart was corroded in a 50 ml tube with 10 ml of Maceration Solution (#07359) at 50°C for 2 ~ 3 hours and then
room temperature for 24 to 48 hours. After digestion, the cast was carefully washed in water and photographed.

**Scanning Electric Microscope (SEM).** Once dry, the coronary vascular casts were carefully mounted on an aluminum stub using double-stick carbon tape. Samples were then introduced into the chamber of the sputter coater and coated with gold in argon gas at 25 mA for 2 min. SEM examination was conducted with a field emission scanning electron microscope (Hitachi, model # S4300) at vacuum (3 kV, 10 µA, Flash 3).

**Histology.** The frozen hearts were cryostated at 8 µm. Masson’s Trichrome, PicroSirius Red and fluorescent staining of collagen fibers and myocytes were performed as previously described(10). For fluorescent imaging of vasculature, frozen sections were fixed with 10% formalin for 5 min; the slides were mounted with DAPI medium after been washed three times in PBS. The capillary diameter was calculated with calibration of standard of Applied Image Analysis Micrometer.

**Statistics.** Variables are expressed as mean ± SEM. Student t-test was performed to compare experimental groups using GraphPad Prism software. P-Values < 0.05 were considered statistically significant.

**RESULTS**
Cardiac dysfunction in ischemic congestive heart failure (CHF). Heart function was significantly decreased in CHF compared with control. The left ventricle (LV) was dilated; ejection fraction (EF) and percentage of fraction of shortening (FS) decreased, but the LV diastolic anterior wall thickness in the CHF group was the same as the control group (Table 1).

Coronary arterial pathological changes in the heart. Overall, coronary vasculature volume increased from 0.055 ml/g of myocardium in control to 0.165 ml/g of myocardium in CHF hearts (Figure 1 A, B and C). Arterial stenosis occurred mostly in post-branch segments (Figure 1 D and E). Neointima formed not only in the main coronary artery (Ø > 350 µm), but also in middle arteries (Ø > 150 - 349 µm) and multiple small arteries (Ø > 50 - 149 µm) (Figure 1 F, G and H). The MI size and degree of fibrosis in remote myocardium do not correlate with arterial stenosis. Neointima formed in the main coronary artery in 24% of CHF hearts, in middle arteries for 12% of hearts and in small arteries for 29% of hearts; thus, the overall incidence of arterial stenosis in all arterial levels is approximately 47% (Table 2).

Capillary density increased in remote myocardium. Utilizing multiple fluorescent imaging for myocytes, collagen fibers and capillaries in heart sections, capillaries of diameter 1 – 5 µm with cast resin were visible (Figure 2). The structural relationship of myocytes and capillaries is not clear—some myocytes have 6 capillaries surrounding the muscle cell, while others have none (Figure 2
There were 99 capillaries (Ø ≤ 10 µm) per mm$^2$ of myocardium in control, while there were 196 capillaries per mm$^2$ of myocardium in CHF.

Capillaries in CHF hearts with diameters of 4 ~ 10 µm increased from 43% to 83%, while capillaries with diameters of 1, 2 and 3 µm were increased 10.6 fold, 3.9 fold, and 1.4 fold respectively (Figure 3 A and B) compared with control. This led to the increase of capillary cross area per mm$^2$ of myocardium in CHF, especially for micro capillaries of diameter ≤ 3 µm (Figure 3 C and D).

