



האיגוד הישראלי לכירורגית לב וחזה  
THE ISRAEL SOCIETY OF CARDIOTHORACIC SURGERY

האיגוד הקרדיולוגי בישראל  
ISRAEL HEART SOCIETY



The 60<sup>th</sup> International Conference of the Israel Heart Society  
in association with the Israel Society of Cardiothoracic Surgery

22-23 April 2013, ICC International Convention Center, Jerusalem

# C-Reactive Protein and Atherothrombosis: Cause or Effect?

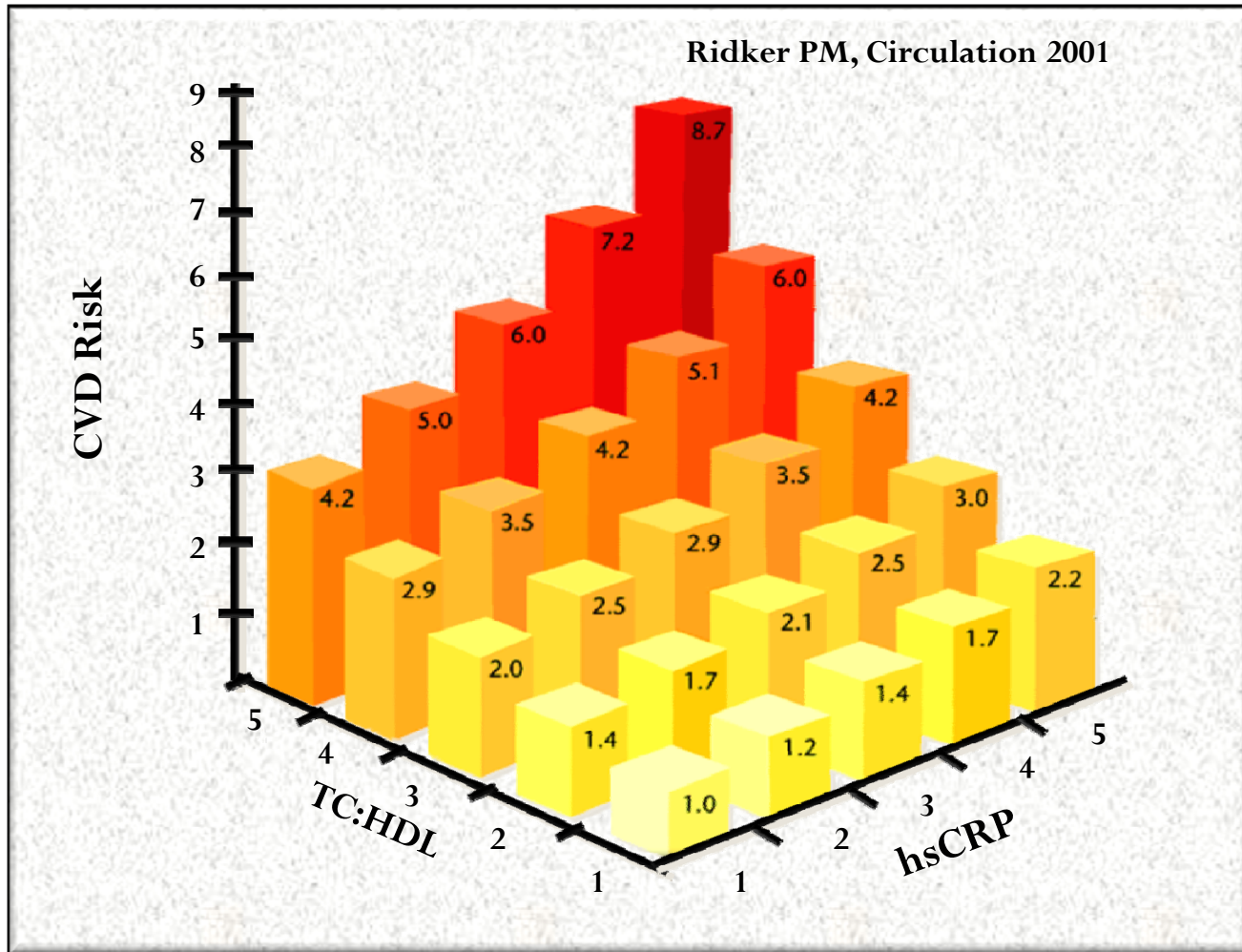
**Etty Grad**, Rachel M. Pachino, Mordechai Golomb, Michal Levy & Haim  
Danenberg.

*Cardiovascular Research Center, Hadassah-Hebrew University Medical Center,  
Jerusalem, Israel.*



# CRP and cardiovascular disease

First line host defense molecule



# The NEW ENGLAND JOURNAL of MEDICINE

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**Table 3. Outcomes According to Study Group.**

End Point	Rosuvastatin (N=8901)		Placebo (N=8901)		Hazard Ratio (95% CI)	P Value
	No. of Patients	Rate per 100 person-yr	No. of Patients	Rate per 100 person-yr		
Primary end point	142	0.77	251	1.36	0.56 (0.46–0.69)	<0.00001
Nonfatal myocardial infarction	22	0.12	62	0.33	0.35 (0.22–0.58)	<0.00001
Any myocardial infarction	31	0.17	68	0.37	0.46 (0.30–0.70)	0.0002
Nonfatal stroke	30	0.16	58	0.31	0.52 (0.33–0.80)	0.003
Any stroke	33	0.18	64	0.34	0.52 (0.34–0.79)	0.002
Arterial revascularization	71	0.38	131	0.71	0.54 (0.41–0.72)	<0.0001
Hospitalization for unstable angina	16	0.09	27	0.14	0.59 (0.32–1.10)	0.09
Arterial revascularization or hospitalization for unstable angina	76	0.41	143	0.77	0.53 (0.40–0.70)	<0.00001
Myocardial infarction, stroke, or confirmed death from cardiovascular causes	83	0.45	157	0.85	0.53 (0.40–0.69)	<0.00001
Death from any cause						
Death on known date	190	0.96	235	1.19	0.81 (0.67–0.98)	0.03
Any death	198	1.00	247	1.25	0.80 (0.67–0.97)	0.02

men

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# CRP: a marker or a maker of atherothrombosis?

