Serelaxin

The recombinant form of Human Relaxin-2 for the treatment of patients with acute heart failure

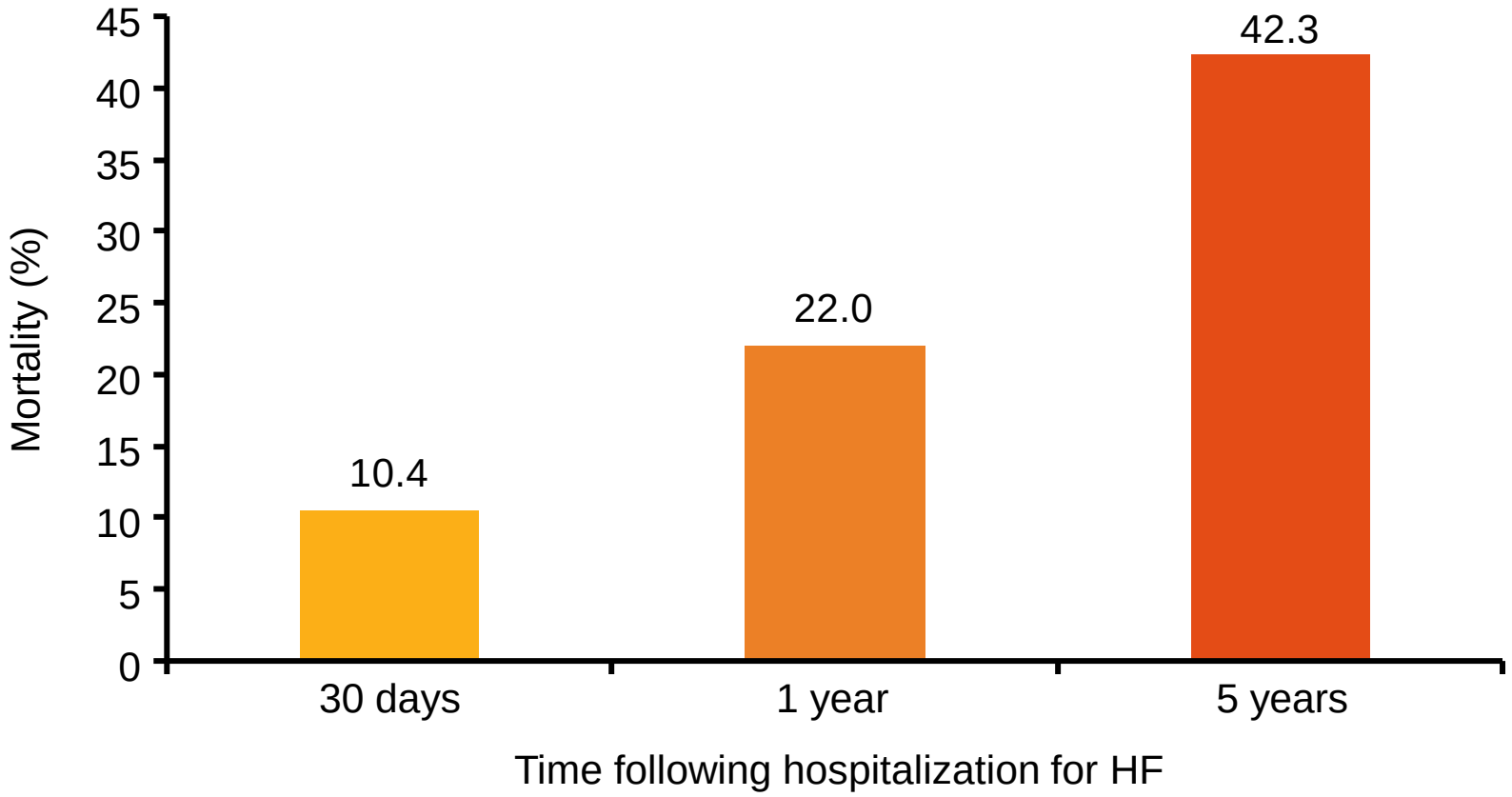
Ricardo Rocha, MD

Basel, Switzerland

NOVARTIS

Patients have a poor prognosis following heart failure hospitalization

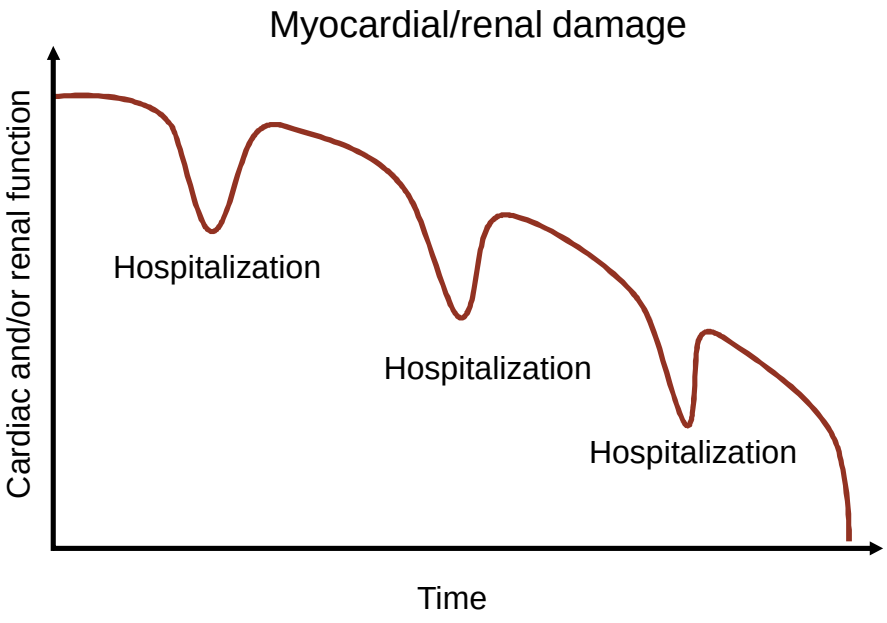
Analysis of HF data from the Atherosclerosis Risk in Communities (ARIC) population-based study from four communities in the USA (1987–2002)

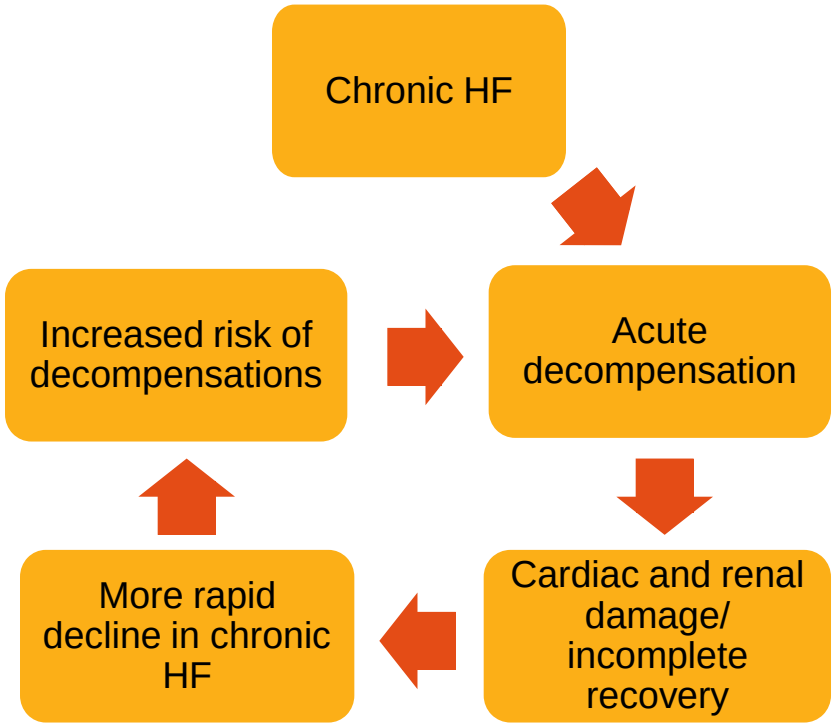


Loehr et al. Am J Cardiol 2008;101:1016–22

The pathophysiology of heart failure results in an increasingly downward spiral

- Acute decompensated HF is associated with frequent hospitalizations[1]
  - after initial stabilization, there are high rates of mortality and rehospitalizations[2]
  - with each hospitalization, there is likely myocardial and renal damage that contributes to progressive LV or renal dysfunction, leading to an inevitable downward spiral[3]
  - current therapies only treat symptoms and do not target the underlying mechanisms leading to cardiac and renal injury

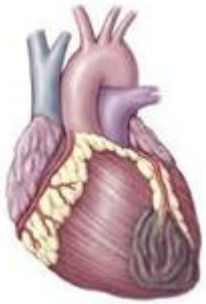




LV=left ventricular

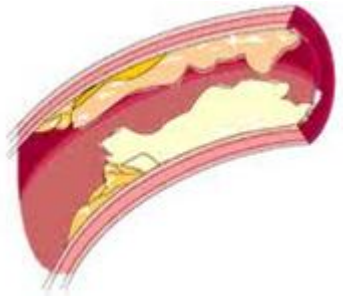
1. Alla et al. Heart Fail Rev 2007;12:91–5; 2. Cleland et al. Eur Heart J 2003;24:442–636;
3. Gheorghiade et al. Am J Cardiol 2005;96:11G–17G