**Characteristics of myocardial capillary morphology.** Fluorescent imaging provided a basic snapshot of capillary cross sectional morphology (Figure 2A), while scanning electric microscope was used to characterize capillaries in more detail longitudinally (Figures 4 to 7). Capillaries in the control group exhibited a primarily uniform arrangement, linear orientation, and straight, consistent shape; when they branch or change direction, they transition smoothly with minimal alterations in diameter. In contrast, capillaries in CHF hearts exhibited irregular arrangement, significant alterations in diameter, and curvy, distorted, inconsistent shape. Capillaries in CHF hearts had frequent narrow (Ø < 2 µm) vessel segments (Figure 4). The length of capillary segments between two nodes where bifurcation occurred was shorter in CHF (46 ± 13 µm) compared with control (76 ± 8.6 µm) (Red lines in Figure 4 A and C and Table 3). Loci of capillary bifurcation in CHF exhibited greater multiplicity than control, with 2 or more branches splitting off a single node (Figure 5). In addition, extremely narrow capillary branches (≤ 3 µm) appeared in CHF. These micro-channels appeared to
bridge between larger capillaries, and contributed to the increase of microvascular density in CHF hearts compared to control (Figure 6). Figure 7 A and B show that vascular stenosis occurred not only in capillaries, but also in arterioles (Ø = 20 ~ 50 µm). Stenosis especially occurred at locations where vessels intersected. Stenosis also occurred along the course of arterioles, which were up to 66% narrowed (Figure 7). Figure 7 C and D shows a rough, coarse texture on the innermost surface of capillaries in CHF; this is likely evidence of endothelial disorder, although endothelial function was difficult to assess. Features and data regarding capillaries gleaned from scanning electric microscope imaging in CHF are summarized in Table 3. Capillary curling and deformation was found in all CHF samples. However, left ventricle EF data from echocardiography is not linearly correlated to the degree of capillary disordering (Table 3).

**Coronary congestion and lower cardiac reserve.** Chronic coronary congestion mostly occurred in capillaries, rather than higher level vessels, because coronary micro-circulation cross-sectional area in myocardium increased significantly with CHF compared to control (Figure 8 A). Left ventricle was dilated in CHF. A transient elevation of preload induced by injecting 50 µl of 30% saline into the left jugular vein raised end diastolic volume (EDV) and significantly enhanced contractility when myocytes were stretched, leading to increased EF in normal hearts. In contrast, in CHF hearts, the transient increase in preload resulted in a much smaller elevation in EDV and EF than control hearts (Figure 8 B - D). This
data indicates that lower cardiac reserve was probably associated with chronic congestion in coronary capillaries, which were likely not functioning well in CHF hearts based on SEM imaging.

DISCUSSION

Vasculature is a continuous system with flow from arteries, to arterioles, to capillaries, and finally back to veins. Pathological changes in the main coronary artery could be detected by angiography, positron emission tomography (PET) and magnetic resonance imaging (MRI) in vivo, while atherosclerosis in small arteries were detectable only by histological staining in vitro(12, 23). However, the role of capillaries in the development of congestive heart failure is not well known. In the present study, we report successive vascular changes of coronary arteries from main, middle and small arterial branches to arterioles and capillaries in CHF which was induced by chronic aortic constriction plus ischemia/reperfusion followed with aortic de-banding. We show that vascular pathological change was widespread, rather than isolated to a large artery such as the coronary left anterior descending (LAD) artery, and successive, spreading from the main arterial stem through the capillaries.

The cardiac pumping function was affected by multiple factors: MI size, myocyte contractibility, interstitial and perivascular fibrosis, vascular pathophysiology and neurohormonal factors(36). Coronary artery disease has long been considered one of the major causes of ischemic congestive heart
failure(22). However, heart failure in the context of coronary artery disease is a heterogeneous condition. Many factors contribute to LV dysfunction, including stenotic severity, quantity of infarct-related arteries, subsequent remodeling hypertrophy, fibrosis, neurohormonal activation, systemic hypertension, myocardial revascularization and endothelial dysfunction(21). Research thus far has focused on pathogenic changes in main coronary branches, such as plaque build-up and clotting in the left anterior descending artery(15, 40). Pathogenic changes occurring in small arterial or capillaries have not been studied closely enough due to shortage of proper animal models of coronary arterial atherosclerosis, especially in rodent hearts(1, 27, 29).

