Oxidative Inhibition of Erythrocyte Na^+-K^+ Pump: A Functionally Relevant Circulating Marker of Oxidative Stress

Dr. Chia-chi Liu

Molecular Cardiac Research Unit

Sydney Medical School

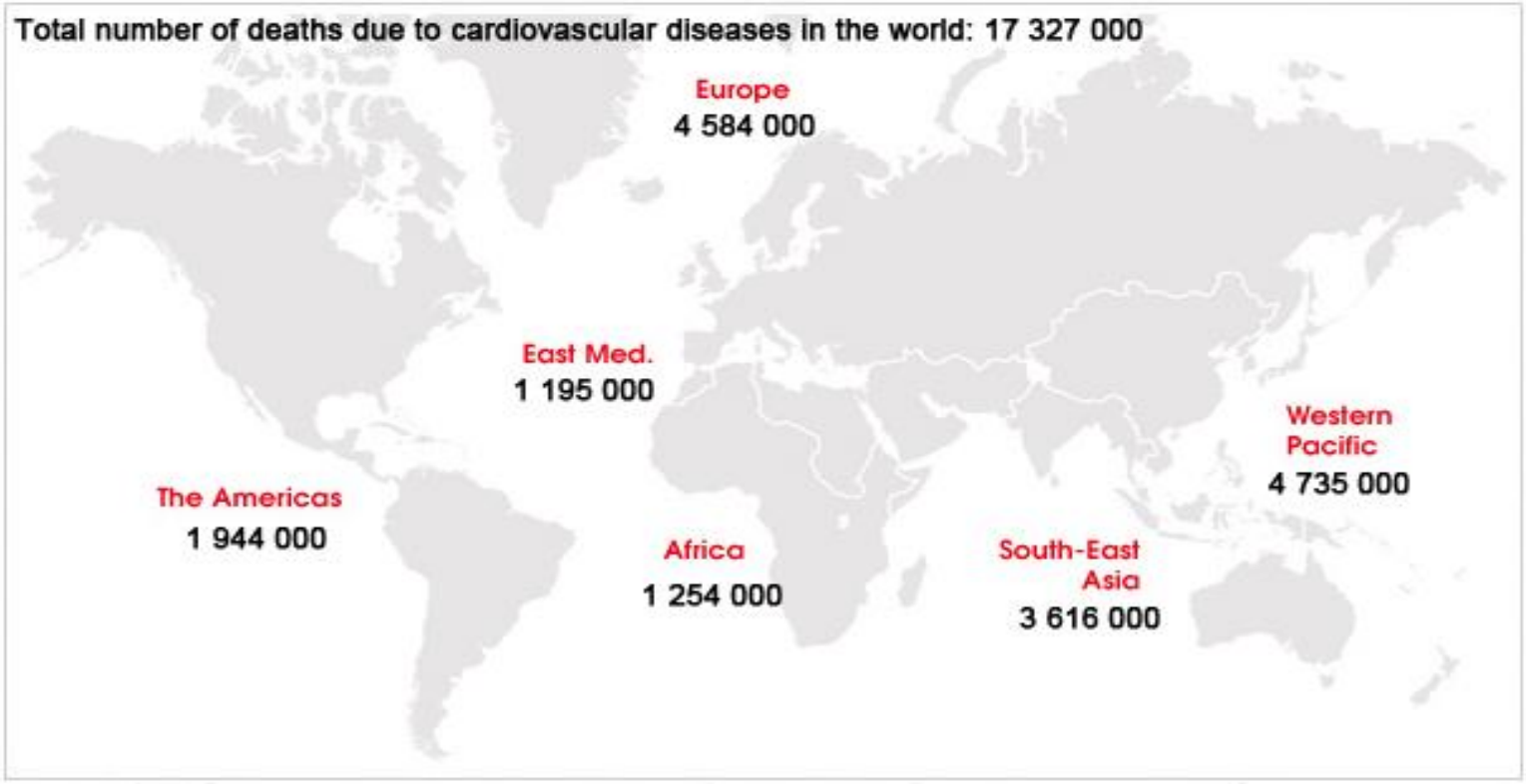
University of Sydney

Australia

Outline

- **Cardiovascular disease (CVD)**
- **CVD and Na pump**
- Background of Na pump
- Oxidative regulation of the pump
- Measurement of oxidative damage of Na pump- a potential biomarker

Cardiovascular disease (CVD)



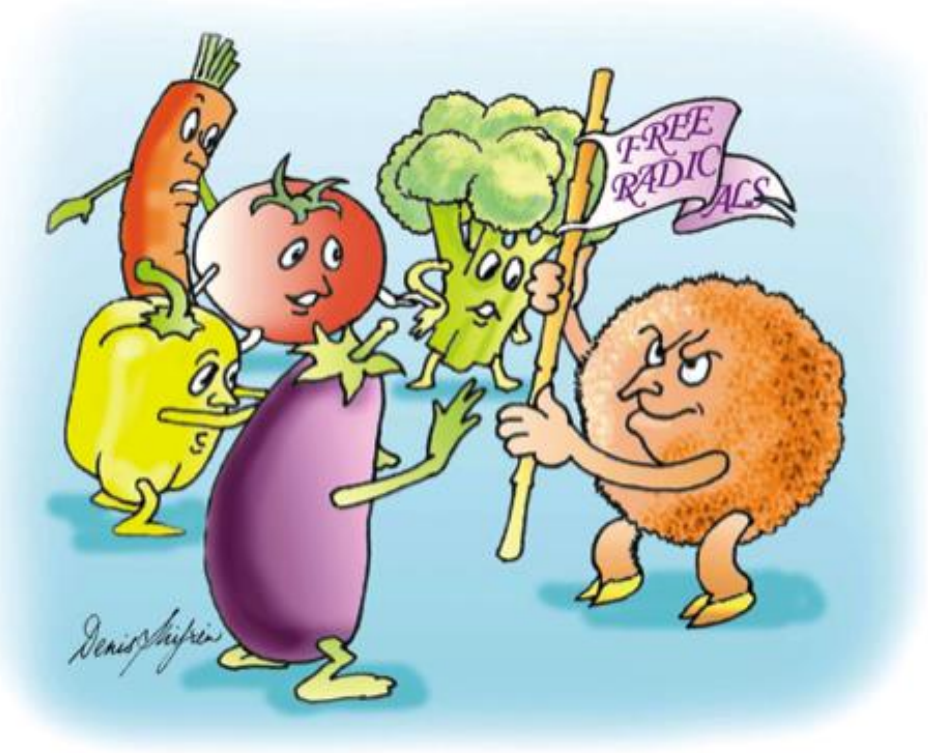
- the number 1 cause of death globally
- accounting for 17.3 million deaths per year
- a number that is expected to grow to >23.6 million by 2030

http://www.who.int/cardiovascular_diseases/en/

Overview

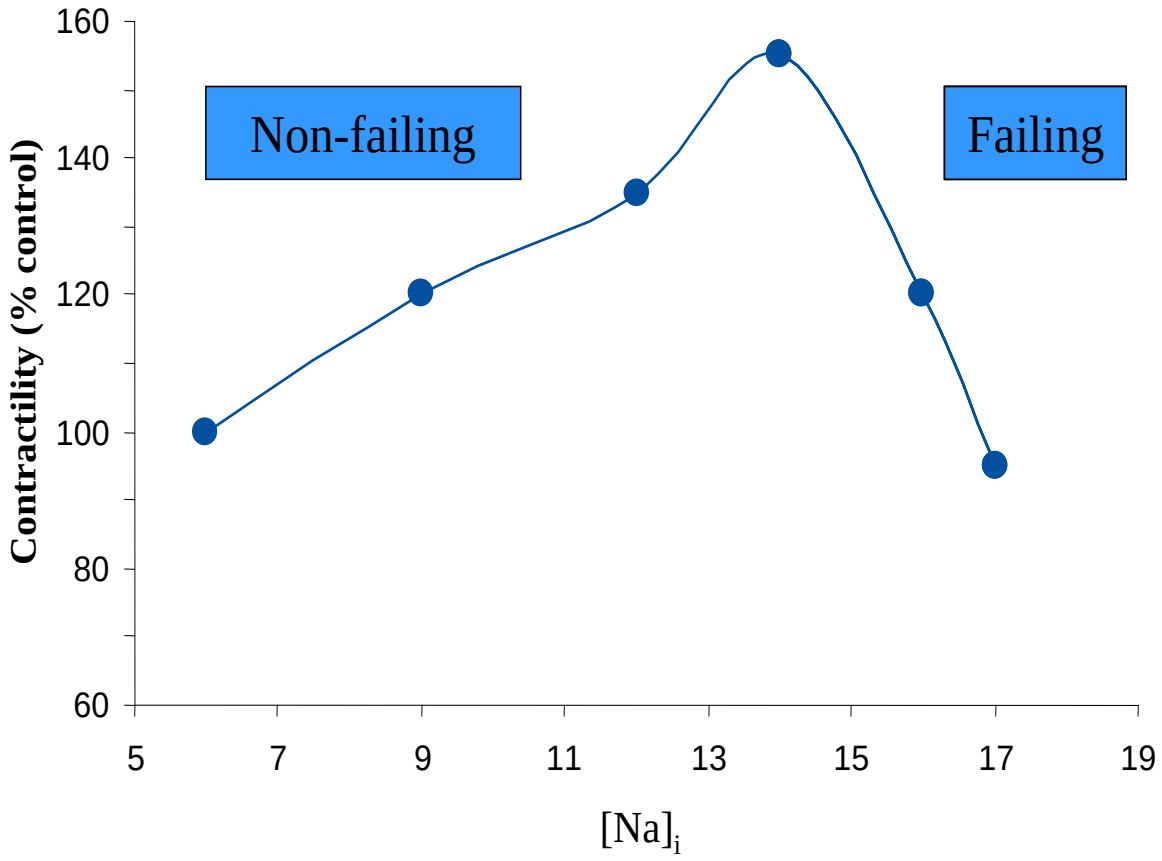
- Reactive oxygen species: key feature of cardiovascular disease

