Remodeling of the chronic severely failing ischemic sheep heart after coronary microembolization: functional, energetic, structural, and cellular responses


The need for continuing pursuit of a suitable animal model of heart failure (HF) is highlighted by the difficulty in studying the underlying basic mechanisms and natural history in the human condition, confounded by pharmacological interventions. Previous emphasis on cardioregional, hemodynamic, and neurohormonal derangements has shifted toward remodeling and the role played by stretch-activated pathways, cytokine activation, and changes in cardiomyocytes and the extracellular matrix.

HF has previously been induced in several large animal species (e.g., dogs, calves, and pigs) using techniques that damage the myocardium, such as coronary microembolization (40, 42), rapid ventricular pacing (7), coronary artery ligation (29), or cardiotoxic drugs such as adriamycin. Alternatively, volume loading the heart by creation of an arteriovenous fistula or aortic regurgitation has also been used, as has pressure loading with aortic banding. From the human perspective, the most relevant methods are those that interfere with coronary blood flow to cause infarction or chronic ischemic damage. The principal advantages of the microembolization approach include homogeneity of damage, which is limited to the left ventricle (LV), ability to titrate the response, and potential avoidance of mitral regurgitation. In contrast to the other approaches, the microembolization model has also proven remarkably stable but is subject to the criticism that it lacks associated large coronary artery disease. Coronary ligation or constriction, on the other hand, fails to involve the small vessels that are commonly diseased in humans, especially diabetics.

We established a large animal model of severe HF that bears close resemblance to human ischemic cardiomyopathy (14), the most common cause of HF (9), and studied a cohort of 23 animals for up to 6 mo. The sheep model showed remarkable stability. In a previous small series, we established the practicality of sequential selective coronary artery microembolization (13), examined the microscopic morphometric picture of remodeling (14), and studied cytokines and related links in the apoptotic death cascade and their relationship to wall stress (15).

With the use of a much larger cohort, the present study extends the scope of previous findings (18, 33) and integrates hemodynamic, energetic, neurohormonal, extracellular matrix, wall stress, cytokine, and apoptosis-related responses in terms of remodeling and natural history in chronic, severe, untreated ischemic HF.

MATERIALS AND METHODS

Sixty-five adult merino sheep of either sex with a body weight of 47 ± 8 kg were used in a study protocol approved by the Institutional Animal Care and Ethics Committee. The animals received humane care in accordance 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). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
care in compliance with the “Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 86-23, Revised 1985).

Study design. The animals were acclimatized for at least 2 wk before being enrolled in experiments that had two major components: selective intracoronary microembolization (ICM) leading to HF and long-term (6 mo) profiling of hemodynamic, energetic, and neurohumoral parameters.

After the final (6 mo) dynamic profiling, the heart was harvested and cellular responses [Fas ligand (FasL)/Fas and caspases] to wall stress as well as extracellular matrix collagen levels and phenotype were assayed (Fig. 1).

Hemodynamic assessment was performed at baseline, when HF was established, and again 3 and 6 mo later and included LV pressure (LVP), LV pressure-volume (P-V) relationship, and cardiac output (CO). LV volume, LV ejection fraction (LVEF), and LV wall thickness were derived echocardiographically. Neurohumoral studies examined plasma atrial natriuretic peptide (ANP), angiotensin II, aldosterone, and plasma renin activity (PRA) on the day of, but before, the hemodynamic studies.

Hearts from 16 sheep, including 11 sheep from the described HF group at 6-mo follow-up and 5 normal controls, were analyzed for collagen content and phenotypic change (Fig. 1). All surgical procedures were performed under anesthesia, which was induced by thio- pentone (15–20 mg/kg) and maintained with 1.5–2% isoflurane in 40% oxygen using a respirator (model 8, Bird; Palm Springs, CA). Expired CO₂ was monitored with a POET II monitor (Criticare Systems; Milwaukee, WI) and maintained at 30–35 mmHg. All hemodynamic measurements(recordings were taken after at least 30 min of stable anesthesia and at end expiration. Surgical procedures were performed under sterile conditions.