In our study, the data shows that coronary structures in CHF exhibit not only vascular stenosis, but also expansion. Both stenosis and expansion occurred not only in main coronary arterial branches, but also in small arteries, arterioles and capillaries. The mechanism of vascular stenosis/expansion in this model is not very clear. In chronic pressure overload (hypertension), a number of vasoconstrictors are released in response to increased sympathetic tone and activation of the renin-angiotensin-aldosterone system in order to maintain cardiovascular compensation in the midst of decreased cardiac output(5). It has been well documented that all those vasoconstrictors (catecholamine, angiotensin(35)) and cytokines (such as endothelins, tumor necrosis factor-a) contributed to neointimal formation(33) which could increase vessel resistance to blood flow(17, 41) to the overloaded myocardium. The compensatory mechanism could be reversed and became deleterious when excess vasoconstrictors were
released in a state of emergency, as these can rapidly increase oxygen demand or lead to insufficient blood supply(18). The compensatory overturn did not occur very often in pressure overload model in rodent if the aorta was barely constricted for a few months(9). When a transient occlusion of the coronary artery was added to the hypertrophic heart, an extra disturbance in coronary vasculature was induced due to the simultaneous action of both systemic vasoconstrictors (neurohormonal factors) and local vasodilators (calcium overload, inflammatory factors, oxygen radicals) which were infused after ischemia/reperfusion incident(19). Ischemic anaerobic glycolysis led to mass opening of capillaries, resulting in reduced resistance and slowed blood flow velocity(44). Vascular expansion and stenosis probably resulted from these paradoxical responses. It is not well known how arteries and capillaries were impacted during or after myocardial I/R injury in the context of chronic pressure overloading. The vascular proliferation induced by hypertrophy and ischemia contributed not only to neointimal formation, but also to angiogenesis of capillaries.

Capillaries in the heart are affected by many factors, including cardiac afterload, ischemia/infarction, exercise training, high altitude, metabolism and heart rate(7, 31). Rakusan et al(39) reported that pressure overload left ventricular hypertrophy in children demonstrated proportional capillary angiogenesis, whereas in adults, hypertrophy seemed to be associated with failure of compensatory angiogenesis(6). Our data show that coronary
vasculature volume increased from 0.055 ml/g of LV in control hearts to 0.165 ml/g of LV in CHF hearts, while capillary density in remote myocardium increased from 3.26% of myocardial cross-sectional area in control to 9.18% of myocardial cross-sectional area in CHF. Capillary growth could be a result of both pressure overload and I/R induced inflammation. Two features which indicate pathogenic capillary growth are: (1) shortened capillary segments between nodes of vascular bifurcation, and; (2) poly-bifurcations arising from one node (Figures 4 and 5). These features could contribute to disturbed laminar flow, which is linked to artherosclerosis(8). Using scanning electric microscope imaging, extremely narrow micro-channels (Figure 6) become visible in CHF hearts. These micro-channels appear to form bridges between capillaries and seem to be distinct from narrowed capillaries (Figure 4 C and D). The function of both micro-channels and narrowed capillaries (diameter ≤ 3 µm) were not clear, but potentially could aggravate ischemia because it would be more difficult for red blood cells (diameter 6.8 – 8.2 µm(13, 16)) to flow through the narrowed capillaries. The consequences of advanced vascular remodeling were detrimental in CHF hearts, not only due to the increase in capillary number, but also due to the alteration of functional structure. Increased capillary volume, caused by expansion and/or angiogenesis, led to cardiac congestion and LV wall higher tension, thus increasing wall stiffness and oxygen consumption, resulting in further ischemia and decrease in cardiac functional reserve (Figure 8). Capillary disorder could be one of the mechanisms of inotropic agent side effects: this drug was initially used to enhance cardiac contractility to overcome heart depression but actually led to
myocyte exhaustion(2), probably due to the capillary stenosis which prevent
myocytes from receiving adequate oxygen even though the main coronary
arterial branches are not narrowed in chronic heart failure with coronary arterial
disease (CAD)(21). In contrast, mitigating capillary burden by reducing myocyte
oxygen consummation, using agents such as β-blockers that inhibit left
ventricular contractility may relieve symptoms of CHF(30). Endothelin-
 antagonists could also potentially improve the prognosis of CHF, since
endothelial dysfunction in coronary capillaries is a crucial component of the
progression of congestive heart failure(3).