Does CRP affect atherogenesis, thrombosis or both components of the atherothrombotic process?

# CRP and atherogenesis

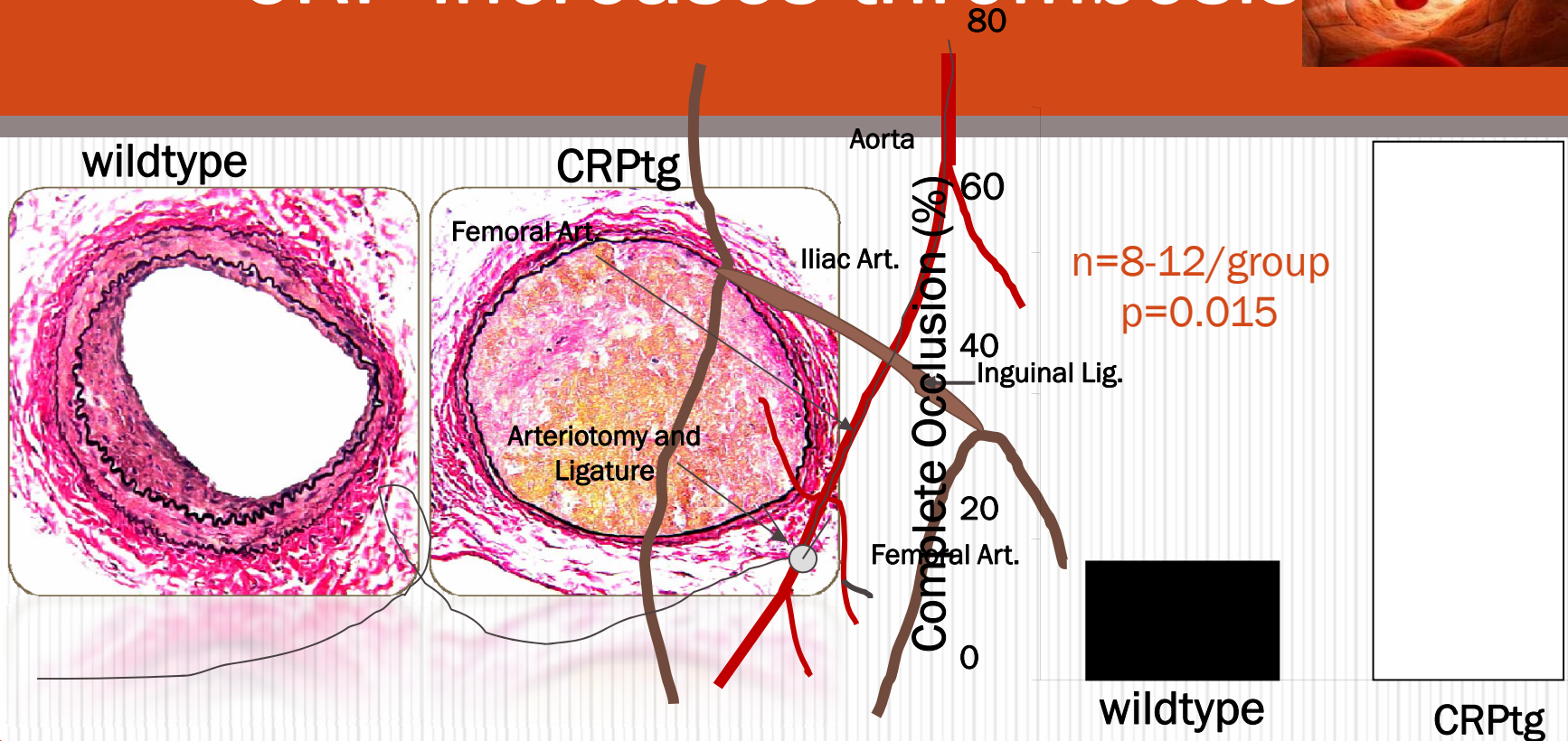
- Histological staining of atherosclerotic lesions consistently demonstrates CRP within the lesion.
- *In vitro* experiments testing the addition of CRP to cultured Ecs, SMCs and monocytes identified several potential pro-inflammatory mechanisms by which CRP promote atherosclerosis.
- Studies in mice prone to atherosclerosis such as *APO-E KO* or *LDLR KO* have not come up with uniform conclusions.
- Accumulating data indicate that CRP is probably not pro-atherogenic in human .



# How does it work?



# CRP increases thrombosis



# Pathways of CRP induced thrombosis

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- 1. Down-regulates nitric oxide production and bioavailability**

