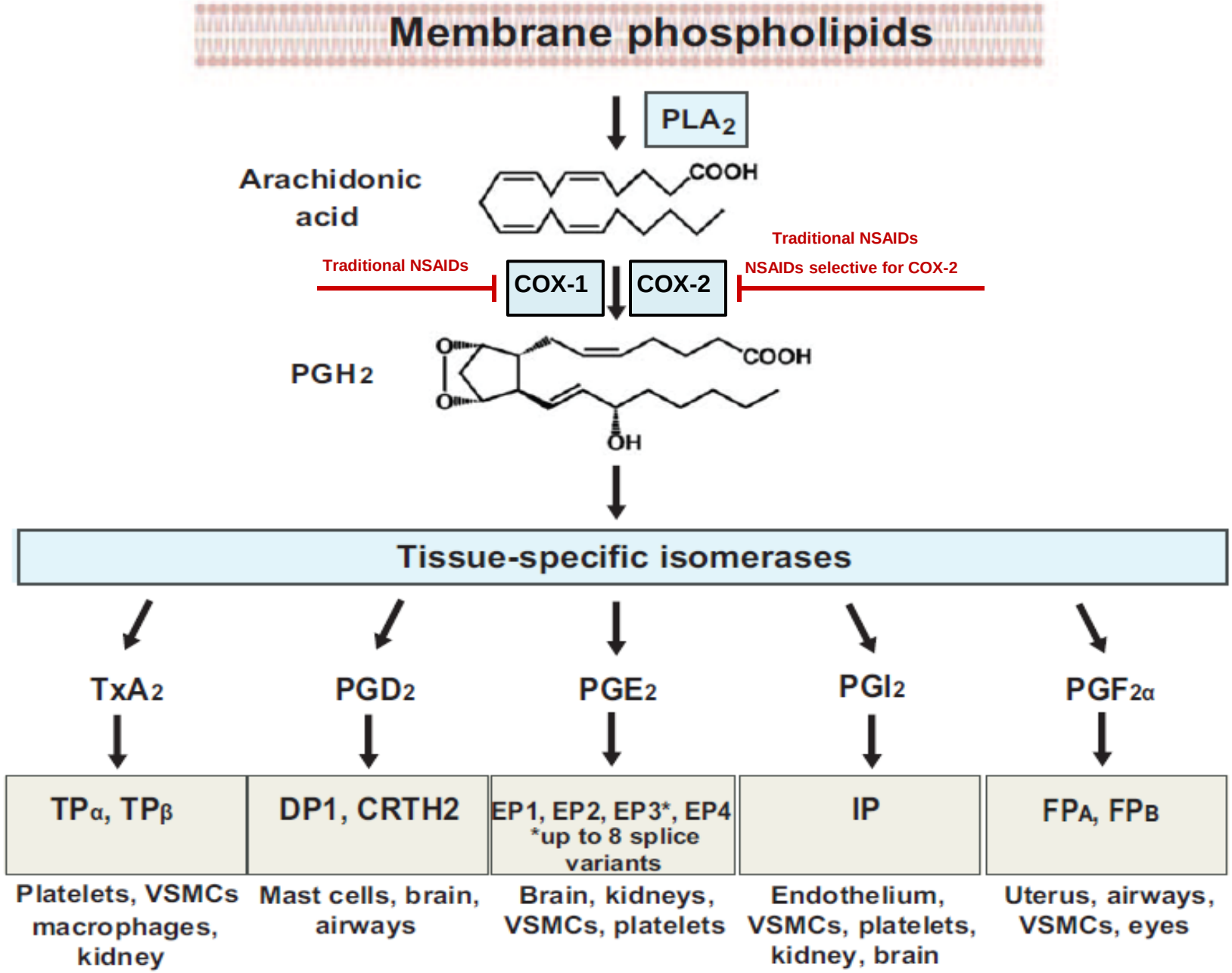
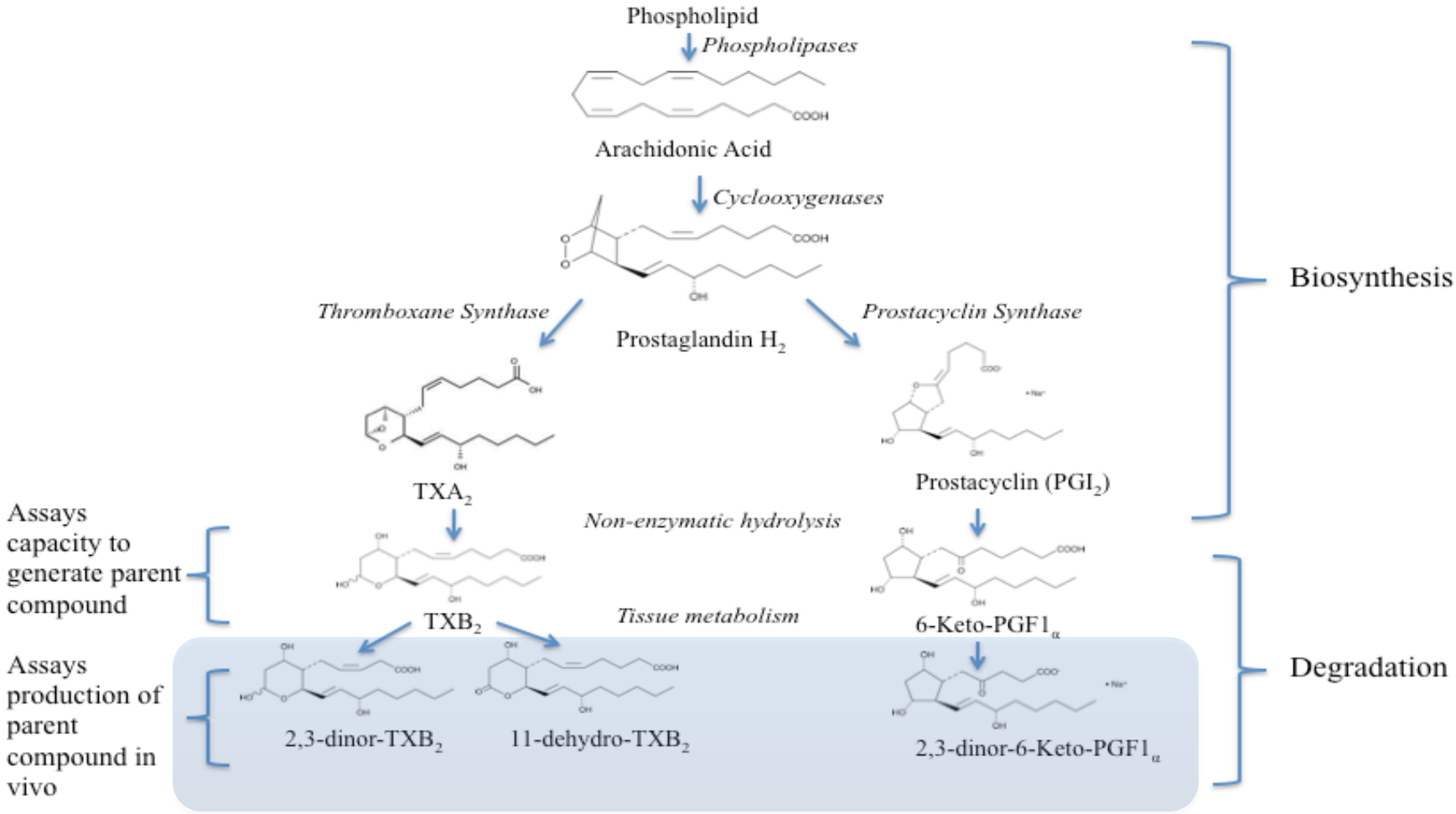
Mechanistic basis for a cardiovascular hazard from NSAIDs

Garret A. FitzGerald M.D.,F.R.S.
Perelman School of Medicine
University of Pennsylvania



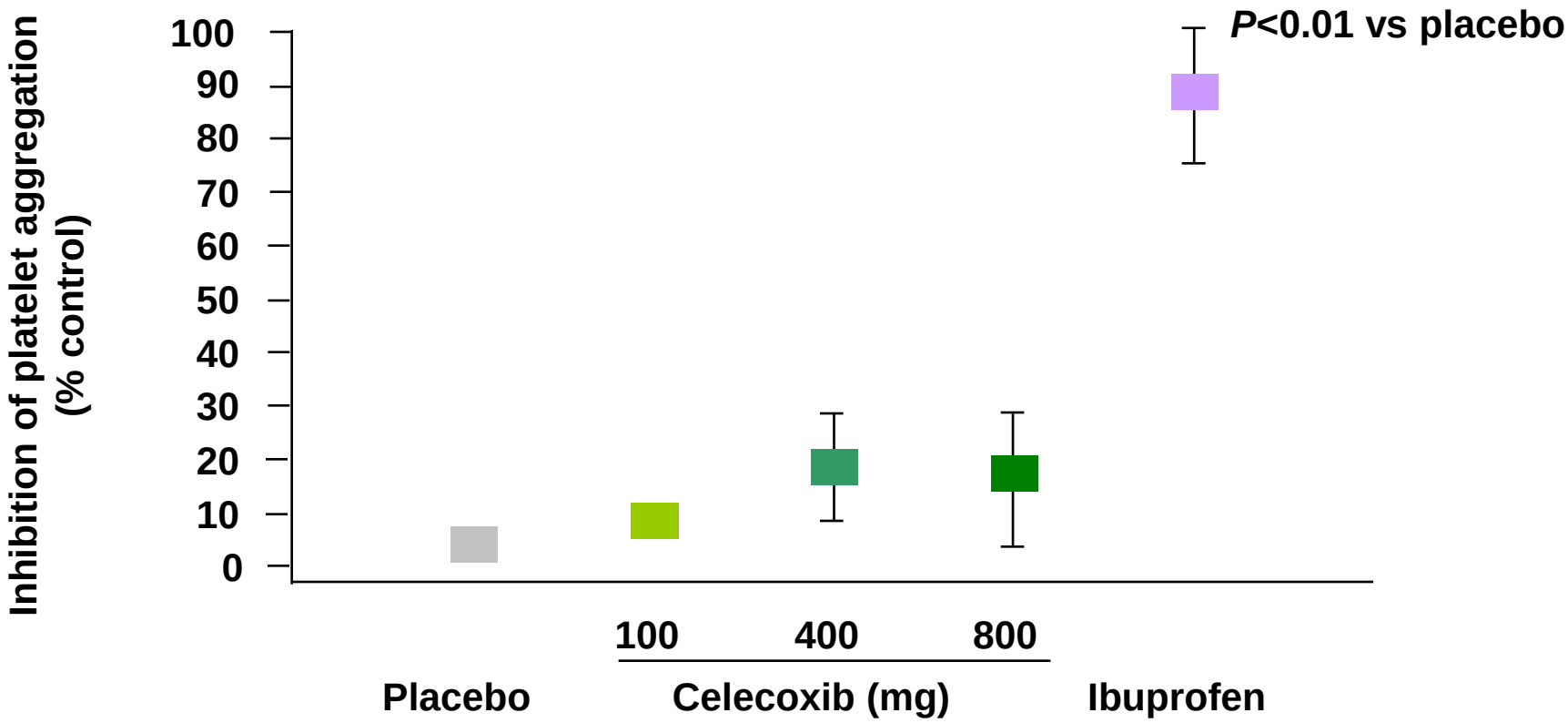
Modified from Ricciotti E & FitzGerald GA., ATVB 2011;31:986-1000

Biosynthesis of TxA_2 and PGI_2



Fitzgerald and FitzGerald Circ Res 112(1):174-94, 2013

Coxibs are not platelet inhibitors

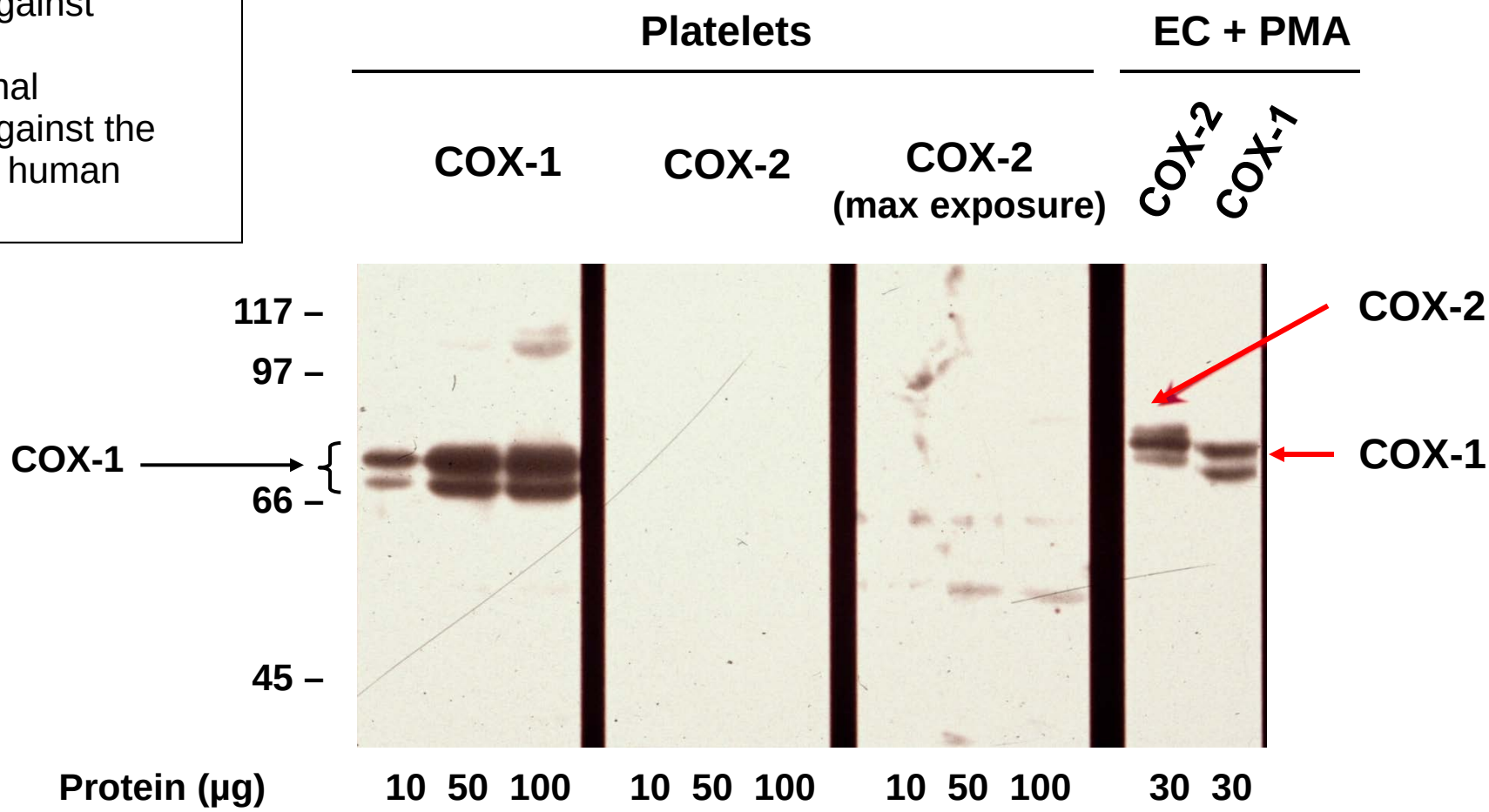


*20 μM arachdonic acid as agonist.