The relationship between myocardial capillary density and coronary flow
reserve in the CHF rats is not clear. This study doesn’t compare the accuracy of
the resin casted fluorescent imaging with methenamine silver or Lectin
immunohistochemical staining for capillary density (39, 43). The data in current
study for capillary blood pressure, flow velocity or resistance in the coronary
vasculature are also not available mainly due to technical barriers. While the
data show that the innermost capillary surfaces are visibly roughened and
textured, the authors could not conclude a cause-and-effect relationship between
endothelial disorder and myocyte dysfunction. The present study also lacked
data regarding the levels of humoral factors such as endothelin, angiotensin II,
norepinephrine and vascular endothelial growth factors which were probably
involved in the pathogenesis of capillary disorder. More work is required, both in
vitro and in vivo, to better understand the role of capillary disorder in CHF. The
The present study’s findings need to be expanded upon utilizing updated cardiac imaging techniques and in-depth analysis of abnormalities on biochemical, molecular and genetic levels.

**In summary:** In a chronic pressure overload plus ischemia/reperfusion model of heart failure, we found that vascular disorder occurs not only in main branches of the coronary artery, but also in arterioles and capillaries. The myocardial capillary density did not directly correlate to cardiac functional improvement because there is an intermediate factor which plays a critical role in material exchange between blood and myocytes: capillary structure. The capillary structural disorders found in CHF hearts include stenosis, non-linear arrangement, curled shape, drastic changes in diameter, proliferation, and roughened surface texture. This disorder might be one of critical contributing factors to the energy starvation which leads to reduction of intrinsic contractility of myocytes. Further research needs to incorporate technological advances of molecular biology in conjunction with physiological measurements, such as PET, MRI and myocardial contrast echocardiography, to best elucidate the role of the coronary microvascular dysfunction in ischemic congestive heart failure.

**ACKNOWLEDGEMENTS**

This work was supported in part by grants from the National Institutes of Health: R01HL093183, and P20HL100396 (RJH) and the Transatlantic Leducq
Foundation (RJH), and K23HL111339-01 (CG).

DISCLOSURES

There are no conflicts of interest with any of the authors.

References:


Figure Legends

Figure 1. The changes of coronary artery in different level in congestive heart failure (CHF). A. control coronary vasculature. B. CHF. C. Increase of coronary vasculature volume in CHF hearts. Animal number, control = 7, CHF = 8. ** p < 0.01, compared with control. D. stenosis in left coronary artery. Arrow indicates arterial narrowing in main branch of left coronary artery. E. arterial narrowing in multiple small arteries (arrow). F. neointima formation in major branch on coronary artery stained by Masson’s Trichrome. Blue = collagen, red = myocytes, black = nuclei. LCA = left coronary artery. G. Picrosirius red stain shows neointima in middle arteries. Red = collagen fibers, pale yellow = myocytes, black = nuclei. H. neointima formed in multiple small arteries (Arrow).

Figure 2. Multiple imaging of myocytes, collagen fiber and capillary in heart. A and B: fluorescent imaging of vessels and myocytes in CHF heart. Red = vessels, green = myocytes, blue = nucleus. C and D: double staining of capillary and collagen fiber, C = control. D = CHF. Green = vessels. Red = collagen fiber. Collagen fiber and capillary were significantly increased in CHF heart.

Figure 3. Increase of capillary density in remote myocardium in ischemic congestive heart failure (CHF). A. capillary number in myocardium (mm²) was increased in CHF. Animal number, control = 6, CHF = 7, observed capillary number, control = 1497, CHF = 5019. B. increase fold of capillary number of (CHF - control)/control was much higher in micro-capillary (diameter 1 – 3 µm). C.
Cross sectional area of capillary (diameter < 10 μm) in myocardium was increased in CHF group. Animal number, control = 6, CHF = 7, ** p < 0.01 compared with control. D. the increase fold of capillary cross area was much higher in micro-capillary (diameter < 3 μm).