The pathophysiology of acute heart failure remains poorly understood



Heart

Acute HF involves a complex set of interactions between systems



Peripheral vasculature

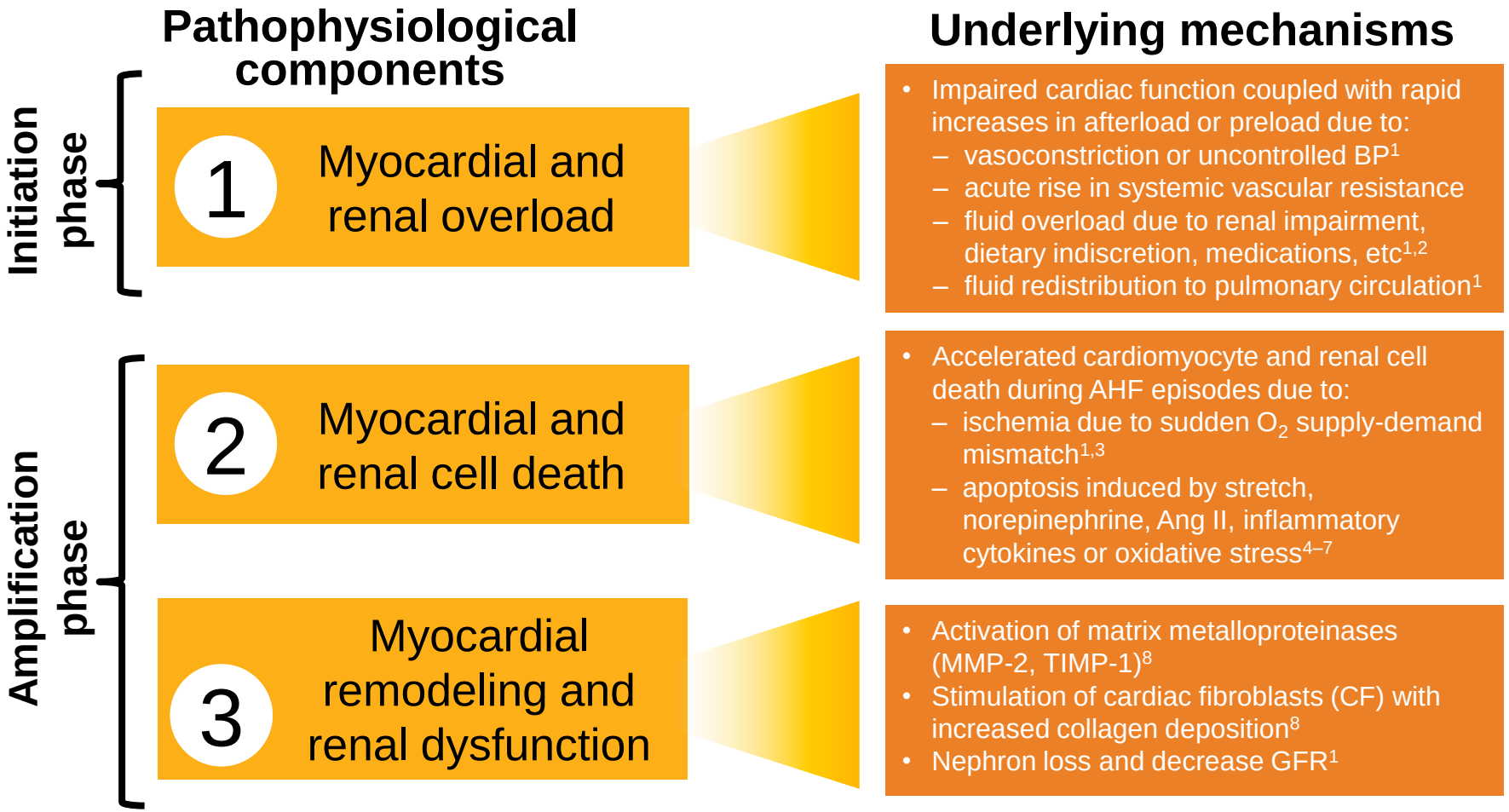
Kidney

The exact mechanisms and the relative contribution of each is uncertain and is likely to vary between patients

Felker et al. Circ Heart Fail 2010;3:314–25

Facing the challenges of AHF

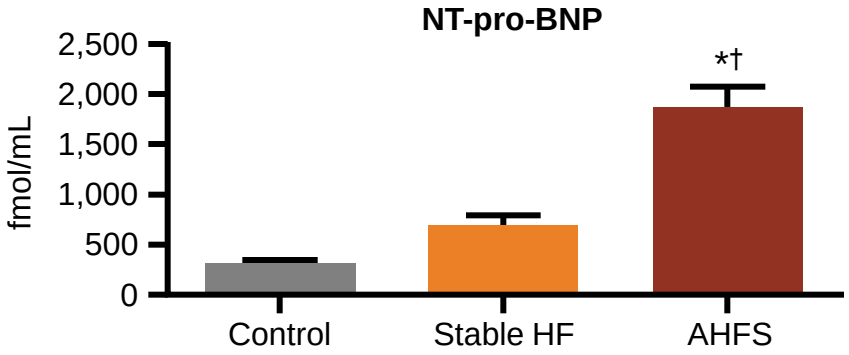
A complex syndrome with three main pathophysiological components

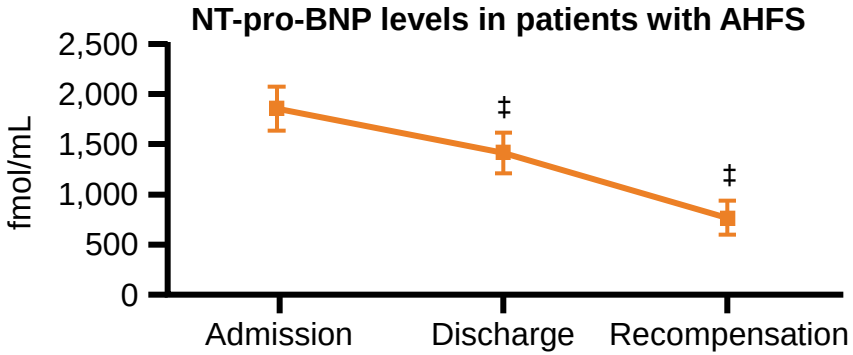


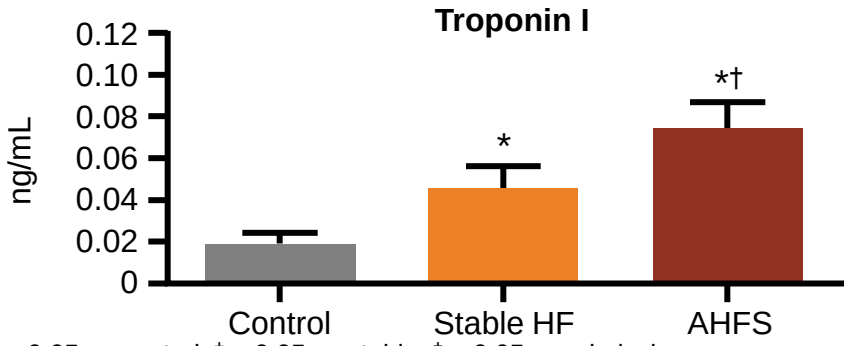
AHF=acute heart failure; Ang II=angiotensin II; BP=blood pressure; GFR=glomerular filtration rate; MMP-2=matrix metalloproteinase-2; NSAIDS=non-steroidal anti-inflammatory drugs; TIMP-1=tissue inhibitors of matrix metalloproteinases-1

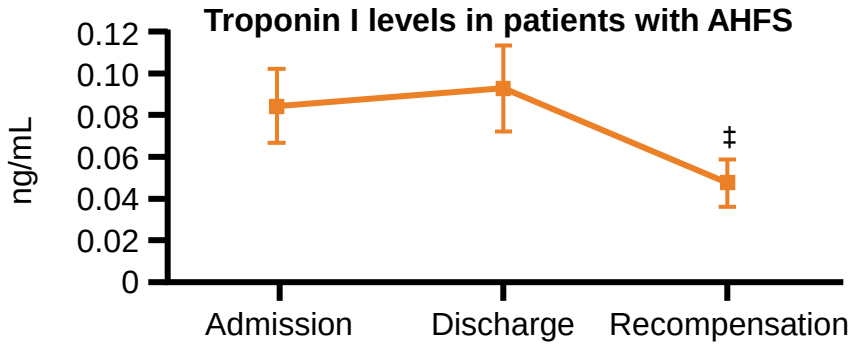
1. Cotter et al. Eur J Heart Fail 2008;10:165–9; 2. Hunt et al. J Am Coll Cardiol 2009;53:e1–e90;
3. Cotter et al. Am Heart J. 2008;155:9–18; 4. Bott-Flügel et al. Eur J Heart Fail 2008;10:129–32;
5. Feng & Wang. J Geriatr Cardiol 2008;5:1–6; 6. Tsutsui et al. Am J Physiol Heart Circ Physiol 2011;301:H2181–90;
7. Oikonomou et al. Hellenic J Cardiol 2011;52:30–40; 8. Biolo et al. Circ Heart Fail 2010;3:44–50

Acute heart failure syndrome episodes are associated with transient increases in NT-proBNP and troponin I, a marker of cell injury/death[1]