Staged ICM. Microsphere delivery was guided by preprocedure echocardiography and by the response of the ECG and LV and arterial pressures that were monitored during the entire procedure. A Judkins (5-Fr) catheter was passed via carotid artery cutdown to engage the orifice of either the left anterior descending or left circumflex coronary artery. After the intravenous injection of 25 mg lidocaine, 0.1–0.4 ml of 90-μm polystyrene microspheres (2.54% solid latex, Polysciences; Warrington, PA) diluted in 2 ml of 0.9% saline were injected into the selected artery. The microsphere suspension was subjected to ultrasonic mixing and manual shaking immediately before the injection. In each procedure, the embolization was repeated until significant myocardial compromise was achieved as evidenced by changes in LV end-diastolic pressure (LVEDP), arterial pressure, and ECG S-T. The procedure was repeated every 2 wk, with the end point of microembolization being the achievement of an echocardiographic LVEF <35%, stable for 4 wk.

In a subgroup of sheep (n = 21), after baseline blood sampling, specimens were taken at 2, 6, and 18 h after ICM for measurement of plasma creatine kinase (CK). Postoperatively, pain was managed with intramuscular buprenorphine (Reckitt and Colman Products; London, UK), and heart rate (HR) and respiration were also closely monitored. Other medications such as supplemental potassium, intravenous furosemide, nitroglycerine, and lidocaine were given as necessary.

Fig. 1. Sheep numbers (N) at start, with heart failure (HF) at enrollment into study, and at 6-mo follow-up, including breakdown of loss due to death. Also, composition of the subgroups reported and the number of coronary microembolizations (Embos) performed in the principal groups are shown. LVEF, left ventricular (LV) ejection fraction.
LVEF, LV volume, and LV wall thickness measurement. Echocardiography was performed in the right lateral decubitus position using an Ausonics Opus I system (Ausonics; New South Wales, Australia). Standard LV short-axis views at the mitral, midpapillary, and apical levels, together with long-axis views, were recorded on videotape. LV areas measured from the transverse sections and the LV long-axis length were used to calculate LV end-systolic (LVESV) and end-diasstolic volumes (LVEDV) using the modified Simpson’s rule formula. The inner endocardial margin defined the LV lumen and the LVESV was derived as follows: 

\[ \text{LVESV} = \frac{1}{2} \pi r^2 h \]

where \( r \) is the internal radius (in cm), and \( h \) is the LV wall thickness (in cm). LV wall thickness was taken to be the average of septal and free wall dimensions (19).

Central venous pressure was measured via the atrial port of the Swan-Ganz catheter with a fluid-filled pressure transducer system (Ohmeda). Pulmonary vascular resistance was calculated as mean pulmonary artery pressure × 80/CO (in dyn·cm⁻²).

Myocardial contractility and contractile reserve. A 12-electrode 7-Fr pigtail conductance catheter (CardioDynamics; Leiden, The Netherlands) and a 5-Fr Millar catheter-tipped pressure transducer were positioned in the LV for measurement of LV volume and LVP. A 22-Fr balloon-tipped catheter (CV 1014 Fogarty Occlusion Catheter, Edwards Lab, American Hospital Supply) was placed in the inferior vena cava via jugular vein cutdown for transient (15–20 s) inferior vena cava occlusion to reduce LV preload while the LV P-V relationship was recorded. Data were acquired by a Levycom Sigma-5-DF signal conditioner processor (CardioDynamics) and recorded on an IBM personal computer. LV end-systolic elastance (Eₚₑₛ) and preload recruitable stroke work (PRSW) were used as load-independent indices of LV contractility (1). These indexes were computed by commercial software (CardioDynamics). To study the cardiac contractile reserve, intravenous dobutamine (0.1 mg/ml) was infused at incremental rates of 18, 27, 36, 45, and 54 ml/h. Each infusion rate was continued for 5 min until the HR reached 140 beats/min.

External work (EW), potential energy (PE), and the P-V area (P-V area = EW + PE) were derived, and the ratio of EW/P-V area used to characterize the work efficiency of the normal and failing heart (35, 33).

Neurohumoral studies. Venous blood samples were obtained (between 6 and 8 AM and before sheep were fed) from an indwelling central venous catheter previously placed via the jugular vein. The samples were chilled and centrifuged immediately, and the plasma was stored at −70°C. Plasma angiotensin II was measured with a double-antibody RIA modified method using the Buhlmann Laboratory (Basel, Switzerland). The sensitivity of the measurement is 0.1 pg/ml with a recovery of 91.0 ± 5.1%. Plasma aldosterone was assayed by a Sorin Biomedica RIA kit (Saluggia, Italy) with sensitivity of 15 pg/ml. The α-ANP assay in plasma used a classical RIA (competitive protein binding) in homogeneous phase using the double-antibody separation technique specific for α-ANP, with a sensitivity of 7 pg/ml (4).

