Nourin-associated miR-137 & miR-106b: Novel Inflammatory Mediators in Heart Failure; Inhibition by Cyclocreatine Phosphate

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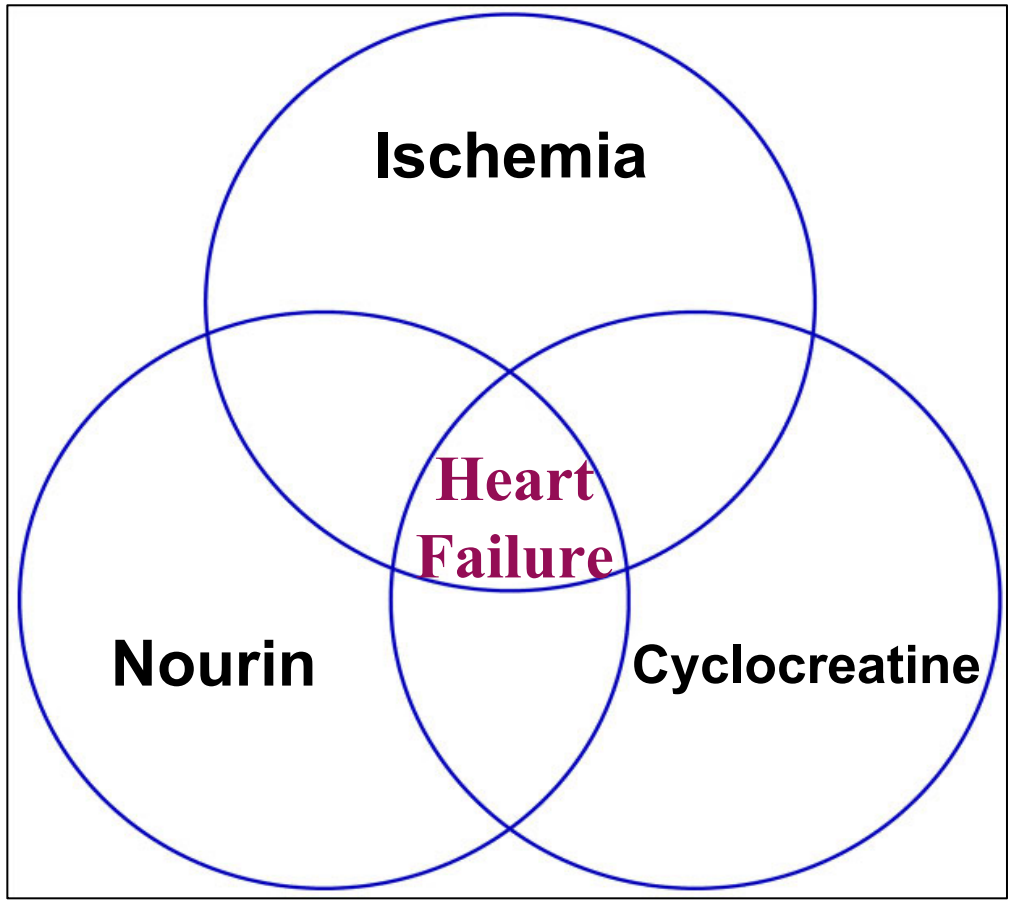
Outline

- What is Nourin?
- What is Cyclocreatine?
- Rationale
- Hypothesis
- Experimental Design
- Results
- Conclusions

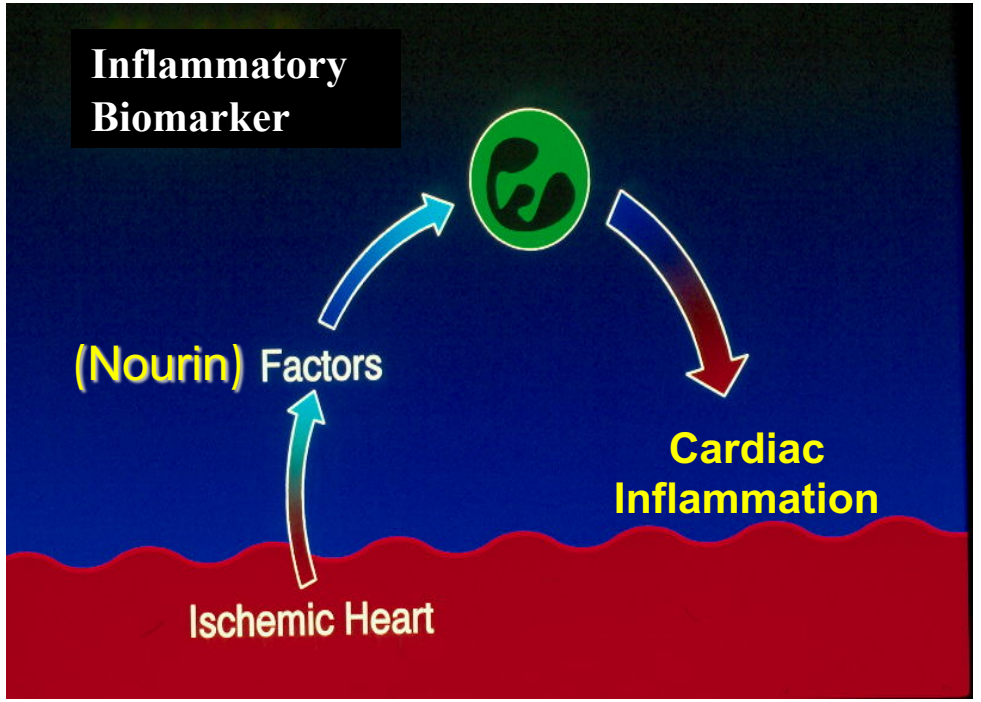


What is Nourin?

A Novel "Injury Response" Molecule!

NOURIN:

- **Released within 5 minutes by ischemic human and animals hearts**
- **A 3 Kda formyl peptide inflammatory mediator, purified from human ischemic hearts**
- **Stimulates leukocyte chemotaxis and is associated with acute cardiac inflammation in early reperfusion, and chronic inflammation**
- **Activates human leukocytes & vascular endothelial cells (VECs) to express:**
  - **Cytokine storm mediators**
  - **Digestive enzymes**
  - **Free radicals**