scular



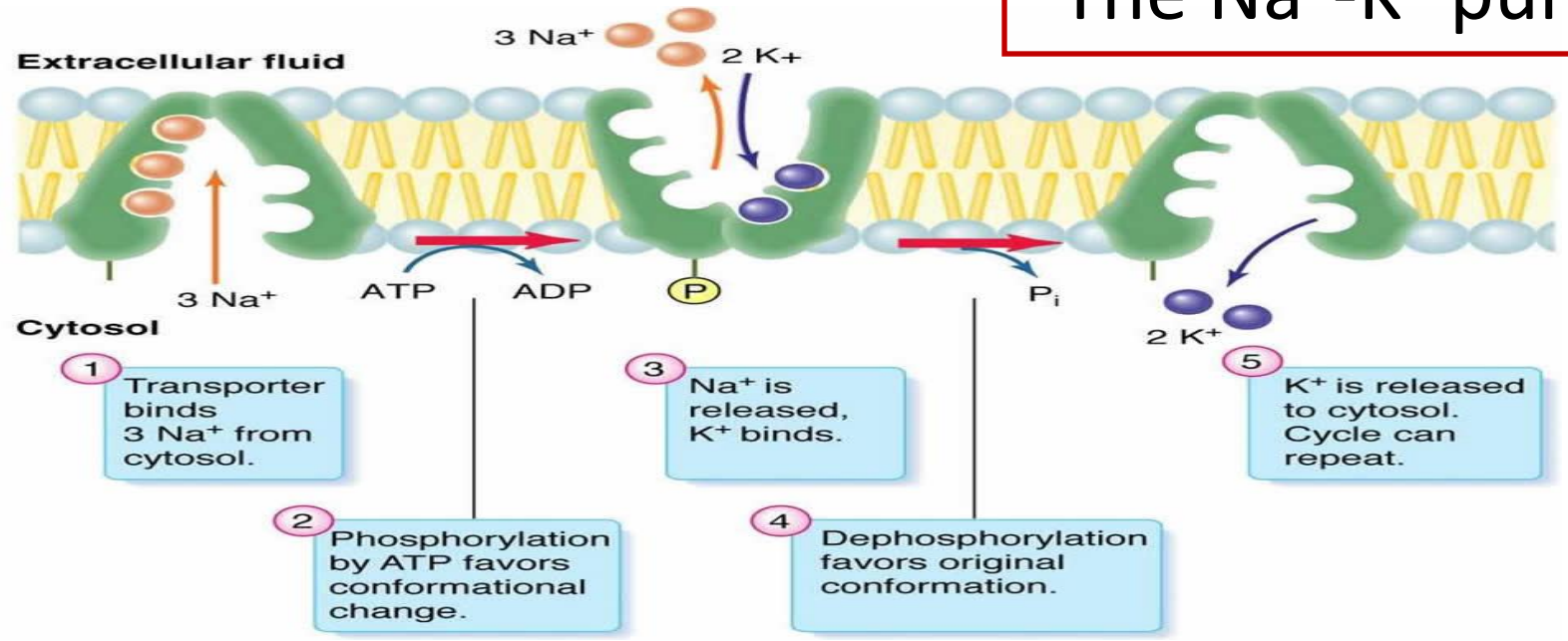
Myung et al. BMJ 2013;346:f10

Raised levels of cardiac myocyte Na^+



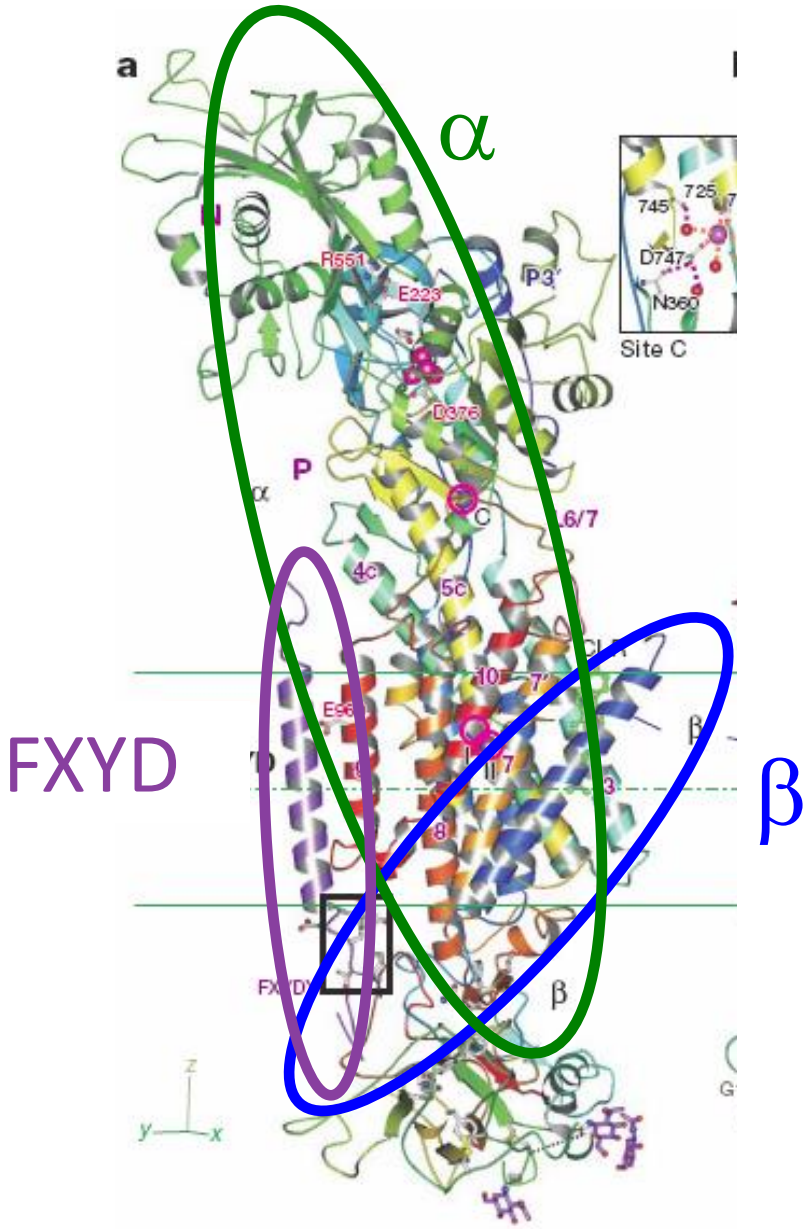
DM Bers. *Cardiac E-C Coupling*. 2001

The Na^+-K^+ pump



- Plasma membrane ion transporter
- Transports $3Na^+$ out- and $2K^+$ into cells
- Uses ~20% of all energy (ATP) in body
- Crucial for normal function and survival of all cells, especially for cardiac myocytes