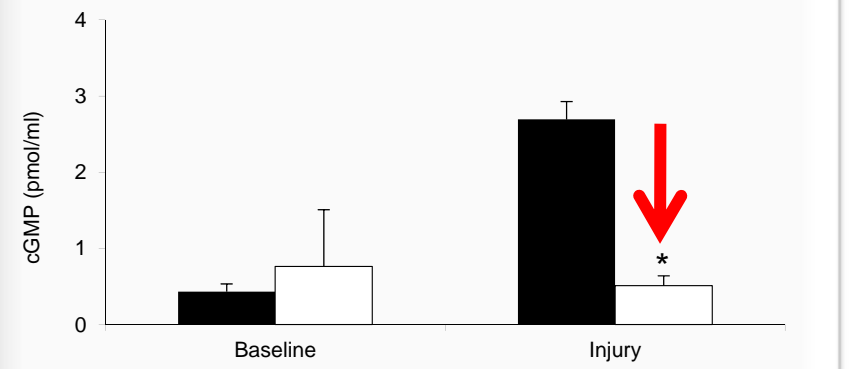
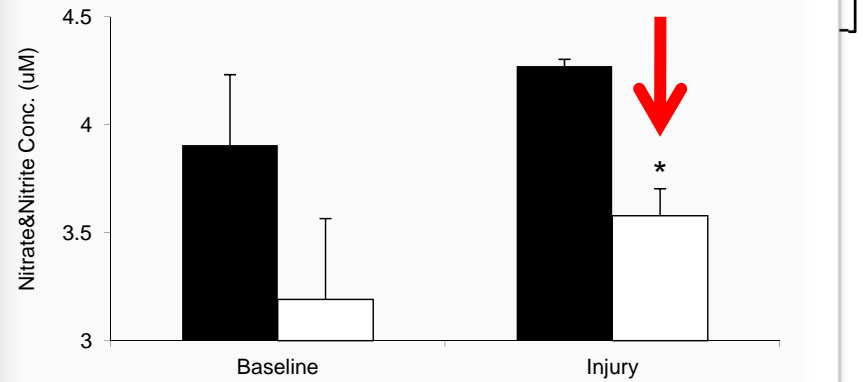
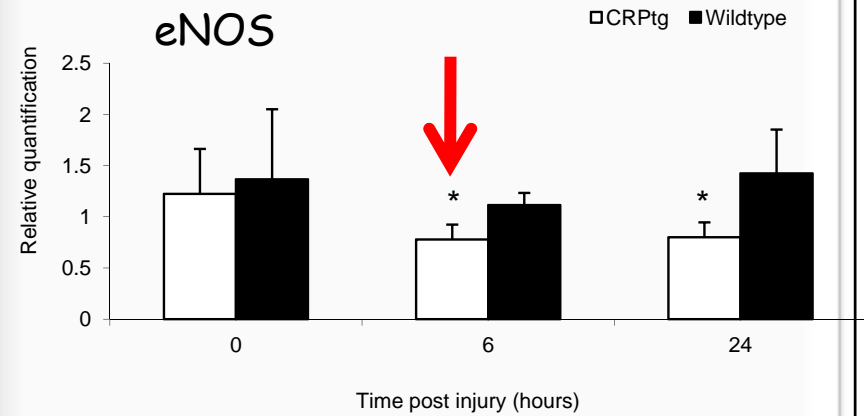
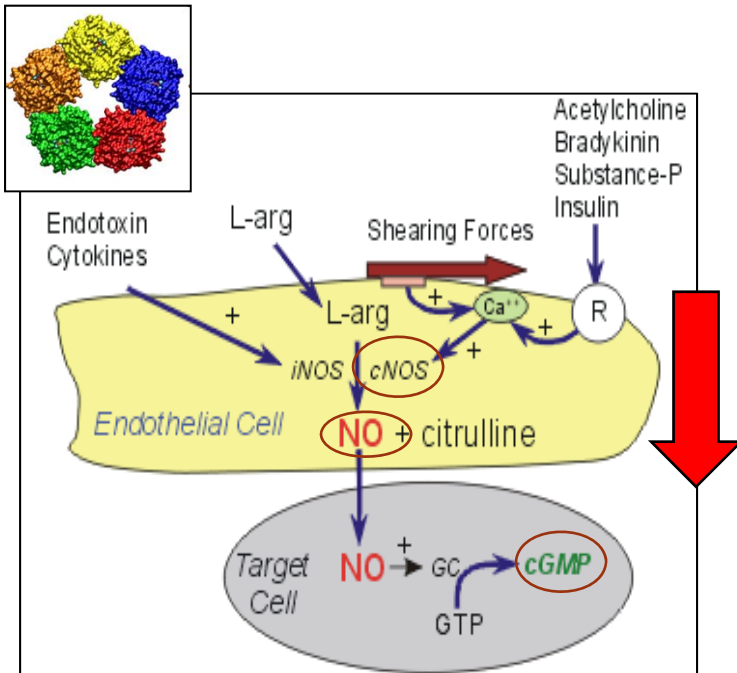


## Transgenic expression of human C-reactive protein suppresses endothelial nitric oxide synthase expression and bioactivity after

Etty Grad,<sup>1</sup> Mordechai Golomb,<sup>1</sup> Irit Mor-Yosef,<sup>1</sup> Nikolay Koroukhov,<sup>1</sup> Chaim Lotan,<sup>1</sup> Elazer R. Edelman,<sup>2</sup> and Haim D. Danenberg<sup>1</sup>

<sup>1</sup>Cardiovascular Research Center, Hadassah Hebrew University Medical Center, of Health Sciences and Technology, Harvard-Massachusetts Institute of Technology