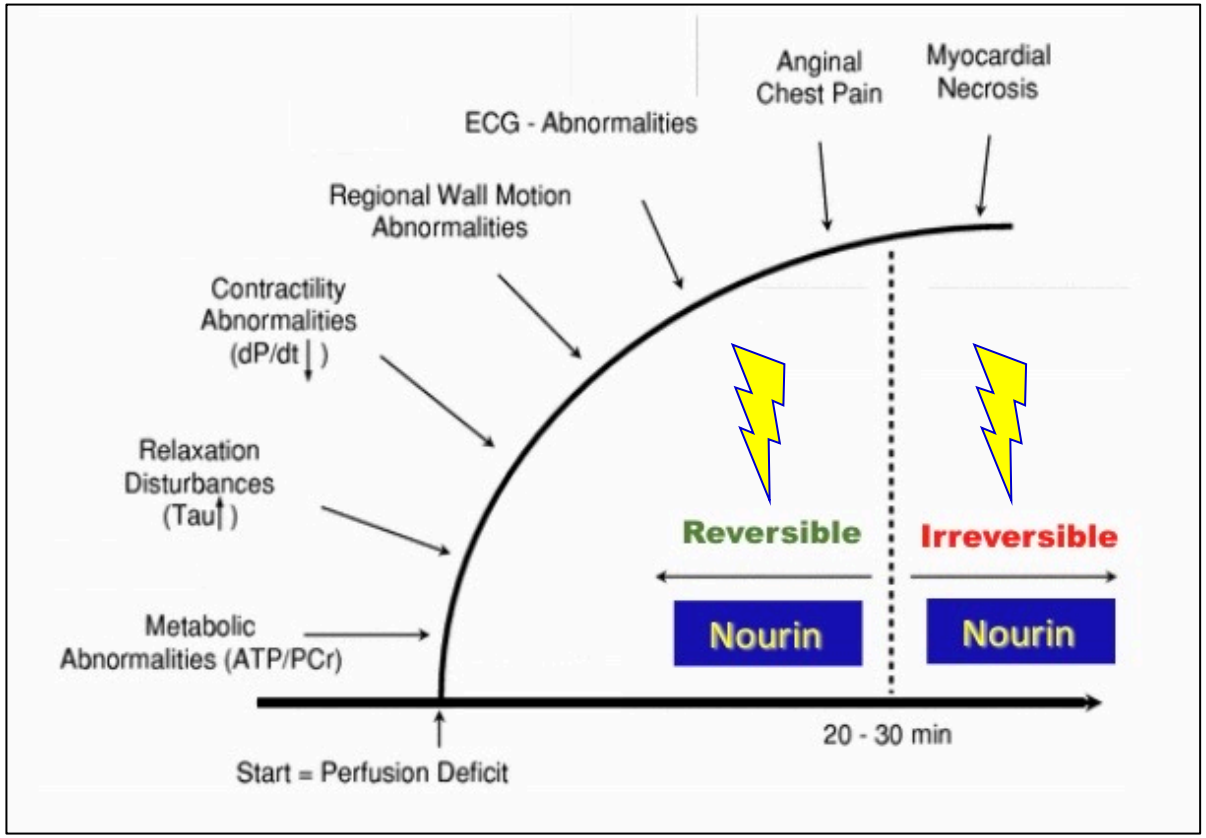


Elgebaly SA, et al. Expert Review of Cardiovascular Therapy – 2019 – REVIEW
Elgebaly SA, et al. Society for Cardiovascular Angiography and Interventions (SCAI) - 2013

What is Unique About Nourin?

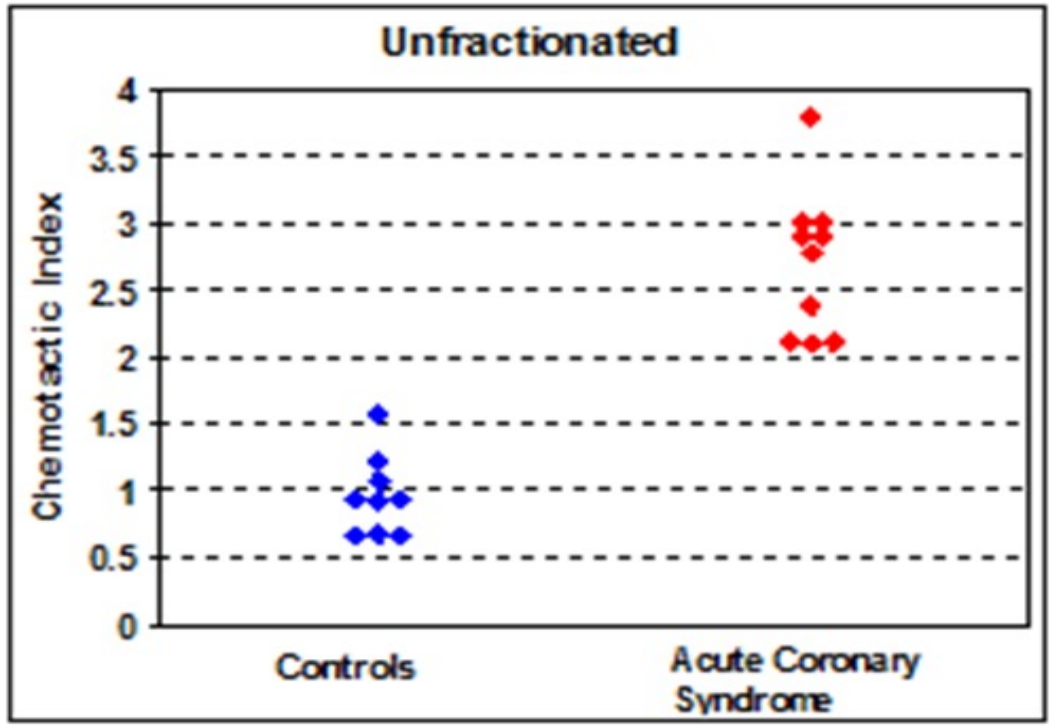
Released by "Reversible" Ischemia!

- **Released after "reversible" ischemic myocardium when cells are "sick", but not dead**
- **Detected "at presentation" to hospital ED in:**
  - **ACS**
  - **STEMI**
  - **NSTEMI**
- **Not detected in:**
  - **Symptomatic Non-Cardiac**
  - **Healthy**
- **Measured by ELISA & Chemotaxis using:**
  - **Serum and plasma samples**
  - **Fresh and frozen (-70 °C for 3 years) samples**

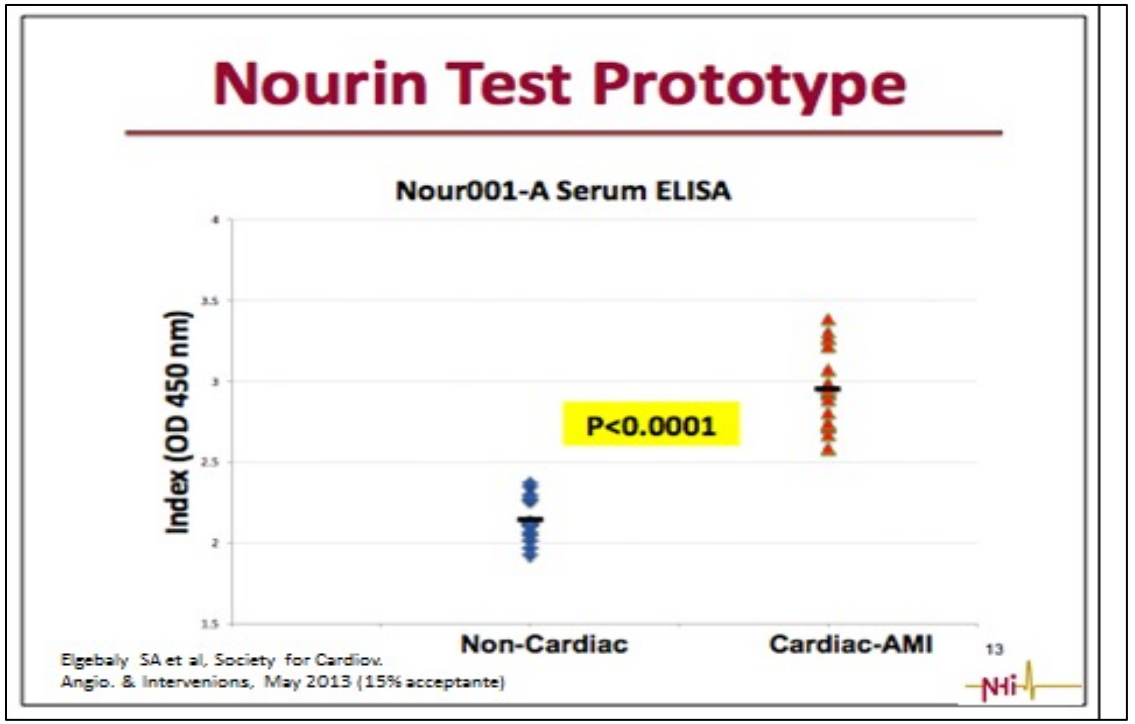


Modified from Dymarkowski S, et al. In Clinical Cardiac MRI 2005 (pp. 173-216). Springer, Berlin, Heidelberg.

Nourin Protein in ACS Patients (first 1.5 to 3.5 hrs.)
Nourin Protein in AMI Patients (first 8 hrs.)