Myocardial collagen determination. In the 16 sheep (including 5 normal controls) where myocardial collagen content and phenotype were assayed (Fig. 1), the heart was quickly removed and the great vessels and atria were dissected free after death by an intravenous injection of thiopentone and potassium chloride. The right ventricle (RV) and LV were separated, blotted dry, and weighed. Sections of the RV and LV free wall and the interventricular septum just below the papillary muscles were dissected, snap frozen in liquid nitrogen, and stored at −70°C until analysis.

Myocardial collagen content was quantified after hydrolysis of the tissues and incubation with Ehrlich’s reagent (5), with absorbency of the solution measured at 558 nm. Hydroxyproline content was calculated from a standard curve and expressed as micrograms per milligrams of dry tissue weight. Collagen content was derived as the hydroxyproline level × 7.46.

Interrupted gel electrophoresis (36) was used to assay collagen phenotype after 1 g of heart tissue had been homogenized and peptic digested (23). The gels were then immersed in a stain solution composed of 1.25% Coomassie brilliant blue for 60 min and then destained. The protein bands were quantitated by scanning densitometry at 570 nm. Most fibrillar collagens have similar cross links, this approach assumes that α-chains alone can be used to characterize collagen type I and III phenotypes and that proportions of β- and γ-chains are similar.

Expression of FasL, caspases-8, -3, and -2. The protein expressions of FasL and caspases were determined by Western blotting as previously described (15, 16). Briefly, protein extracts from the sheep LV were separated by SDS-PAGE. After the proteins were transferred onto a polyvinylidene difluoride membrane, FasL, or caspases were probed using primary antibodies from Santa Cruz Biotechnology (Santa Cruz, CA). Positive controls of cell lysates were from Transduction Laboratories (Lexington, KY). The antibody-labeled bands were visualized using the NEN BLAST method (NEN Life Science Products; Boston, MA). Laser scanning densitometry was used to determine volume densities (in arbitrary units) of the bands detected by the corresponding antibody.

Determination of caspase activities. The caspase activity assay was performed as previously described (15) using a fluorescent 7-amino-4-trifluoromethycoumarin (AFC) substrate/inhibitor QuantiPak (BioMol; Plymouth Meeting, PA). With the use of this technique, a linear correlation of f(x) = 21.9x − 3.1 was found between the change in fluorescence intensity due to the cleavage of the AFC fluorophore from the substrates by the corresponding caspase activity.

Statistical data analysis. Data are presented as means ± SD unless otherwise stated. Comparison of hemodynamic parameters was performed by the appropriate one-way ANOVA, followed by a post hoc t-test. A P value <0.05 was considered statistically significant.

RESULTS

Effect of stepwise coronary microembolization. Myocardial damage due to intracoronary injection of microspheres was evidenced by acute ECG changes and a peak increase in the CK level at 6 h after the procedure (Fig. 2A). The baseline LVEF of the 65 sheep was 58.7 ± 6.9%. Of these 65 sheep, 38 sheep were successfully induced into HF with a LVEF decrease of 50% (58.3 ± 7.4% at baseline vs. 29.1 ± 4.4% at HF establishment). The LVEF remained stable when reexamined 3 mo (n = 19 of 38 sheep) and 6 mo (n = 21 of 38 sheep) later (Fig. 2B).

Echocardiographic parameters and calculated LV wall stress are detailed in Table 1. LVEDV increased 73% with the induction of HF and remained 55% above baseline, LV end-
diastolic diameter showed a stable 30% increase, and stable thinning of the LV wall was observed. Accordingly, LV wall stress increased markedly in response to the embolization-induced HF and remained elevated.

Between 1 and 10 embolization procedures (median 3, total of 149 in 38 sheep) were needed to induce HF. Sixty-five procedures involved microsphere injection into the left circumflex coronary artery, 56 procedures into the left anterior descending coronary artery, and 28 procedures into the left main coronary artery, with median doses of 0.4 ml (range 0.1–1 ml), 0.5 ml (range 0.1–1.7 ml), and 0.5 ml (range 0.1–0.9 ml), respectively. The total volume needed to induce HF was a median of 1.6 ml (range 0.5–9.5).