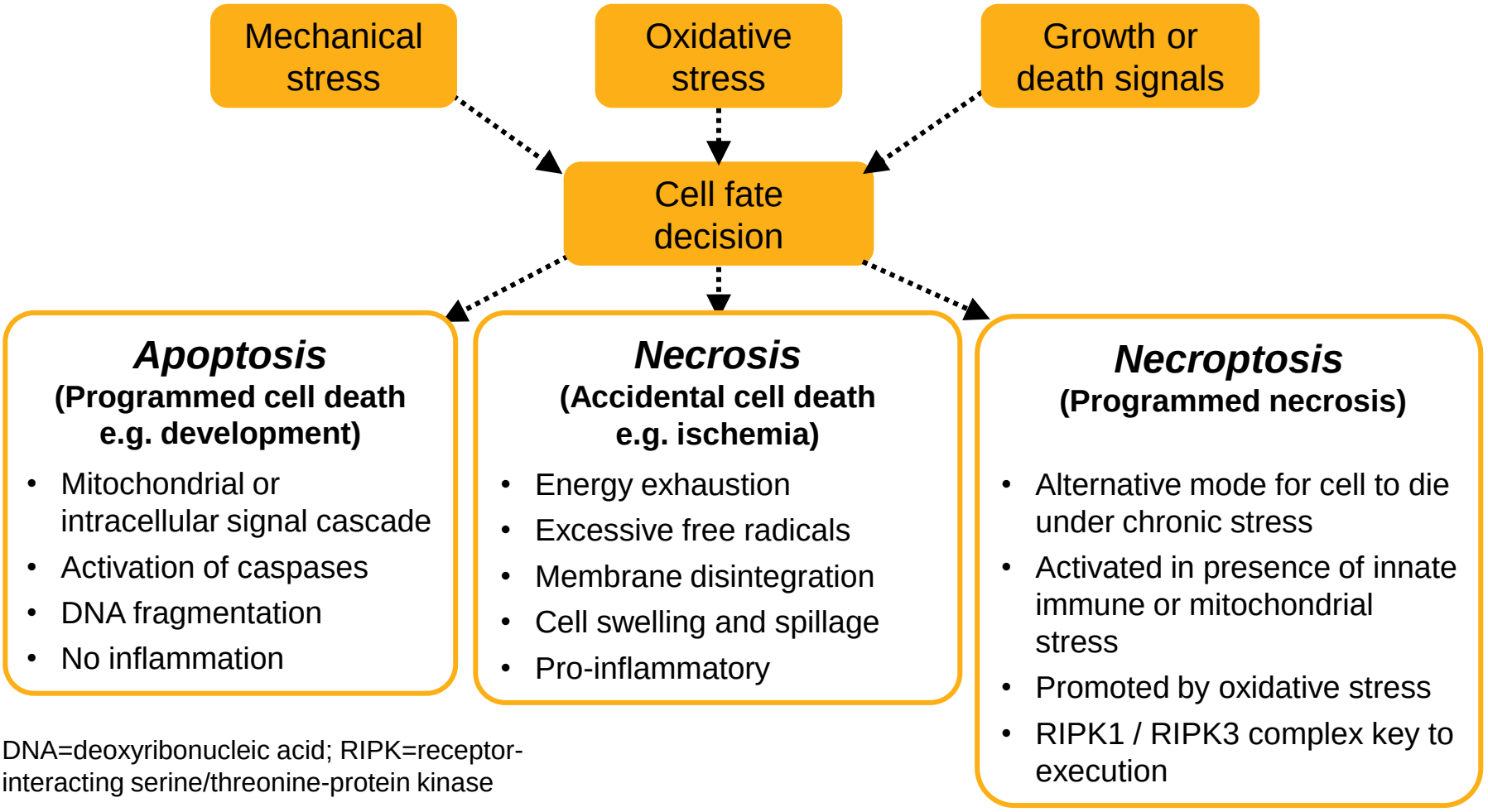
*p<0.05 vs control; †p<0.05 vs stable; ‡p<0.05 vs admission

- Troponin, a marker of myocyte injury and/or death, is known to be elevated in patients with HF without evidence of acute myocardial infarction[2]
- Episodes of AHFS are associated with non-ischemic processes known to cause myocyte death, including mechanical strain and oxidative stress[3]
- These data suggest that episodes of acute HF decompensation may be associated with cardiac myocyte injury and/or death

AHFS=acute heart failure syndrome; NT-pro-BNP=N-terminal pro-B-type natriuretic peptide

1. Biolo et al. Circ Heart Fail 2010;3:44–50; 2 Missov et al. Circulation 1997;96:2953–58;
3. Cheng et al. J Clin Invest 1995;96:2247–59

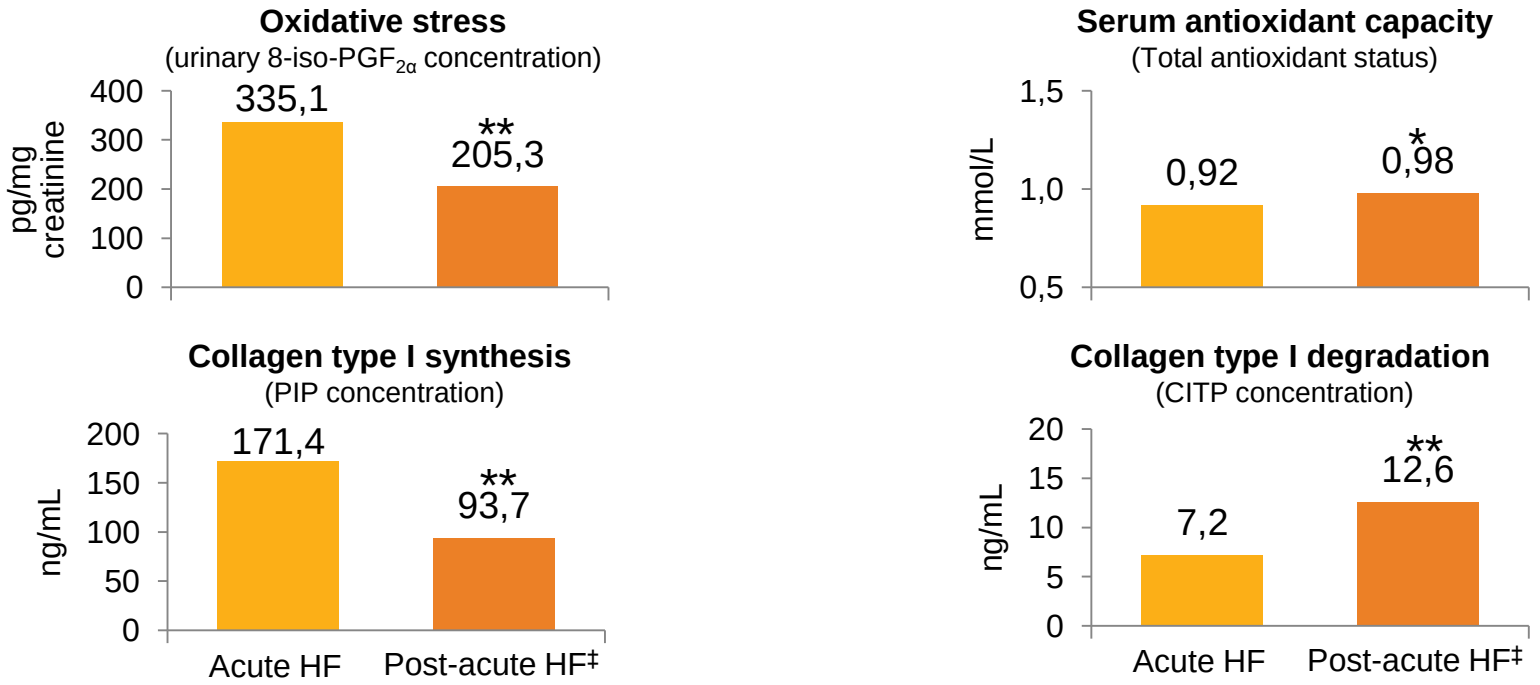
Cell death and injury in heart failure: multiple mechanisms and evolving concepts



DNA=deoxyribonucleic acid; RIPK=receptor-interacting serine/threonine-protein kinase

Liu. Can J Cardiol 1999;15:8–10B; Li et al. Circ Res 2009;104:896–904; Li et al. Hypertension 2010;56:1109–17; Li et al. Cell 2012;150:339–50; Vandenabeele et al. Nat Review Molec Cell Biol 2010;11:700–14

Episodes of acute heart failure are associated with increased oxidative stress and collagen synthesis



*p<0.01; **p<0.0001 vs acute phase; ‡Post-acute HF measurements taken ~2 weeks after admission

- Myocardial remodeling involves rebuilding of the myocardial extracellular matrix, which is predominantly composed of collagen fibers
- Free radical generation has also been linked with myocardial remodeling
- These data from a study in 43 patients with acute HF demonstrate increased markers of collagen type I synthesis and oxidative stress, and reduced collagen type I degradation and total antioxidant status during the acute HF phase compared with ~2 weeks after admission

CITP=carboxy-terminal telopeptide of collagen type I; 8-iso-$PGF_{2\alpha}$=8-iso-prostaglandin $F_{2\alpha}$;
PIP=procollagen type I carboxy-terminal peptide

Kunishige et al. Circ J 2007;71:1893–7

Large randomized controlled trials in acute heart failure have failed to demonstrate outcome benefits