McAdam et al. *Proc Natl Acad Sci USA*. 1999;96:272.

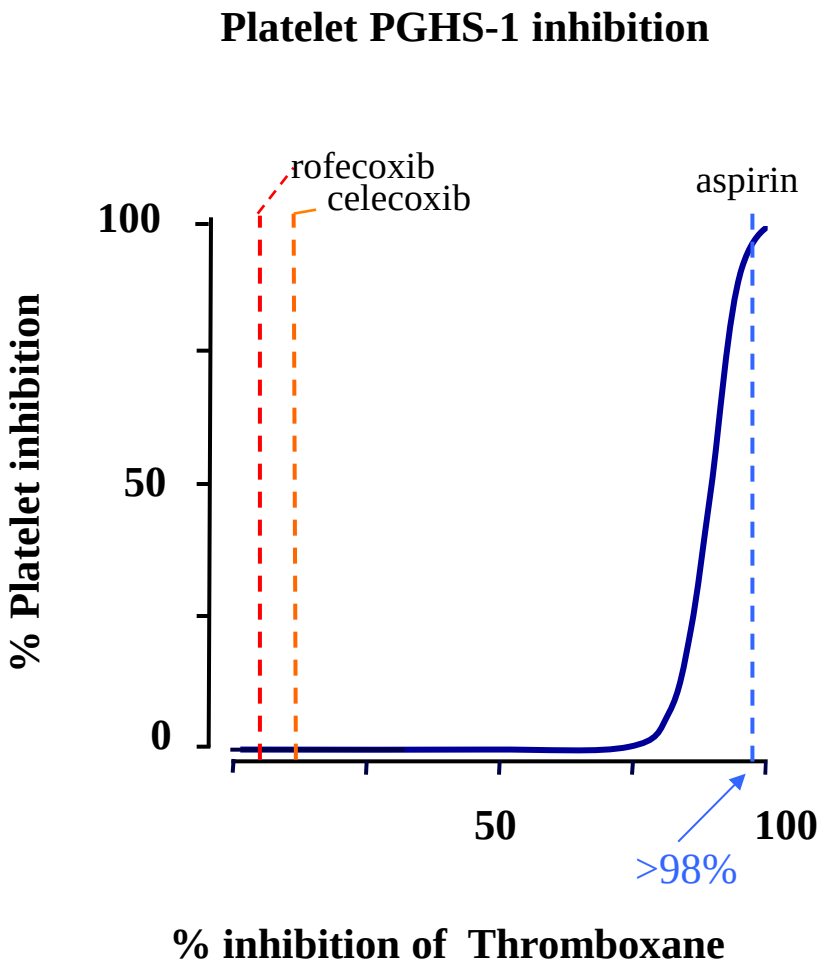
COX in Human Platelets: Western Blot Analysis

Anitbodies
COX-1: monoclonal antibody raised against purified ram COX
COX-2: monoclonal antibody raised against the COOH peptide of human COX-2

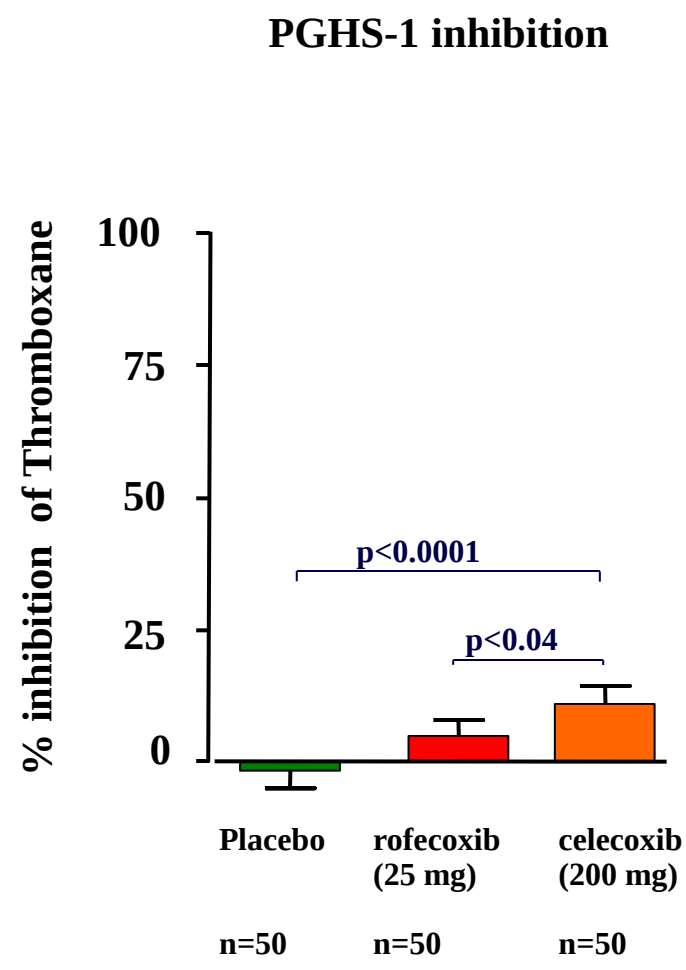


Habib A 2000

Selective inhibitors of PGHS-2 are not platelet inhibitors

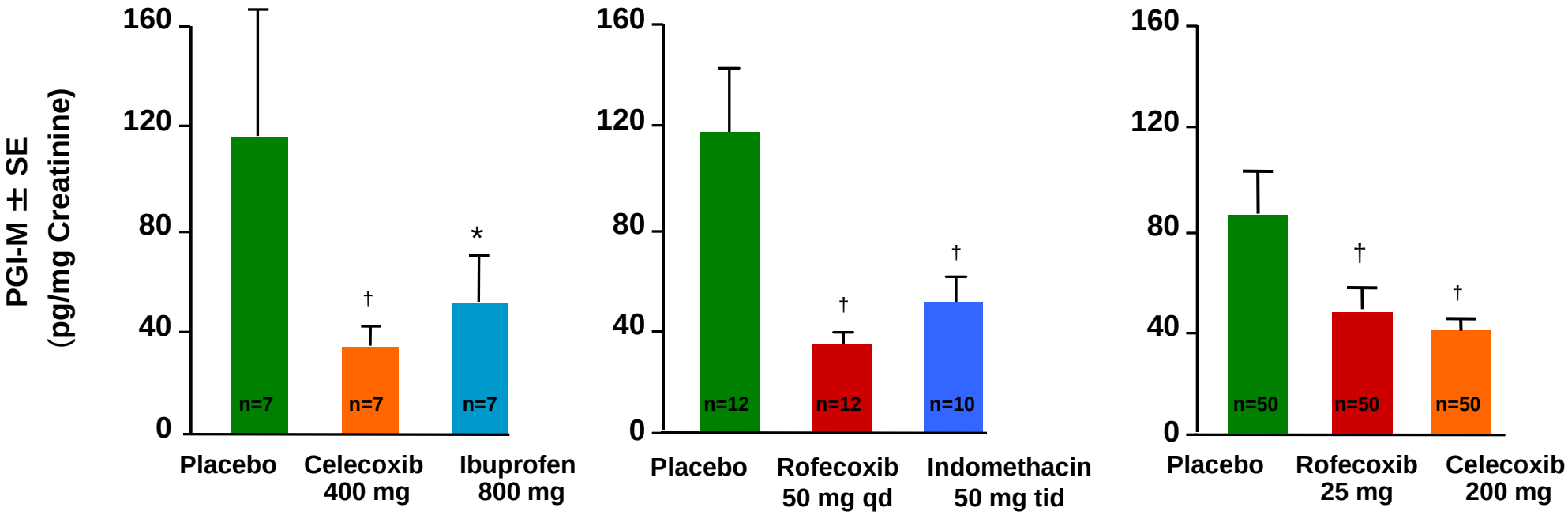


Reilly and FitzGerald Blood 69: 180 – 6, 1987



Fries et al Gastroenterology 130:55-64, 2006

Inhibition of prostacyclin synthesis by celecoxib and rofecoxib



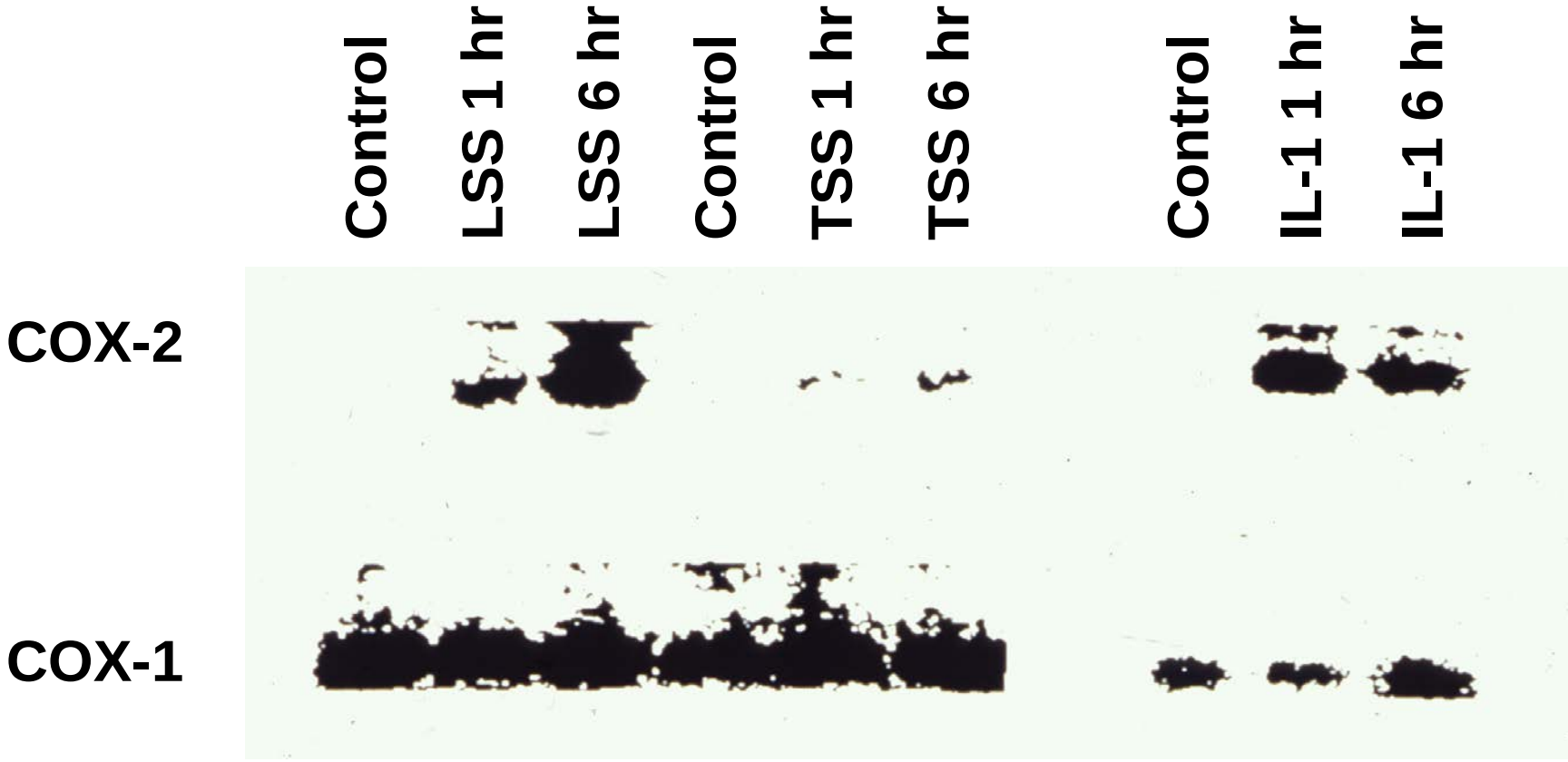
McAdam et al.
PNAS. 1999;96:272

Catella-Lawson et al.
JPET. 1999;289:735.

Fries et al Gastroenterology
130:55-64, 2006

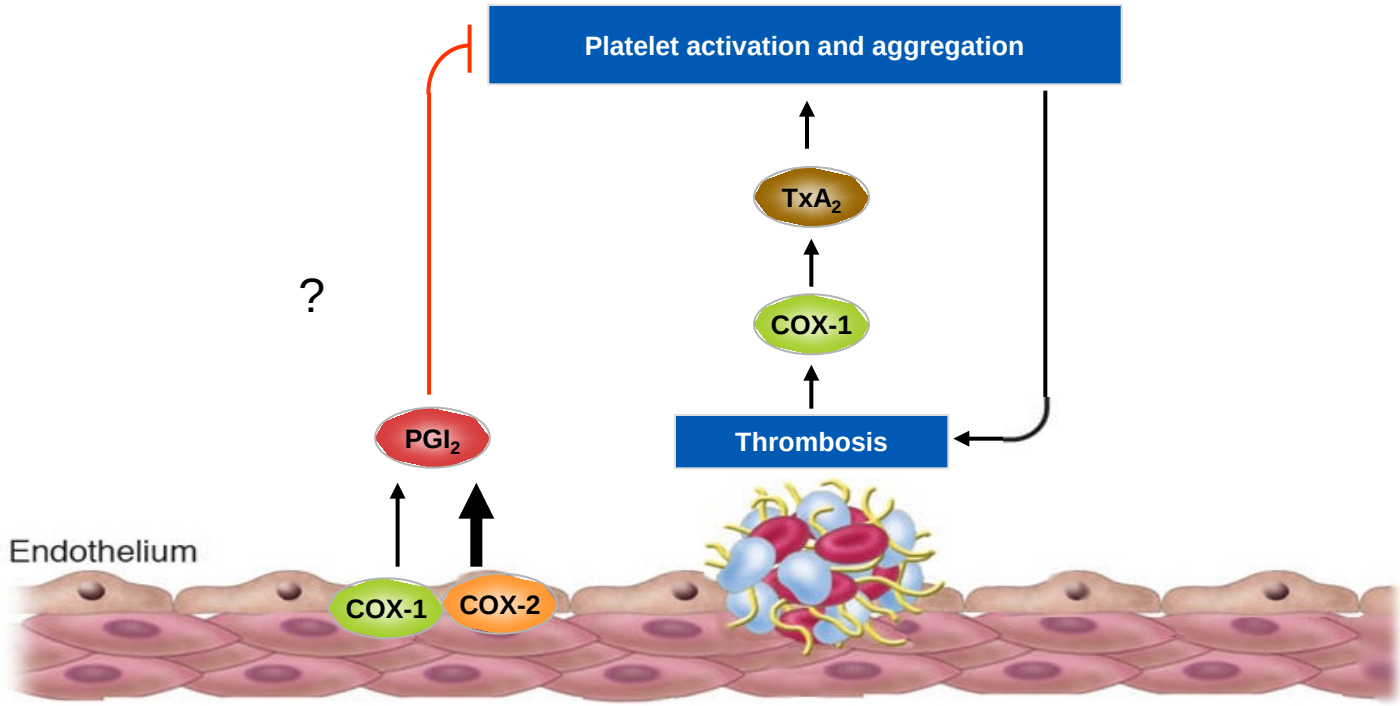
[1]PGI-M = 2,3-dinor-6-keto-$PGF_{1\alpha}$; † $P<0.01$ vs Placebo; * $P<0.05$ vs Placebo.