Figure 4. Characteristics of capillaries in congestive heart failure (CHF). Images are taken with scanning electric microscope. A. Control. Capillary thickness is uniform, changes in diameter are smooth. B. CHF. Capillaries are curled, changes in diameter are frequent and lack smooth transition. C. CHF. Capillary thickness varies drastically in CHF. The change in capillary diameter is irregular. Arrows indicate extremely narrow capillary branches. D. Significant increase in number of smaller capillaries (Ø < 3 µm) in CHF compared to control. Total number of images = 209, of 7 control hearts and 7 CHF hearts. Segments between loci of capillary bifurcation are of significantly shorter length in CHF than in control (red line in A and C. See Table 3).

Figure 5. Increase of capillary bifurcation in congestive heart failure (CHF). A. Control. Capillary distribution is regular and smooth. B. CHF. Segments between bifurcation loci in CHF are much shorter than in control. C. Two capillary branches off of one node. D. Five capillary bifurcations off of one node in CHF.

Figure 6. Micro channel formation between capillaries in congestive heart failure (CHF). A. There are very few micro channel (Ø < 3μm) connection between
capillaries in control. B – D. micro channel were opened in CHF. It was not clear that those micro channels were “opened” or “angiogenesised”.

Figure 7. Stenosis in arterioles and endothelial disorder in congestive heart failure (CHF). A. Stenosis formation in arterioles and intersecting vasculature. B. Stenosis was also seen in the middle of arteriole, as severe as 66% narrowed. C and D. Capillaries have textured, rough surfaces, likely indicative of endothelial disorder in CHF although assaying endothelial function was difficult.

Figure 8: Coronary congestion and lower cardiac reserve. A. Increase of coronary capillary cross sectional area in remote myocardium indicated chronic congestion in CHF. Capillary = vessels with diameter ≤ 20 µm. Animal number, control = 6, observed area = 12.7 cm², CHF = 7, observed area = 24 cm². B. Preload response of LV induced by injection 50 µl of 30% NaCl through left jugular vein led to transient increase of LV end diastolic volume (EDV). EDV in CHF heart is much bigger than control. Increase of EDV by injection of the saline led to much more elevation of EF in control vs CHF, while in CHF, the P-V loop shifts to right slightly, EF didn’t change very much, indicated cardiac functional reserve is lower in CHF. C. Illustration of preload response in every individual sample. C = control. CHF = congestive heart failure. D. Average of C. EDV in CHF group is significant higher than control. Injection a bolus of NaCl results in increase of 208 ± 17 µl of EDV, along with elevation 10 ± 2% of EF in normal
heart. EDV and EF were increased $73 \pm 12.5 \, \mu l$ and $1.7 \pm 0.3\%$ separately in CHF, $a =$ before injection, $b$ and $c =$ after injection, $\Delta =$ increase.

Table 1. Cardiac function by echocardiography in CHF rats. $N =$ number, $AW_d =$ anterior wall, diastolic. $AW_s =$ anterior wall, systolic. $PW_d =$ posterior wall, diastolic. $PW_s =$ posterior wall, systolic. $EDV =$ end diastolic volume, $ESV =$ end systolic volume, $EF =$ ejection fraction, $FS =$ fraction of shortening. $HR =$ heart rate, $BPM =$ beat per minute. $BW =$ body weight, $g =$ gram. ** $p < 0.01$ compared with control.

Table 2. Characteristics of I/R injury, fibrosis and arterial stenosis in congestive heart failure. $N =$ animal number. MI = myocardial infarction, $T =$ transmural, Sub = no transmural, $\ast \ast \ast =$ MI $> 30\%$, $\ast \ast =$ MI $15 - 30\%$, $\ast =$ MI $< 15\%$ of LV. Fibrosis = interstitial fibrotic area, $\ast \ast \ast \ast \ast =$ $> 5\%$, $\ast \ast =$ $3 - 5\%$, $\ast =$ $2 - 2.9\%$, control $< 1\%$ of non-ischemic myocardium. MA-S = main arterial ($\phi > 350 \, \mu m$) stenosis. Mid A-S = middle arterial ($\phi > 150 - 349 \, \mu m$) stenosis, SA-S = small arterial ($\phi > 50 - 149 \, \mu m$) stenosis, $\ast \ast \ast \ast = >30\%$, $\ast \ast = 15 - 30\%$, $\ast = < 15\%$ of arterial internal area. Multi-S = multiple arterial stenosis, $\# \# \# =$ vessel number $> 5$, $\# \# =$ vessel number $4 - 5$, $\# =$ vessel number $2 - 3$.