ACS
Chemotaxis Assay



AMI
ELISA

Nourin Regulatory Network

Nourin-associated miRNA-137

- **Using Nourin amino acid sequence, an integrated bioinformatics analysis was conducted and the interaction network was constructed:**

lncRNA	miRNA	mRNA
CTB89H12.4	miR-137	FTHL-17

miR-137 is a marker of cell damage and a hypoxia responsive autophagy-signaling pathway linked to myocardial ischemia and Coronary Artery Disease (CAD)

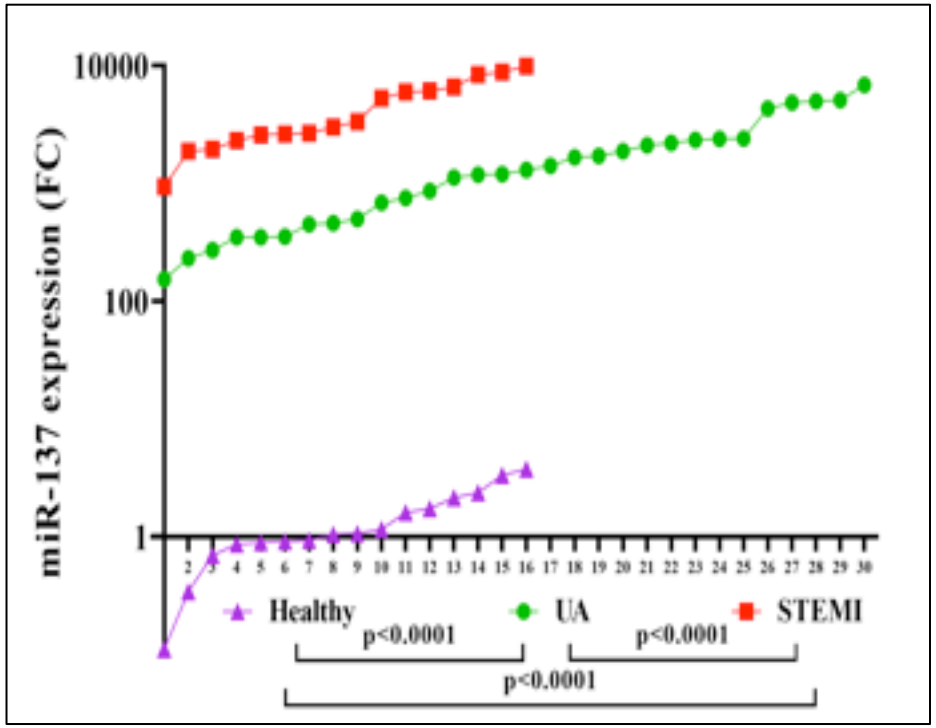
Nourin-associated miRNA-106b

- **Using Nourin amino acid sequence, an integrated bioinformatics analysis was conducted and the interaction network was constructed:**

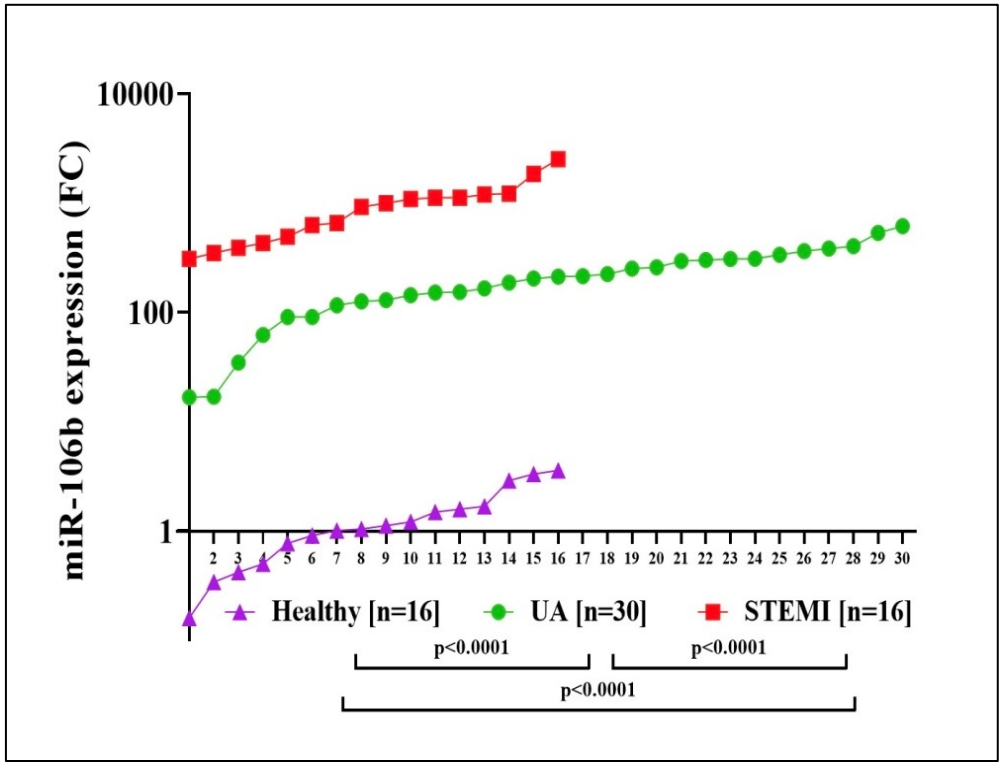
lncRNA	miRNA	mRNA
CTB89H12.4	miR-106b	ANAPC11

miRNA-106b is an inflammatory-signaling pathway linked to myocardial ischemia

Expression Pattern of miR-137 and miR-106b in UA, STEMI & Healthy



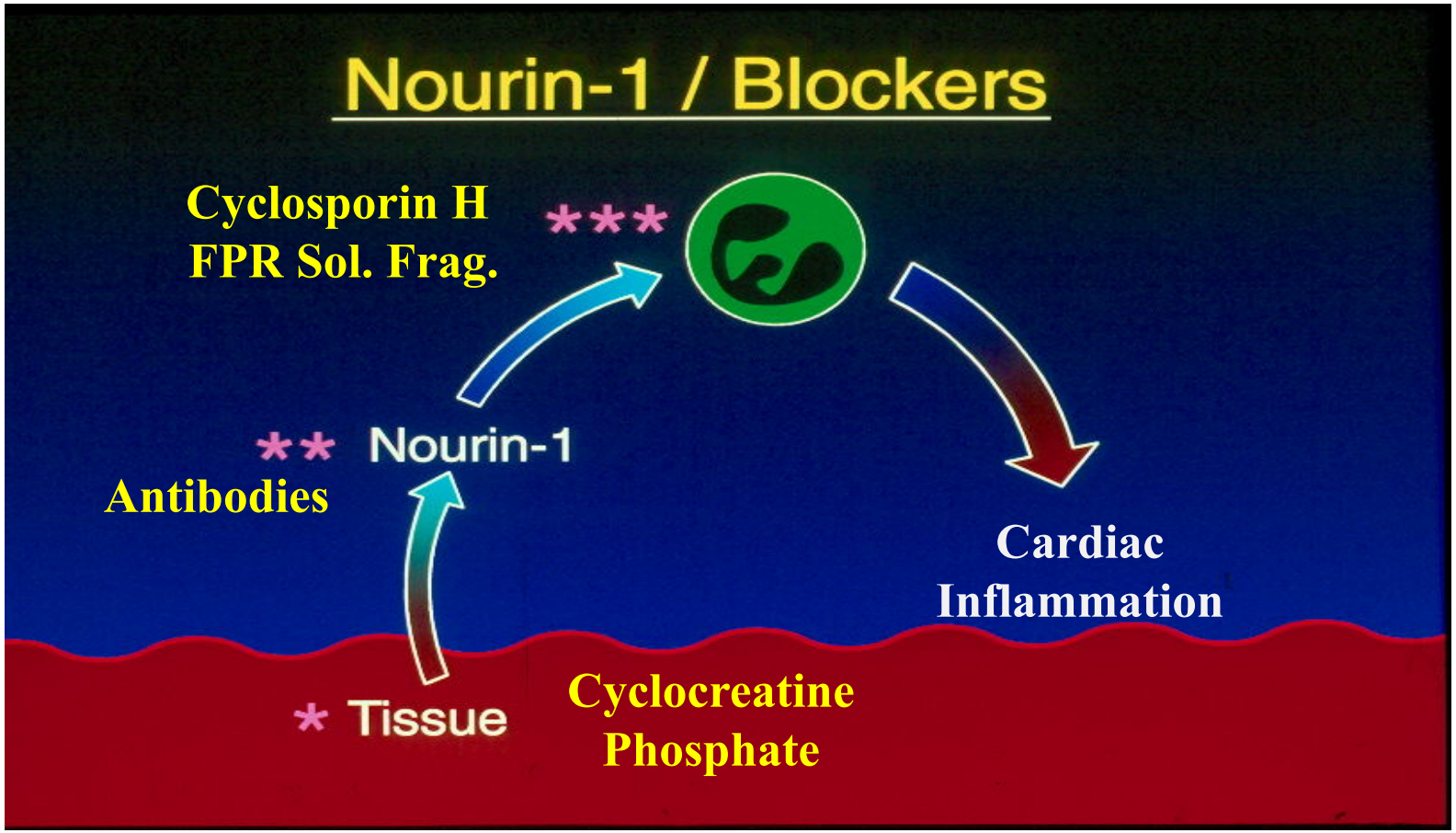
High expression level of miR-137 was detected in STEMI, followed by Unstable Angina (UA) patients. Healthy subjects showed baseline low level of miR-137 expression