REGULATED EXPRESSION OF COX-2 IN ENDOTHELIUM BY LAMINAR SHEAR

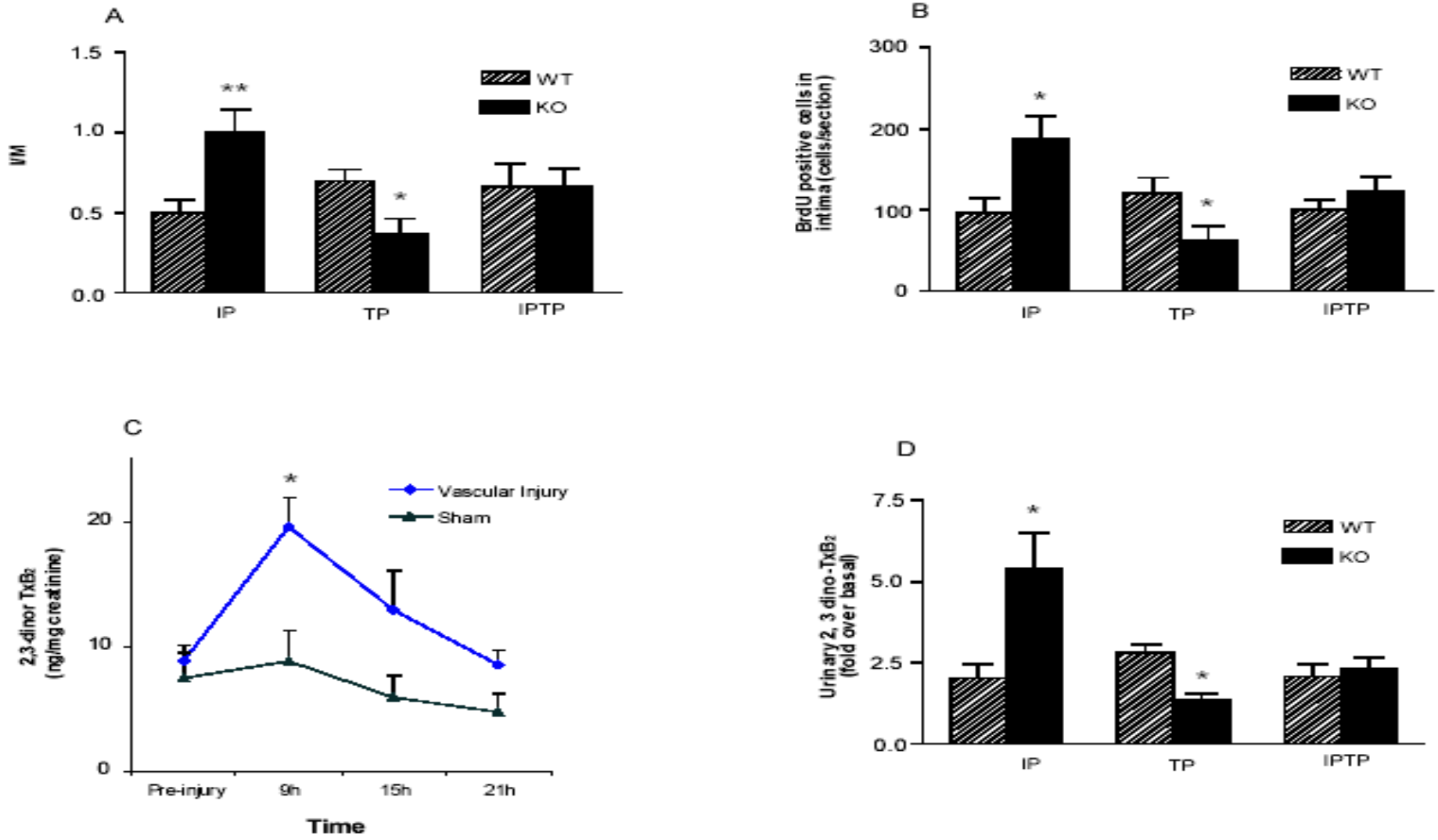


Topper et al. *Proc Natl Acad Sci USA.* 1996

Hypothesis:
Prostacyclin (PGI_2) formed in the vessel wall acts to restrain clot formation



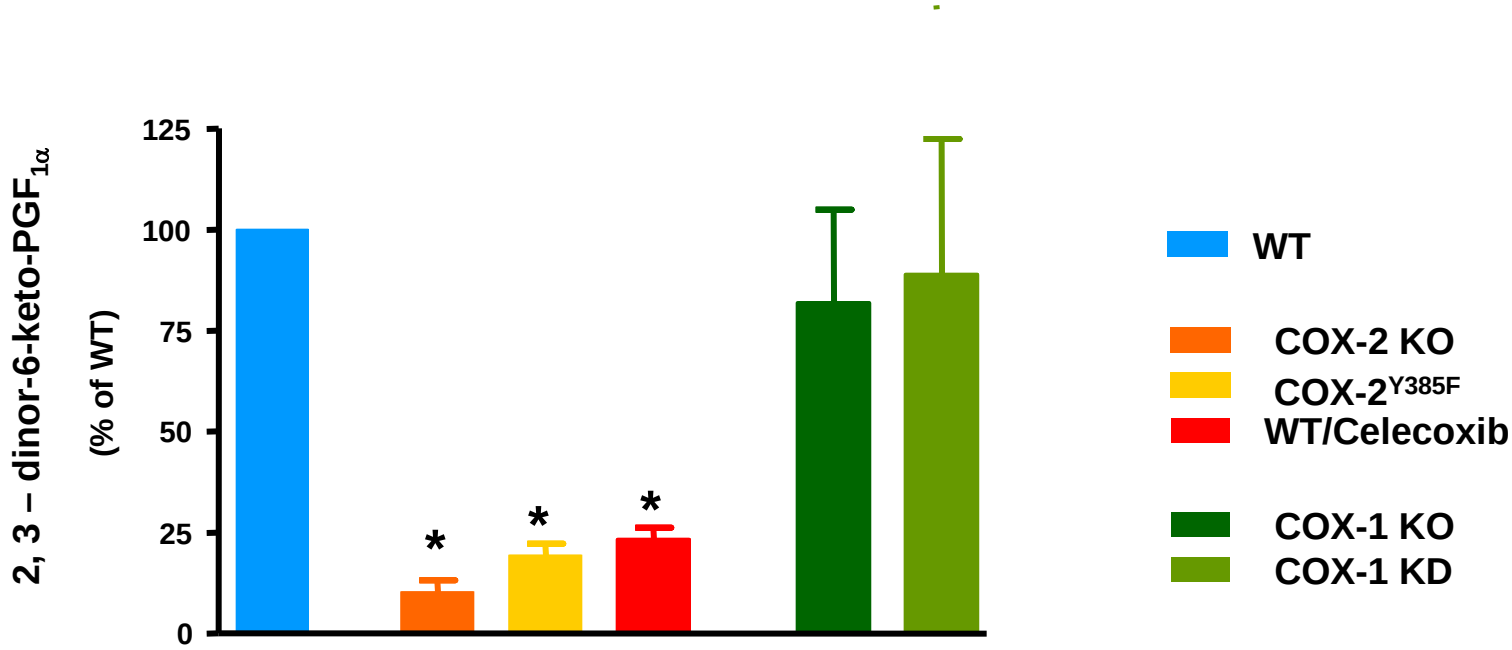
PGI_2 modulates the cardiovascular response to TxA_2 *in vivo*



Cheng et al *Science*. 296: 539 – 541, 2002.

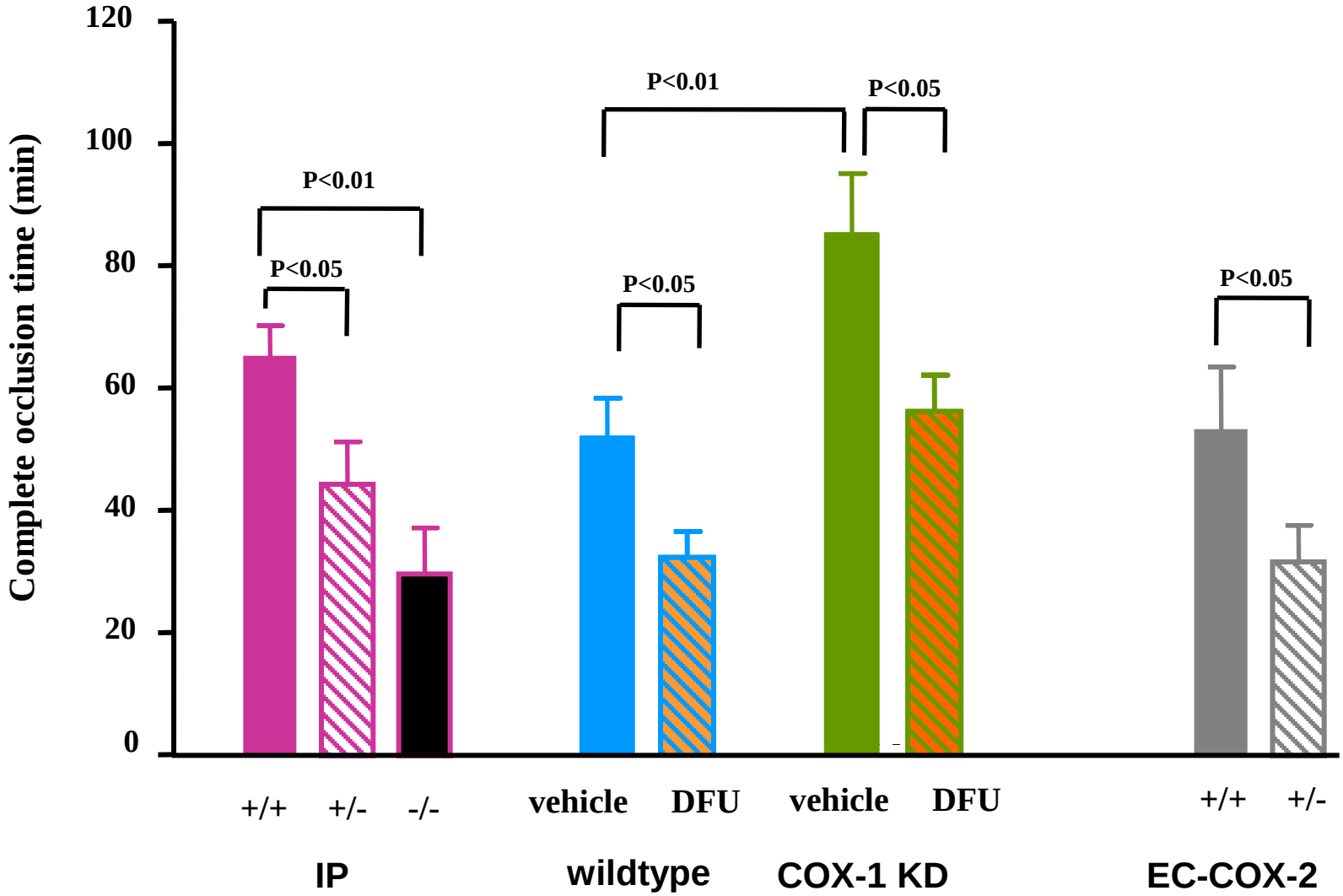
Murine COX-2 derived PGI_2

Prostacyclin
(PGI_2)



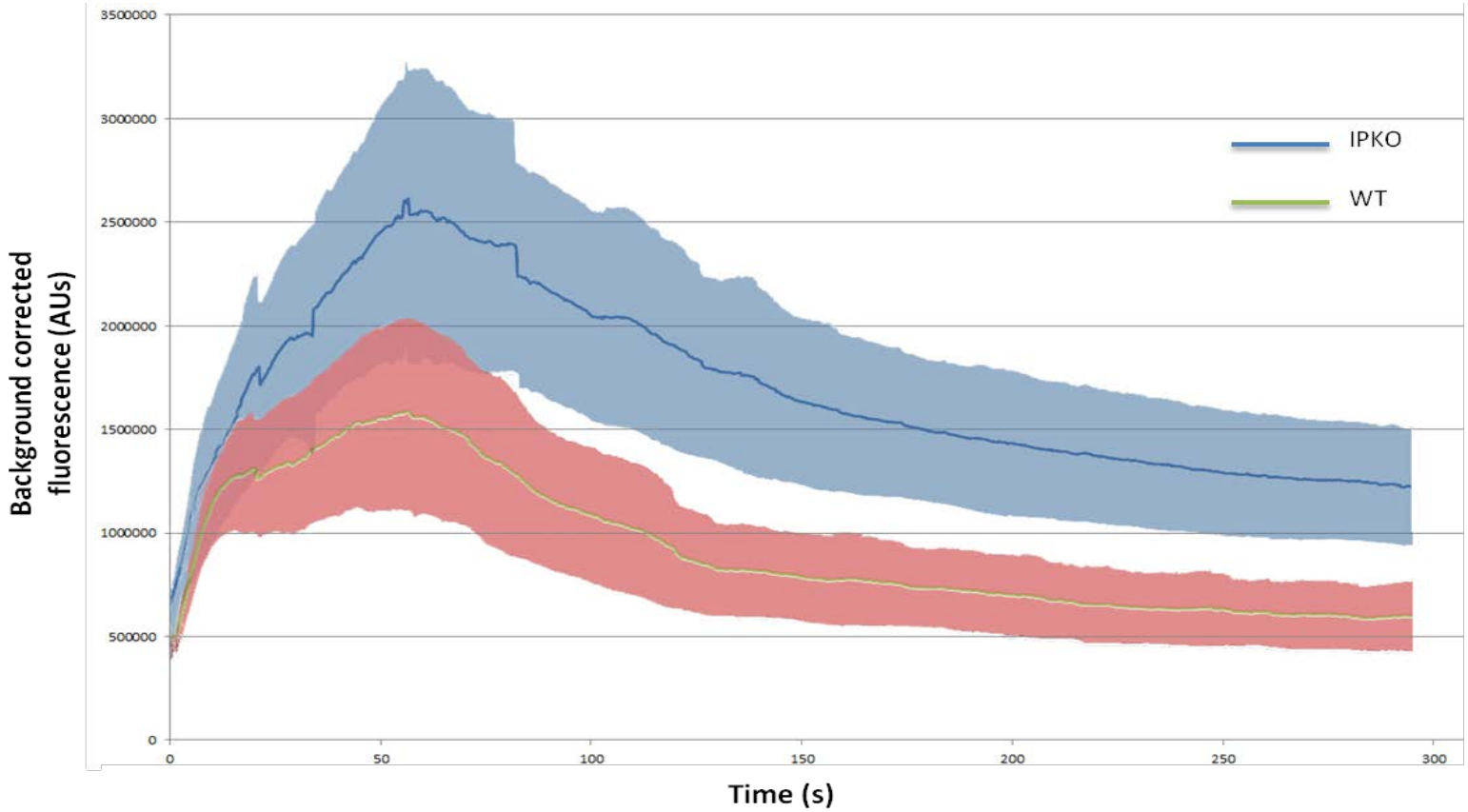
Cheng, 2006 and Yu et al JCI 115; 986-995, 2005

COX-2 derived prostacyclin modulates macrovascular thrombosis dose dependently



Cheng, Wang Science 2002
Yu et al JCI 2005
Yu, Riciotti et al STM 2012

Microvascular thrombosis: Deletion of the prostacyclin receptor augments platelet deposition and reduces disaggregation