3D structure of the Na^+-K^+ pump



Nature 459:446-50, 2009

Na^+-K^+ pump Regulation

- Kinases mediate Na^+-K^+ pump regulation

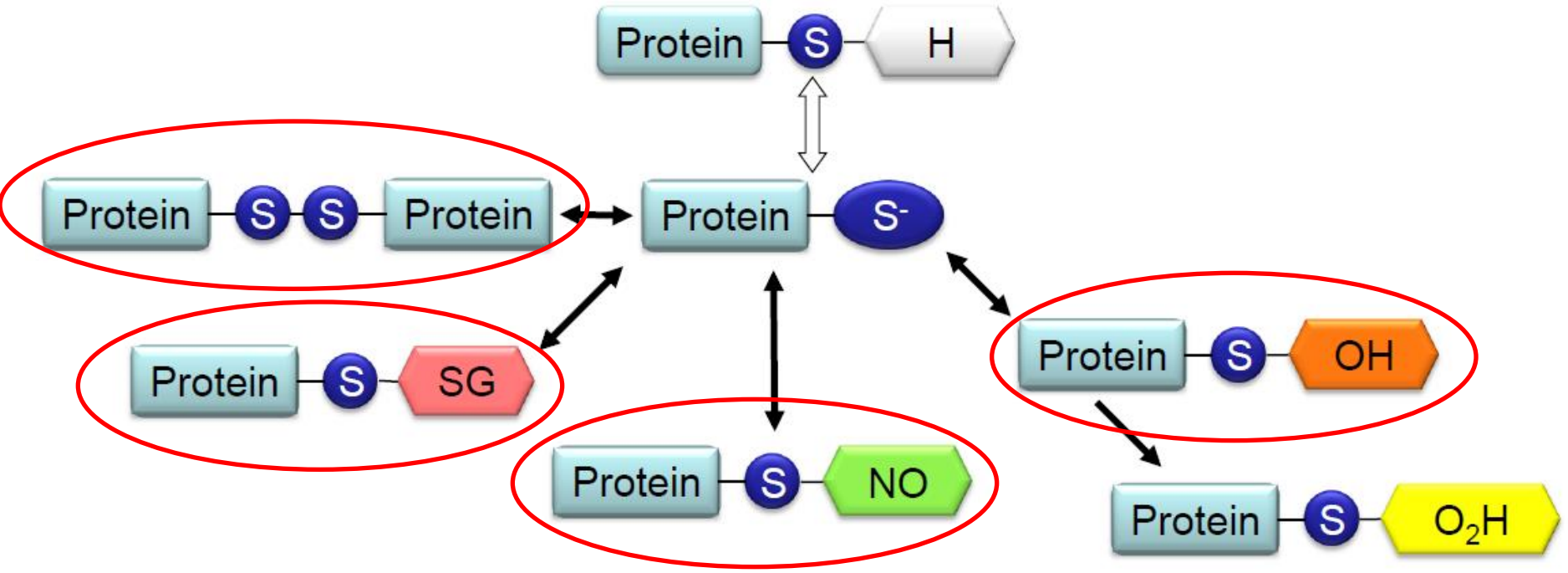
  - *however,* kinases have poor access to phosphorylation sites on the pump molecule

  -Bibert S & Geering K. J Biol Chem 2008
  -Sweadner & Feschenko. Am J Physiol Cell Physiol 2001
  -Cornelius, et al. J Bioenerg Biomembr 2001

- Chemical oxidants decrease Na^+-K^+ pump activity

  -Ellis DZ, Rabe J, Sweadner KJ. J Neurosci 2003
  -White CN et al. Am J Physiol Cell Physiol 2008