Twenty-seven sheep died before HF could be established (Fig. 1). Their baseline LVEF (59.1 ± 6.6%) did not differ from those who attained HF status. The total volume of the microsphere suspension injected in this group was a median of 0.8 ml (range 0.3–3.7 ml). The causes of death in these animals were as follows: ventricular fibrillation in 10 sheep, severe acute HF in 12 sheep, sudden cardiac death when not monitored in 3 sheep, and lung infection in 2 sheep. The 10 instances of witnessed ventricular fibrillation occurred intraoperatively shortly after microsphere injection, whereas most of the fatal acute HF episodes (8 of 12) occurred postoperatively (>24 h after surgery). The latter subgroup may represent animals with severe HF who died before hemodynamic confirmation of their status. In the other one-third of severe acute HF cases, the condition manifested within 24 h of microembolization, either at the induction of anesthesia or immediately after injection of microspheres. Three sheep died suddenly without a definitive diagnosis of cause of death; these were classified as “sudden cardiac death.” Two other sheep died of postoperative pneumonia (4 and 16 days after surgery) resistant to antibiotic treatment.

Fifteen sheep in the HF group died prematurely before the projected 6-mo follow-up (Fig. 1). Ventricular fibrillation (4 intraoperative), acute HF (2 intraoperative and 1 immediate postoperative), high-grade intraoperative atrioventricular conduction block in 2, and postoperative sudden cardiac death in 2 were the causes of mortality. Conditions such as loss of appetite (2 animals), respiratory obstruction (1 animal), and bad general condition (1 animal) were other causes of death.

The success rate of HF induction increased with experience, especially in relation to procedural deaths. n, No. of animals.

Table 1. Time course of echocardiographic findings in heart failure sheep

<table>
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<th>Baseline</th>
<th>Establishment</th>
<th>Heart failure</th>
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<tr>
<td></td>
<td></td>
<td>3 mo</td>
<td>6 mo</td>
</tr>
<tr>
<td>LVEDV, ml</td>
<td>82 ± 24 (38)</td>
<td>143 ± 36* (38)</td>
<td>137 ± 30* (18)</td>
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<tr>
<td>LVW, cm</td>
<td>0.8 (0.7–0.9) (37)</td>
<td>0.6 (0.5–0.8)* (38)</td>
<td>0.7 (0.6–0.7)* (17)</td>
</tr>
<tr>
<td>IVS, cm</td>
<td>0.9 ± 0.2 (37)</td>
<td>0.7 ± 0.15* (38)</td>
<td>0.7 ± 0.2* (17)</td>
</tr>
<tr>
<td>LVEDID, cm</td>
<td>4.3 ± 0.6 (37)</td>
<td>5.6 ± 0.6* (38)</td>
<td>5.5 ± 0.6* (17)</td>
</tr>
<tr>
<td>LVEDW stress, dyn/cm²·10⁻³</td>
<td>14.6 ± 10.6 (35)</td>
<td>56.4 ± 26.8* (38)</td>
<td>55.3 ± 26.9* (17)</td>
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Values are means ± SD; nos. in parentheses are nos. of sheep. LVEDV, left ventricular (LV) end-diastolic volume; LVW, LV free wall thickness (data are expressed as medians with nos. in parentheses as percentiles); IVS, interventricular septum thickness; LVEDID, LV end-diastolic internal diameter; LVEDW stress, LV end-diastolic wall stress. Kruskal-Wallis one-way analysis of variance on ranks was used for statistical analysis, followed by Dunn's test. *P < 0.05 compared with baseline.
Hemodynamic and neurohumoral changes. With the establishment of HF, LVEDP had doubled from baseline (Table 2). This change was maintained to 6 mo, although a trend for LVEDP to decrease was observed. In HF sheep, the cardiac index was reduced by 16% and stroke volume by 20%, and no improvement occurred during the entire 6-mo period. Myocardial contractile function was compromised in the HF animals (Fig. 3), as shown both by load-independent indexes such as $E_{es}$ (40% decrease) and PRSW (49% decrease) and also by the load-dependent index LV dp/dt$_{max}$ (35% decrease). The compromised contractile function showed no improvement with time. LV diastolic function also deteriorated, as indicated by a 41% increase in $t$ and a 40% decrease of LV dp/dt$_{min}$. These changes were maintained over the study period. The HR under anesthesia was no different after the onset of HF. The early decrease of LV systolic pressure at the time of HF establishment was subsequently largely reversed.