Trial name	Patient population	Intervention	Primary endpoint	Significant effect?
OPTIME-CHF[1]	951 patients admitted with exacerbation of systolic HF	i.v. **milrinone** vs pbo for 48 hours	Length of hospitalization for CV causes	✘
VERITAS[2]	1,448 patients hospitalized with AHF	i.v. **tezosentan** vs pbo for 24–72 hours	Change in dyspnea, incidence of death and worsening HF at 7 days	✘
SURVIVE[3]	1,327 patients hospitalized with AHF	i.v. **levosimendan** vs dobutamine	All-cause mortality at 180 days	✘
EVEREST[4]	4,133 patients hospitalized with AHF	**Tolvaptan** 30 mg once-daily vs pbo for 60 days	All-cause mortality and CV death or hospitalization for HF	✘
ASCEND-HF[5]	7,141 patients hospitalized for AHF	i.v. **nesiritide** vs pbo for 24 hours–7 days	Change in dyspnea and 30-day all-cause mortality or HF hospitalization	✘
PROTECT[6]	2,033 patients hospitalized for AHF	i.v. **rolofylline** vs pbo for up to 3 days	Composite of survival, HF status and renal function	✘

pbo=placebo

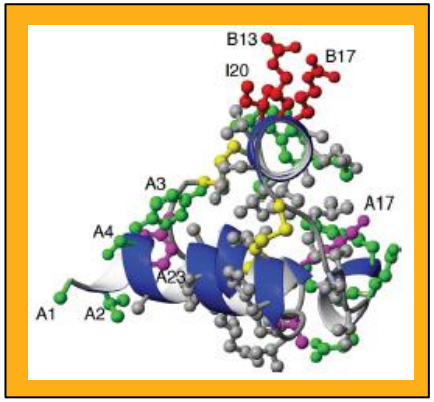
1. Cuffe et al. JAMA 2002;287:1541–7; 2. McMurray et al. JAMA 2007;298:2009–19;
3. Mebazaa et al. JAMA 2007;297:1883–91; 4. Konstam et al. JAMA 2007;297:1319–31;
5. O'Connor et al. N Engl J Med 2011;365:32–43; 6. Massie et al. N Engl J Med 2010;363:1419–28

Serelaxin
Mechanism of action and clinical data

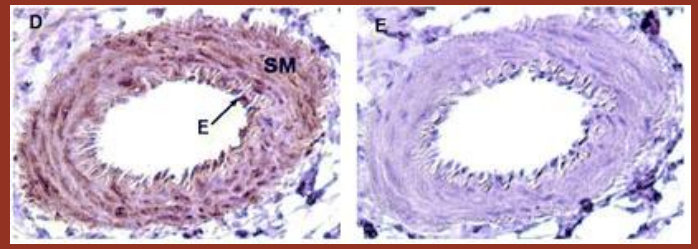
NOVARTIS

Serelaxin is a recombinant form of the human hormone relaxin-2 that acts directly on CV tissues

Structure of native and manufactured human relaxin-2



Relaxin acts directly on CV tissues

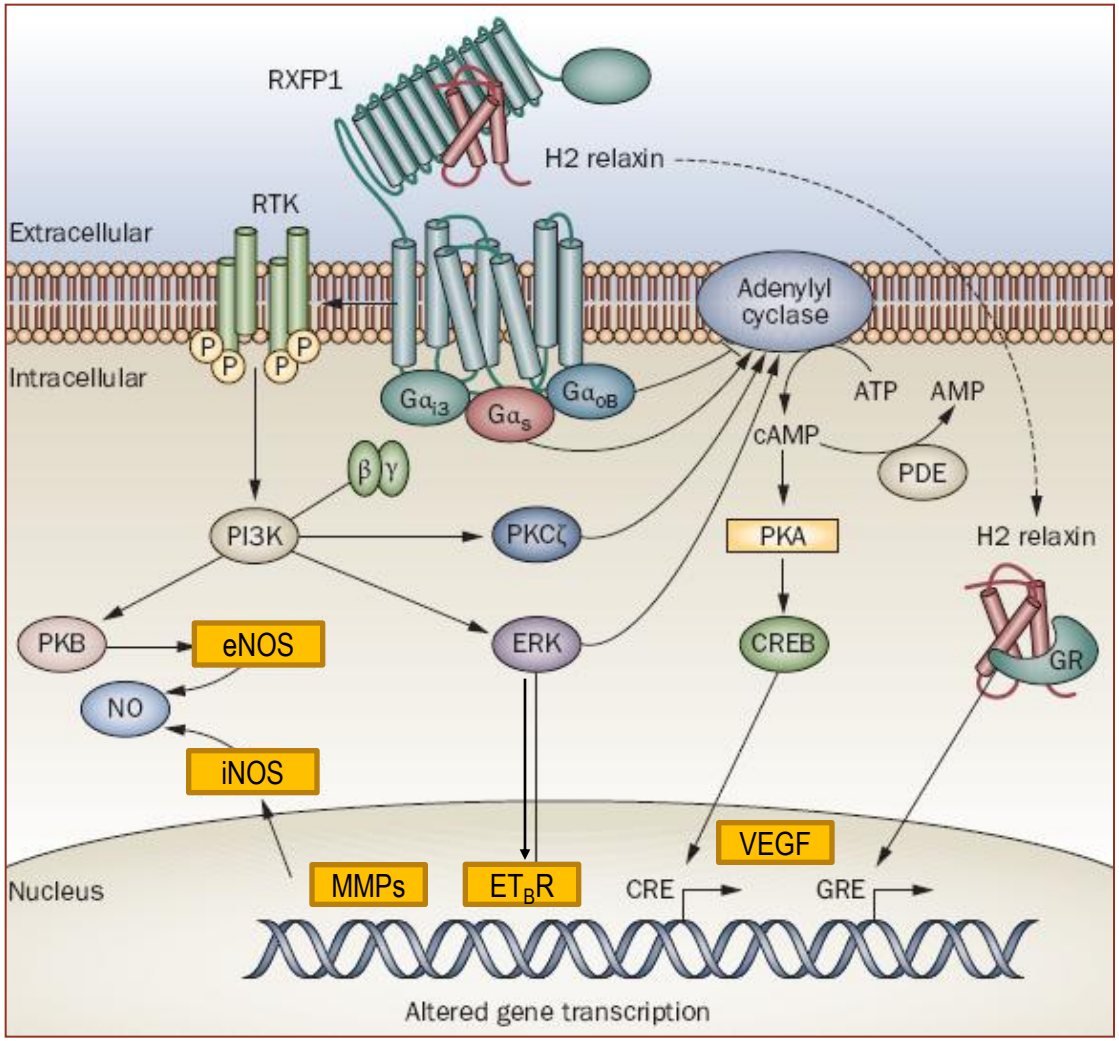


Positive immunostaining for relaxin and precursor forms in both the endothelium and vascular smooth muscle of a small renal artery from a virgin female rat

- Naturally occurring peptide hormone , discovered in 1929
- Human relaxin-2 is one of seven peptides in the relaxin family of hormones
- Structure of human relaxin-2:
  53 amino acids (2 chains connected by 2 disulphide bonds)
- Relaxin-2 mediates its effects
  via specific G-protein-coupled receptors:
  RXFP1 (LGR7) and RXFP2 (LGR8)
- Cardiovascular tissues are equipped with relaxin receptors that are activated by circulating or regionally generated relaxin -2 to mediate diverse signaling pathways

Teichman et al. Heart Fail Rev 2009;14:321–9; Jeyabalan et al. Adv Exp Med Biol 2007;612:65–87
Kong et al. Moll Cell Endocrinol 2010;320:1–15; Du et al. Nat Rev Cardiol 2010;7:48–58
Novak J. et al. FASEB J. 2006; 20: 2352–2362

Serelaxin triggers multiple pathways following binding to its receptor



NOS=nitric oxide synthase; ET_BR=endothelin type B receptor; MMP=matrix metalloproteinase; VEGF=vascular endothelial growth factor

Adapted from Du et al. Nat Rev Cardiol 2009;7:48–58

Relaxin mediates maternal hemodynamic improvements during pregnancy

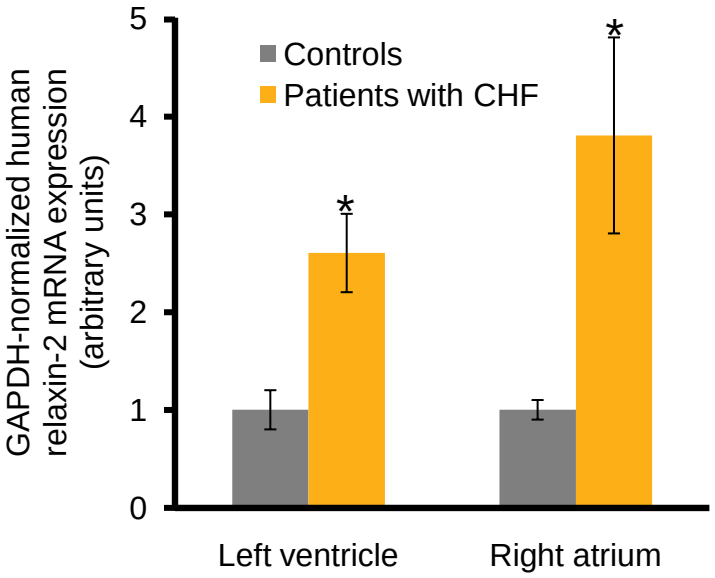
PARAMETER	PREGNANCY
Systemic vascular resistance (dyn.s.cm^2)	↓
Cardiac output (L/min)	↑
Global arterial compliance (mL/mm Hg)	↑
Renal vascular resistance (dyn.s.cm^2)	↓
Renal blood flow (mL/min/1.73m^2)	↑
Creatinine clearance (mL/min/1.73m^2)	↑

- Onset of hemodynamic changes coincident with relaxin elevation during 1st trimester of pregnancy; similar but smaller changes observed during the luteal phase of menstrual cycle
- The beneficial effects of relaxin see in pregnancy may be beneficial in acute heart failure

Baylis. Am J Kid Dis 1999; 34:1142–4; Schrier et al. Am J Kid Dis 1987; 284–9;
Dschietzig et al. Pharmacol Therap 2006;112:38–56 ; Jeyabalan et al. Adv Exp Med Biol 2007;612:65–87