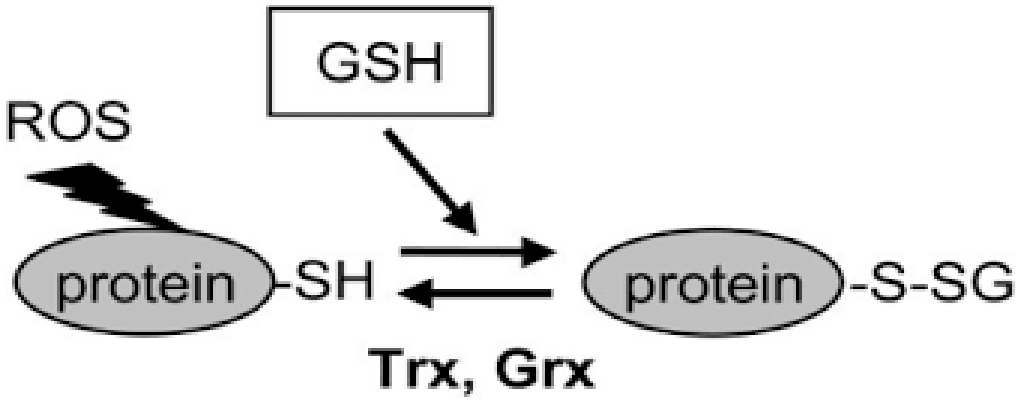
Oxidative protein modifications



Trends in Cardiovascular Medicine. 20(3):85-90, 2010

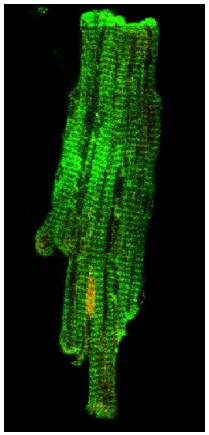
Protein Glutathionylation

- Protein glutathionylation -

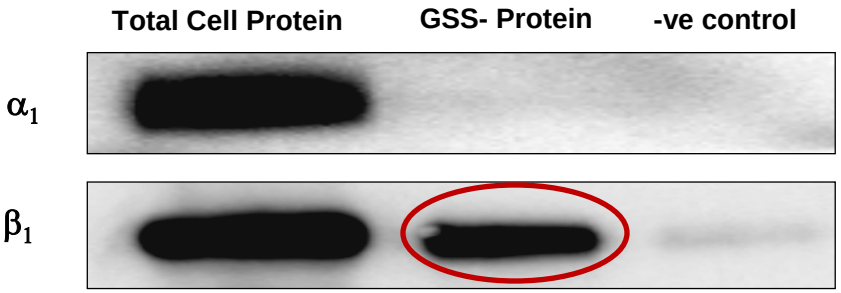


  - adduct with –ve charge
  - is stable but reversible

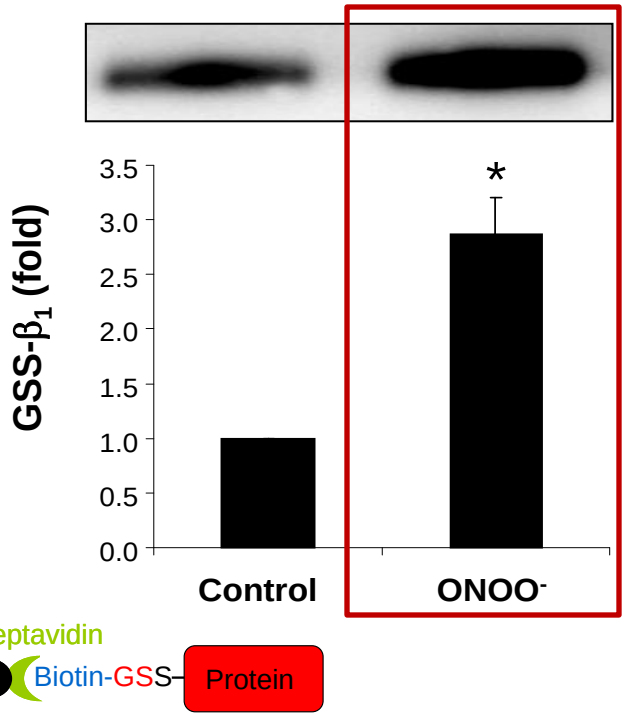
Liu, C.C. Circ Res 105, 693-700; 2009

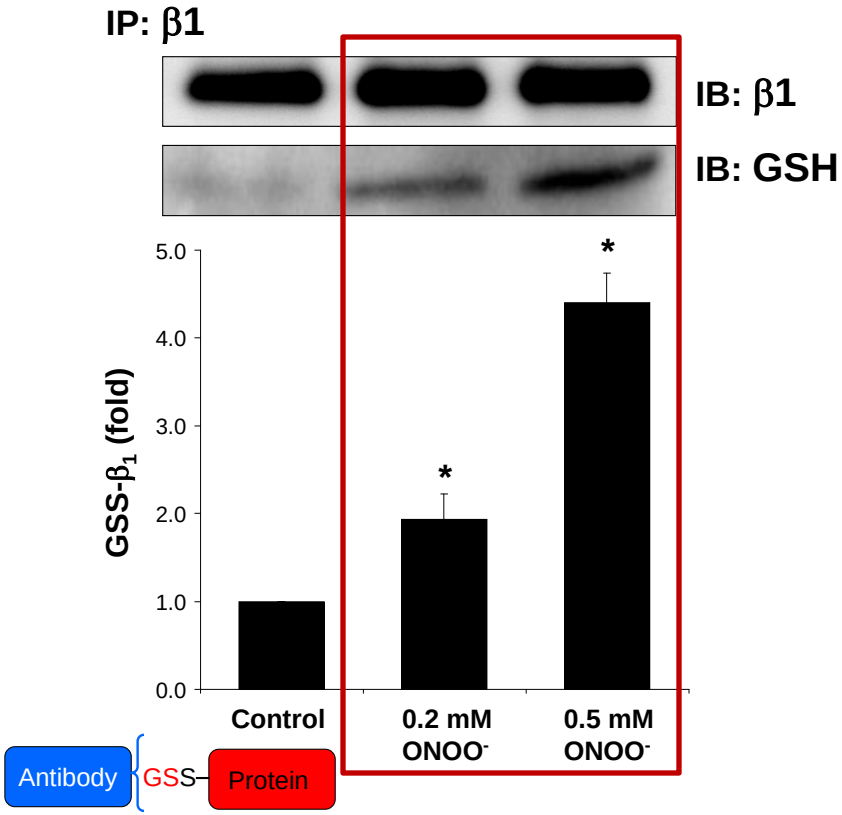


Glutathionylation of Na^+-K^+ pump



Baseline



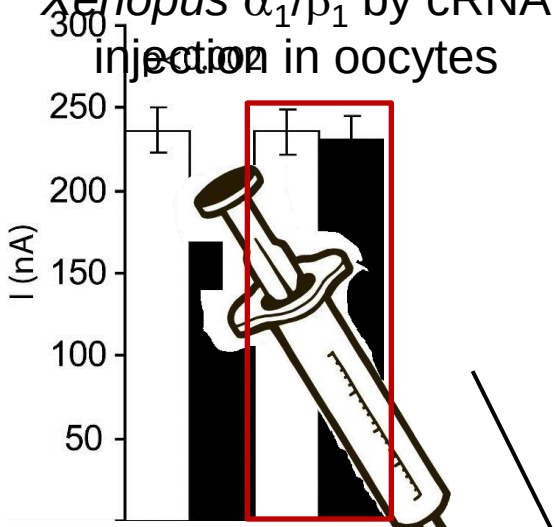


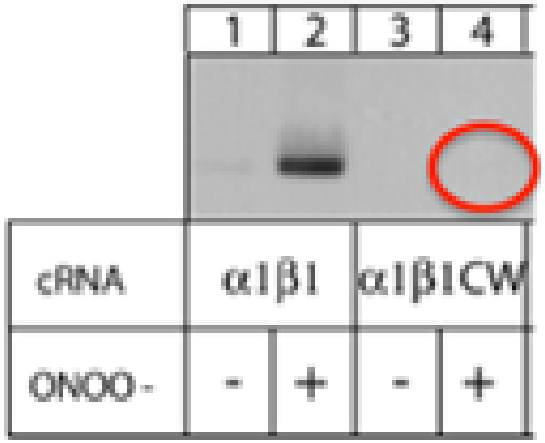
Liu, C.C. Circ Res 105, 693-700; 2009

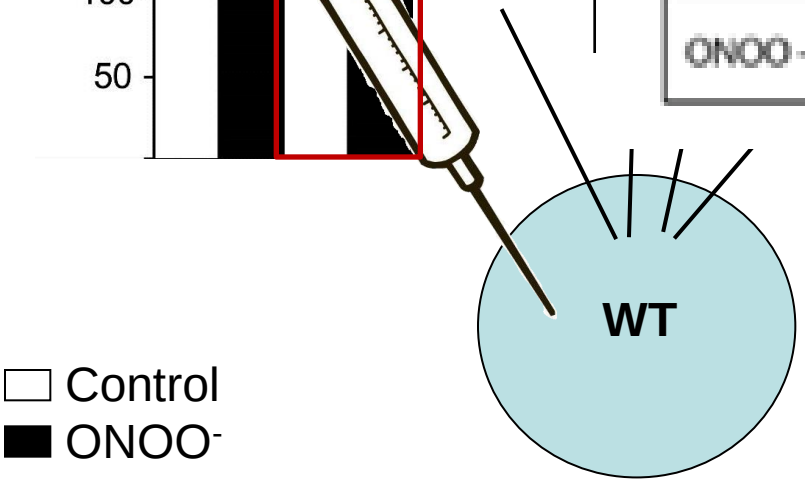
Mutation of Cys45 in β_1 subunit

Cys→Trp mutation eliminates ONOO⁻-induced potentiation

Overexpression of *Xenopus* α_1/β_1 by cRNA injection in oocytes







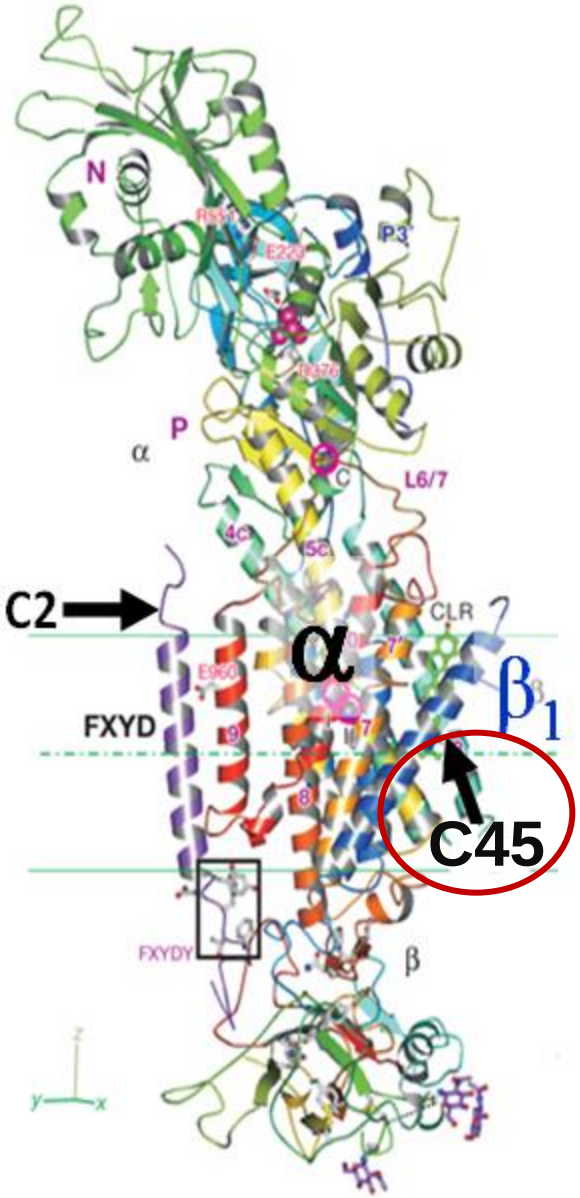
WT

vs.

Cys45→Trp mutation

Liu, C.C. Circ Res 105, 693-700; 2009

Only Cys 45 in β_1 subunit



Circulation Research
Journal of the American Heart Association

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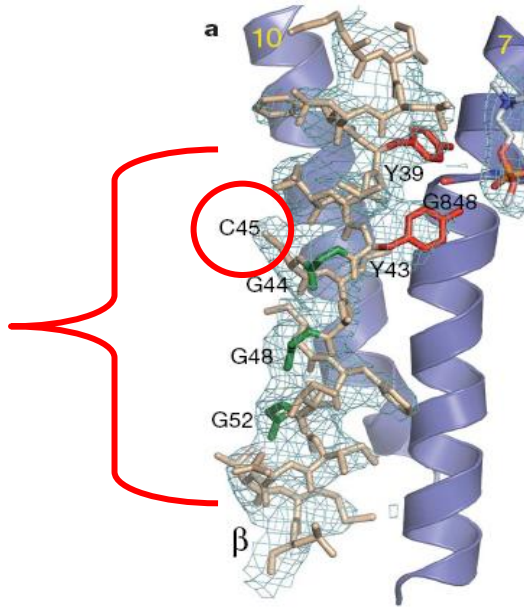
Reversible Oxidative Modification. A Key Mechanism of Na+-K+ Pump Regulation

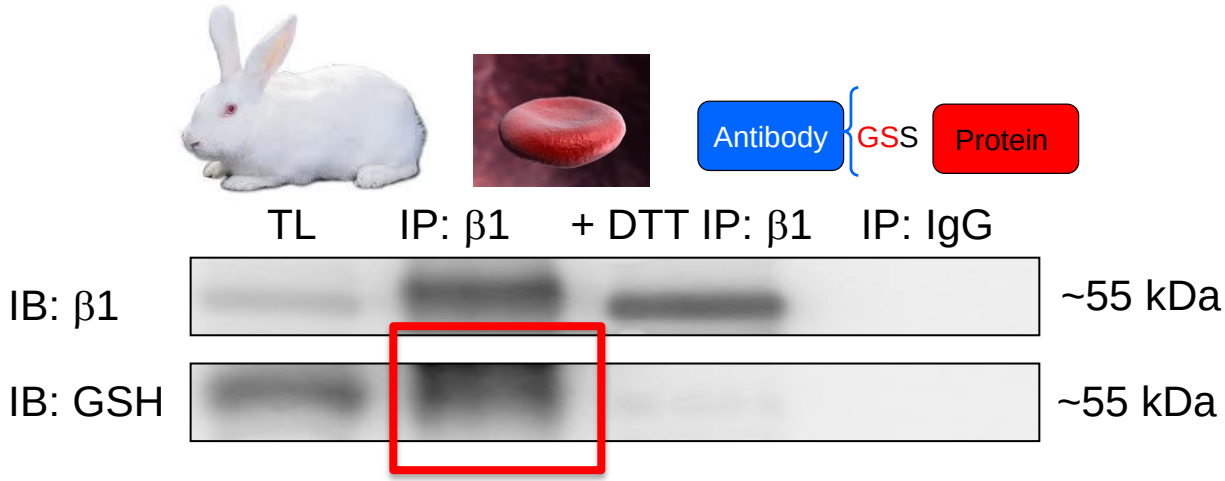
Gemma A. Figtree, Chia-Chi Liu, Stephanie Bibert, Elisha J. Hamilton, Alvaro Garcia, Caroline N. White, Karin K.M. Chia, Flemming Cornelius, Kaethi Geering and Helge H. Rasmussen

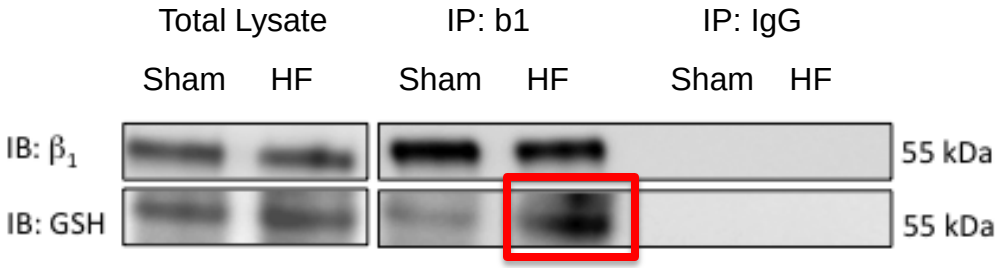
Circ. Res. published online Jun 18, 2009;