After dobutamine infusion, when the HR had reached 140 beats/min, the LV P-V relationship was again established and $E_{es}$ was analyzed. Dobutamine increased $E_{es}$ significantly in both baseline and HF conditions. However, in HF animals, the $E_{es}$ resulting from dobutamine stimulation did not exceed the original unstimulated $E_{es}$ value (Figs. 3 and 4A).

PE of the LV increased slightly but not significantly after HF had been established (from 2,434 ± 913 to 3,184 ± 920, 2,976 ± 1,441, and 2,972 ± 746 mmHg·ml at 0, 3, and 6 mo, respectively). The work efficiency (EW/P-V area) of the failing heart was significantly decreased at each of the time points studied (Fig. 4B).

Figure 5 shows neurohumoral changes in response to HF and their progress over the next 6 mo. ANP was significantly increased by HF and remained so for the 6-mo observational period. When ANP levels were matched with right atrial pressure over the 6 mo, a significant correlation was observed (Fig. 6). Plasma aldosterone levels, on the other hand, rose sharply but returned to normal by 6 mo. The increase in PRA was even more short lived, showing only a remaining trend toward increase at 3 mo ($P = 0.08$). Plasma angiotensin II was not changed.

Myocardial collagen changes. The total collagen content of the LV in HF animals was increased by 170%, whereas in the RV remained unchanged (Fig. 7A). Analysis of the collagen subtypes showed a twofold increase in the type I-to-type III collagen ratio in HF sheep, again confined to the LV (Fig. 7B). An inverse relationship between LV total collagen content and LVEF was established (Fig. 7C).

Expression of FasL and expression and activities of caspases-8, -3, and -2. A close relationship was found between myocardial FasL expression, the ligand that couples to Fas, the death receptor, and caspases-3 and -2, which together with other members of this group are held to be responsible for the execution of apoptosis (Fig. 8A). A close relationship was also seen between the activities of the upstream protease caspase-8 (a member of the group 2 caspases responsible for the initiation of apoptosis) and caspase-3 (Fig. 8B) and between level of caspase-3 expression and LV wall stress (Fig. 8C).

**DISCUSSION**

A chronic ischemic model of HF. Understanding cardiorenal, hemodynamic, and neurohumoral derangements in HF has provided substantial benefits (17, 20), but emphasis has shifted to remodelling and the role played by stretch-activated pathways, cytokine activation, and changes in cardiomyocytes and the extracellular matrix (8, 12, 31). A realistic animal model of chronic HF can provide insights into this complex interplay of factors. Our ovine model uses stepwise selective coronary microembolization and has hallmarks of the most common type of severe HF in humans (14). It results in LV dilatation, deterioration of systolic and diastolic function, changes in fibrillar collagen, and neurohumoral activation. It shows increased wall stress, enhanced cytokine activity, and activation of the caspase pathway with a possible effect on apoptosis. We report on 21 untreated sheep followed for 6 mo with severe HF. HF is a major cause of morbidity, mortality, and expenditure (30). After the initial pathological insult, there is neurohumoral activation and remodeling of the LV, including changes in interstitial collagen and continued loss of myocytes (20). Therefore, a model that traces this path aids in the understanding of pathophysiology and development of new treatment strategies.