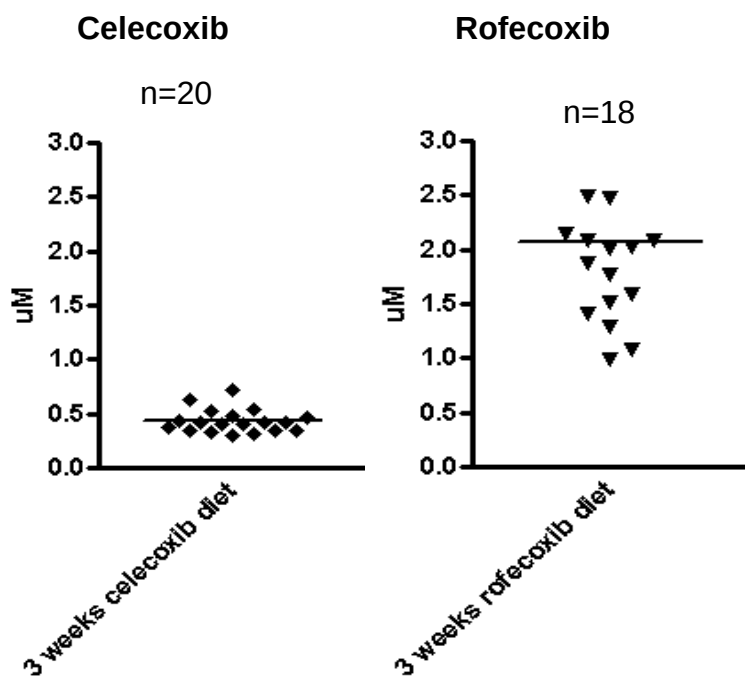
IPKO

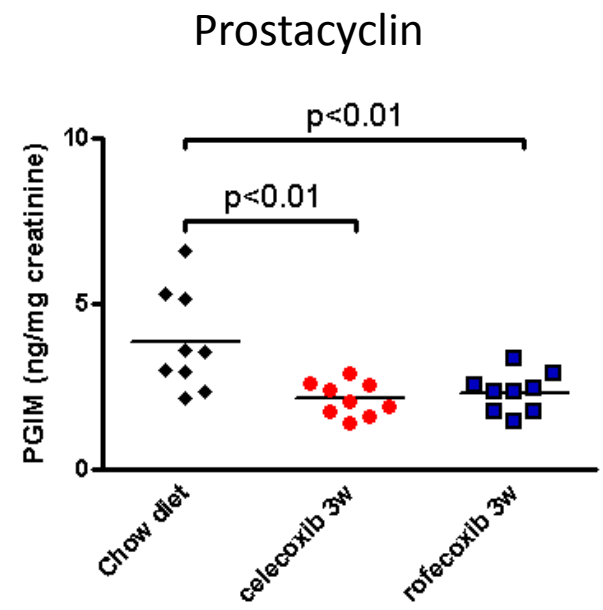
WT

WT: 10 mice 112 injuries
IPKO: 10 mice, 135 injuries

Mike Adam, Emanuela Ricciotti, 2013

Plasma concentrations and urinary metabolites measured after celecoxib (100 mg/kg/day) or rofecoxib (50 mg/kg/day).

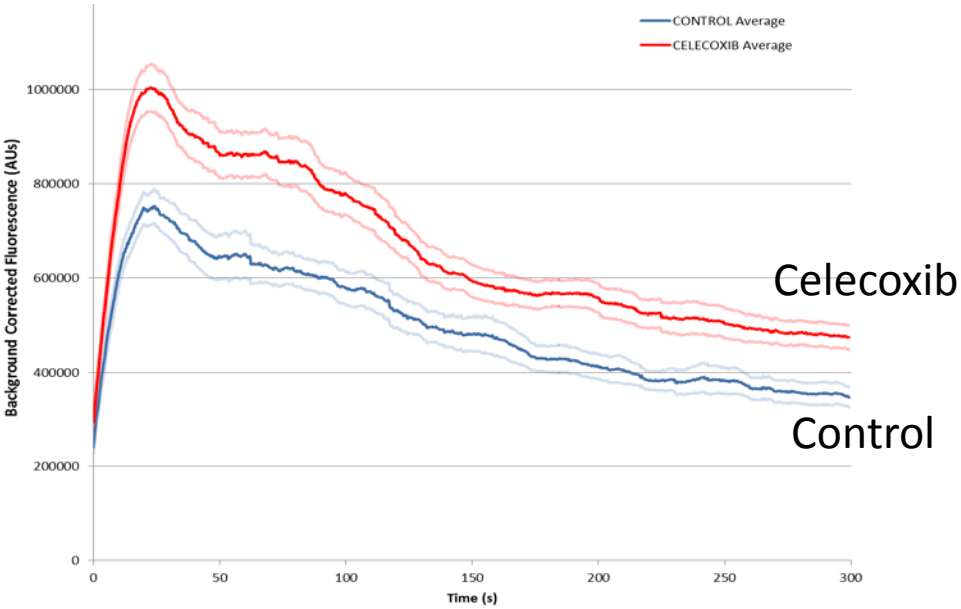




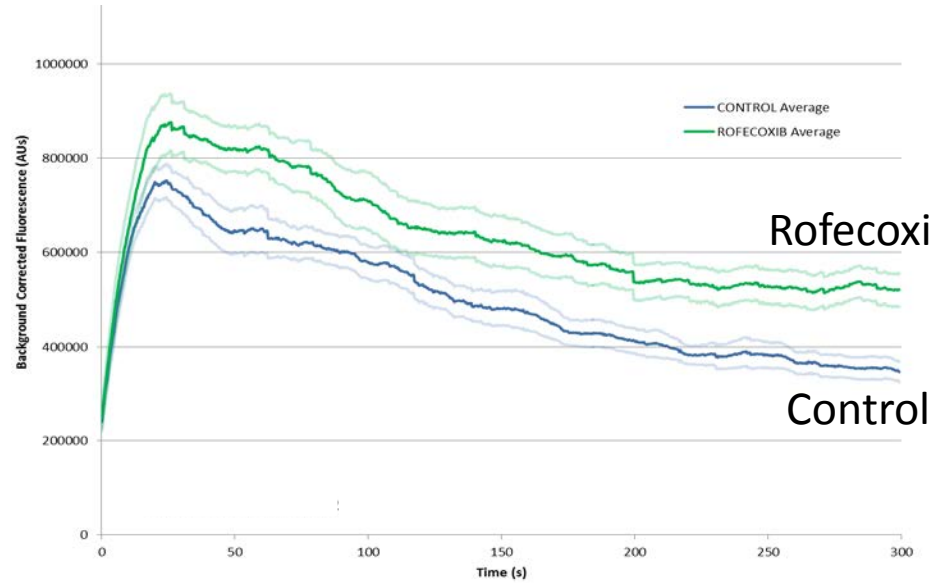
Emanuela Ricciotti

Celecoxib and rofecoxib augment platelet deposition and reduce disaggregation

Celecoxib



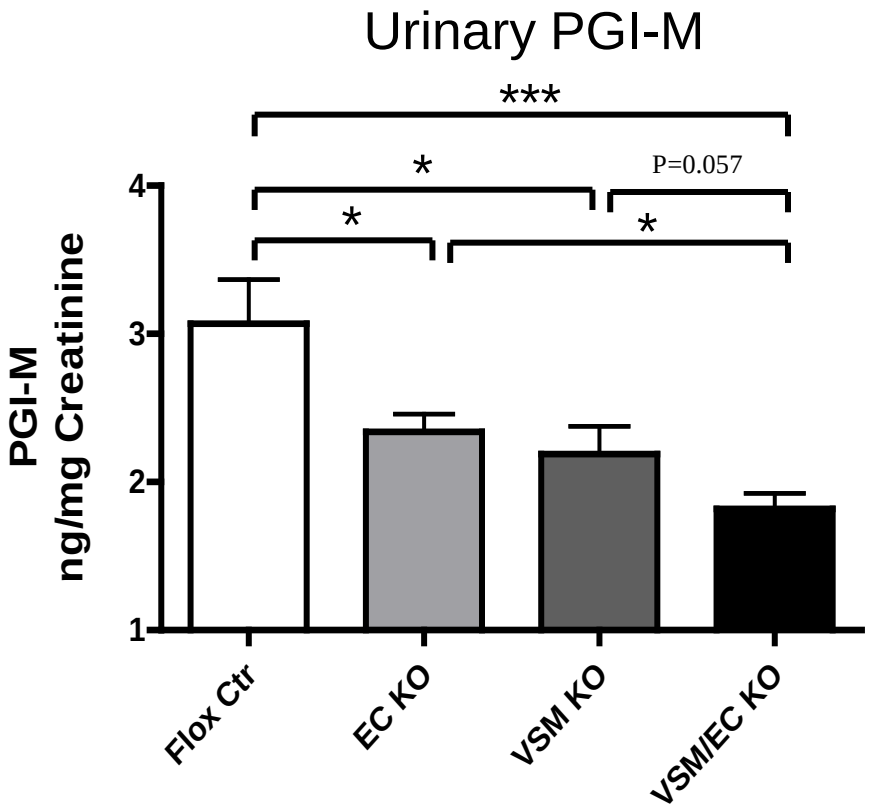
Rofecoxib

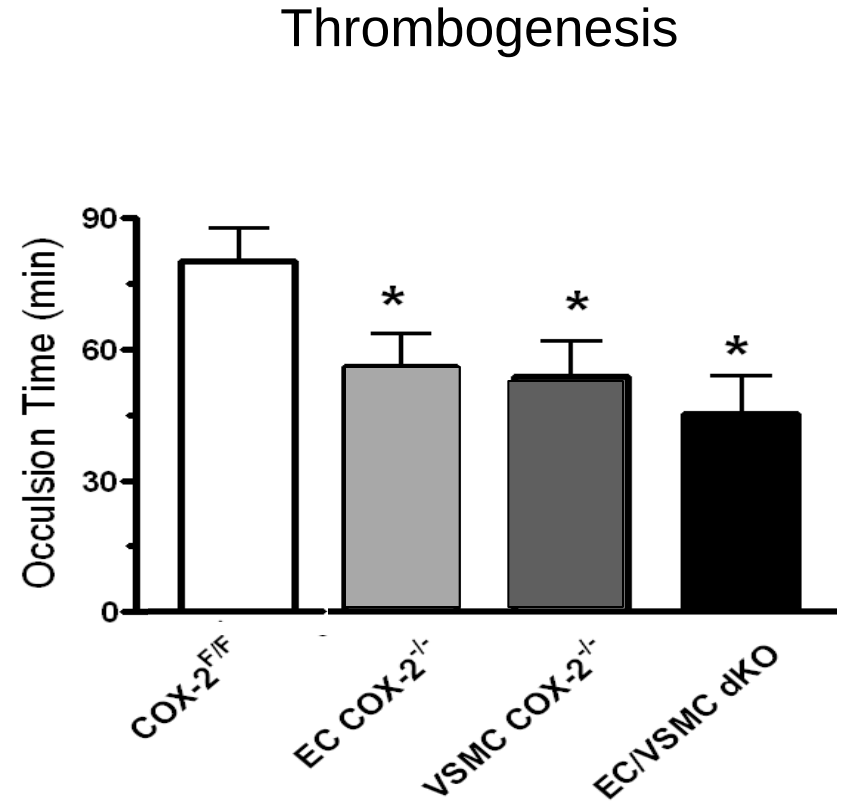


Control: n=10 mice, 110 injuries
Celecoxib: n=10 mice, 122 injuries
Rofecoxib: n=8 mice, 96 injuries

Mike Adam, Emanuela Ricciotti, 2013

Vascular COX-2 contributes to urinary PGI-M and restrains thrombosis

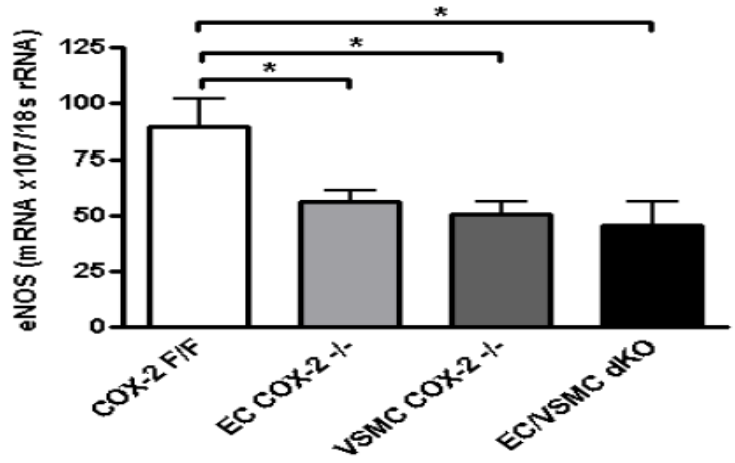


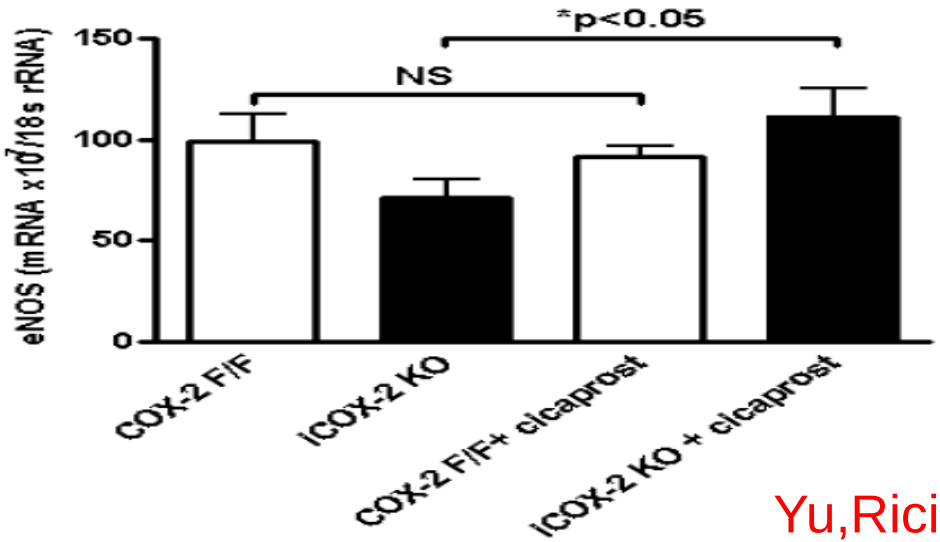


*, p<0.05; ***, p<0.001, n=16-22

Yu et al Science Trans Med 2012

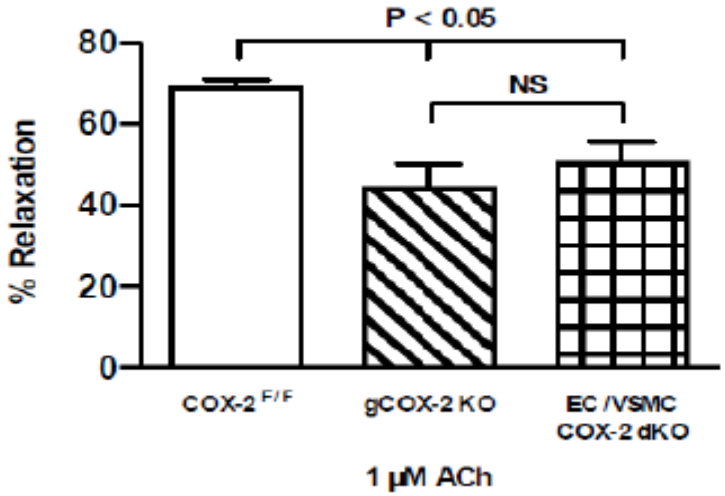
Vascular COX-2 regulates expression of eNOS