Table 3. Characters of capillaries by scanning electric microscope imaging in congestive heart failure (CHF). $AW_d =$ anterior wall, diastolic. $EF =$ ejection fraction. $FS =$ fractional shortening. $BW =$ body weight. $CNM =$ capillary number
of measured. §§§ = capillary curled, distorted in most observed area. Ø Ø Ø = Capillary diameter irregular in > 50% of imaging area. Ø Ø = Capillary diameter irregular in around 30 ~ 50% of imaging area. +++ = micro channel could be seen in most of imaging, ++ = micro channel could be seen in around half of imaging. + = micro channel could be seen in around third of imaging. @@@ = coarse surface could be seen in most imaging at high magnification (≥ 1 k), @@ = in around half imaging at high magnification, @ = in around third of imaging. **p < 0.01 compared with control. Total imaging numbers = 244.
Coronary vasculature

Volume/LV (ml/g)

C

Control

CHF

**
A. Control

B. CHF

C. CHF

D. CHF

3.8 µm

2 µm

3.2 µm

1 µm

6 µm

4.5 µm

0.7 µm
Coronary circulation cross area in myocardium (%)

B

C

D

\[ y = 0.0471 \times + 48 \]
\[ y = 0.023 \times + 25 \]

\[ \Delta = 208 \mu l \]
\[ \Delta = 10\% \]
\[ \Delta = 73 \mu l \]
\[ \Delta = 1.67\% \]
<table>
<thead>
<tr>
<th>Animal N.</th>
<th>AWd, cm</th>
<th>AWs, cm</th>
<th>PWd, cm</th>
<th>PWs, cm</th>
<th>EDV, ml</th>
<th>ESV, ml</th>
<th>EF, %</th>
<th>% FS</th>
<th>HR, BPM</th>
<th>BW, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>0.21 ± 0.01</td>
<td>0.39 ± 0.01</td>
<td>0.21 ± 0.01</td>
<td>0.39 ± 0.01</td>
<td>0.55 ± 0.03</td>
<td>0.01 ± 0.00</td>
<td>97 ± 0.31</td>
<td>74 ± 2</td>
<td>379 ± 12</td>
</tr>
<tr>
<td>CHF</td>
<td>17</td>
<td>0.21 ± 0.01</td>
<td>0.34 ± 0.02</td>
<td>0.23 ± 0.01</td>
<td>0.36 ± 0.02</td>
<td>0.97 ± 0.08**</td>
<td>0.18 ± 0.02**</td>
<td>80 ± 1.200**</td>
<td>45 ± 1.2**</td>
<td>351 ± 15</td>
</tr>
<tr>
<td>Serial No</td>
<td>MI</td>
<td>Fibrosis</td>
<td>MA-S</td>
<td>Mid A-S</td>
<td>SA-S</td>
<td>Multi -S</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------</td>
<td>------</td>
<td>----------</td>
<td>------</td>
<td>---------</td>
<td>------</td>
<td>----------</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>T***</td>
<td>+++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>T**</td>
<td>+++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>T*</td>
<td>+</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>T*</td>
<td>++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>T**</td>
<td>++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Sub</td>
<td>+++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Sub</td>
<td>++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>T**</td>
<td>+++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Sub</td>
<td>+++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Sub</td>
<td>++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>T**</td>
<td>+++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>T*</td>
<td>++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Sub</td>
<td>++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>Sub</td>
<td>+++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Sub</td>
<td>++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>T***</td>
<td>+++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>Sub</td>
<td>++</td>
<td>@</td>
<td>@</td>
<td>@</td>
<td>@@</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N = 17  
2T*** (12%)  
8+++ (47%)  
2©©© (12%)  
1©©© (6%)  
1©©© (6%)  
2@@@ (12%)  
4T** (24%)  
8++ (47%)  
2© (12%)  
1© (6%)  
3©© (18%)  
2@@ (12%)  
3T* (17%)  
1+ (6%)  
1© (6%)  
1@ (6%)  
8Sub (47%)  