Table 2. Time course of hemodynamic changes

<table>
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<tr>
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<th>Baseline</th>
<th>Establishment</th>
<th>Heart failure</th>
</tr>
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<tbody>
<tr>
<td>LVEDP, mmHg</td>
<td>7.2±3.9 (38)</td>
<td>15.4±5.8* (38)</td>
<td>13.2±5.6* (20)</td>
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<tr>
<td>LVESP, mmHg</td>
<td>91.6±15.9 (37)</td>
<td>78.4±12.4* (36)</td>
<td>83.7±13.4 (19)</td>
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<tr>
<td>$LV E_{es}$, mmHg/ml</td>
<td>2.17±0.77 (13)</td>
<td>1.31±0.28* (13)</td>
<td>1.47±0.4* (12)</td>
</tr>
<tr>
<td>$LV PRSW$, mmHg</td>
<td>74.4±28.8 (13)</td>
<td>38.1±13.3* (15)</td>
<td>47.7±17.3* (13)</td>
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<tr>
<td>LV $dp/dt_{max}$, mmHg/s</td>
<td>1.278±346 (37)</td>
<td>829±242* (36)</td>
<td>820±177* (19)</td>
</tr>
<tr>
<td>LV $dp/dt_{min}$, mmHg/s</td>
<td>1.815±447 (37)</td>
<td>1,062±264* (36)</td>
<td>1,255±281* (19)</td>
</tr>
<tr>
<td>CI, l/m²·min⁻¹</td>
<td>3.23±0.67 (25)</td>
<td>2.63±0.76* (25)</td>
<td>2.7±0.51* (19)</td>
</tr>
<tr>
<td>SV, ml</td>
<td>77.5±17 (25)</td>
<td>62.7±15.4* (25)</td>
<td>63.8±14.3* (19)</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>97±12 (25)</td>
<td>94±12 (25)</td>
<td>97±11 (19)</td>
</tr>
<tr>
<td>$t$, ms</td>
<td>27±7 (13)</td>
<td>38±7 (13)</td>
<td>37±5 (13)*</td>
</tr>
<tr>
<td>RAP, mmHg</td>
<td>3.4±1.6 (19)</td>
<td>6.1±1.1 (21)</td>
<td>6.8±3* (18)</td>
</tr>
<tr>
<td>PulVR, dyn·s·cm⁻⁵</td>
<td>887±279 (17)</td>
<td>980±272 (22)</td>
<td>971±258 (18)</td>
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</table>

Values are means ± SD; nos. in parentheses are nos. of sheep. LVEDP and LVESP, LV end-diastolic and end-systolic pressure; $LV E_{es}$, LV end-systolic elastance; $LV PRSW$, LV preload recruitable stroke work; $LV dp/dt_{max}$ and $LV dp/dt_{min}$, first derivatives of LV pressure rise and decay; CI, cardiac index; SV, stroke volume; HR, heart rate; $t$, time constant of LV pressure decay; RAP, right atrial pressure; PulVR, pulmonary vascular resistance. *P < 0.05 compared with baseline.
Fig. 3. Representative LV pressure-volume (LVP) loops of a sheep in response to a transient reduction of preload resulting from brief inferior vena cava occlusion. A: establishment of HF caused a marked rise in heart volume and end-diastolic pressure and a decrease in EF and end-systolic pressure. LV end-systolic elastance (E_s) decreased from 2.1 to 1.3. B: dobutamine increased baseline LV E_s to 4.3. However, when the failing heart was dobutamine stimulated, its LV E_s approached only the baseline nonstimulated level (1.7), indicative of a decrease in the contractile reserve. Note that the data shown here should be seen in the context of data displayed in Fig. 1 and Tables 1 and 2.

The sheep in this study demonstrated a persistent decrease of stroke volume (20%) and the cardiac index (16%) (Table 2), a characteristic not consistently reproduced by coronary ligation or single microembolization. There was also LV dilatation, global impairment of wall movement, and thinning of the LV wall (Table 1). LVEF remained below 30% during the study (Fig. 2B). Our previous work suggests that these changes result from myocyte loss due to necrosis, possible myocyte slippage (14, 41), and ongoing apoptosis (15). There was also evidence of myocyte hypertrophy (14) and localization of FasL to the intercalated discs, where adjacent cardiomyocytes transmit mechanical stress. These “compensatory” changes are detrimental, causing increasing myocardial oxygen consumption and reduced energy efficiency (35). Our findings also suggest that increased wall stress may activate the apoptosis signaling

Fig. 4. A: effect of dobutamine infusion with stable chronotropic response (heart rate of 140 beats/min) on LV E_s in the normal and the microembolized failing heart at various time points. *P < 0.05 comparing with and without dobutamine; **P < 0.05 compared with dobutamine baseline. B: effect of microembolization-induced HF on mechanical energy efficiency over 6 mo. EW, external work; PVA, pressure-volume area. *P < 0.05 compared with baseline. n, No. of animals.

Fig. 5. Plasma neurohumoral changes at various times after the establishment of HF. A: atrial natriuretic peptide (ANP); B: aldosterone (Aldo); C: plasma renin activity (PRA); D: ANG II. *P < 0.05 compared with baseline. n, No. of animals.
cascade involving the interaction of the FasL/FasL complex with procaspase-8 (15, 37). The lack of deterioration in the majority of our untreated sheep lends support to the heart’s potential for myocyte regeneration (2). However, the early deaths suggest that the capacity for remodeling and cellular regeneration may vary.