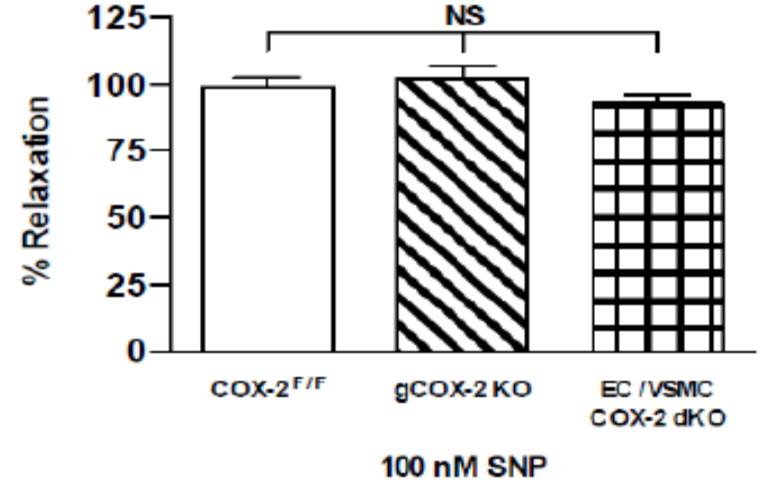


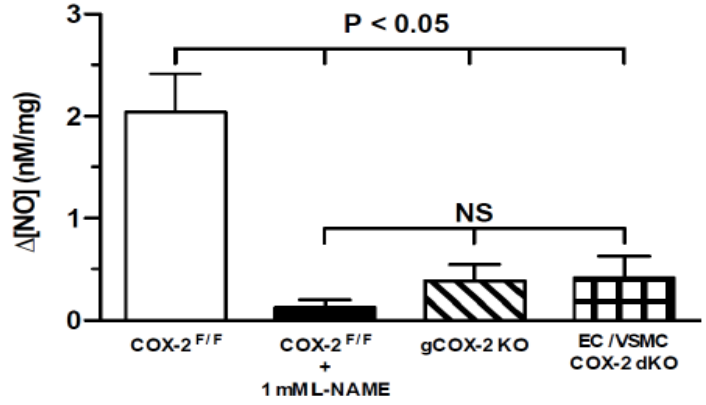


Yu,Riciotti et al STM 2012

Vascular COX-2 deletion not compensated for by increased release of NO; au contraire





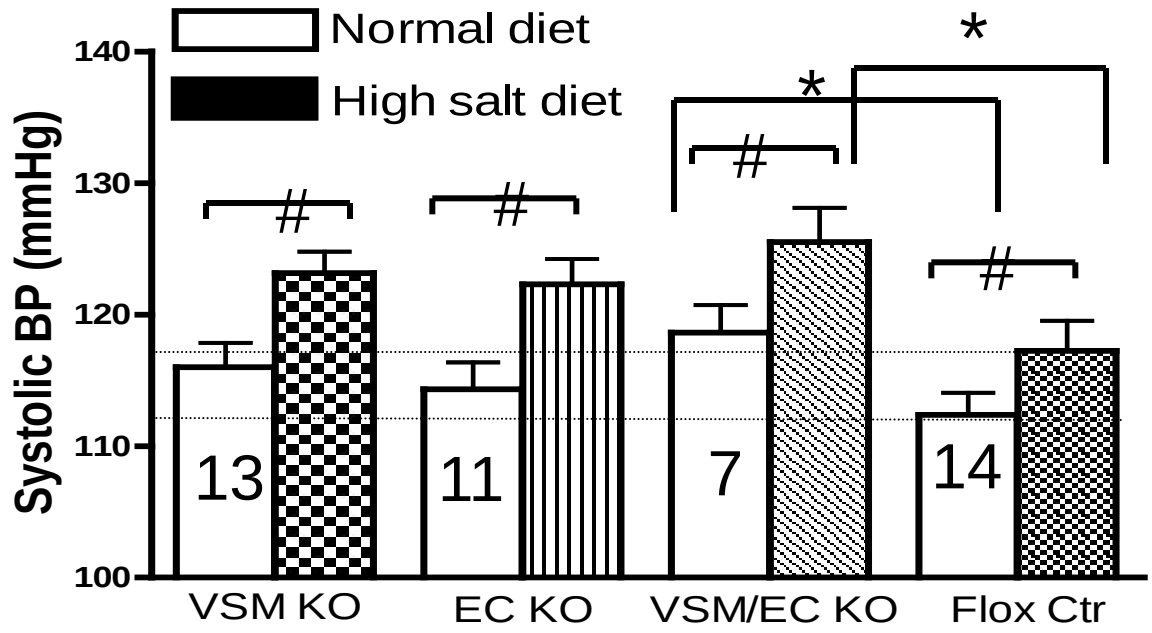


Yu,Riciotti et al STM 2012

MECHANISM BASED CARDIOVASCULAR HAZARD - 1

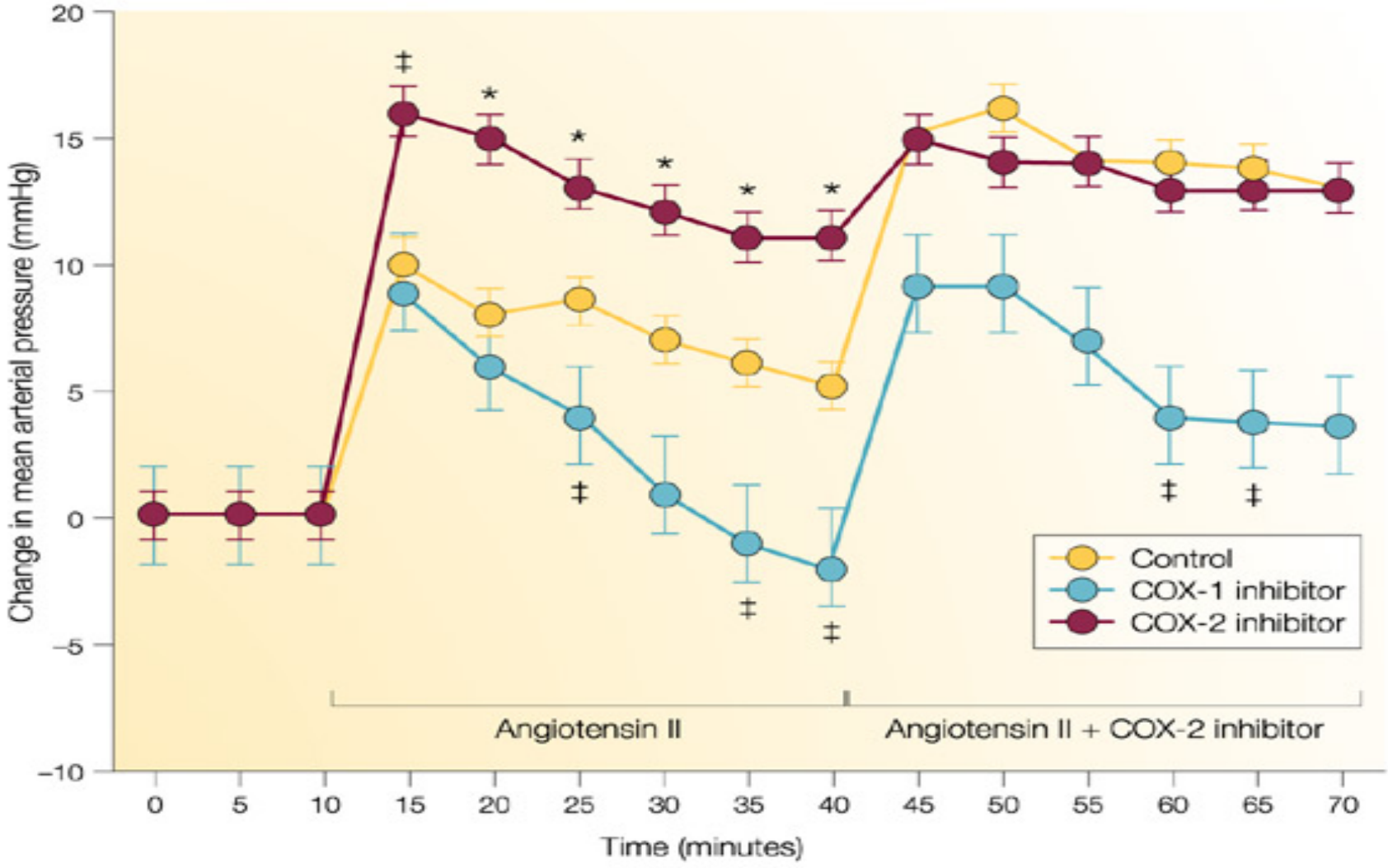
- PROSTACYCLIN RESTRAINS PLATELET ACTIVATION AND THROMBOGENESIS IN VIVO
- SUPPRESSION OF VASCULAR PROSTACYCLIN DOES NOT CAUSE SPONTANEOUS THROMBOSIS , BUT AUGMENTS THE RESPONSE TO THROMBOGENIC STIMULI IN VIVO
- NONLINEAR RELATIONSHIP BETWEEN INHIBITION OF THE CAPACITY OF PLATELETS TO MAKE Tx AND Tx DEPENDENT PLATELET FUNCTION
- A SIMILAR THROMBOTIC HAZARD FROM CELECOXIB AND ROFECOXIB DESPITE SOME DIFFERENCE IN SELECTIVITY

Deletion of Vascular COX-2 elevates Blood Pressure



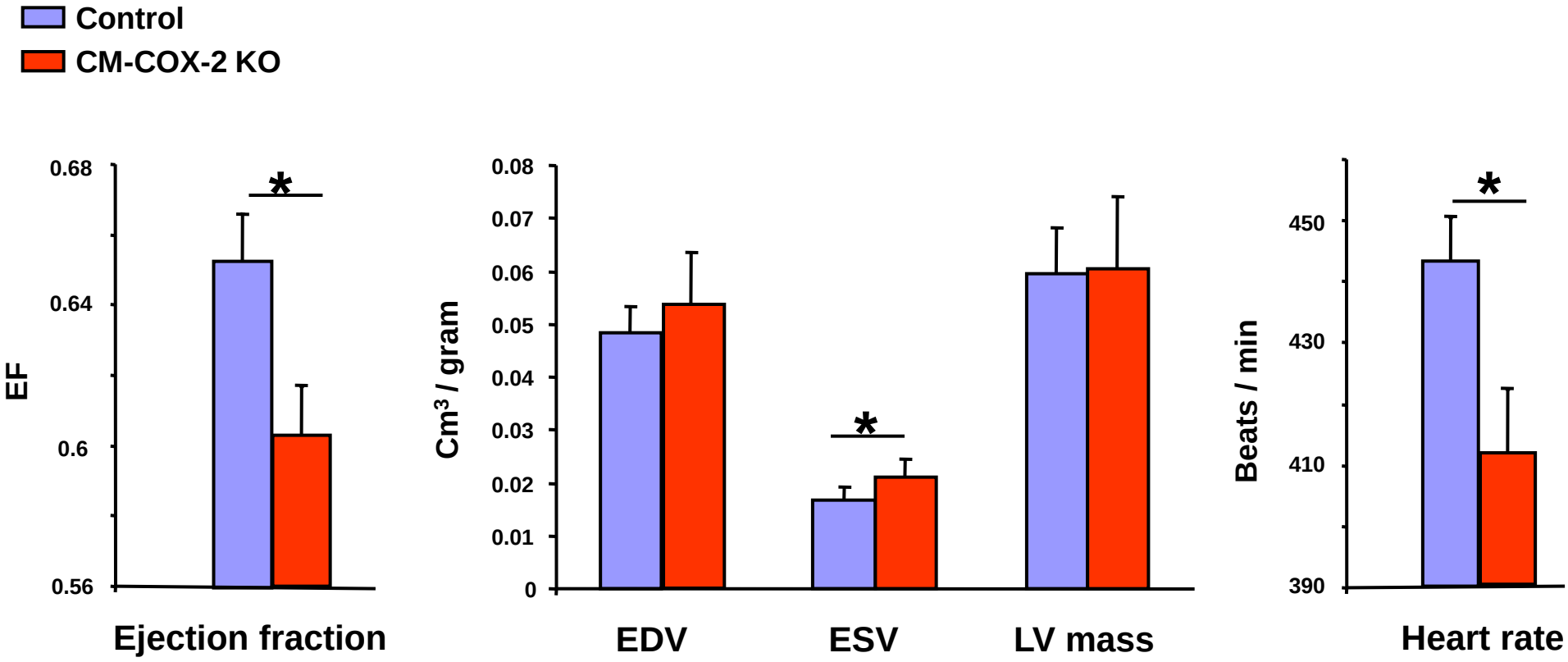
Yu, Riciotti et al STM 2012

#, p<0.01 *, p<0.05



Breyer and colleagues 2002

Mild Cardiac Failure in Cardiomyocyte Specific Deletion of COX-2



Wang D et al PNAS 2009 106(18):7548-52

n=9-11, * p<0.05

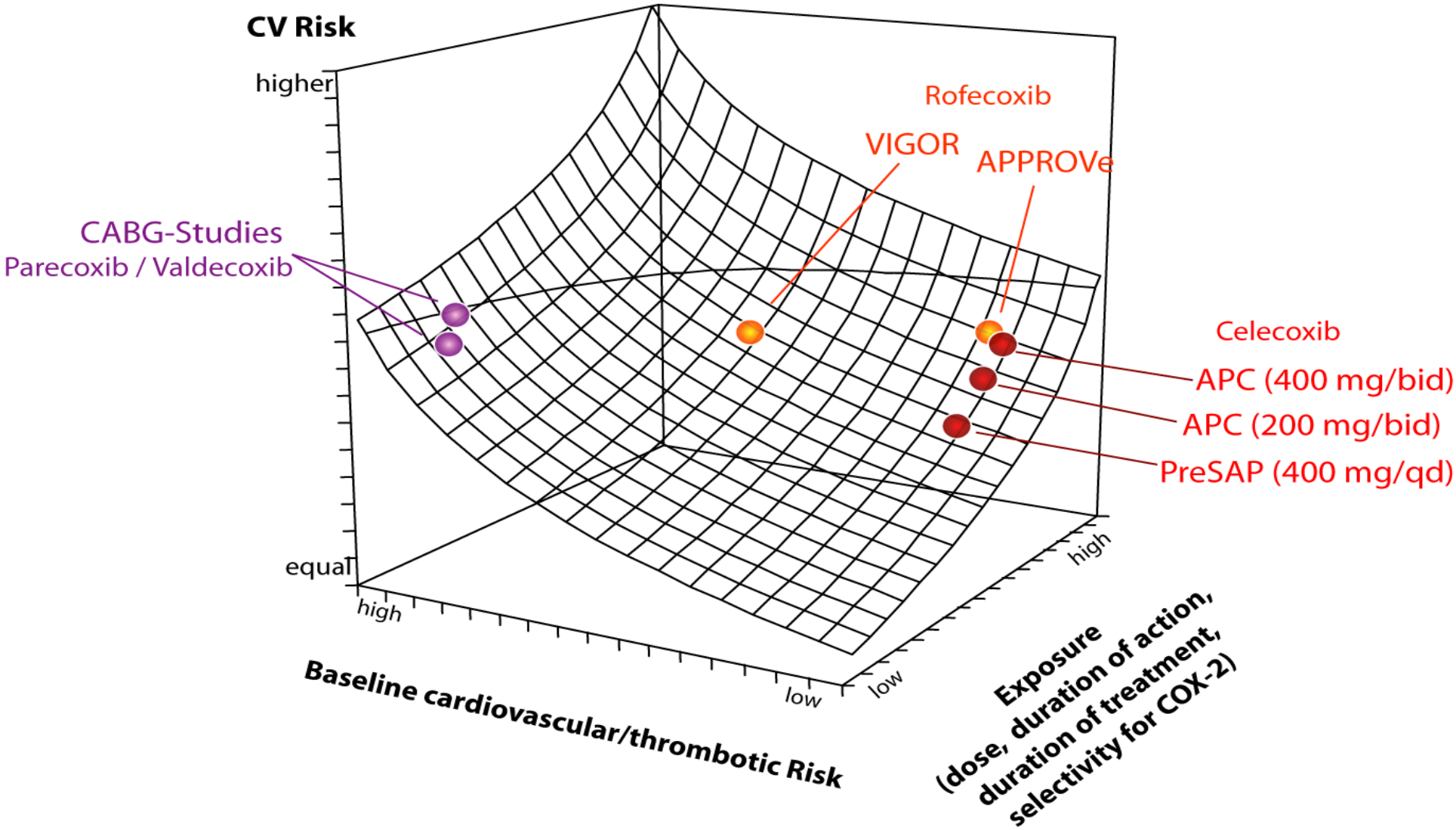
Arrhythmia Inducibility in *COX-2* CKO Mice