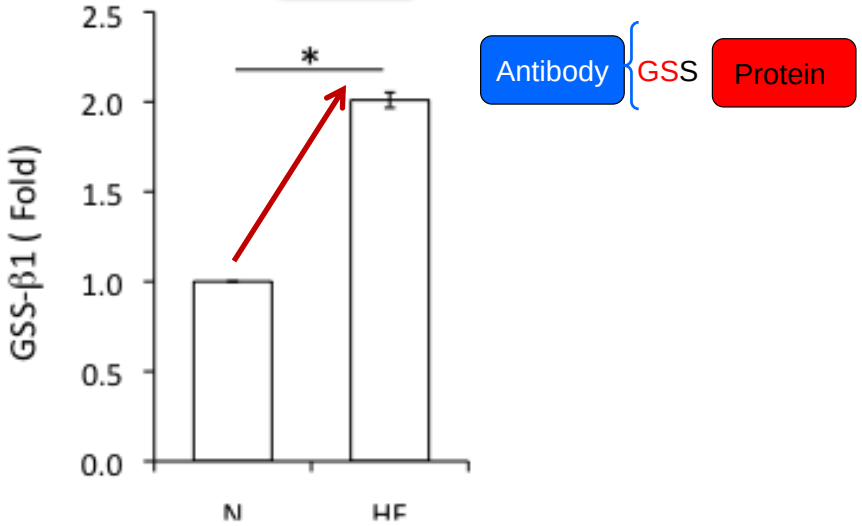


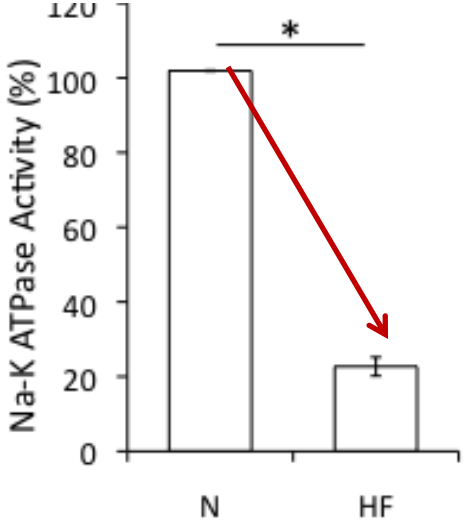
Would knowing the degree of oxidative inhibition of the Na^+-K^+ pump in erythrocytes help to monitor the heart disease progress?

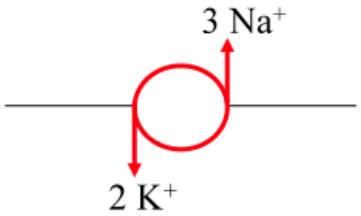
β1 subunit is detectable in erythrocytes, and is glutathionylated







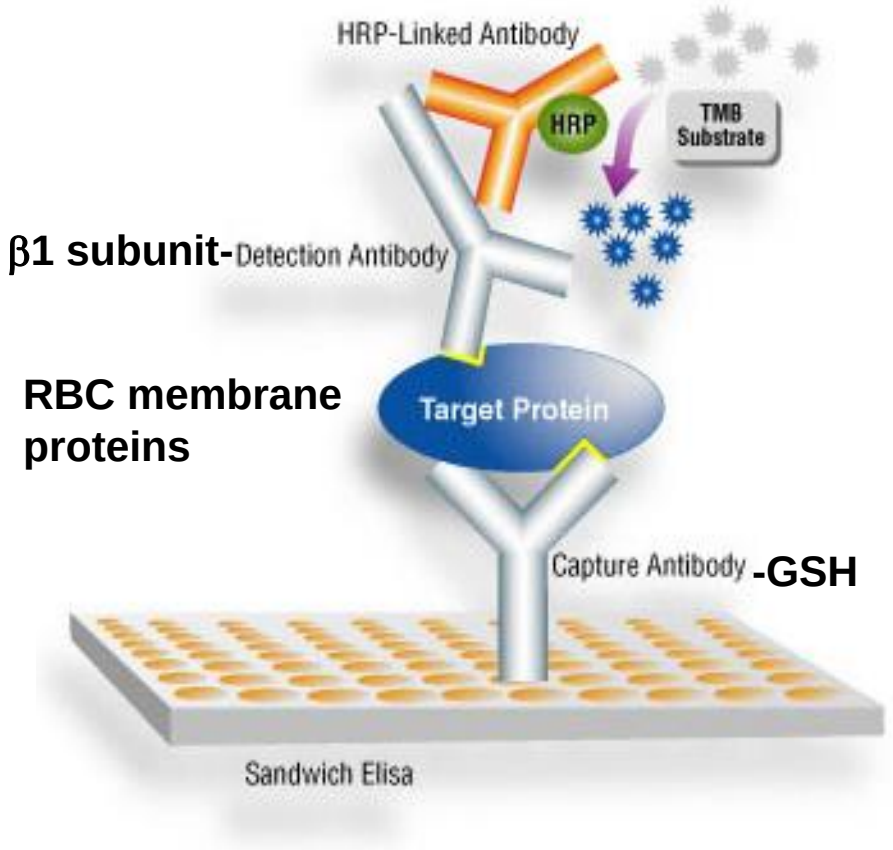




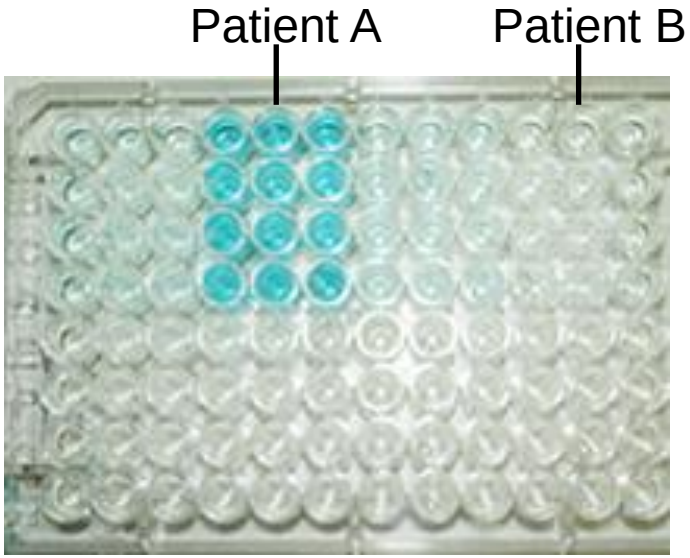
Natasha Fry PhD in progress; Unpublished, 2014