We measured wall stress rather than myocyte stretch but consider them to be interchangeable with respect to cellular responses. In vivo wall stress may have advantages because it integrates the extracellular matrix with the cytoskeleton via integrins, which are believed to be important stretch mediators (8).

Sheep have advantages over dogs, which have an extensive coronary collateral supply and much faster HRs than humans. Our HF model also contrasts with those involving large myocardial infarcts by producing numerous tiny infarcted and peri-infarct zones leading to relatively homogeneous wall stretch.

ANP peaked after 6 mo, whereas aldosterone and plasma renin activity (but not angiotensin II) showed an early rise before declining. This raises the possibility of varied time courses of response of different stretch-sensitive mediators or, alternatively, variation from the extent and homogeneity of stretch distribution. Alternatively, the stretch response in the architecturally complex myocardium may be directionally sensitive, exerting a favorable influence on gene reprogramming.

This study is the first to effectively characterize LV contractility in large animals with chronic, untreated, severe HF and shows significant impairment of native contractility (LV $E_{es}$ and LV PRSW) and contractile reserve (Table 2). Previously abnormalities of the cardiac force-frequency relationship and response to exogenous β-adrenergic stimulation were demonstrated in patients and experimental animals with HF (27, 42). We achieved a constant HR of 140 beats/min with dobutamine to exclude the Bowditch phenomenon and found increased LV $E_{es}$ in the normal but not failing heart (Fig. 4A). This is consistent with a disordered sympathetic drive resulting from reduced cardiomyocyte adenylate cyclase activity (22) and downregulation of adrenergic receptors (3, 10).

Myocardial energy efficiency (EW/P-V area) decreased with chronic HF, indicative of an energy-wasting state (Fig. 4B). In contrast, in a canine model of ischemic HF, it did not change (37).

Plasma ANP levels peaked at 6 mo (Fig. 5), indicative of a continued rise in wall stretch (Fig. 6). On the other hand, PRA and aldosterone rose when HF was established and then declined. However, no increase of plasma angiotensin II was observed, in contrast to a recent coronary ligation model of HF (29).
Changes in the extracellular matrix (the “myocardial skeleton”) are important to the pathophysiological processes that drive the development of HF (25, 28, 39). Matrix metalloproteinases (MMPs) and their tissue inhibitors are differentially expressed in the failing hearts of humans and pigs, in a pattern favoring matrix degradation and turnover (34, 39). We previously reported replacement as well as interstitial fibrosis in this model (14) and now confirm that both content and phenotype of myocardial fibrillar collagen remain altered over 6 mo, with a more stiff type I phenotype (Fig. 7). As collagen content increased, both systolic (LVEF) and diastolic (τ and LV dP/dt max) LV function deteriorated.

Neurohumoral activation causes cardiac dilatation and ECM remodeling with increased collagen turnover and breakdown of the normal collagen network (21, 26). Such disruption may result from catecholamine and aldosterone triggered transcription of mRNAs for MMPs. Dispersed interstitial fibrosis can also result from enhanced angiotensin II activity, which is the basis of some of the beneficial effect of HF treatment with angiotensin-converting enzyme (ACE) inhibitors, and β-adrenergic and aldosterone antagonists. Such treatment has demonstrated a protective effect in those with LV dysfunction even before heart failure supervenes (6).

This sheep model has the advantage of a clear ischemic etiology that is common in humans, although there is no occlusion of the principal coronary trunks. The shee’s size makes it suitable for multiple diagnostic and therapeutic strategies, and it has consistent coronary anatomy, with the left coronary artery and coronary sinus responsible for over 90% of LV blood supply and drainage. Also, similar to humans, sheep have few collaterals, rendering the effect of embolization predictable and the study of LV myocardial metabolism more reliable than in dogs. The sheep did not require medication during the 6-mo observation and study, ensuring homogeneity and stability and allowing investigation of basic mechanisms and interventions.

Method of induction and natural history of HF. Despite diverse methods of inducing HF, such as coronary artery ligation, coronary embolization, fast ventricular pacing, and myocardial intoxication (7), only the first two mimic the most common human etiology. However, coronary ligation requires thoracotomy, produces ventricular dysfunction that is segmental rather than global, and frequently causes mitral regurgitation. Also, the ventricular damage cannot be titrated.