High expression level of miR-106b was detected in STEMI, followed by Unstable Angina (UA) patients. Healthy subjects showed baseline low level of miR-106b expression

Nourin Antagonists

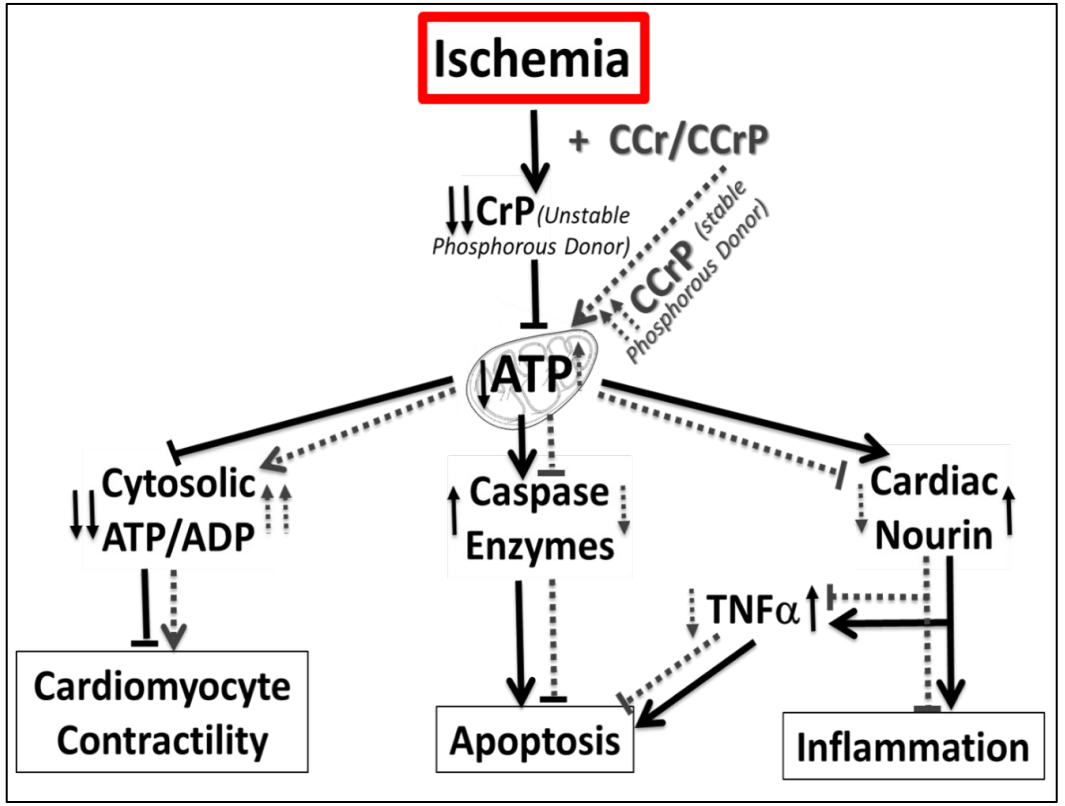
Specifically “Cyclocreatine Phosphate”!



What is Cyclocreatine?

A Novel "Bioenergetic" Compound!

- **Demand ischemia causes irreversible myocardial injury through exhaustion of cellular ATP**
- **Cyclocreatine Phosphate (CCrP) is a "bioenergetic" compound provides cellular energy ATP during ischemia**
- **Prevents myocardial ischemic injury, therefore, reduced:**
  - **Nourin intracellular formation and circulating levels**
  - **Post-ischemic cardiac inflammation**
  - **Apoptosis**
- **Restored contractile function immediately after reperfusion in dog models of:**
  - **AMI**
  - **Cardiopulmonary bypass**
  - **Heart transplantation**



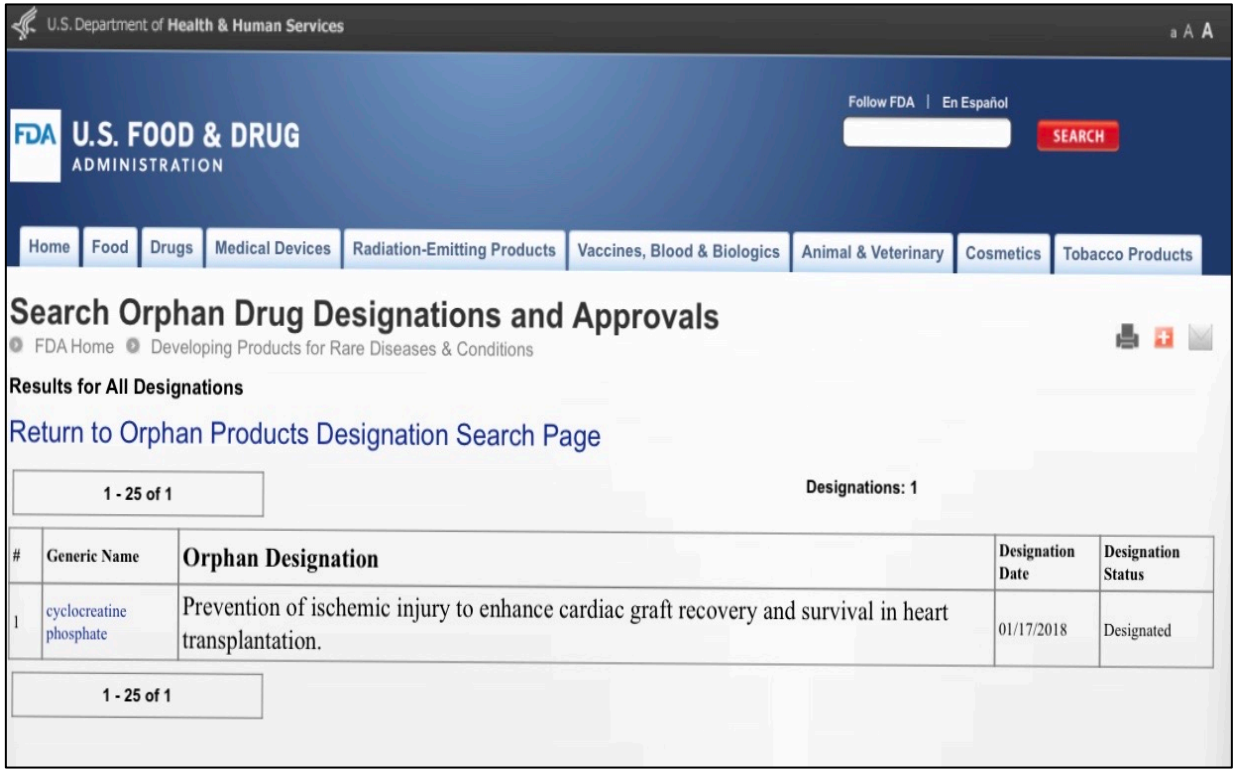
Elgebaly SA, et al. Expert Review of Cardiovascular Therapy – 2019 – REVIEW

What is Unique About Cyclocreatine?