Enzyme Linked Immunosorbent Assay - to quantify eβ1-GSS

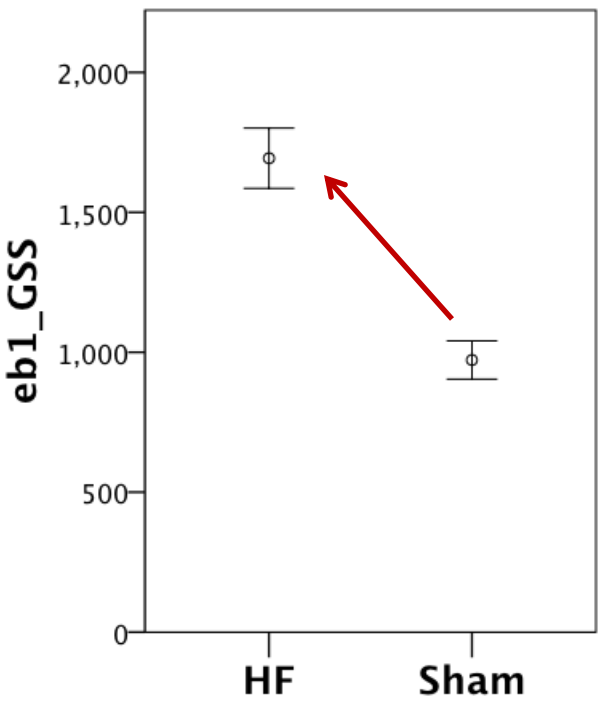
A. Method

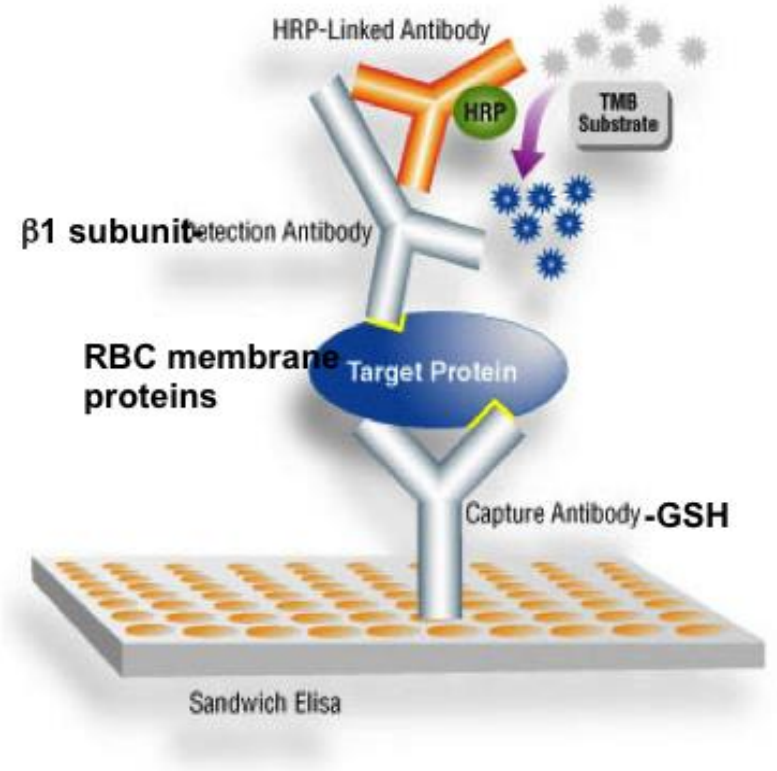


B. Plate assay

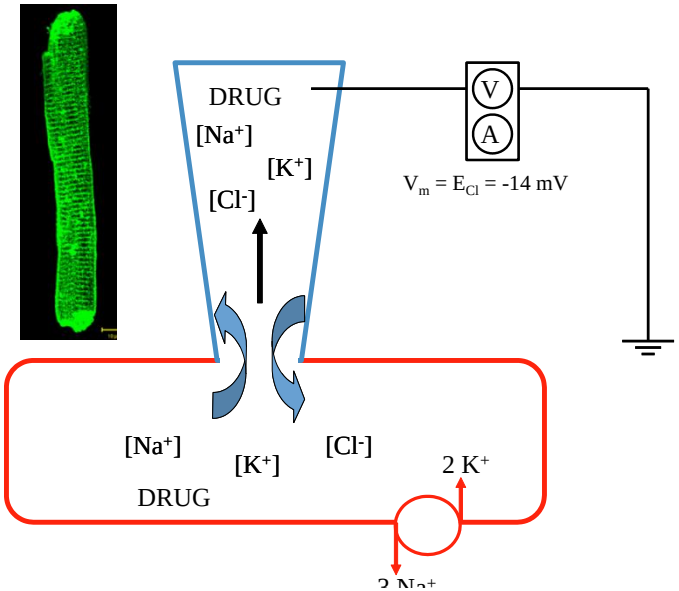


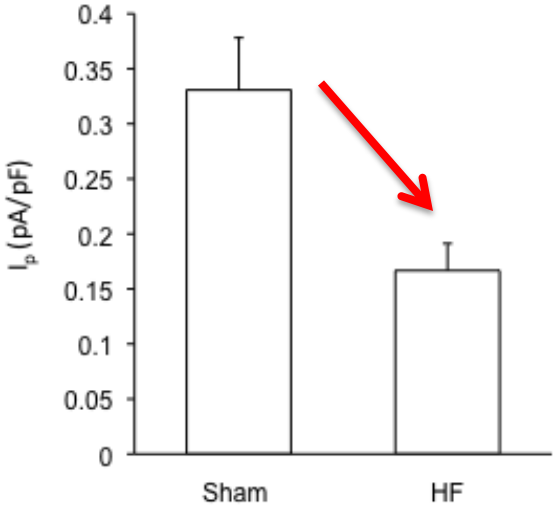
eβ1-GSS in HF vs sham rabbits



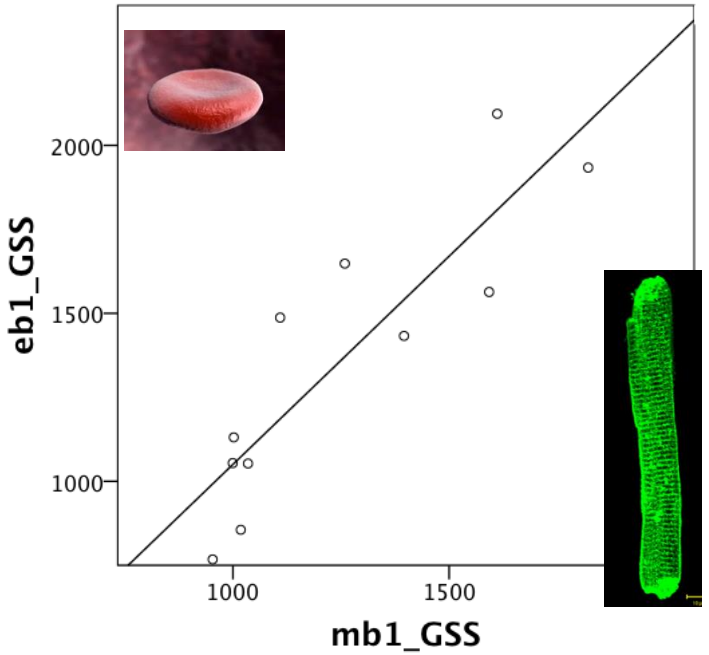


How does eβ1-GSS relate to what's happening in the heart?





Correlation of eβ1-GSS with β1 subunit glutathionylation in cardiac myocytes

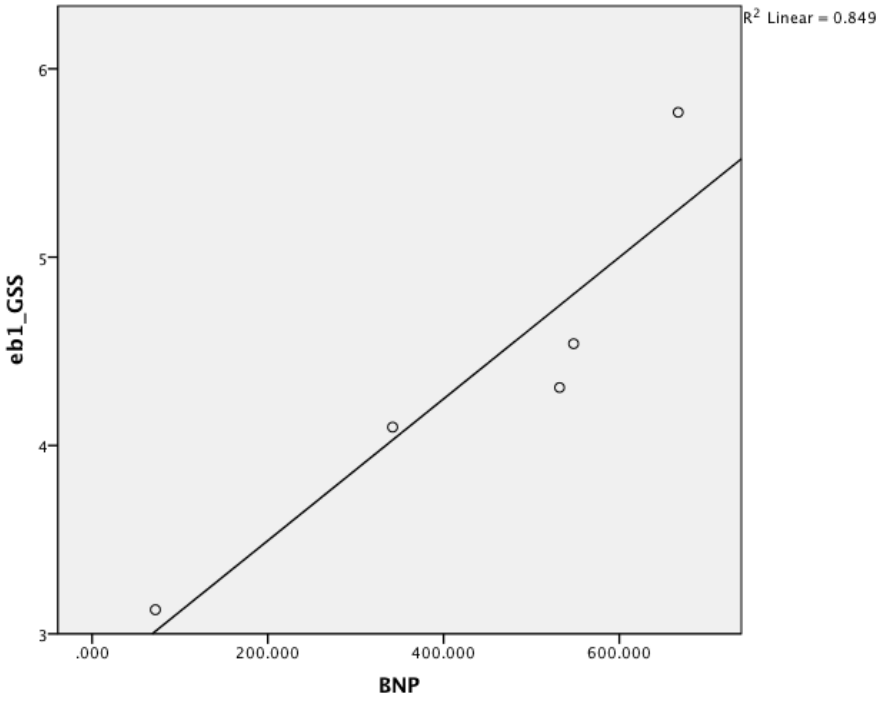


r=0.851; p<0.001

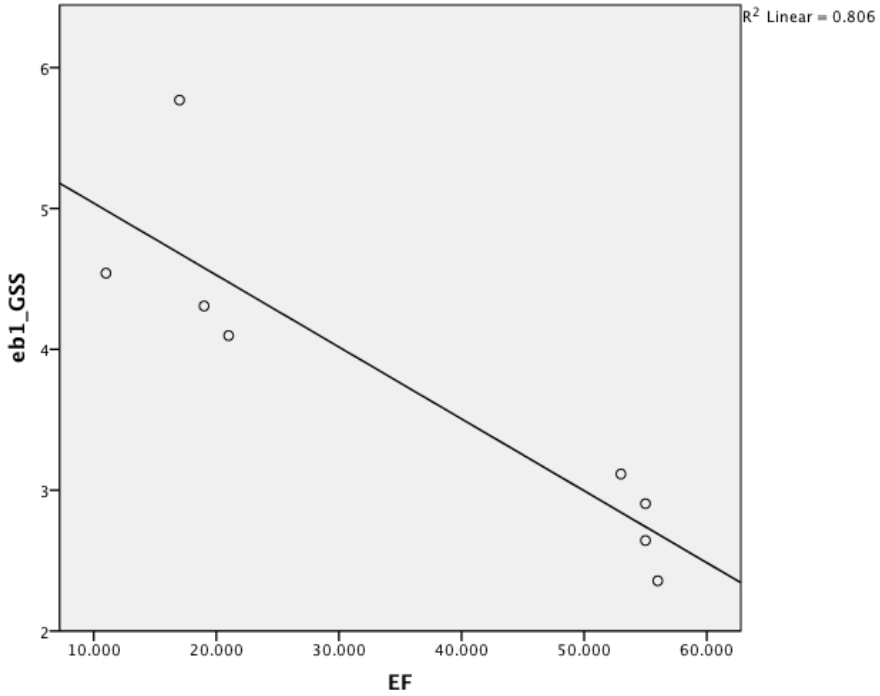
Liu, Unpublished, 2014

How does eβ1-GSS relate to what's happening in the heart?