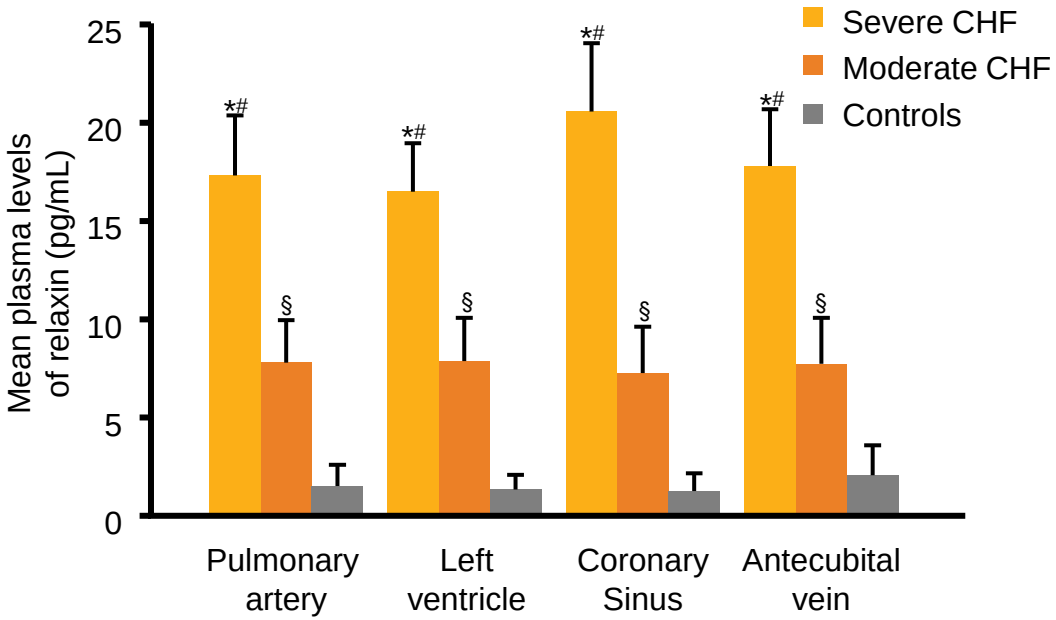
Relaxin is expressed in myocardial tissues and levels are elevated in patients with heart failure

Expression of human relaxin-2 mRNA in myocardial tissues determined by RT-PCR



*p<0.05 vs controls

Plasma levels of relaxin determined by ELISA



p<0.05; *severe CHF vs controls; §moderate CHF vs controls; #severe vs moderate CHF

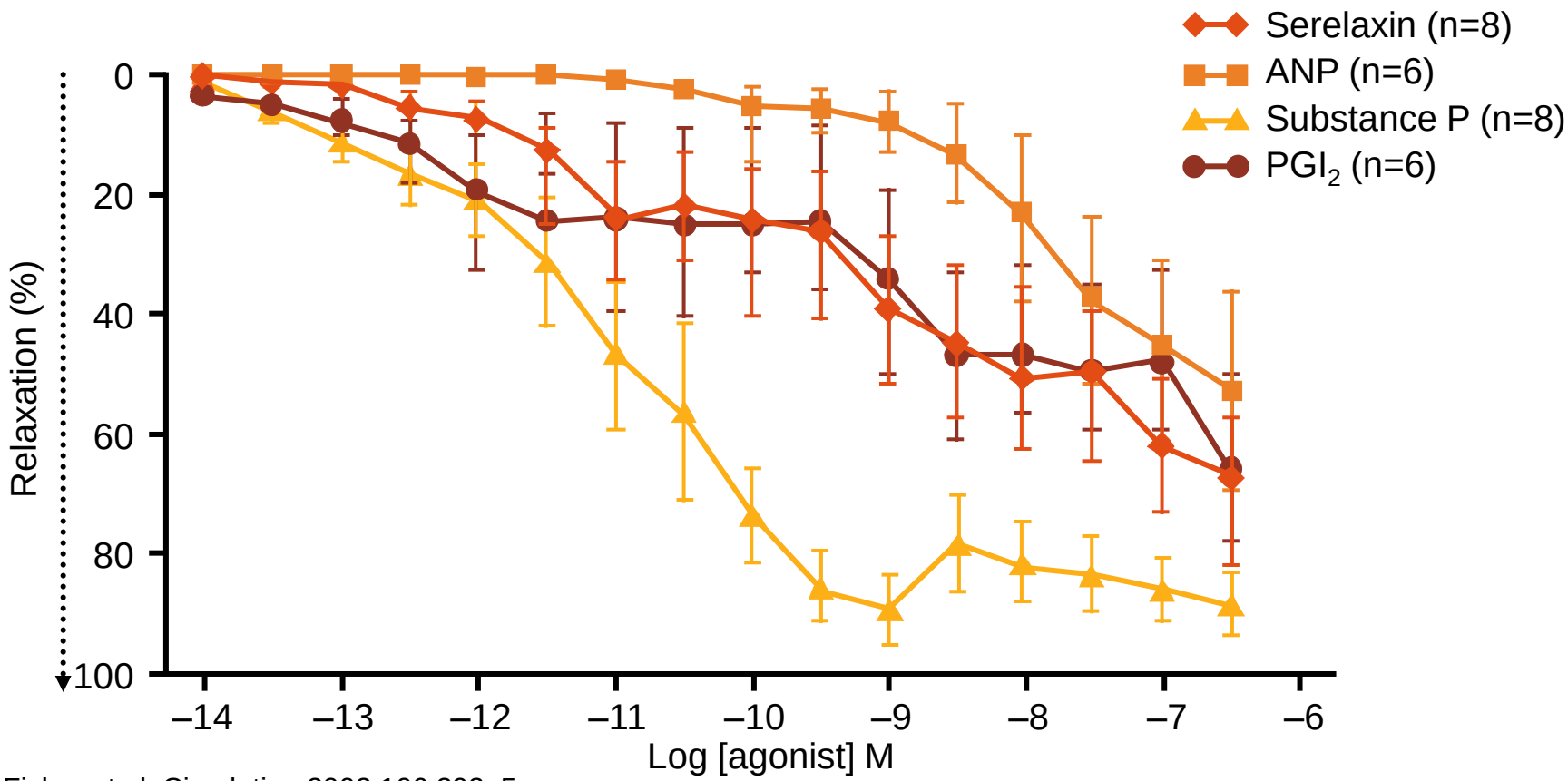
- Relaxin is constitutively expressed in human myocardial tissues
- Expression of relaxin is elevated in patients with CHF compared with individuals with no evidence of structural cardiovascular disease

CHF=congestive heart failure; ELISA=enzyme-linked immunosorbent assay; GAPDH=glyceraldehyde-3-phosphate dehydrogenase; mRNA= messenger ribnucleic acid; RT-PCR=reverse transcriptase polymerase chain reaction; vs=versus

Dschietzig et al. FASEB J 2001;15:2187–95

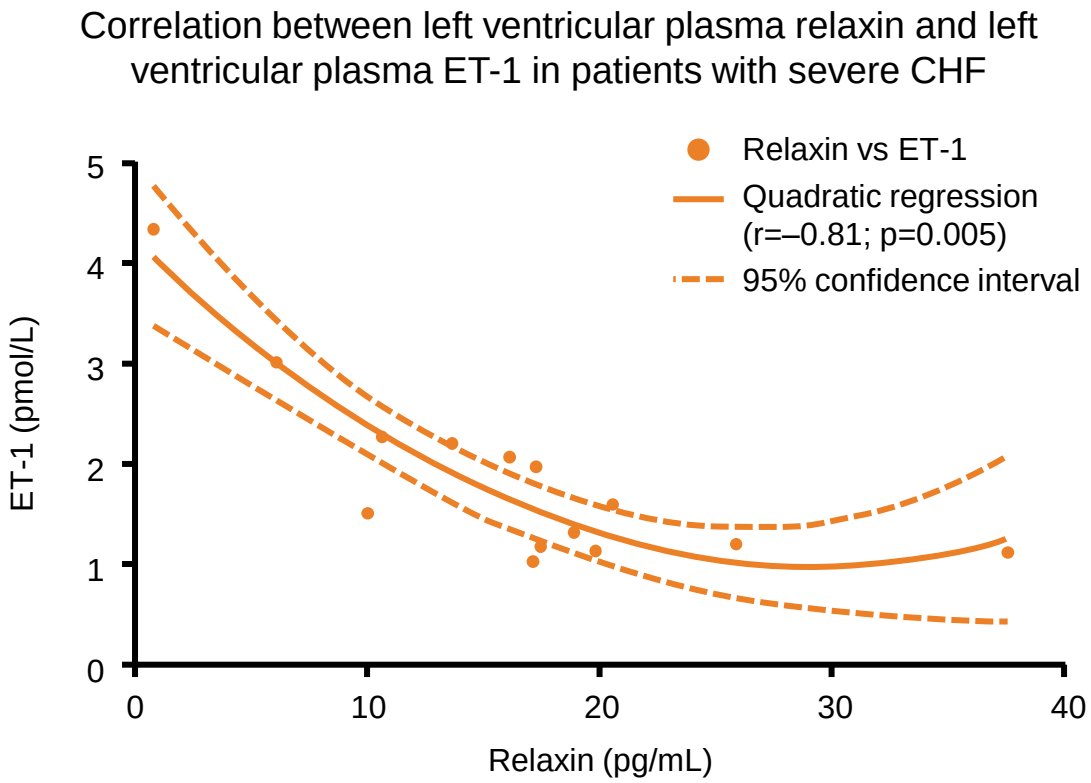
Serelaxin dilates isolated human small resistance arteries *in vitro*

- Resistance vessels from gluteal biopsies were preconstricted with norepinephrine (10^{-5} mol/L)
- Serelaxin was as potent as prostacyclin (PGI_2) in causing vessel relaxation



Fisher et al. Circulation 2002;106:292–5

There is an inverse correlation between plasma endothelin-1 and relaxin in patients with severe heart failure