"Prevents" Myocardial Ischemic Injury!

FDA Awarded Cyclocreatine Phosphate (CCrP) an Orphan Drug Status with Designation of:

"Prevention of Ischemic Injury to Enhance Cardiac Graft Recovery and Survival in Heart Transplantation" (DRU-2015-4951)



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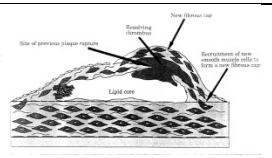
Designations: 1

#	Generic Name	Orphan Designation	Designation Date	Designation Status
1	cyclocreatine phosphate	Prevention of ischemic injury to enhance cardiac graft recovery and survival in heart transplantation.	01/17/2018	Designated

1 - 25 of 1

Pathophysiology of Myocardial Ischemia
Overview of Current Biomarkers & Nourin

AGE →

Fatty streaks →  → Plaque Accumulation

Acute Coronary Syndromes

Pathophysiology

RISK (Low → High)

Plaque Rupture

amyloid A protein
C-reactive protein

Intracoronary Thrombosis

soluble fibrin
P-selectin

Reduced Blood Flow

perfusion imaging (MIBI)
angiography

Myocardial Ischemia

electrocardiogram
echo / MIBI with gating

Myocardial Necrosis

markers of necrosis

Asymptomatic

Unstable Angina

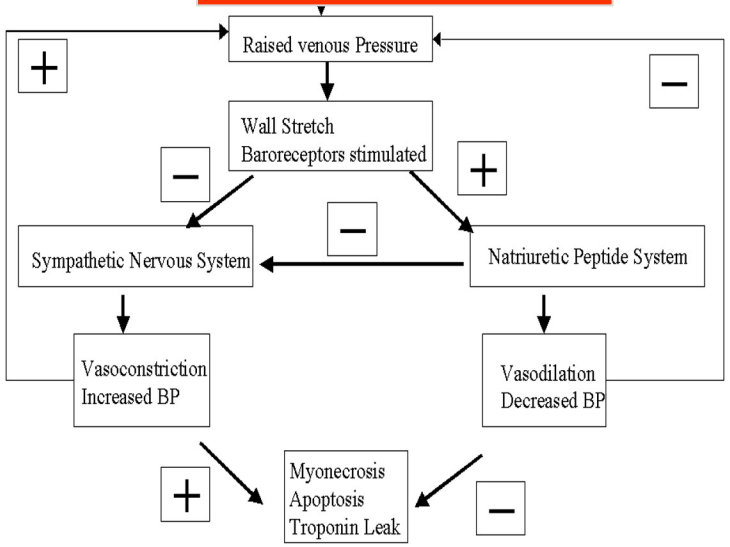
Myocardial Infarction

Nourin

Troponin I & T

Clinical Diagnosis

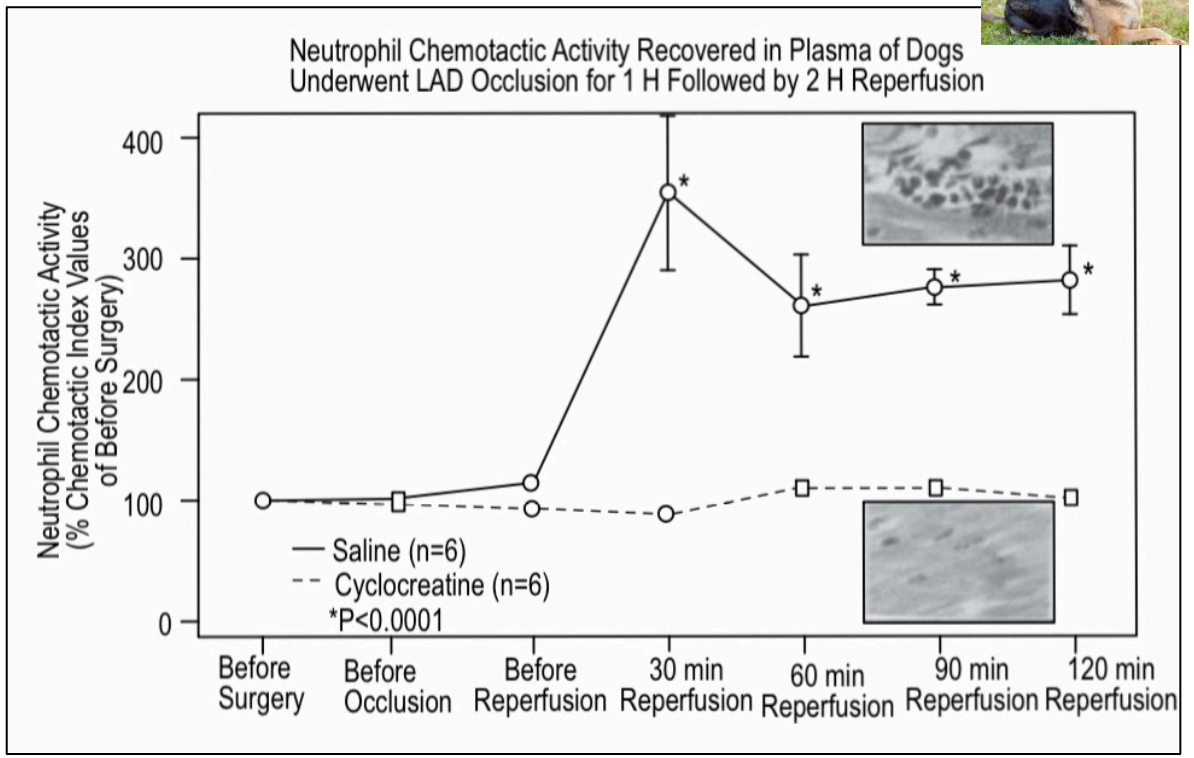
Heart Failure



Rationale

Nourin Levels in Heart Failure

- **Nourin protein participates in early pathogenesis of cardiac inflammation in experimental AMI (figure)**
- **CCr inhibited Nourin & cardiac inflammation (figure)**
- **miR-137 and miR-106b, as a marker of cell damage and inflammation, regulate Nourin**
- **Nourin-associated miR-137 and miR-106b are highly expressed in AMI patients, and not healthy**
- **Questions:**
  1. **Whether Nourin participates in subsequent chronic inflammation associated with remodeling in heart failure (HF)?**
  2. **Whether CCrP reduces Nourin miRNAs?**