B-type Natriuretic Peptide (BNP)



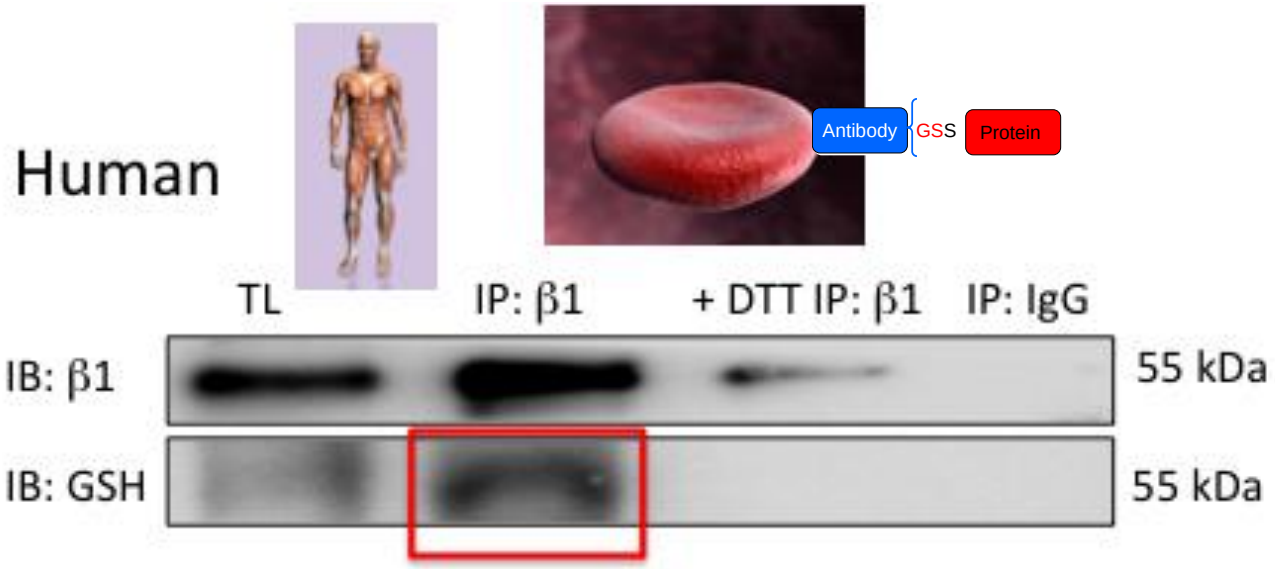
LV systolic function



Natasha Fry PhD in progress; Unpublished, 2014

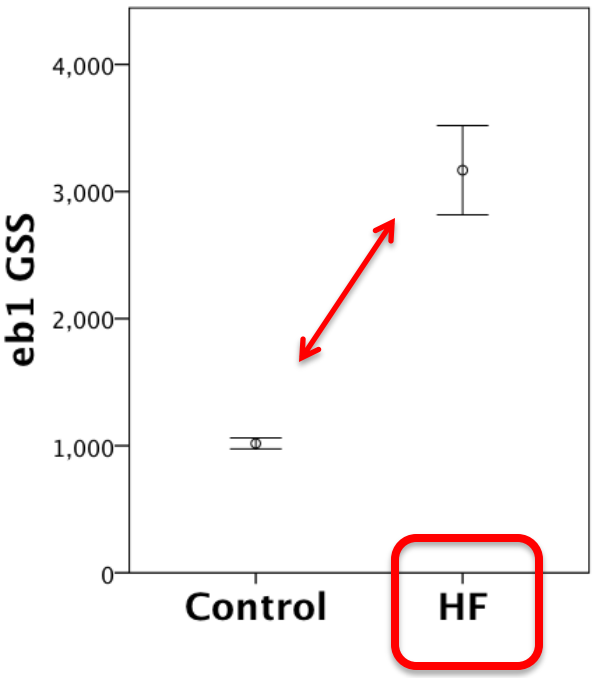
What about in humans?

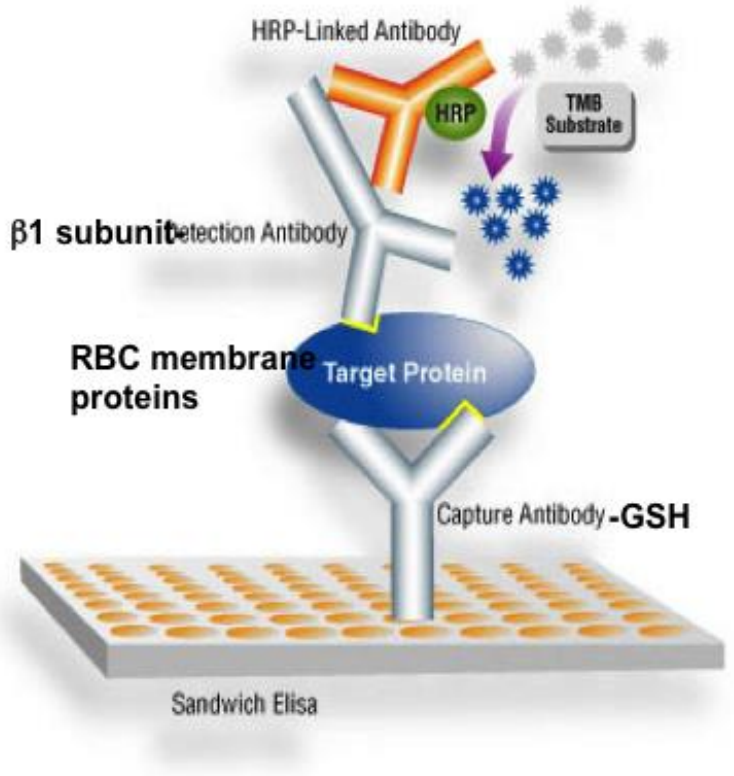
Detection of eβ1-GSS in humans



What about in humans?.....eβ1-GSS

eβ1-GSS HF patients vs. control

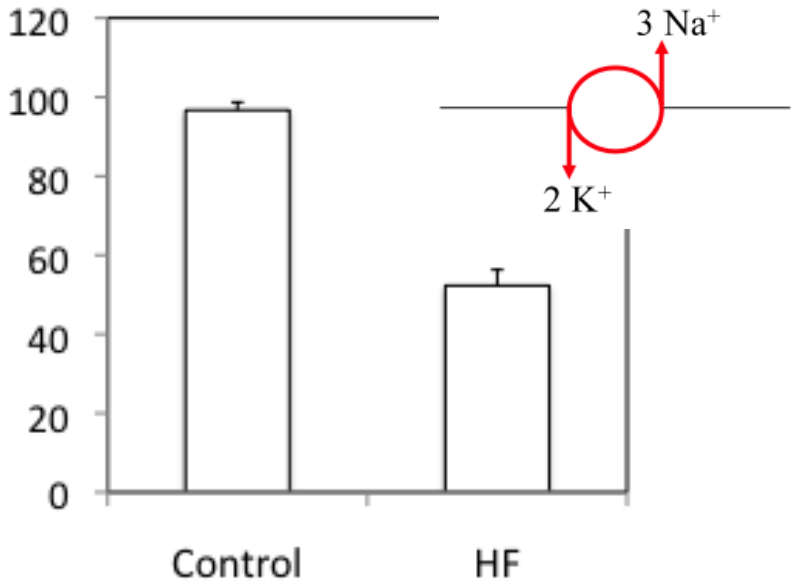




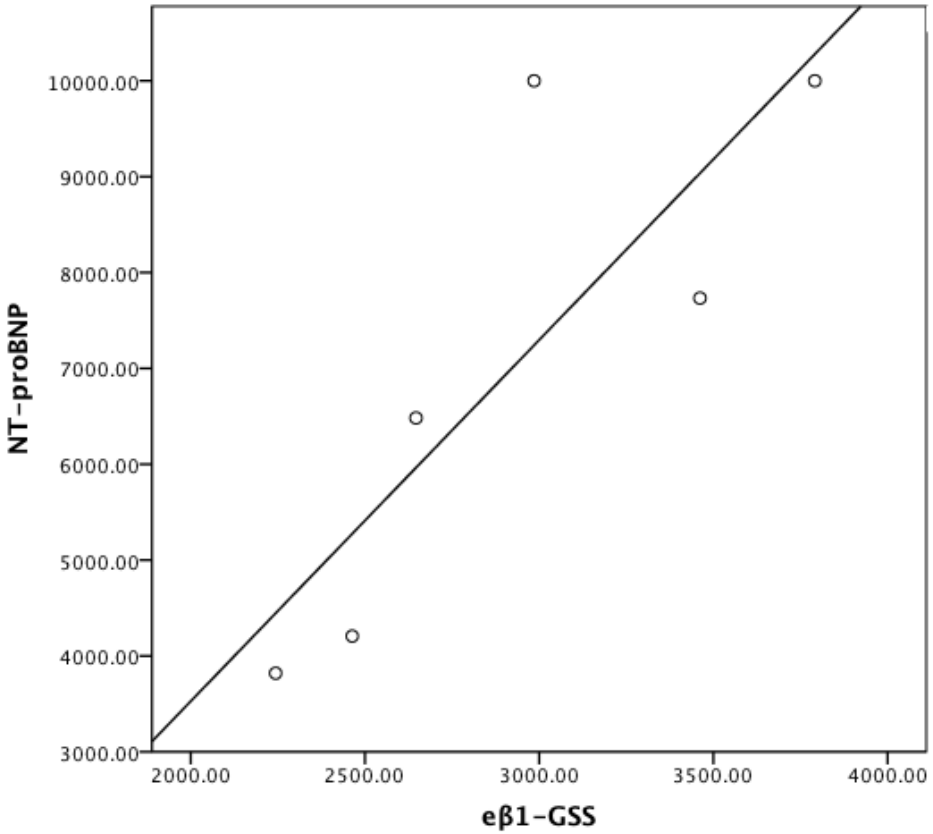
3167 ± 164 U vs 1018 ± 20 U; n=16; p<0.001
Independent of age, gender, bmi.