Sum  
TMI 53%  
100%  
24%  
12%  
29%  
29%
<table>
<thead>
<tr>
<th>Animal serial number</th>
<th>AWTd (cm)</th>
<th>EF (%)</th>
<th>FS (%)</th>
<th>Coronary vessel/BW (mg/kg)</th>
<th>Capillary number increase</th>
<th>Capillary internode length (µm)</th>
<th>Capillary curled, distorted</th>
<th>Capillary narrowed irregularly</th>
<th>Capillary bridge increase</th>
<th>Capillary coarse surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHF-1</td>
<td>0.20</td>
<td>81</td>
<td>45</td>
<td>0.50</td>
<td>↑↑↑</td>
<td>40 (CNM = 196)</td>
<td>§§§</td>
<td>Ø Ø Ø</td>
<td>+++</td>
<td>@@</td>
</tr>
<tr>
<td>ChF-2</td>
<td>0.19</td>
<td>75</td>
<td>41</td>
<td>0.45</td>
<td>↑↑↑</td>
<td>47 (CNM = 324)</td>
<td>§§§</td>
<td>Ø Ø Ø</td>
<td>+++</td>
<td>@@</td>
</tr>
<tr>
<td>CHF-3</td>
<td>0.17</td>
<td>80</td>
<td>44</td>
<td>0.19</td>
<td>↑↑</td>
<td>42 (CNM = 161)</td>
<td>§§§</td>
<td>Ø Ø Ø</td>
<td>+++</td>
<td>@</td>
</tr>
<tr>
<td>CHF-4</td>
<td>0.16</td>
<td>74</td>
<td>38</td>
<td>0.31</td>
<td>↑</td>
<td>68 (CNM = 89)</td>
<td>§§§</td>
<td>Ø Ø Ø</td>
<td>+</td>
<td>@</td>
</tr>
<tr>
<td>CHF-5</td>
<td>0.17</td>
<td>79</td>
<td>43</td>
<td>0.15</td>
<td>↑↑</td>
<td>58 (CNM = 110)</td>
<td>§§§</td>
<td>Ø Ø Ø</td>
<td>++</td>
<td>@@</td>
</tr>
<tr>
<td>CHF-6</td>
<td>0.21</td>
<td>86</td>
<td>50</td>
<td>0.73</td>
<td>↑↑↑</td>
<td>32 (CNM = 577)</td>
<td>§§§</td>
<td>Ø Ø Ø</td>
<td>+++</td>
<td>@@</td>
</tr>
<tr>
<td>CHF-7</td>
<td>0.23</td>
<td>68</td>
<td>34</td>
<td>1.43</td>
<td>↑↑↑</td>
<td>33 (CNM = 479)</td>
<td>§§§</td>
<td>Ø Ø</td>
<td>++</td>
<td>@@</td>
</tr>
<tr>
<td>CHF Mean ± SD</td>
<td>0.19 ± 0.01</td>
<td>78 ± 2.2**</td>
<td>42 ± 1.9**</td>
<td>0.53 ± 0.17**</td>
<td>1 – 3 fold</td>
<td>46 ± 13**</td>
<td>See Figure 4B</td>
<td>See Figure 4C</td>
<td>See Figure 4 &amp; 6</td>
<td>See Figure 7C</td>
</tr>
<tr>
<td>Control (N = 7)</td>
<td>0.21 ± 0.01</td>
<td>96 ± 0.62</td>
<td>75 ± 1.73</td>
<td>0.11 ± 0.02</td>
<td>_</td>
<td>76 ± 8.6</td>
<td>no</td>
<td>no</td>
<td>_</td>
<td>no</td>
</tr>
</tbody>
</table>