	COX-2 CKO (n=10)	Wild-type (n=9)
Mice with AT	1/10	0/9
Episodes VT	17*	3
Duration VT (s)	1.024 ± 0.957	0.410 ± 0.297
VT CL (ms)	50.7 ± 2.82	50.3 ± 0.40
Mice with VT	4/10*	1/9
Age (d)	50.5 ± 3.9	49.3 ± 4.7
Weight (g)	23.1 ± 2.8	23.6 ± 3.0

*p<0.05 compared to Wild-type

PNAS 106(18):7548-52, 2009

Factors apparently conditioning CV-risk detection in randomized controlled trials



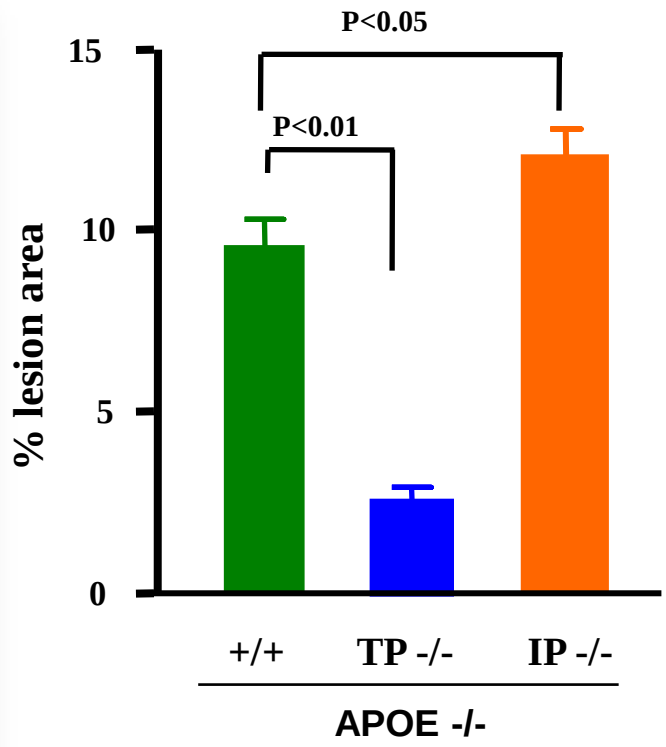
Modified from Grosser, Fries, FitzGerald, J Clin Invest, 116:4-15, 2006

PGI_2 and COX-2 deletion accelerate the initiation of Atherosclerosis

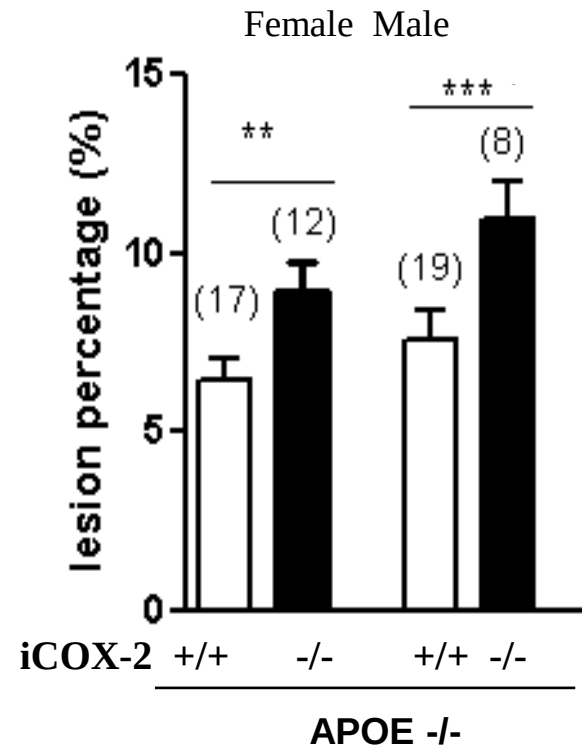
LDLRKO

LDLRKO/IPKO

Egan et al,
Science 10;306(5703):1954-7, 2004

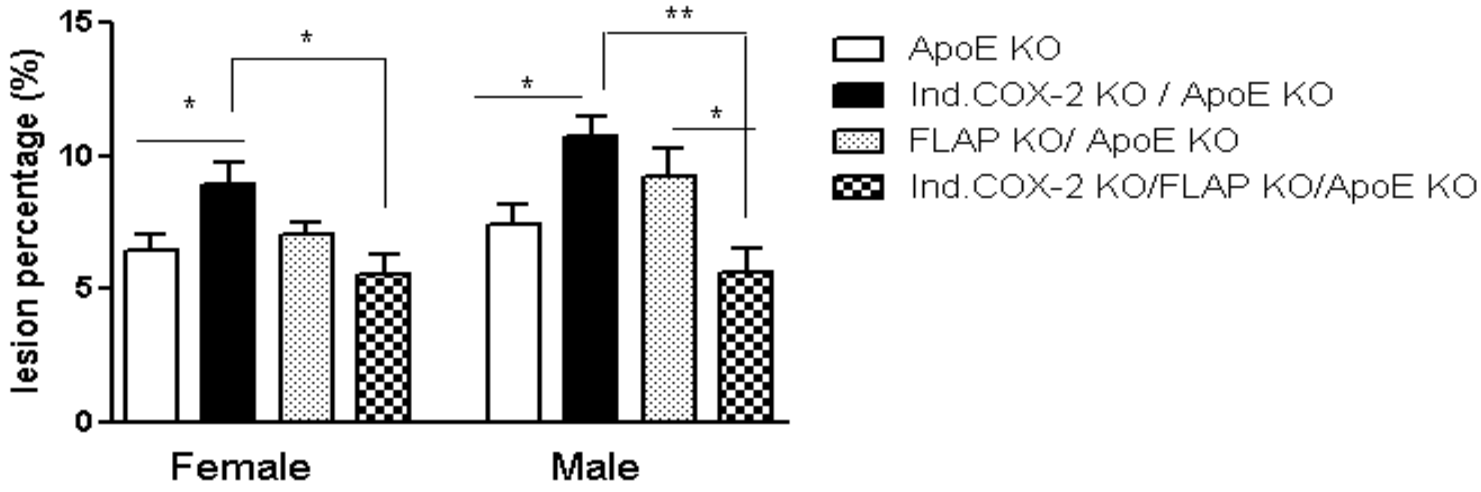


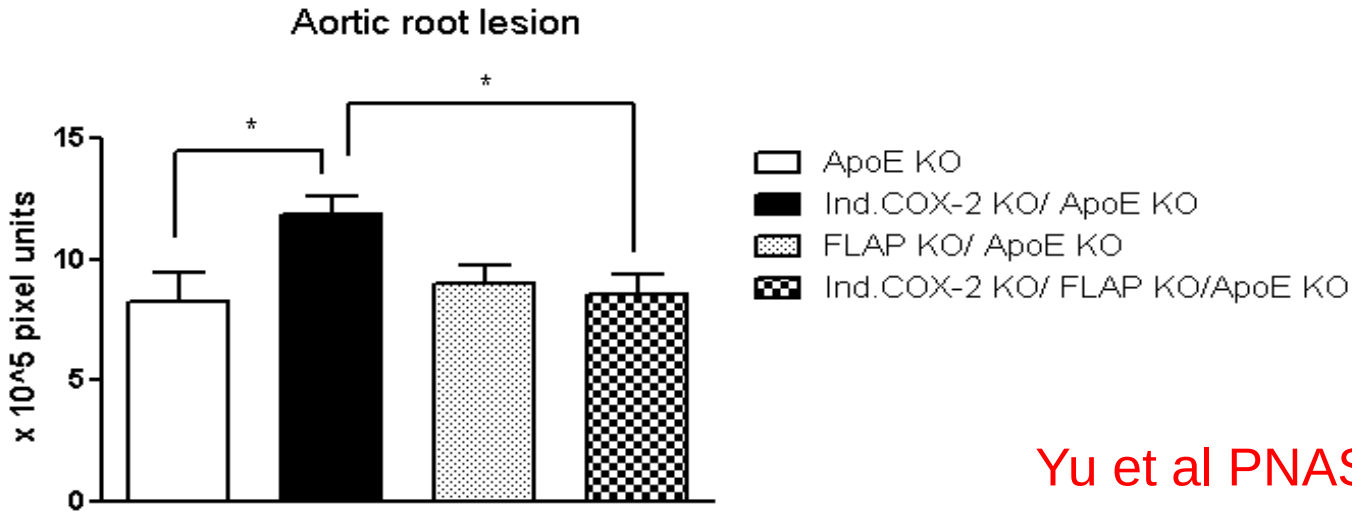
Kobayashi et al,
JCI 114: 784-94, 2004



Yu et al, PNAS 109(17):6727-32, 2012

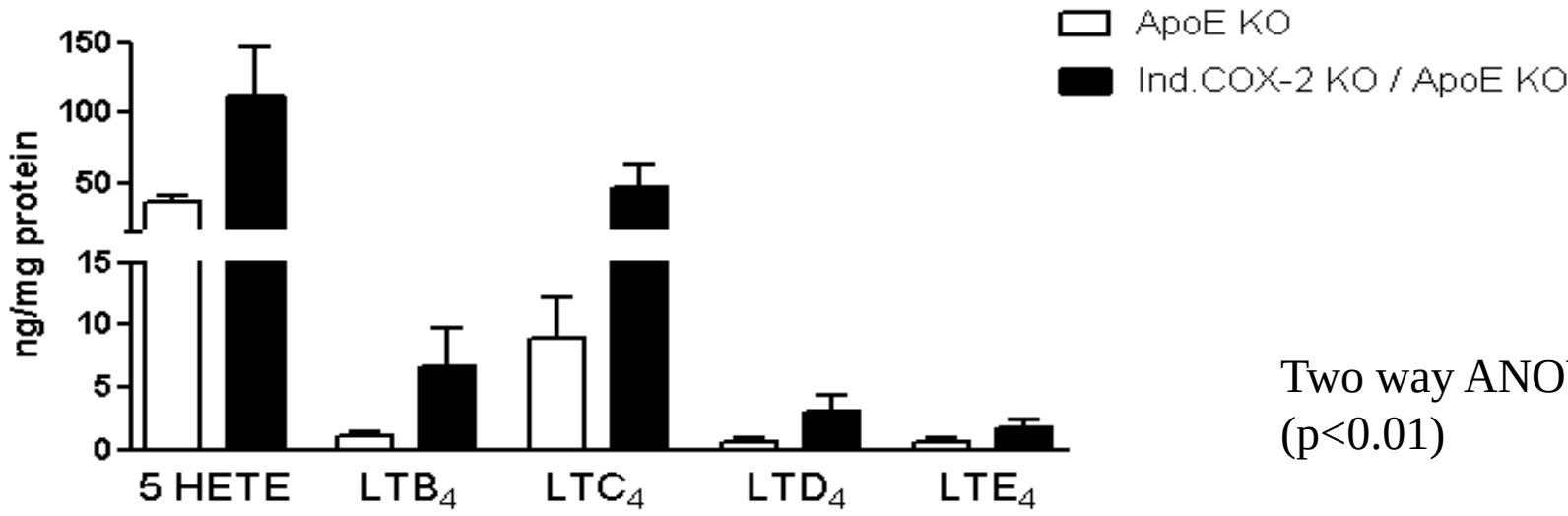
FLAP deletion attenuates atherogenesis consequent to COX-2 deletion





Yu et al PNAS 2012

Substrate shift to LT formation in COX-2 KO mice



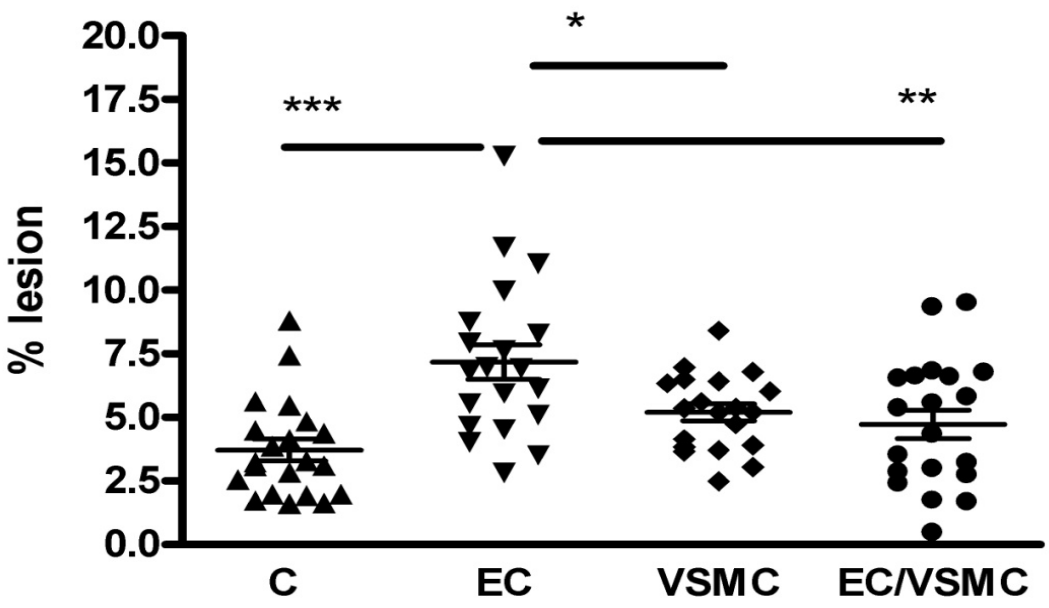
Two way ANOVA
($p<0.01$)

Yu et al PNAS 2012

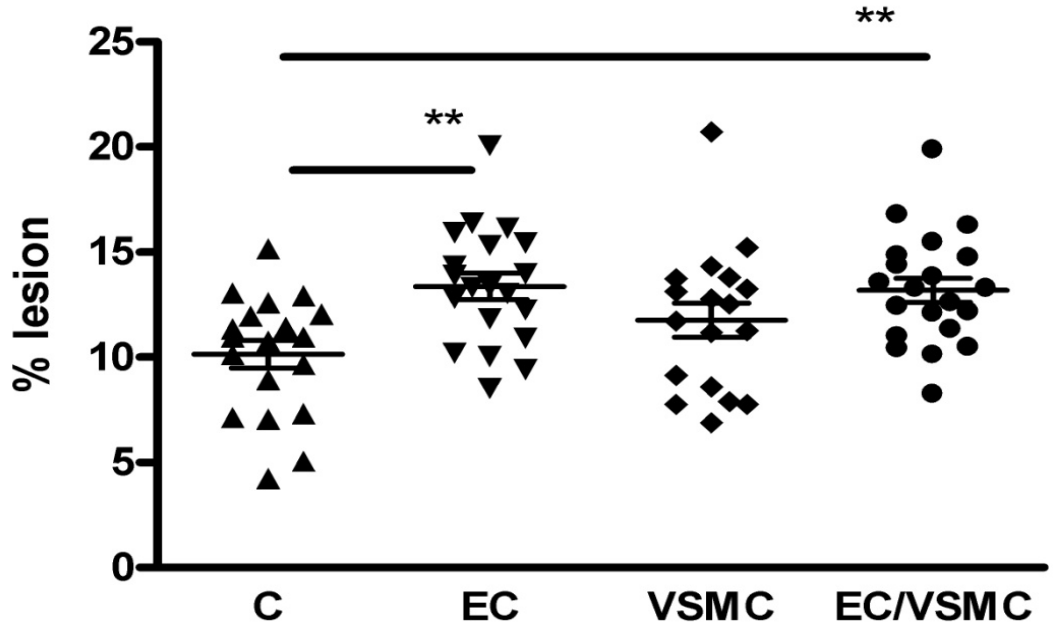
Both Endothelial and Vascular Smooth Muscle Cell COX- 2 restrain Atherogenesis in Hyperlipidemic Mice

En face lesion analysis
(means ± S.E.M.)