Submitted 27 December 2006; accepted in final form 14 March 2007



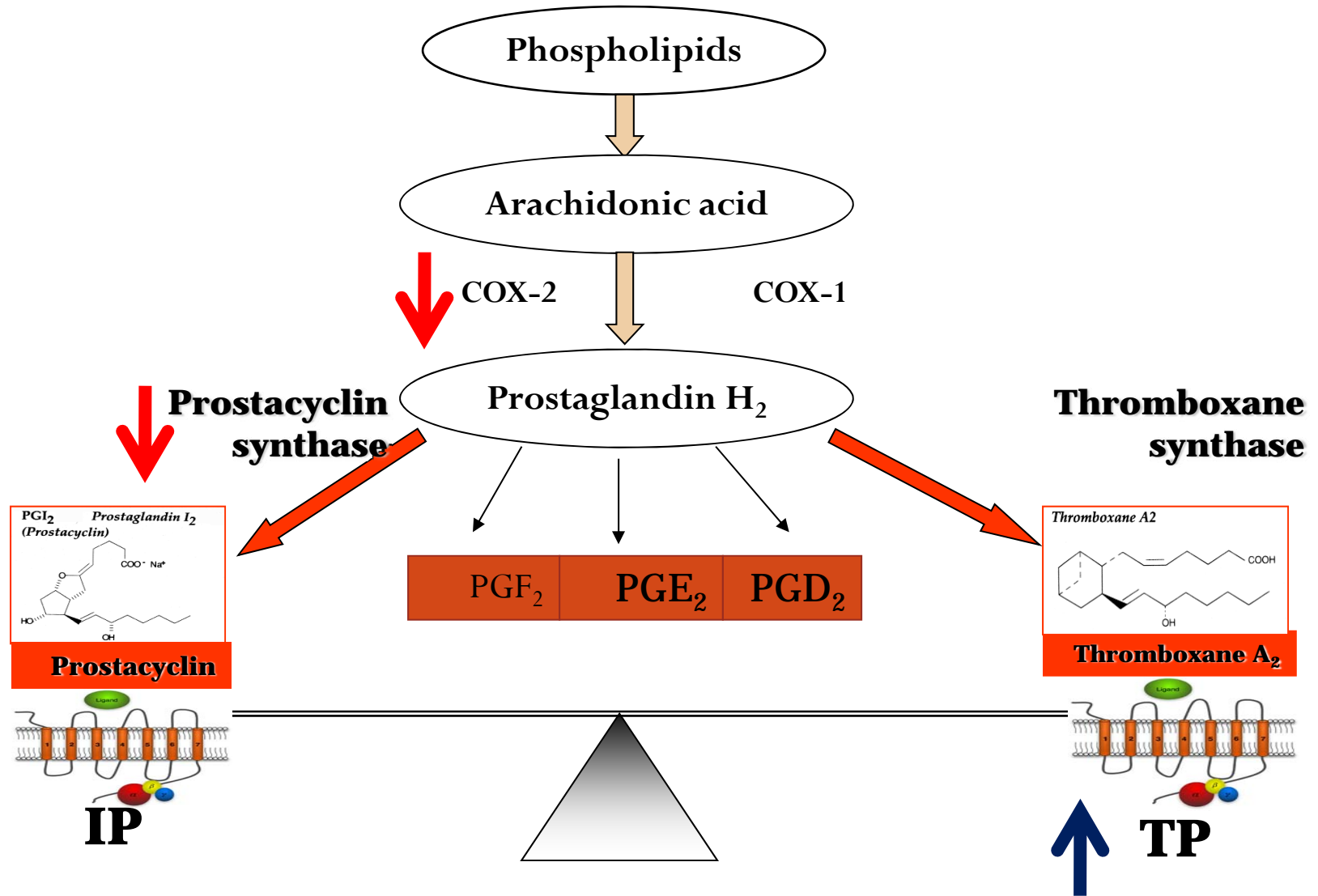
\*p<0.05 n=5 per group

# Pathways of CRP induced thrombosis

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## **2. Modifies prostanoid balance and activity**

# CRP modifies prostanoid pathway

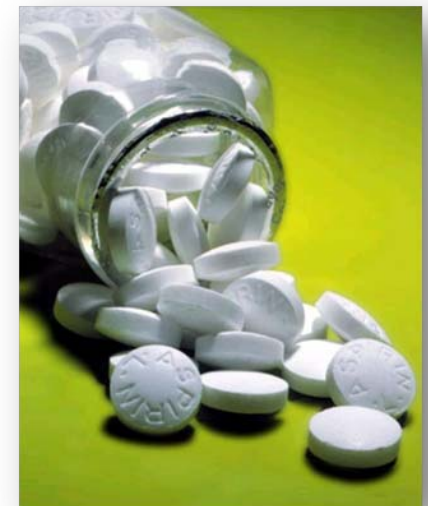
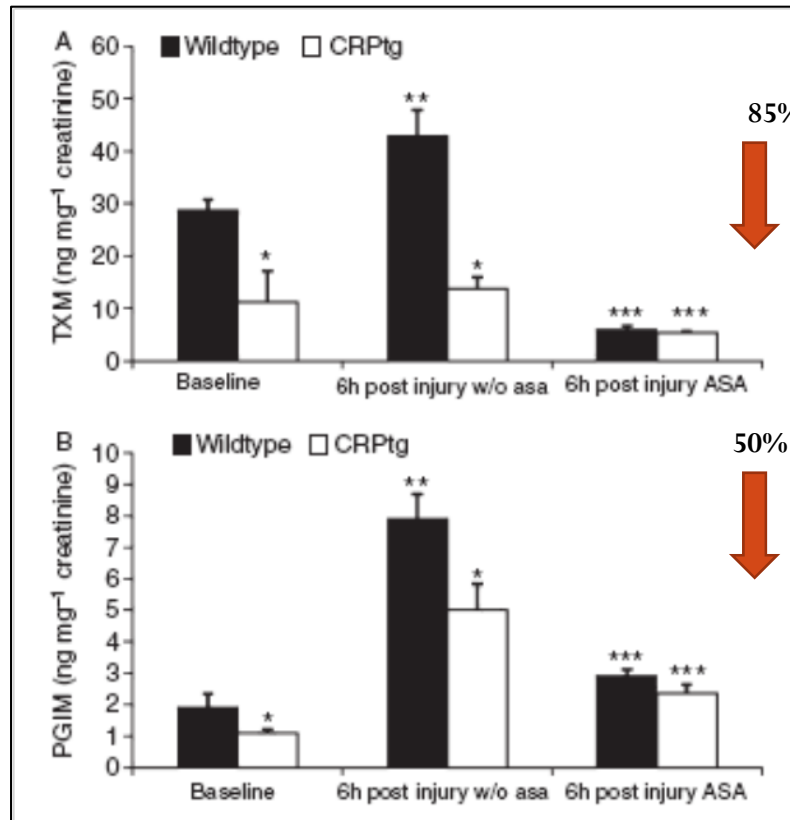
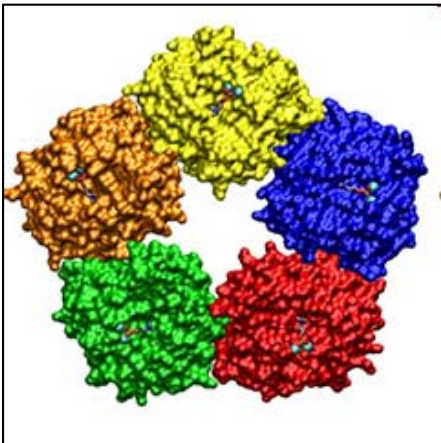