What about in humans?.....Na^+-K^+-ATPase activity

Na-K ATPase activity in erythrocytes from HF patients vs. control

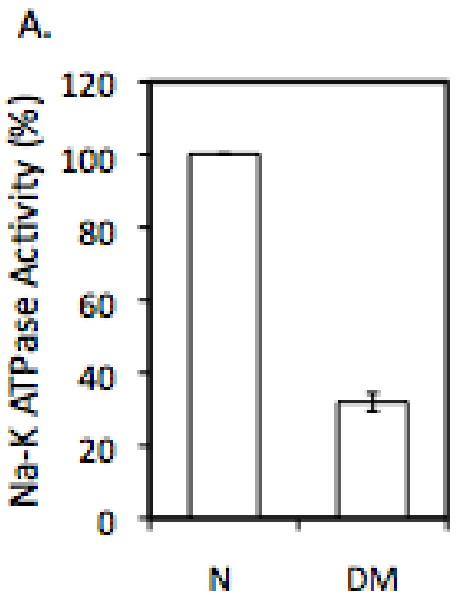


What about in humans? BNP

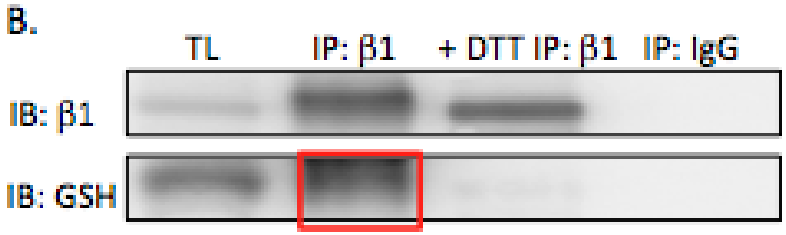


Diabetes and eb1-GSS in animal models

Animal Model: Rabbit



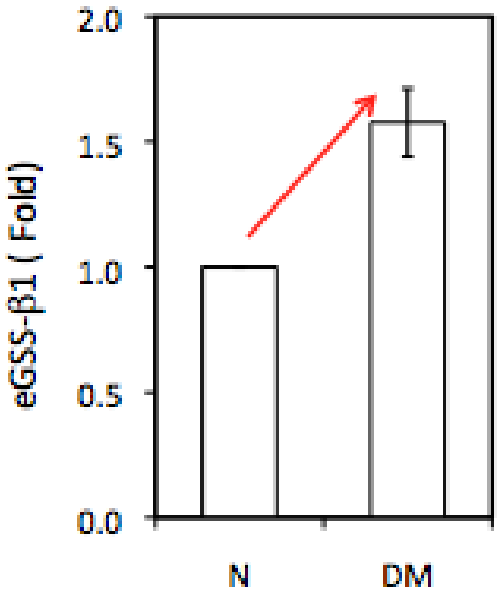
NaK ATPase activity decreased in DM erythtocytes.



S-glutathionylation of erythrocyte β_1 subunits detected.

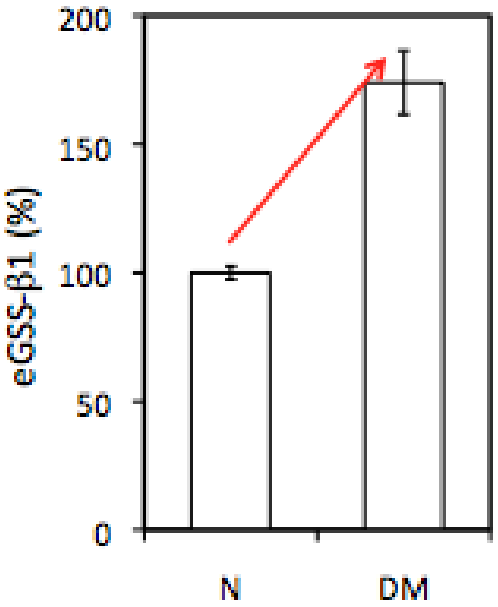
Diabetes and animal models

C. Western blot
-Slow
-Small sample sizes



S-glutathionylation of erythrocyte β_1 subunits detected by CO-IP. *S-glutathionylation* was significantly increased in DM erythrocytes.

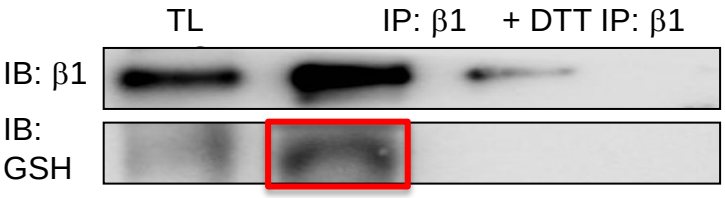
D. ELISA
-Rapid
-Screen Large sample size in one go



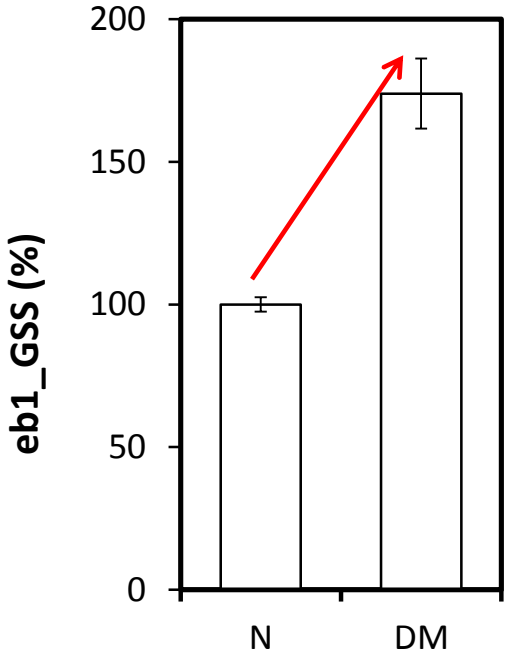
S-glutathionylation of erythrocyte β_1 subunits detected by ELISA. *S-glutathionylation* was significantly increased in DM erythrocytes.

Diabetes and eb1-GSS in humans

Human Model:



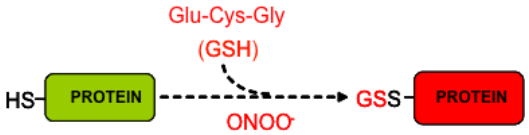
S-glutathionylation of erythrocyte β_1 subunits detected in human.

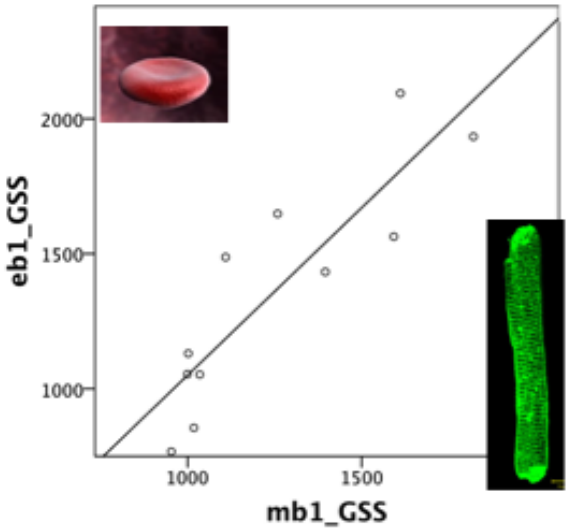


S-glutathionylation of erythrocyte β_1 subunits detected by ELISA. *S-glutathionylation of* erythrocytes was significantly increased in DM patients compared to Normal .)

Summary: eβ1-GSS

- occurs and is detectable!
- ELISA assay is rapid and quantitative
- parallels oxidative inhibition of cardiac Na^+-K^+ pump
- increases in patients with HF and reflects severity
- increases in diabetics





Liu, Unpublished, 2014

Potential prognostic value of eβ1-GSS?

- For HF: important if being used as diagnostic tool, but less so if combined with clinical and laboratory biomarkers for prognostic purposes

- Ongoing work:
  - Prognostic significance of eβ1-GSS over conventional biomarkers and risk factors:
    - in hospitalized HF/DM
    - in community subjects at high risk of HF (SCREEN-HF ~ 4000 subjects)

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-Dr Stephanie Bibert (Switzerland)
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-A/Prof Kathy Sweadner (USA)
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