Male (3 months HFD)



Female (6 months HFD)

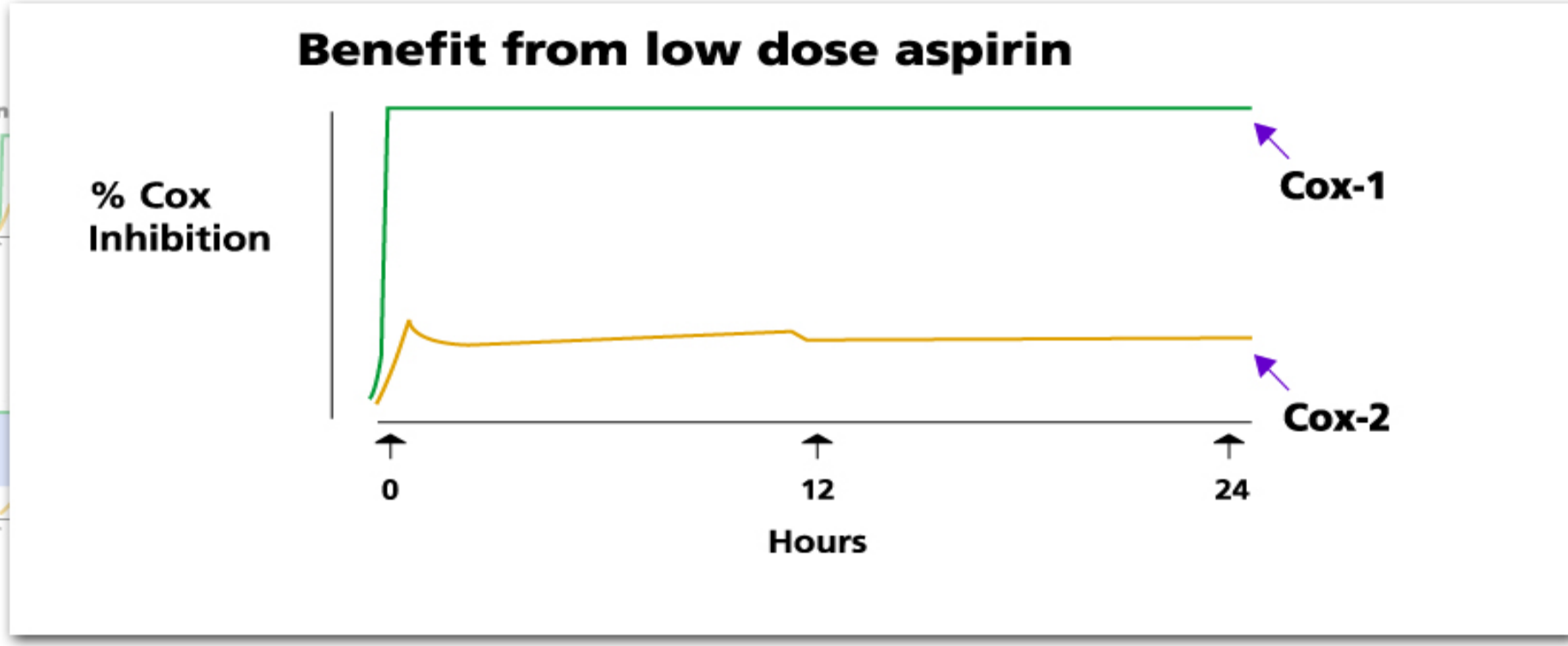


Tang et al Circulation (in press) 2014

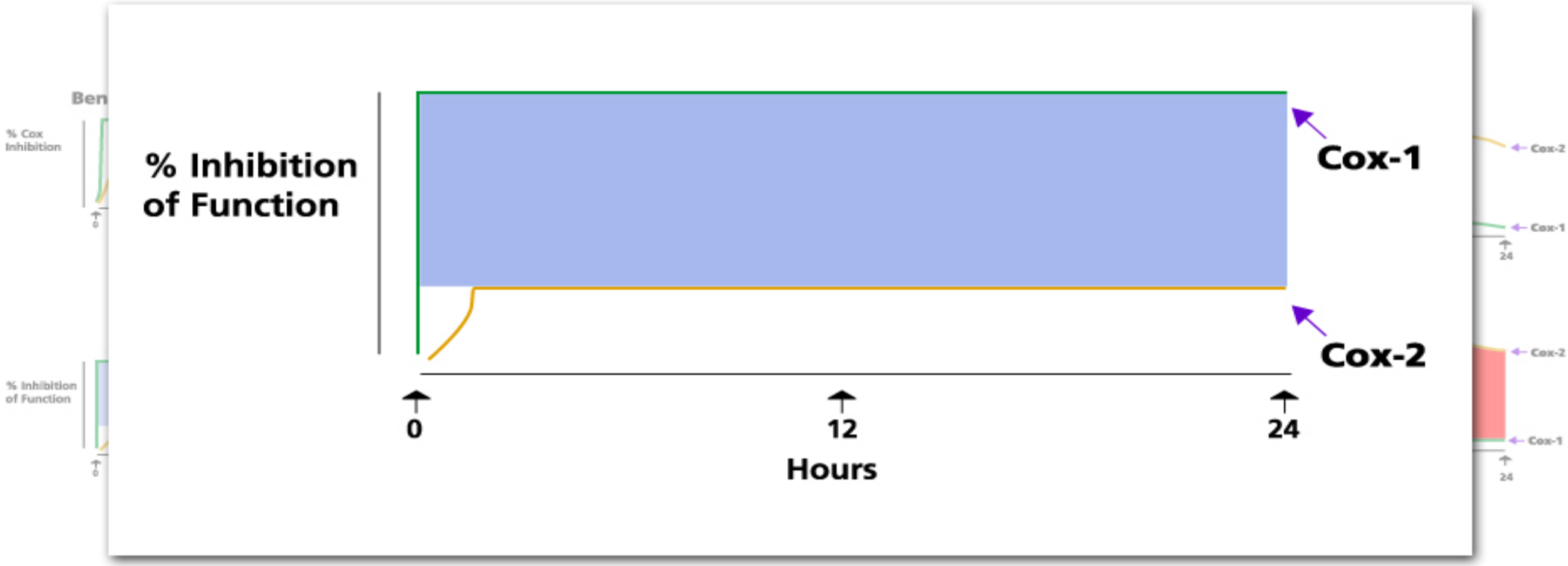
MECHANISM BASED CARDIOVASCULAR HAZARD - 2

- HYPERTENSION DUE TO SUPPRESSION OF COX-2 OFFSET BY INHIBITION OF COX-1
- CARDIOMYOCYTE COX-2 INHIBITION PREDISPOSES TO FAILURE AND SUDDEN DEATH
- INDUCTION AND PROGRESSION OF ATHEROSCLEROSIS MAY EXPLAIN RISK TRANSFORMATION IN SOME RCTs
- RELEVANCE TO ATHEROPROTECTION IN FEMALES

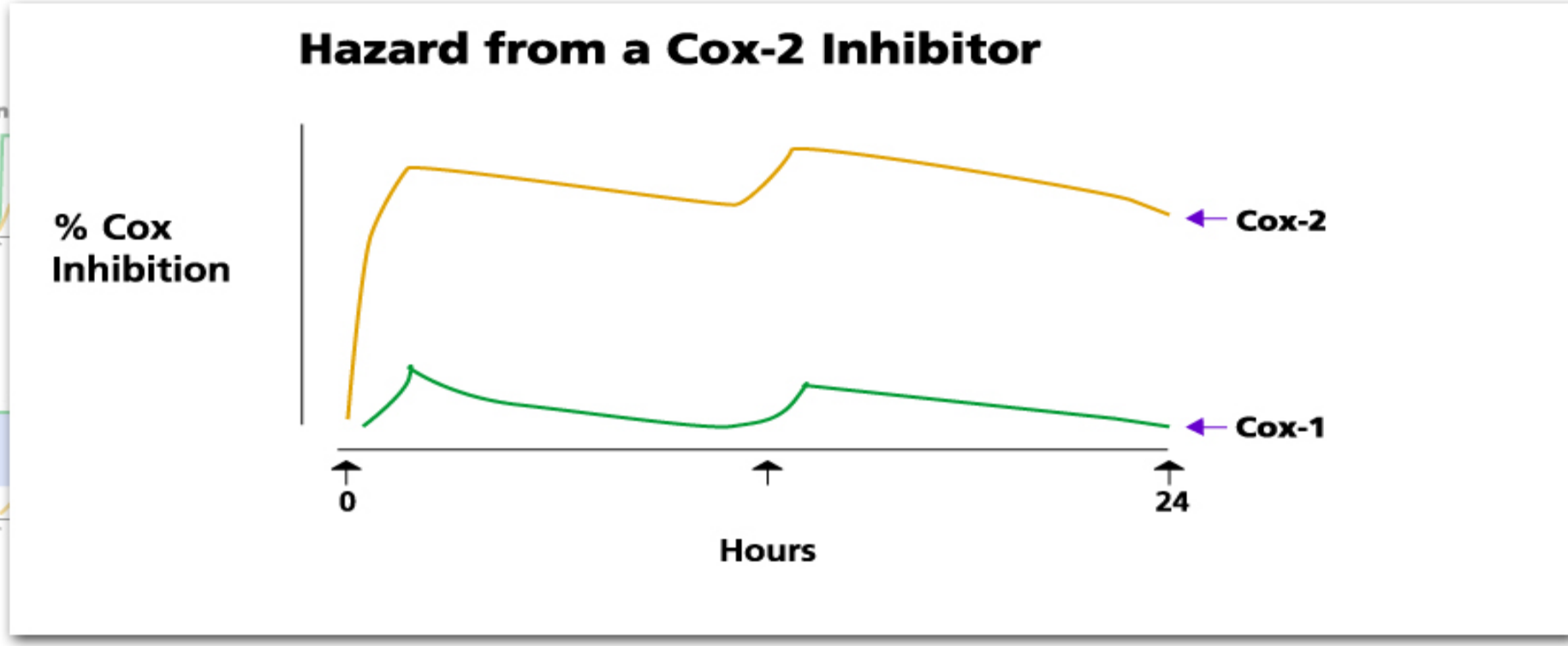
Benefits and Hazards from Coxs Inhibitors



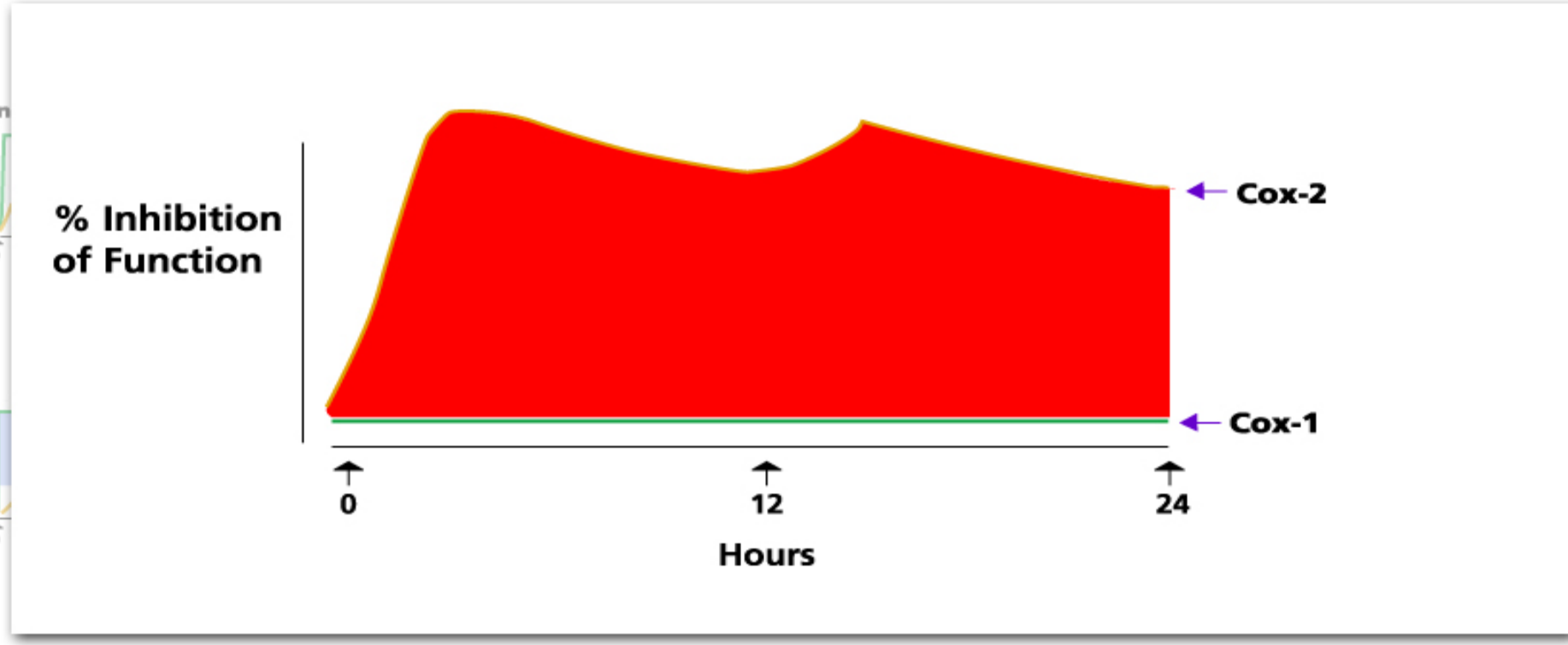
Benefits and Hazards from Coxs Inhibitors