ORIGINAL ARTICLE

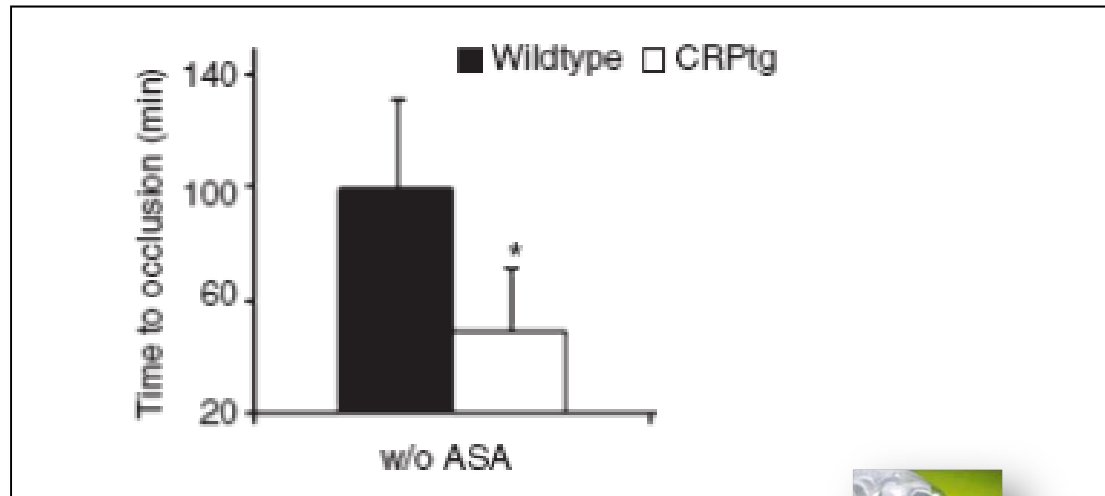
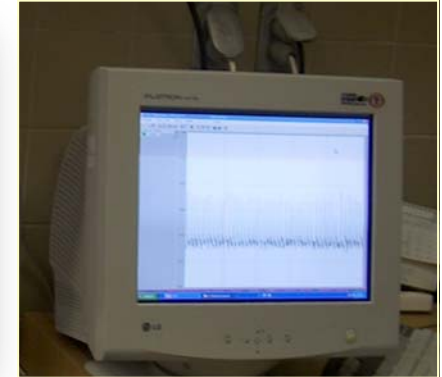
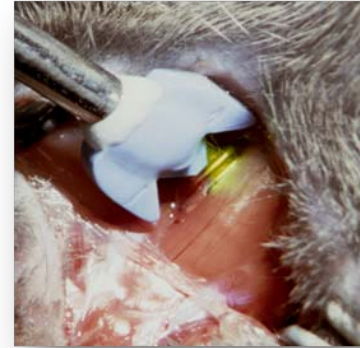
# Aspirin reduces the prothrombotic activity of C-reactive protein

E. GRAD,\* M. GOLOMB,\* N. KOROUKHOV,\* J. A. LAWSON,† C. LOTAN,\* G. A. FITZGERALD† and H. D. DANENBERG\*

\*Heart Research Centre, Hadassah Hebrew University Medical Centre, Jerusalem, Israel; and †The Institute for Translational Medicine and Therapeutics, The University of Pennsylvania, Philadelphia, PA, USA



# Photochemical injury – realtime thrombus formation



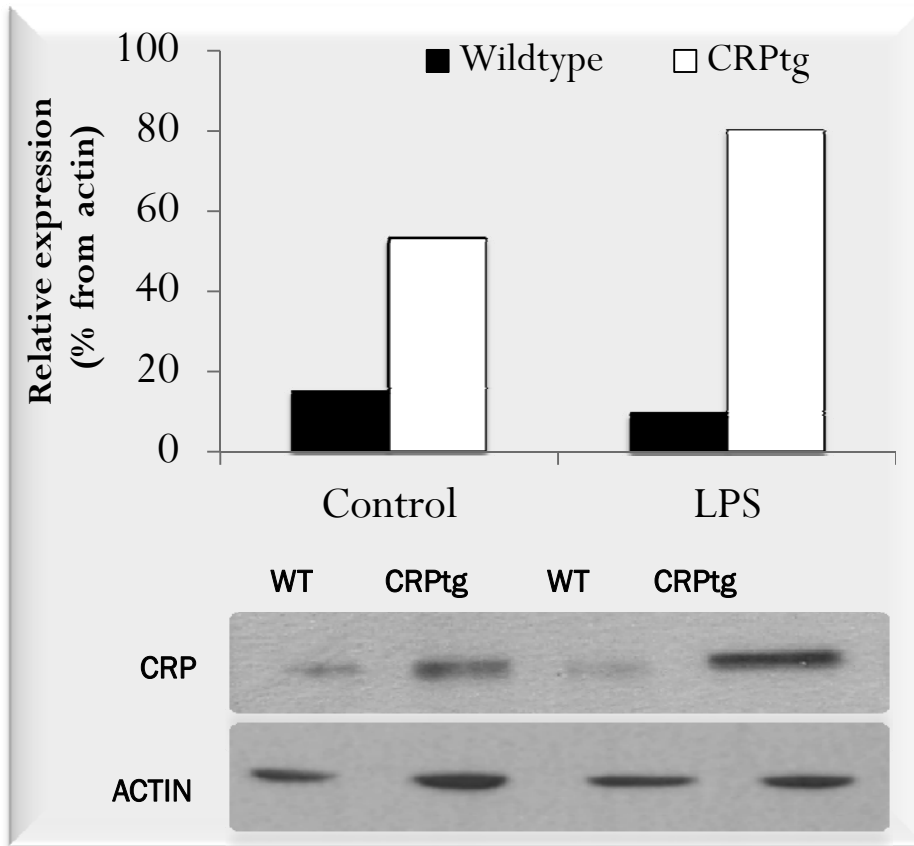
# Pathways of CRP induced thrombosis

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## **3. Endothelial CRP increases platelet adhesion**

# Is local CRP secretion of any patho-biological significance?

MHEC derived from CRPtg mice express human CRP



Real time PCR analysis for human CRP show that MHEC derived from human CRP transgenic mice express mRNA for human CRP, while control wildtype MHEC do not.