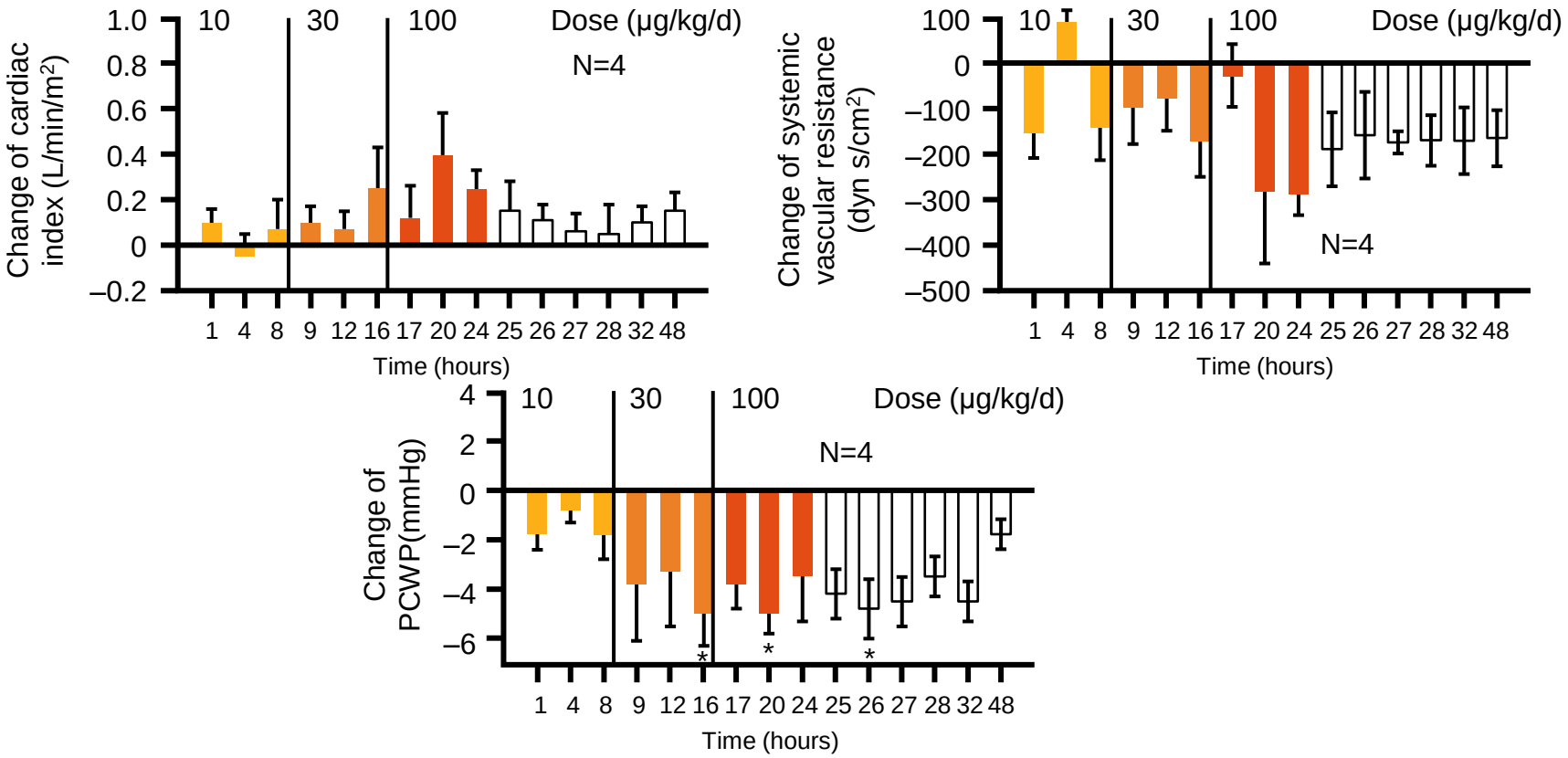


- Among patients with severe CHF, individuals with the highest plasma levels of relaxin had the lowest circulating levels of ET-1

CHF=congestive heart failure; ET-1=endothelin-1; vs=versus

Dschietzig et al. FASEB J 2001;15:2187–95

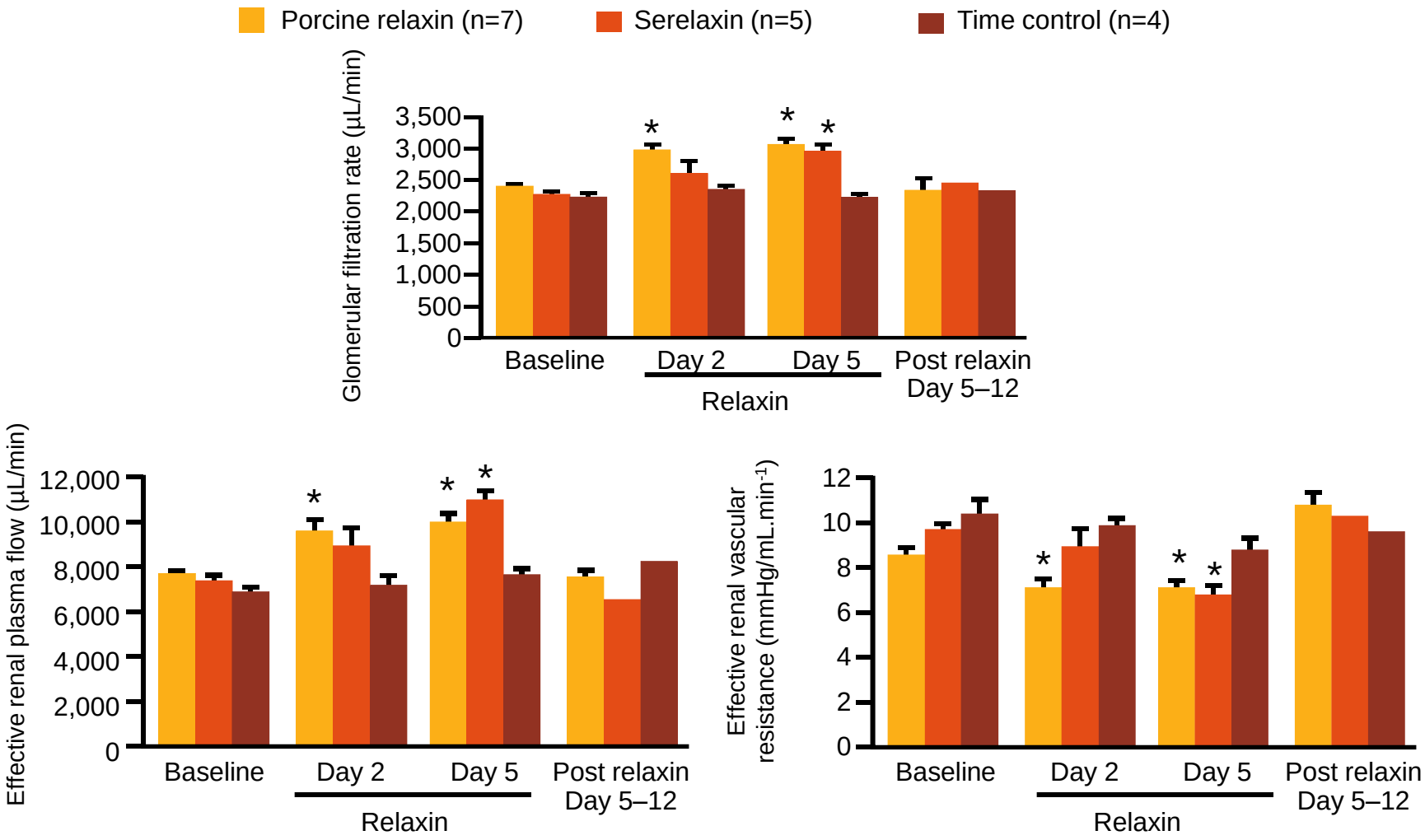
Serelaxin demonstrated favorable hemodynamic effects in a pilot study in patients with chronic heart failure



- Hemodynamic effects of serelaxin during a continuous 24-hour, dose escalating infusion (colored bars) and during the 24-hour post-infusion (white bars)
- Hemodynamic measurements, including cardiac Index (by thermodilution method), systemic vascular resistance and pulmonary capillary wedge pressure, were serially performed using Swan–Ganz and arterial catheters

Dschietzig et al. Ann N Y Acad Sci 2009;1160:387–392

Serelaxin increases GFR and renal plasma flow and reduces renal vascular resistance in rats compared with time control



*p<0.05 serelaxin vs baseline and vehicle

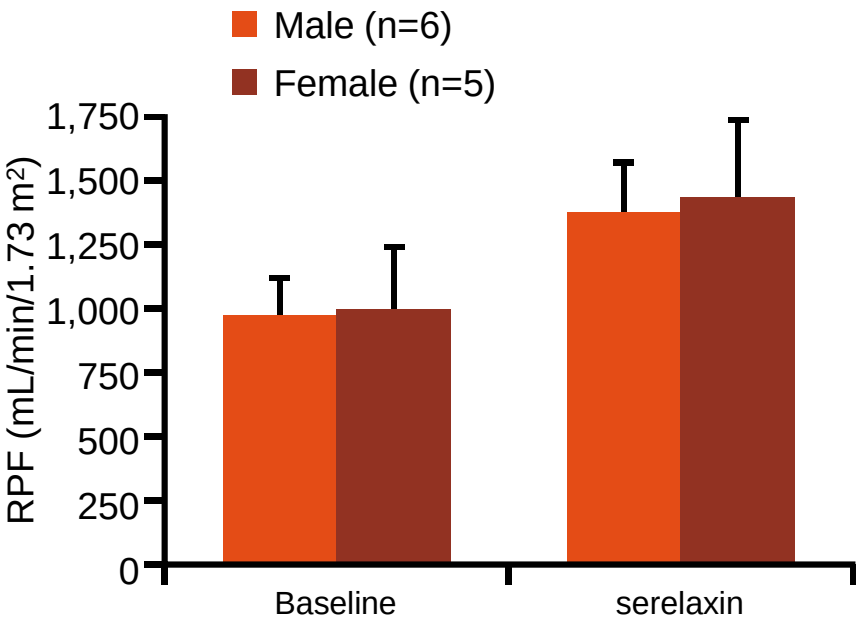
Danielson et al. J Clin Invest 1999;103:527–33