Microsphere injection to induce heart attack in dogs has involved aortic root injection, selective coronary artery delivery, and refinement with repeated injections (32). There are no previous data on microsphere dose needed to induce stable, severe, chronic HF in sheep.

The only previous study of the longer-term hemodynamic and neurohumoral course of experimental severe HF in large animals is that of Sabbah et al. (32), who followed 15 dogs for an average of 3 mo (range 7–19 wk) after coronary embolizations. This corresponds to 2 mo after the establishment of stable HF in our sheep. Our study extends over a threefold longer period and also examines cardiac contractility and energetics. The extensive coronary collateral supply in dogs required greater doses of microspheres (~2 ml during each of the first 3 embolizations and 3–6 ml subsequently) compared with our total of 1.6 ml on average. The myocardial damage in our sheep appears similar (LVEF was 29% at the establishment of HF, corresponding to 26% in dogs 1 mo after the last embolization). However, whereas in sheep the LVEF remained stable over 6 mo, in dogs it continued to decline to 21% over 2 mo, although the fall in the cardiac index was identical. Contrary to expectation, LVEDV in dogs continued to increase over the same period, corresponding to 26% in dogs 1 mo after the last embolization. However, whereas in sheep the LVEF remained stable over 6 mo, in dogs it continued to decline to 21% over 2 mo, although the fall in the cardiac index was identical. Contrary to expectation, LVEDV in dogs continued to increase on follow-up despite the extensive collateral supply, whereas...
our sheep showed a peak increase of LVEDV by 75% at 1 mo, which declined to 55% at 6 mo. This finding may relate to the higher mortality in our sheep (59% of 65 sheep were induced into stable HF, of whom 55%, but only 32% of the original candidates, survived to 6 mo compared with the overall 30% mortality in dogs at 2 mo equivalent follow-up), due to earlier death in the more severely affected animals. One notable difference was the absence of mitral regurgitation in sheep, underscored by the absence of atrial fibrillation, whereas 26% of the dogs had this arrhythmia.

Stepwise coronary embolization causes repeated myocardial infarction and therefore poses a high mortality risk. Our experience shows that ventricular fibrillation and acute LV failure are major causes of mortality, accounting for 37% and 44%, respectively, of the deaths before stable, severe HF could be established. Reduction of intraoperative ventricular fibrillation was achieved by immediate correction of critical hypotension (blood pressure <60 mmHg systolic), administration of prophylactic lidocaine, and use of adequately mixed body temperature microsphere injectate. The quantity of beads injected in any one procedure was based on preoperative echocardiographic LV function and intraoperative arterial pressure and LVEDP. We found that intraoperative LVEDP, with a cutoff of 20 mmHg, was a reliable predictor of postoperative acute HF and the resulting mortality. The success rate of HF induction doubled and intraoperative procedural death declined threefold with the adoption of such precautions (Fig. 2C). Death among animals after HF establishment occurred mostly during anesthesia performed for hemodynamic assessment.

Potential shortcomings of this model. It is recognized that damage is confined to the LV myocardium in the sheep HF model described in the present study and that the model does not include disease of large coronary arteries, nor are the animals affected by concomitant disease. It is likely that early deaths occurred in animals with severe myocardial damage, and thus the surviving cohort was self-selected. In an earlier microscopic morphometric study (14), we showed good correlation between microsphere count and replacement fibrosis. Also, the diffuse, small microinfarctions make precise localization and study of the peri-infarct zones virtually impossible. However, the advantages of this model outweigh these shortcomings and allow the methodological pursuit of pathophysiological mechanisms and assessment of various treatment strategies.

In conclusion, the ovine HF model produced by stepped coronary embolization mimics severe HF in humans with respect to etiology, hemodynamics, and neurohumoral activation, histology, changes in the extracellular matrix, and ventricular remodeling. It provides insights into potentially damaging cytokine activation and the resulting cellular mechanisms of injury leading to apoptosis. It integrates knowledge of cellular processes with the hemodynamic and structural changes that express themselves in altered wall stress. The stable nature of the untreated HF is consistent with a delicate balance between apoptotic cardiomyocyte death, perhaps triggered by myocardial wall stress, and myocyte regenerative capacity coupled with other compensatory mechanisms. The model appears suitable for the investigation of HF, including its cellular and molecular basis, and for the development and testing of medical and surgical interventions.

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