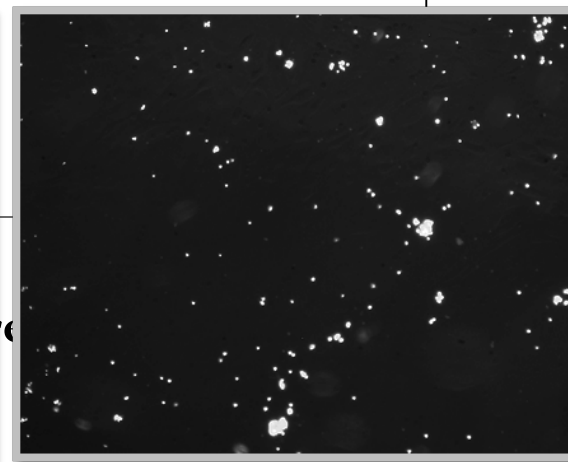
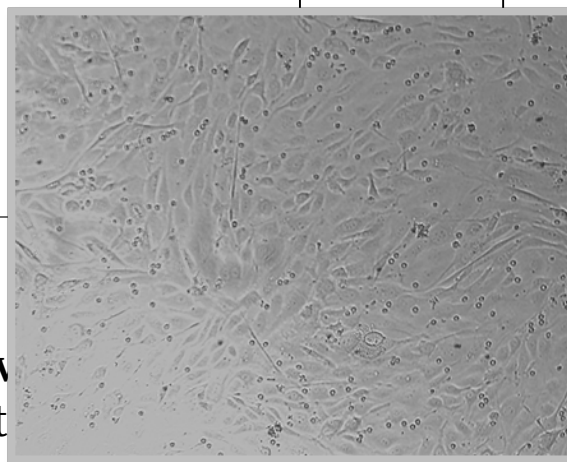
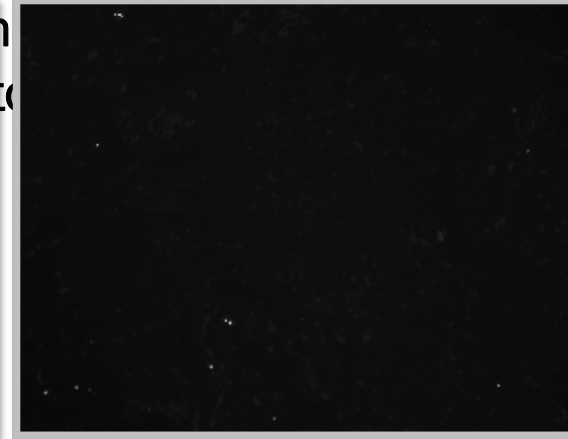
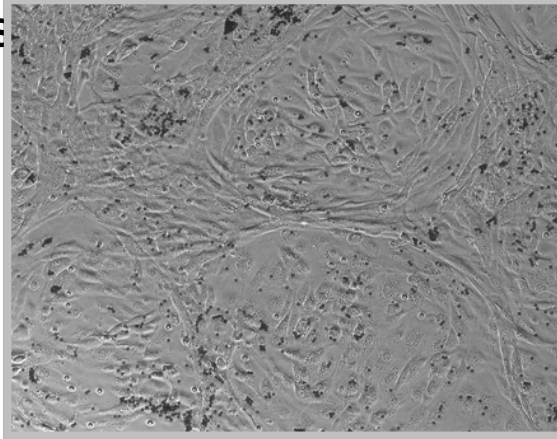
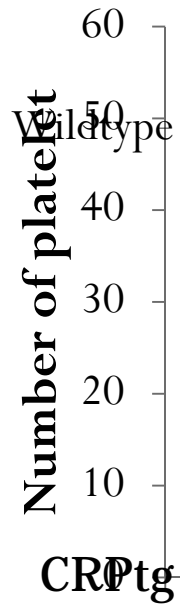
**Endothelial C-reactive protein increases platelet adhesion under flow conditions**

Etty Grad, Rachel M. Pachino and Haim D. Danenberg

*Am J Physiol Heart Circ Physiol* 301:H730-H736, 2011. First published 17 June 2011;

doi:10.1152/ajpheart.00067.2011

Platelet adhesion

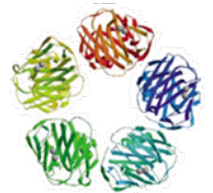


significantly increased

N=6

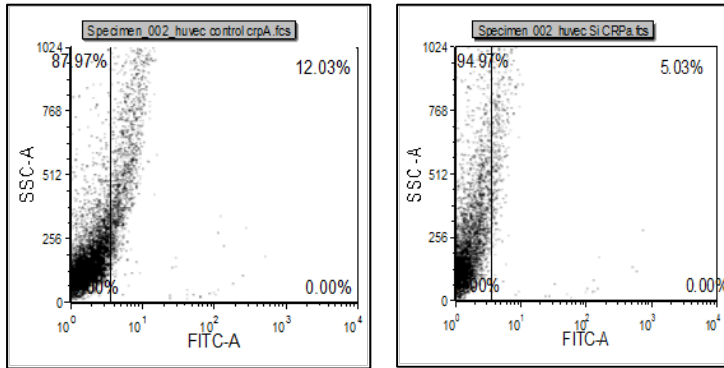
\*p<0.01, WT v

\*\*p<0.01 cont



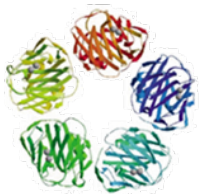


# siRNA reduces hCRP expression in HUVEC by 60%



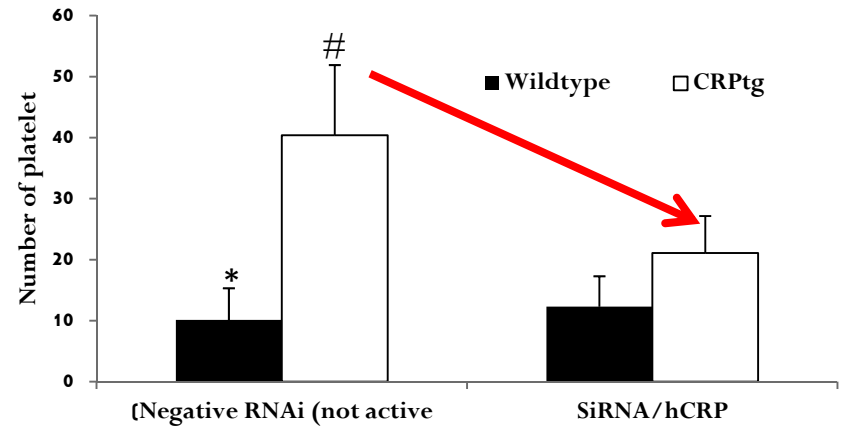
Negative (IC)