Serelaxin increases renal plasma flow in healthy volunteers

Hemodynamic measurements before and after 4 h intravenous infusion with serelaxin in healthy human volunteers

Parameter	Baseline	Serelaxin
RPF (mL/min per 1.73m^2)	983±133	1403±165*
GFR (mL/min per 1.73m^2)	117.7±9.7	115.6±7.8
MAP (mmHg)	114.7±1.7	117.0±3.0
PR (beats per min)	68±1.8	67±1.9

Data are means ± SEM.
*p<0.0001 baseline vs serelaxin



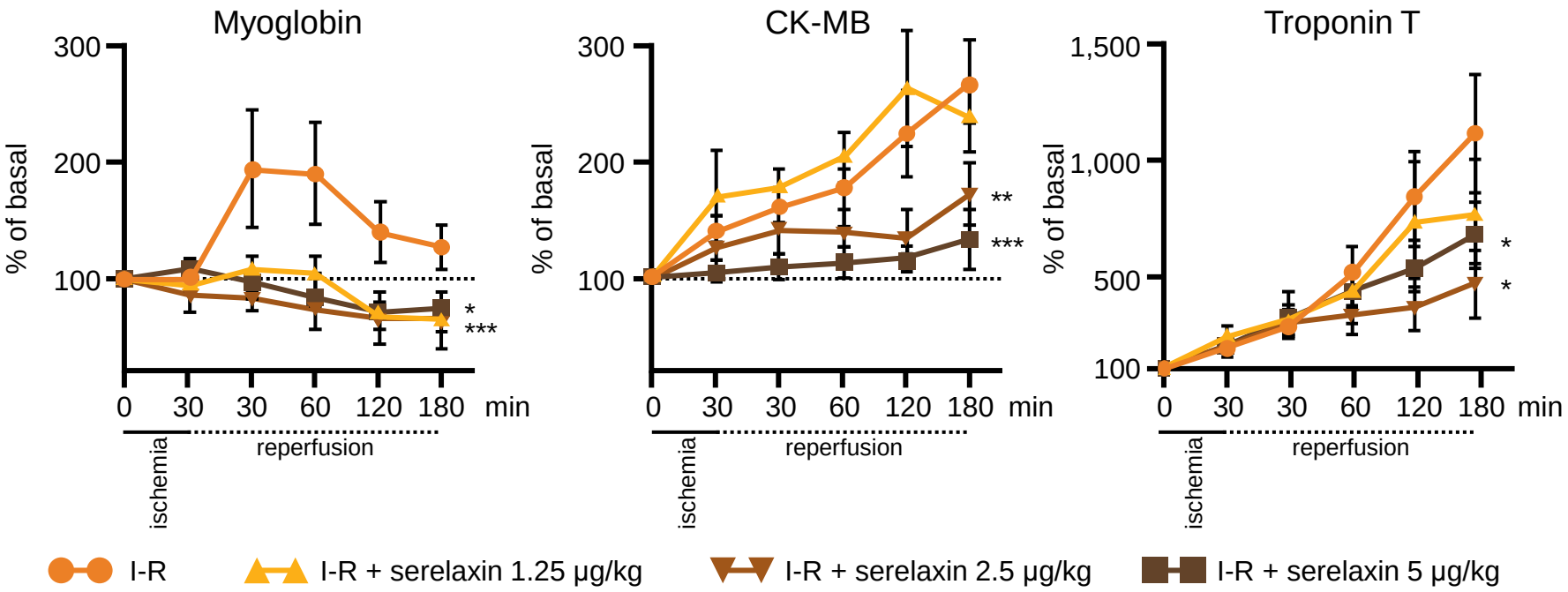
No significant difference in RPF response between male and female volunteers.
Data are means ± SEM

- Administration of serelaxin increased RPF in male and female healthy volunteers without significant effects on GFR. Other effects included significant natriuresis.

MAP=mean arterial pressure; PR=pulse rate; RPF=renal plasma flow; SEM=standard error of the mean

Smith et al. J Am Soc Nephrol 2006;17:3192–7

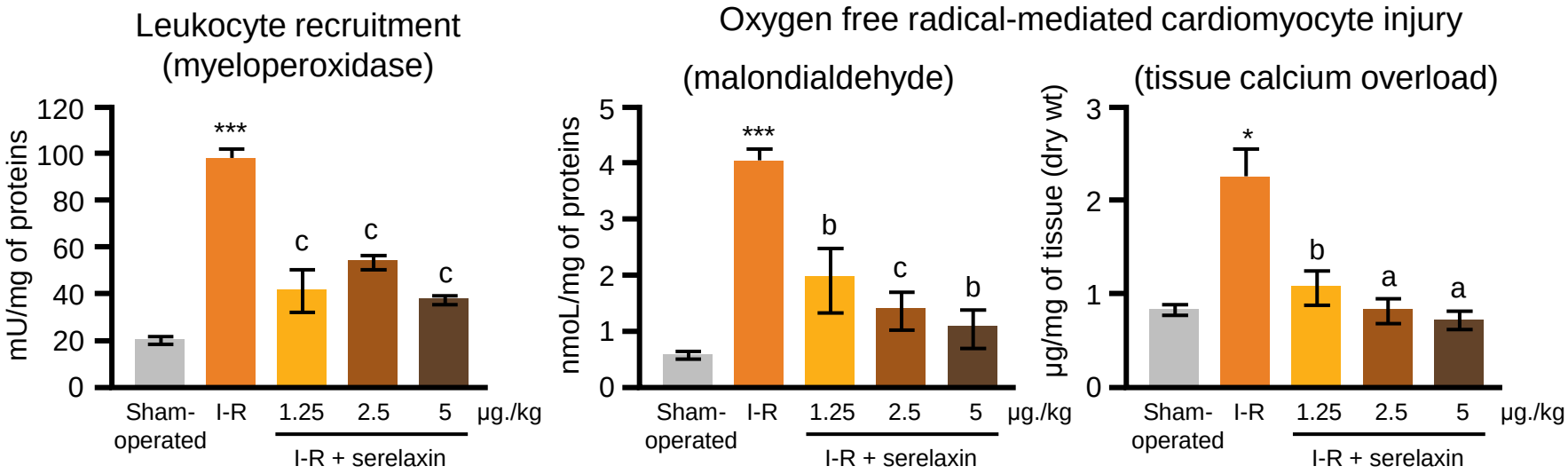
Serelaxin reduces markers of myocardial damage in an *in vivo* porcine model of ischemia/reperfusion



- Serelaxin (1.25, 2.5 and 5.0 µg/kg body weight) was administered following a 30-minute ischemia
- Serelaxin caused a dose-related reduction in key markers of myocardial damage (serum myoglobin, CK-MB, troponin T)
- The greatest reductions were observed with the highest two doses of serelaxin

I-R=ischemia-reperfusion with no serelaxin (vehicle alone)
*p<0.05; **p<0.01; ***p<0.001 vs vehicle alone
Perna et al. FASEB J. 2005;19:1525–7

Serelaxin reduces inflammatory leukocyte recruitment and oxygen free radical-mediated cardiomyocyte injury *in vivo*

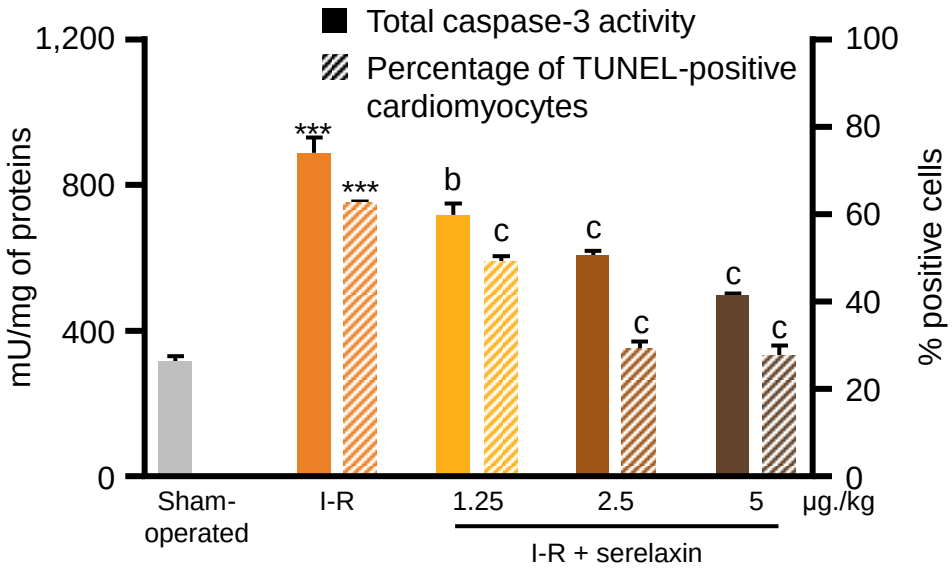


- In an *in vivo* porcine model of myocardial ischemia/reperfusion, three increasing doses of serelaxin (1.25, 2.5 and 5.0 µg/kg body weight) were administered following 30 minutes of ischemia
- Serelaxin caused a dose-dependent reduction in inflammatory leukocyte recruitment (measured by myeloperoxidase concentration) and oxygen free radical-mediated cardiomyocyte injury (measured by malondialdehyde concentration and tissue calcium overload)