Elgebaly SA, et al, J Pharmacol Exp Therap 266(3):1670-1677, 1993
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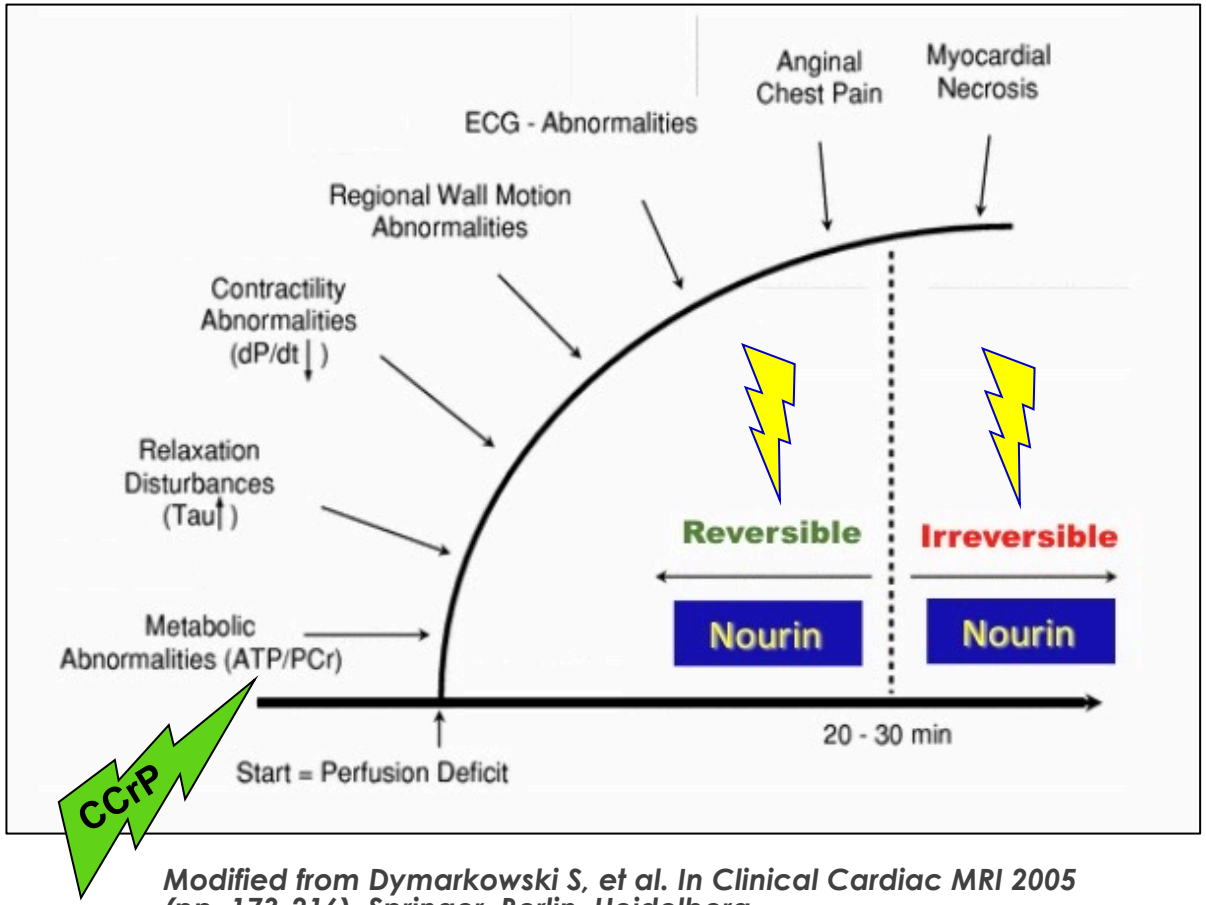
Cyclocreatine and Nourin

Cyclocreatine Phosphate Preserves Myocardial ATP During Ischemia Resulting in Reduction of Serum Nourin Level and Cardiac Inflammation.

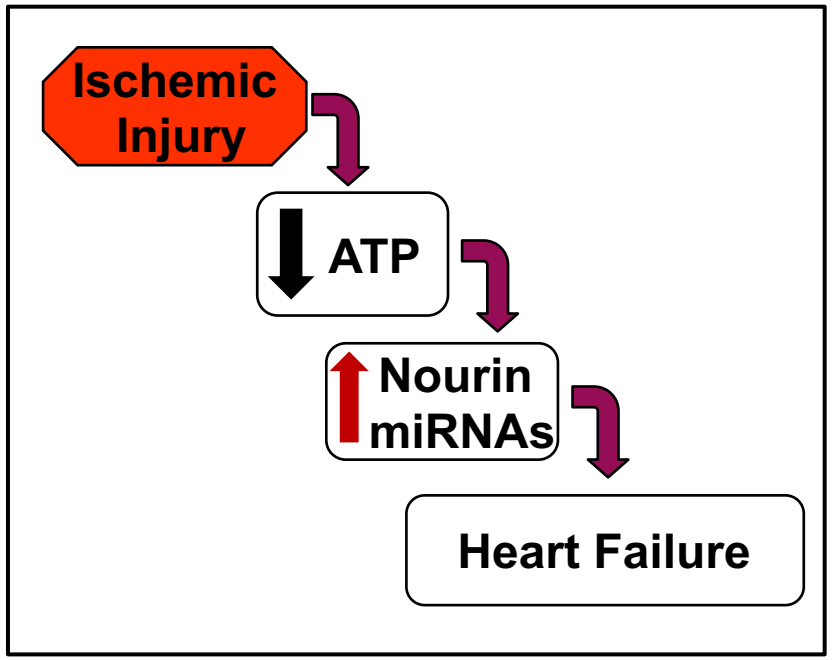


Modified from Dymarkowski S, et al. In Clinical Cardiac MRI 2005 (pp. 173-216). Springer, Berlin, Heidelberg.

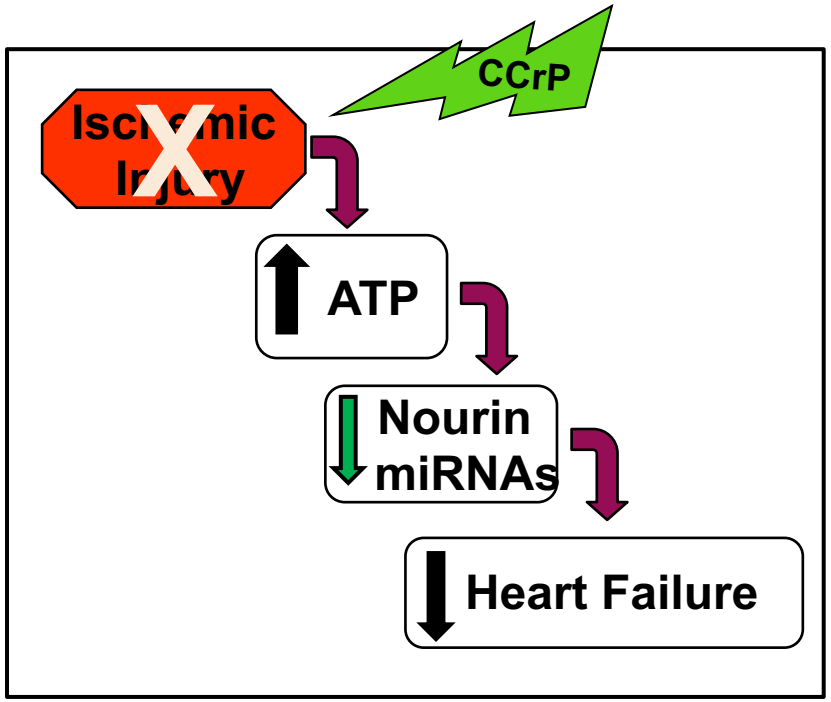
Hypothesis

Role of Nourin miRNAs in Heart Failure

1. ***Nourin-associated miRNAs (markers of cell damage and inflammation) will continue to be highly expressed in ischemia-induced experimental HF.***



2. ***CCrP administration will prevent ischemic injury and inhibit serum expression level of Nourin-associated miRNAs in HF.***