siRNA to hCRP



## siRNA to hCRP significantly reduces platelet adhesion to endothelial cells

N=3-6

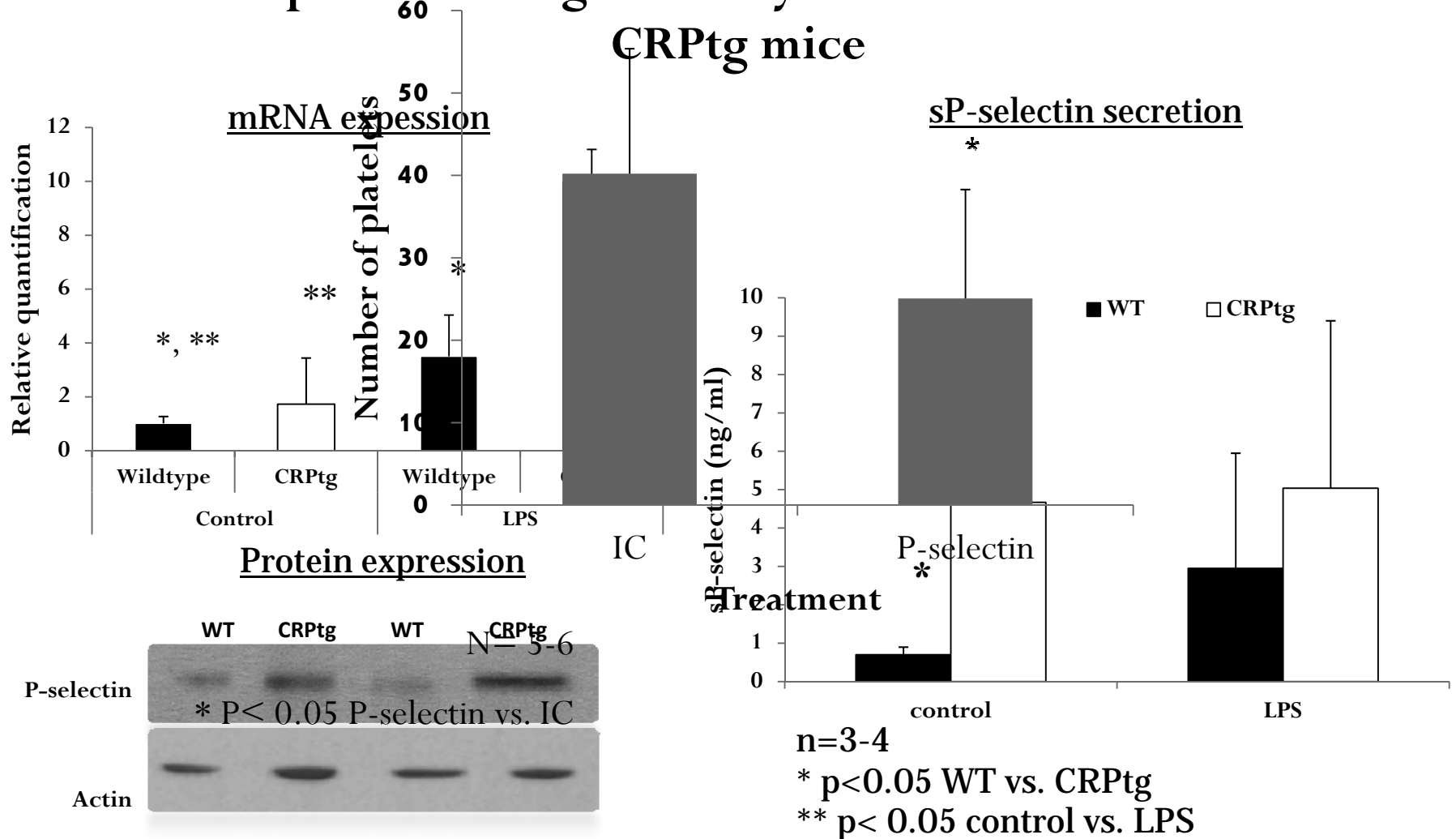


\*  $p < 0.01$  WT vs. CRPtg

#  $p < 0.05$  negative RNAi vs. siRNA/CRP

# P-selectin mediates the pro-adhesive effect of CRP

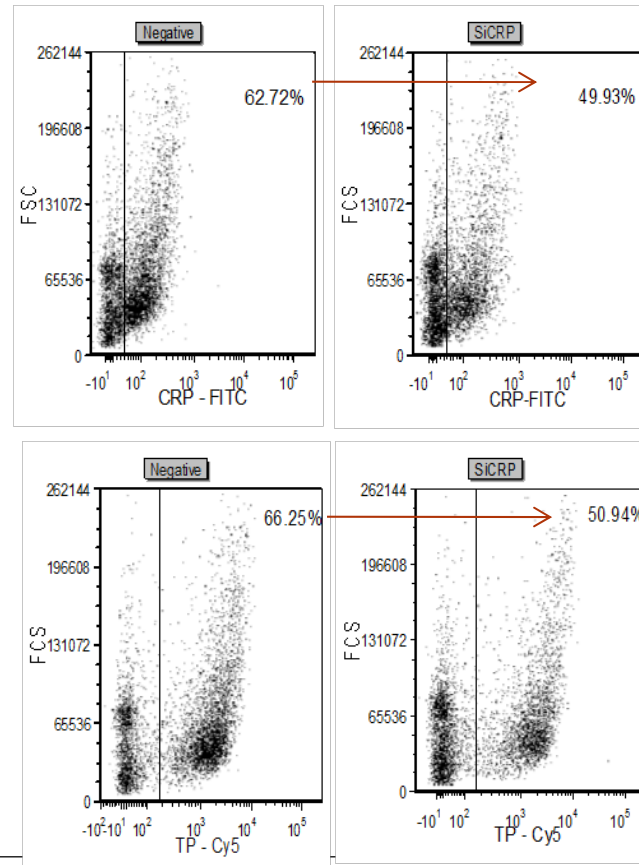
P-selectin expression is significantly increased in MHEC derived of