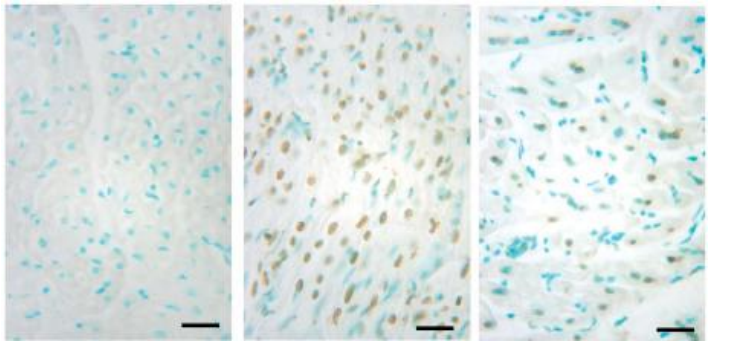
Sham-operated=negative control ; I-R=ischemia-reperfusion=positive control
*p<0.05; ***p<0.001 vs sham-operated; a=p<0.05 vs I-R; b=p<0.01 vs I-R; c=p<0.001 vs I-R
Perna et al. FASEB J. 2005;19:1525–7

Serelaxin reduces myocardial apoptosis in an *in vivo* porcine model of ischemia/reperfusion

Total caspase-3 activity and
percentage of TUNEL-positive cardiomyocytes



Photomicrographs of TUNEL
-positive cardiomyocytes

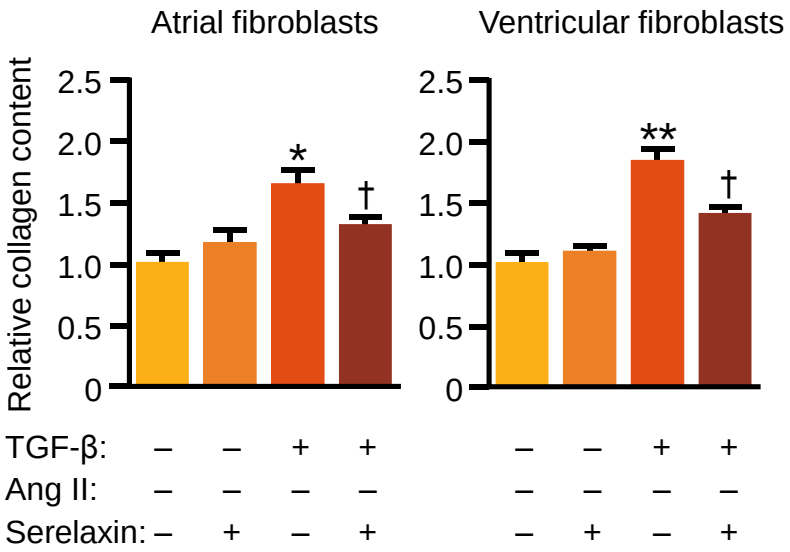


Brown nuclei indicate TUNEL-positive nuclei
(i.e. apoptotic cells)

- Three increasing doses of serelaxin (1.25, 2.5 and 5.0 µg/kg body weight) were administered following a 30-minute ischemia
- Serelaxin caused a dose-related reduction of key markers of cardiomyocyte apoptosis (caspase 3, TUNEL assay)

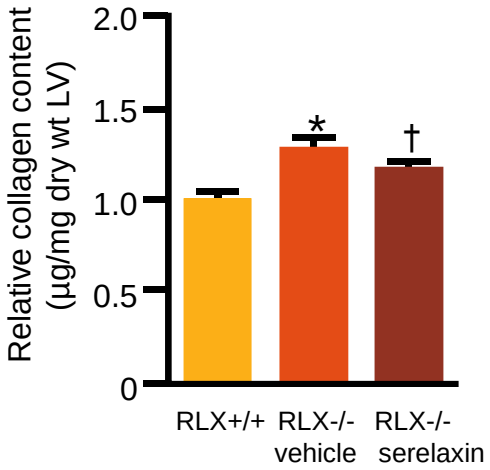
Sham-operated=negative control ; I-R=ischemia-reperfusion=positive control (vehicle only)
***p<0.001 vs sham-operated; b=p<0.01 vs I-R; c=p<0.001 vs I-R
Perna et al. FASEB J. 2005;19:1525–7

Serelaxin modulates collagen deposition *in vitro* and fibrosis *in vivo*



Collagen content of fibroblasts, untreated/treated with serelaxin (100 ng/mL) alone or with TGF-β (2 ng/mL), for 72 h of culture. Results are mean data from 3–4 separate experiments

*p<0.05, **p<0.01 vs untreated cells
†p<0.05 vs TGF-β--treated cells

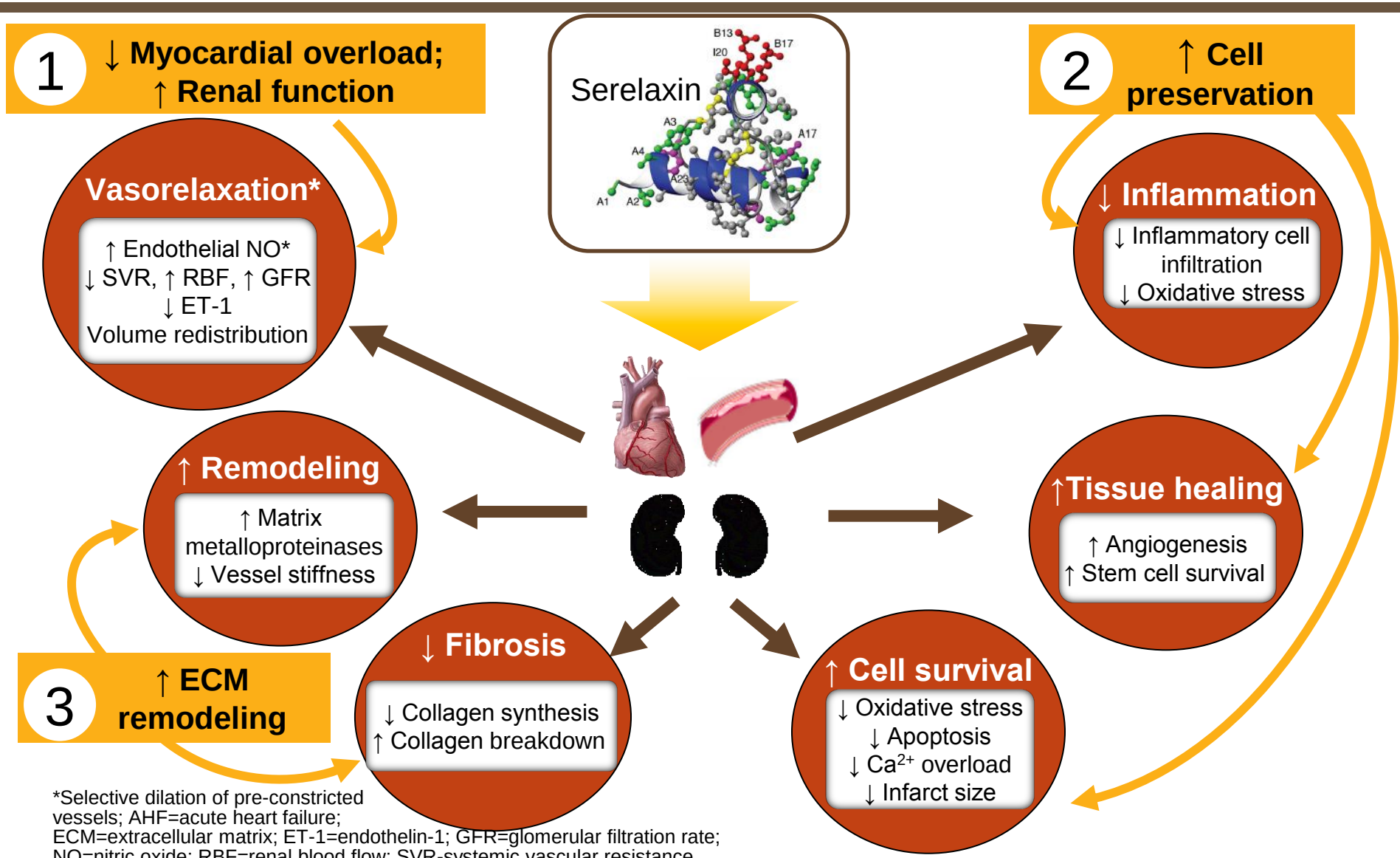


Collagen content/dry weight ventricular tissue was determined from 12-month-old relaxin wild-type (RLX+/+) mice (n=8), relaxin knockout (RLX-/-) mice treated with vehicle alone (n=4), and RLX-/- mice treated with 500 µg/kg/day serelaxin (n=4) for 14 days

*p<0.05 vs RLX+/+
†p<0.05 vs RLX-/- mice treated with vehicle alone

Samuel et al. Endocrinology 2004;145:4125–33

Serelaxin has potential multi-mechanistic effects which may address the pathophysiology of AHF



*Selective dilation of pre-constricted vessels; AHF=acute heart failure; ECM=extracellular matrix; ET-1=endothelin-1; GFR=glomerular filtration rate; NO=nitric oxide; RBF=renal blood flow; SVR-systemic vascular resistance

Adapted from Du et al. Nat Rev Cardiol 2010;7:48–58