Experimental Design

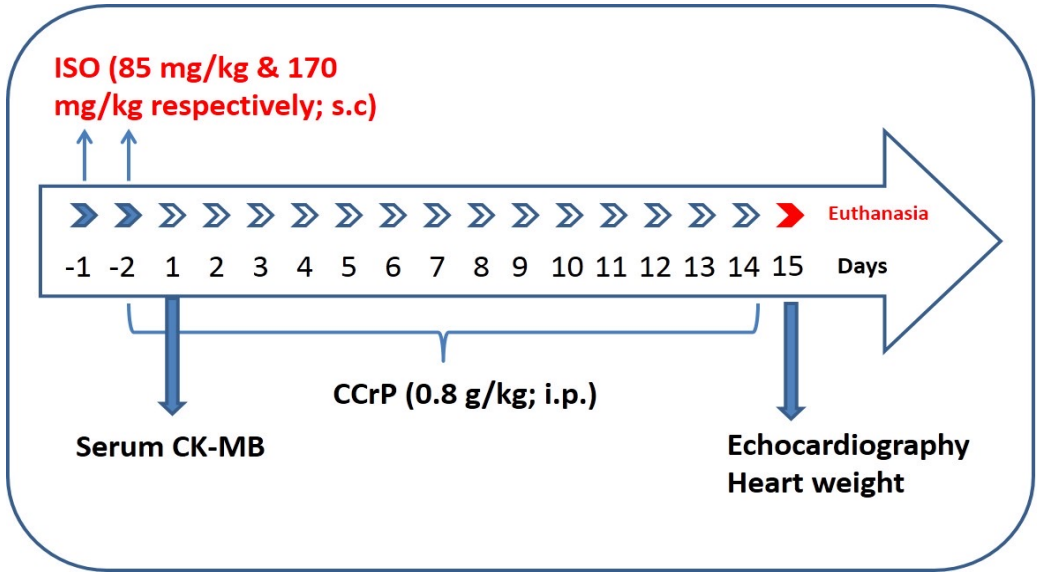
Rat Model of Heart Failure

Isoproterenol (ISO) is a beta-adrenergic agonist which in high doses cause pathologic and molecular changes in rat heart that are similar to myocardial injury in humans. ISO produces subendocardial ischemia and cellular ATP depletion.

25 male Wistar rats (180-220 g)

GROUPS:

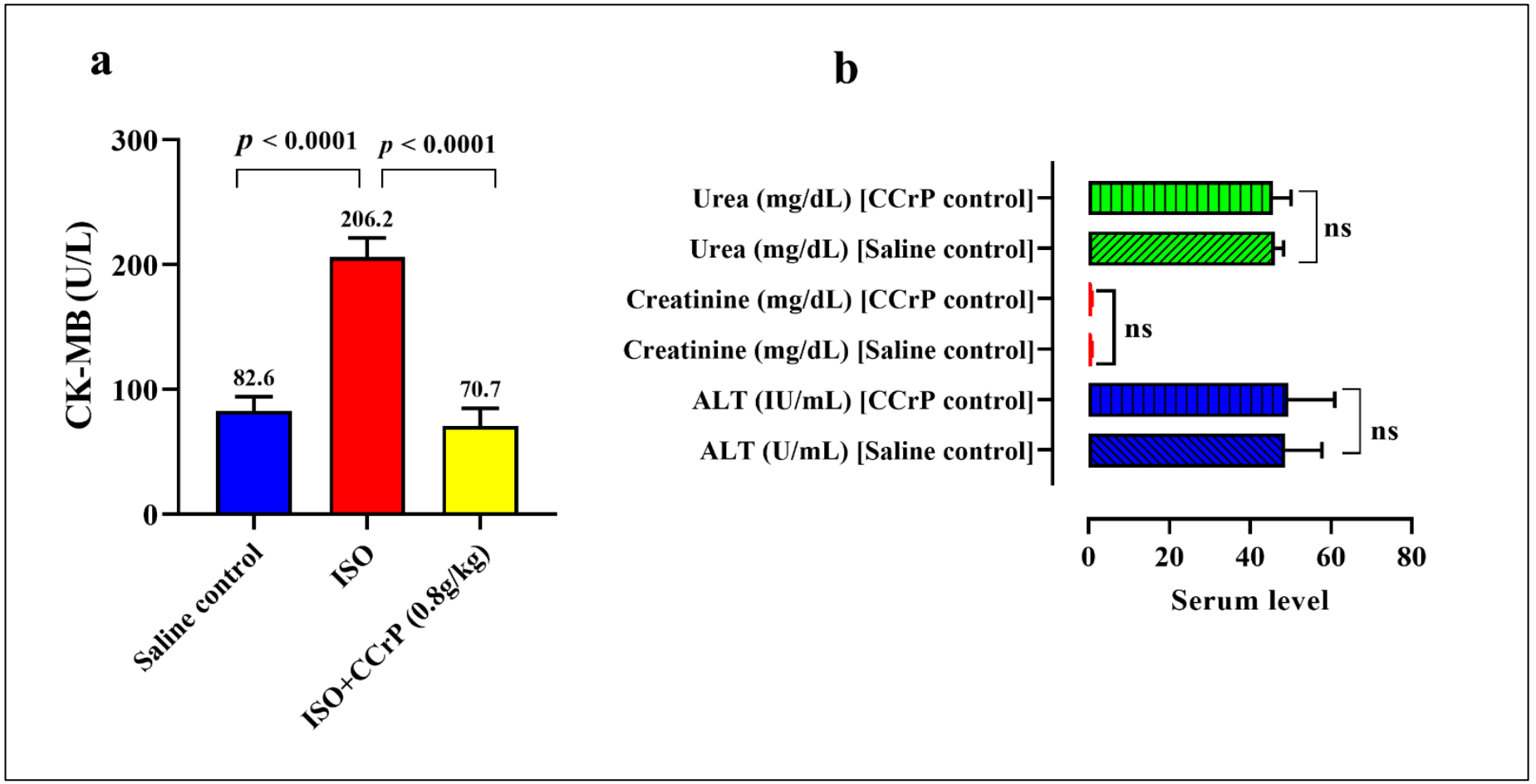
1. **ISO/Saline (n=6)**
2. **ISO/CCrP 0.8 gm/kg/day (n=5)** (effective dose)
   ISO/CCrP 0.4 gm/kg/day (n=3) (limited study)
   ISO/CCrP 1.2 gm/kg/day (n=2) (limited study)
3. **Control/Saline (n=5)**
4. **Control/CCrP 0.8 gm/kg/day (n=4)**