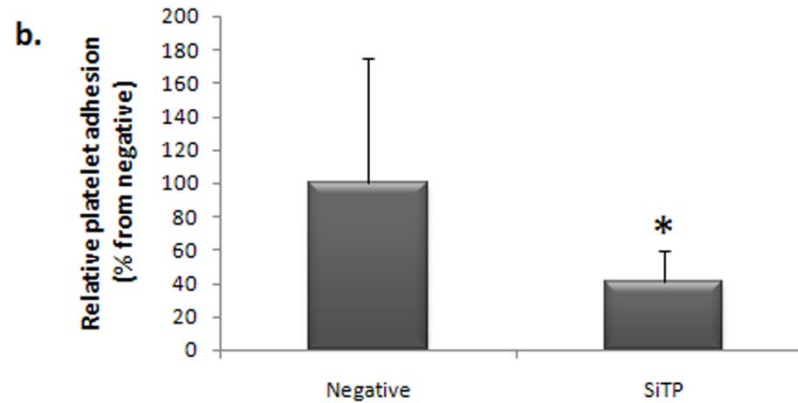
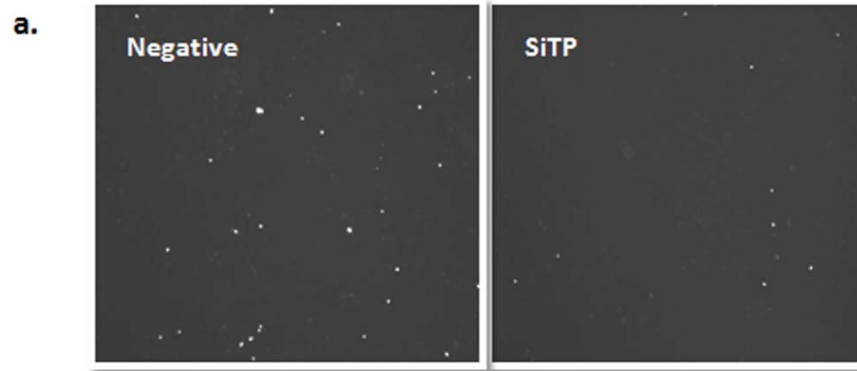
## Role of Thromboxane Receptor in C-Reactive Protein-Induced Thrombosis

Ettv Grad, Rachel M. Pachino, Garret A. FitzGerald, Haim D. Danenberg  
*Arterioscler Thromb Vasc Biol.* 2012;32:2468-2474

CRP SiRNA reduced CRP & TP expression



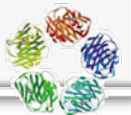
# SiRNA against TP in CRPtg MHECs reduced human CRP proadhesiveness



# Conclusions

## **C reactive protein increases thrombosis:**

1. CRP **decreases nitric oxide** production and activity
2. CRP **modulates the COX-prostanoid pathway:**  
suppresses PGI<sub>2</sub> synthase and augments TXA<sub>2</sub> activity by increasing TP. Aspirin treatment reverses the prothrombotic effect of CRP
3. Human CRP is **locally secreted by endothelial cells:** Local hCRP increases platelet adhesion to murine and human endothelial cells under normal shear flow conditions. The pro-adhesive effect of hCRP is mediated by P-selectin and TP





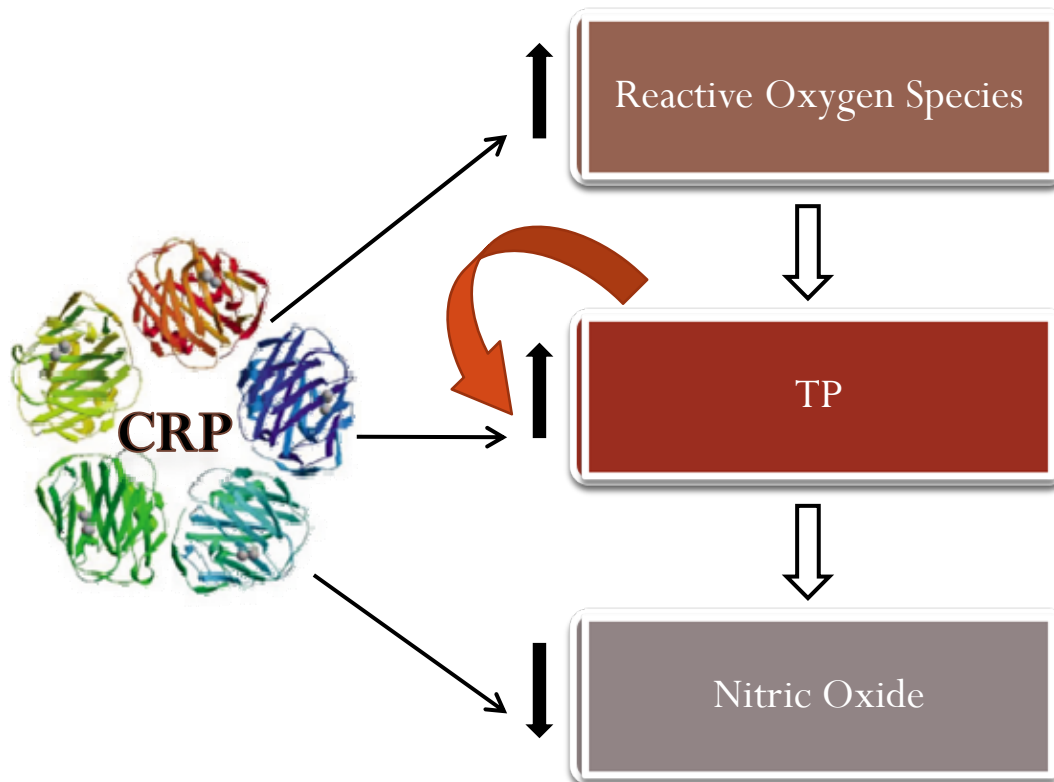
REVIEW

C-reactive protein and atherothrombosis: Cause or effect?

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# CRP increase thrombosis in a several interconnected pathways



Thank You