After 14 days, serum expression of Nourin miRNAs were Measured by real-time PCR

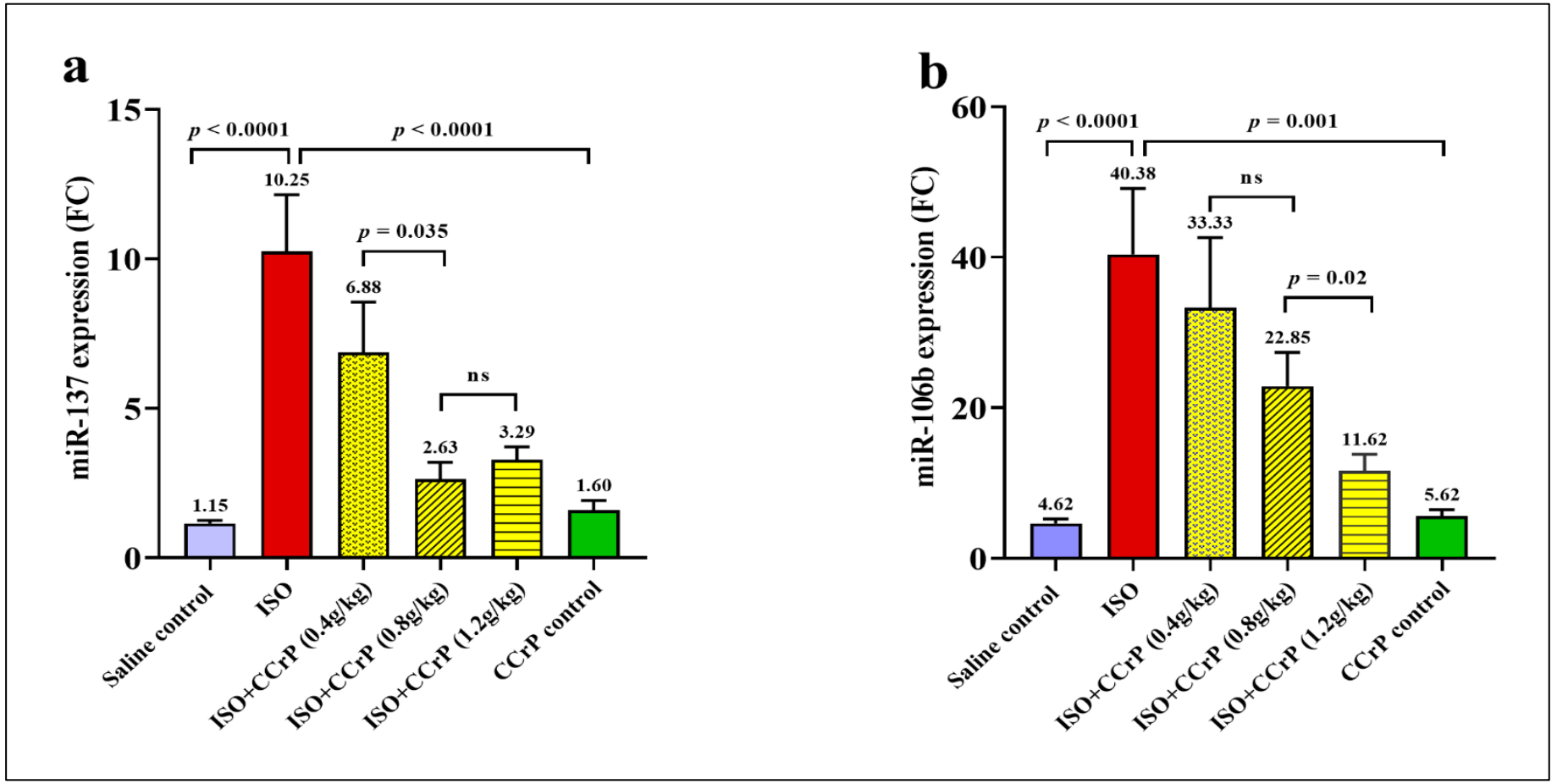
Results 1

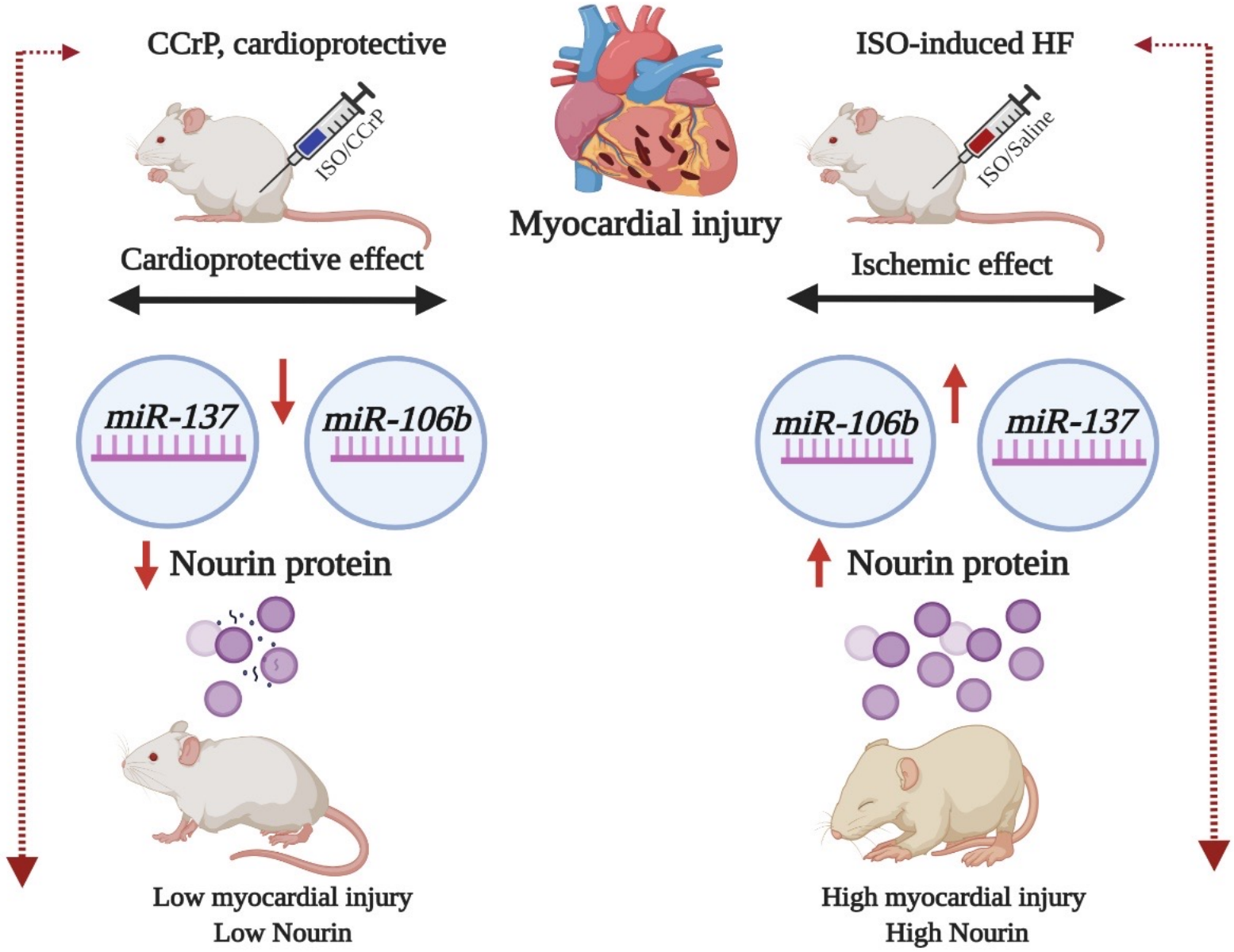
High CK-MB Level in ISO rats only after 24 hours
Lack of Toxicity by CCrP after 14 days



Results 2

High Levels of Nourin-associated miR-137 and miR-106b after 14 Days – Significant Inhibition by CCrP




Nourin-associated miRNAs in ischemic injury
CCrP, cardioprotective
ISO/CCrP
Cardioprotective effect
Myocardial injury
ISO-induced HF
ISO/Saline
Ischemic effect
miR-137
miR-106b
Nourin protein
miR-106b
miR-137
Nourin protein
Low myocardial injury
Low Nourin
High myocardial injury
High Nourin

Conclusions

Results Support Hypothesis

- Nourin-associated miR-137 and miR-106b are highly expressed in rat heart failure model
- Cyclocreatine Phosphate reduced the expression of Nourin-associated miRNAs in rat HF model
- Nourin-associated miRNAs are not expressed in healthy rats

Potential Clinical Applications

- Nourin-associated miRNAs can be used in ischemia-induced heart failure as a:
  - "Therapeutic Target" to control cardiac inflammation
  - "Diagnostic Biomarker" to monitor drug therapy response in HF
- The bioenergetic Cyclocreatine Phosphate is a promising first-in-class "cardio-protective" drug with potential application as a preventive therapy of HF due to ischemia.

Thank You.

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