Benefits and Hazards from Coxs Inhibitors



Benefits and Hazards from Coxs Inhibitors

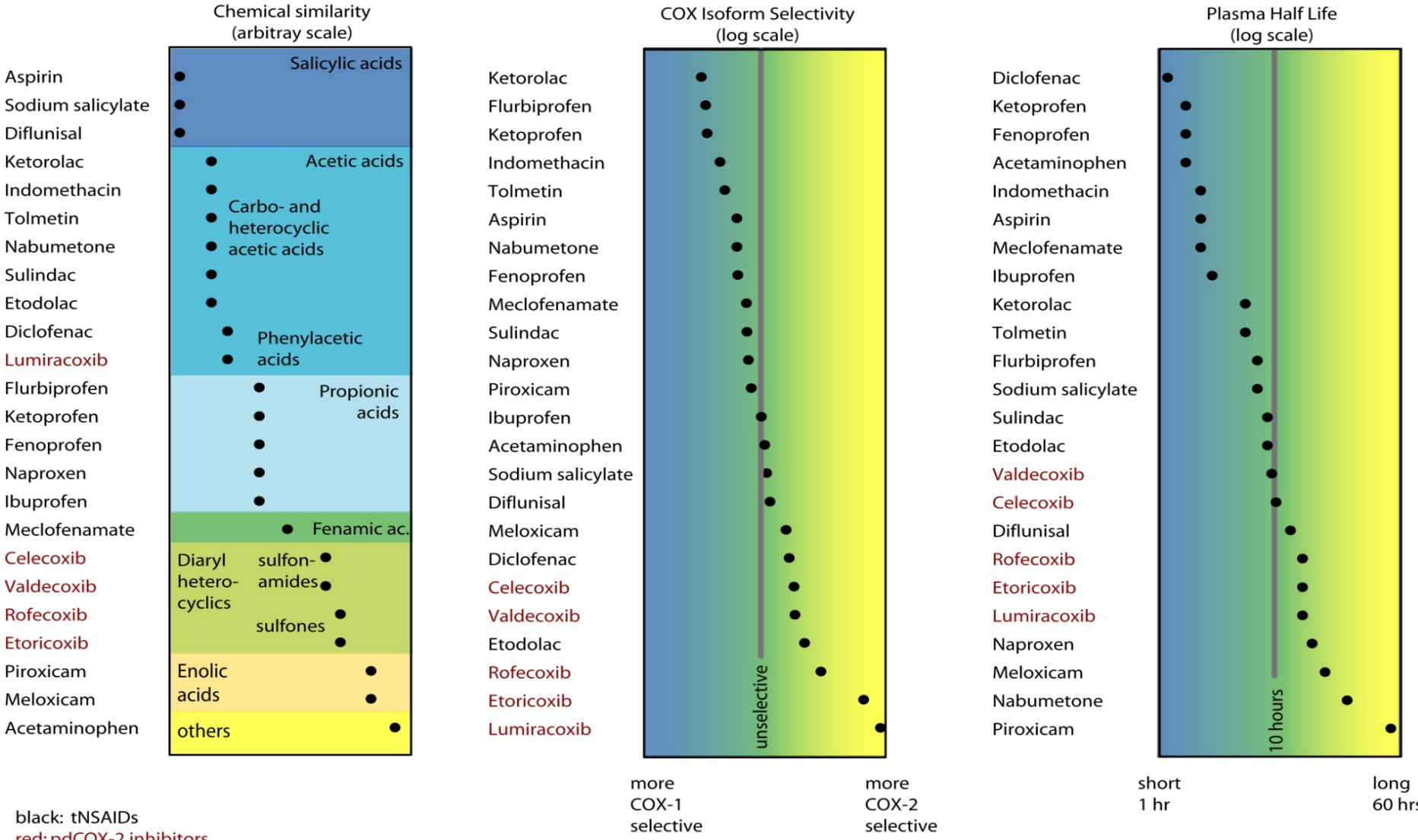


Mechanism based Predictions

- *NSAIDs selective for inhibition of COX-2 will increase the risk of thrombosis*
- *The risk from Celebrex and Vioxx will be similar*
- NSAIDs will confer a risk of hypertension that will relate to the degree of selectivity for COX-2
- *All NSAIDs will confer a risk of heart failure*
- Prolonged administration of selective inhibitors will predispose to CV risk transformation

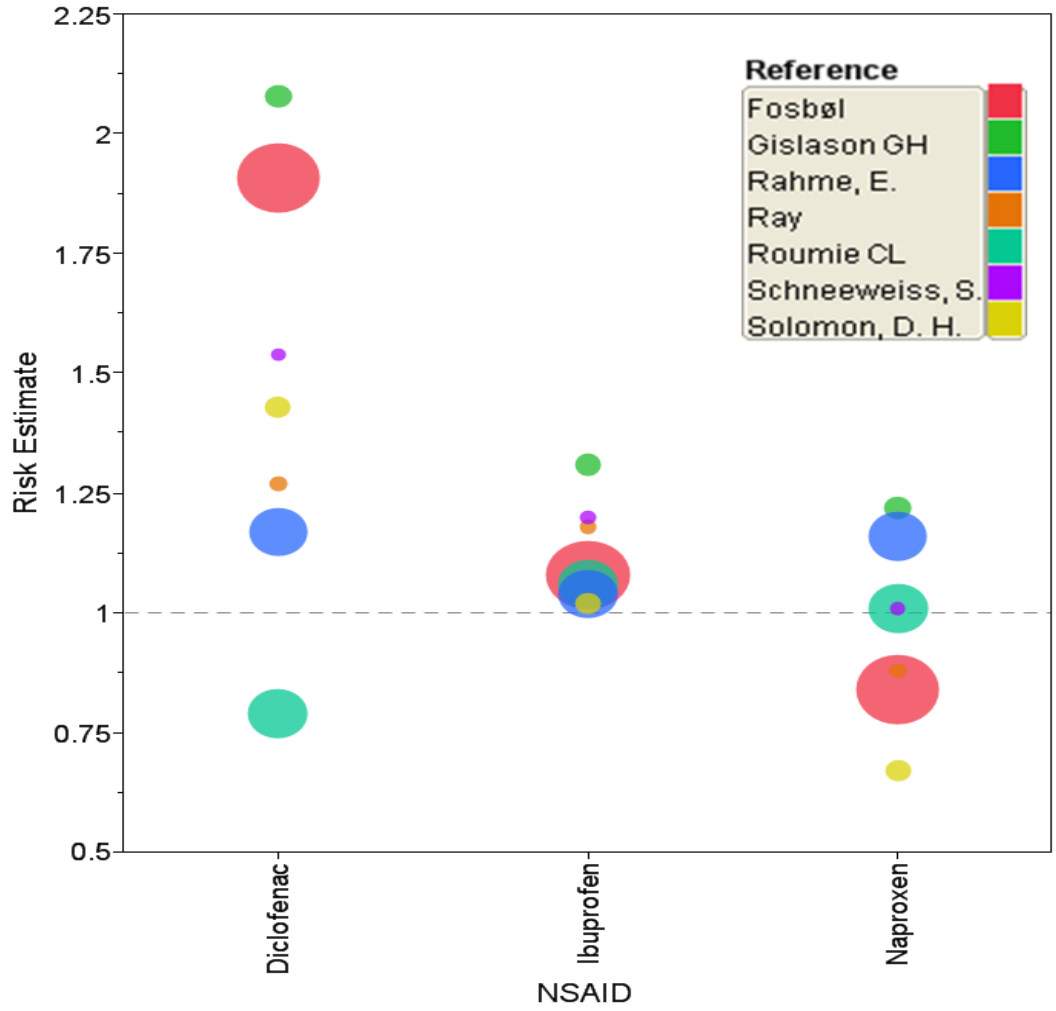
WHAT ABOUT

TRADITIONAL NSAIDs?



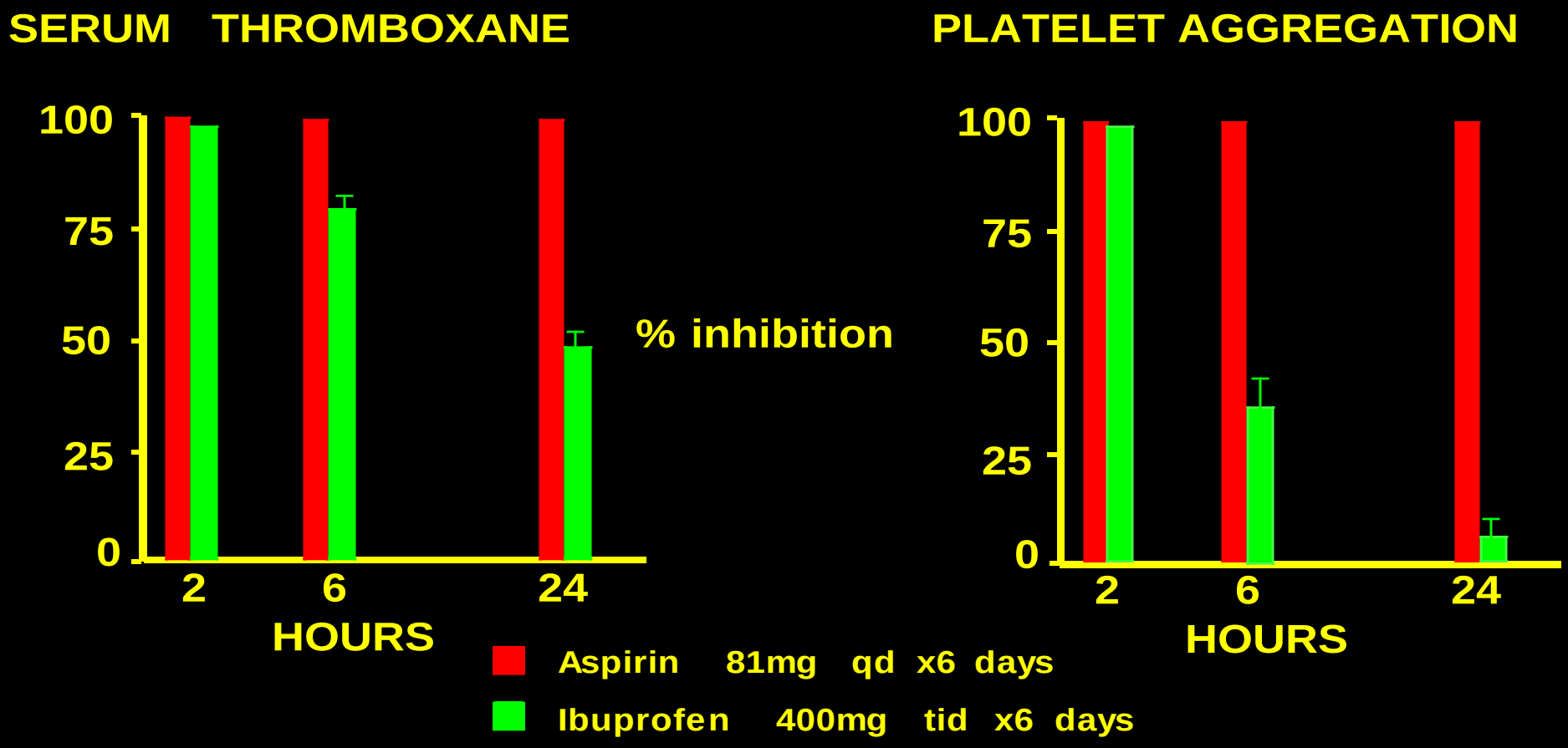
black: tNSAIDs
red: pdCOX-2 inhibitors

Heterogeneity in cardiovascular risk of traditional NSAIDs

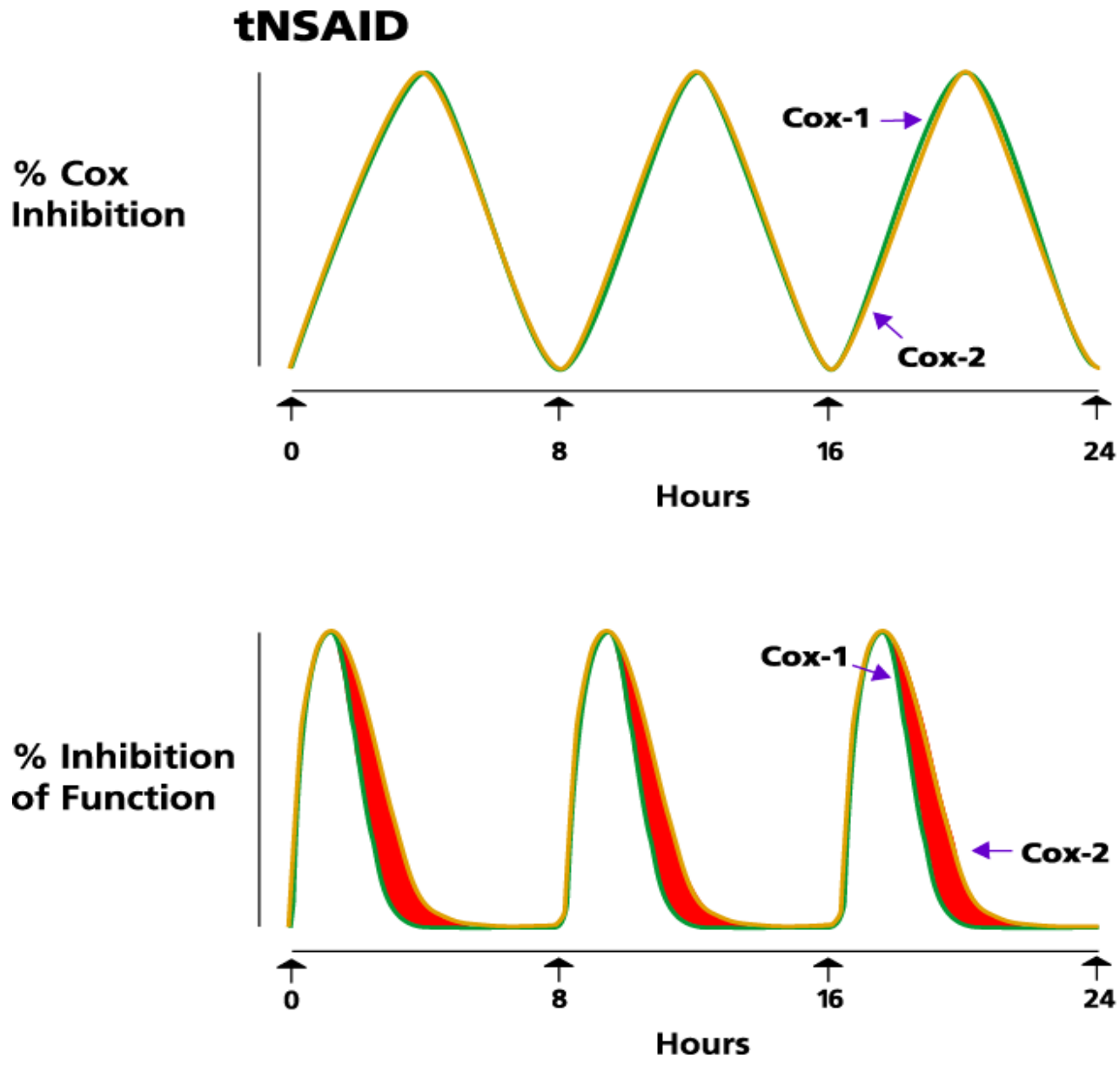


Carsten Skarke, 2013

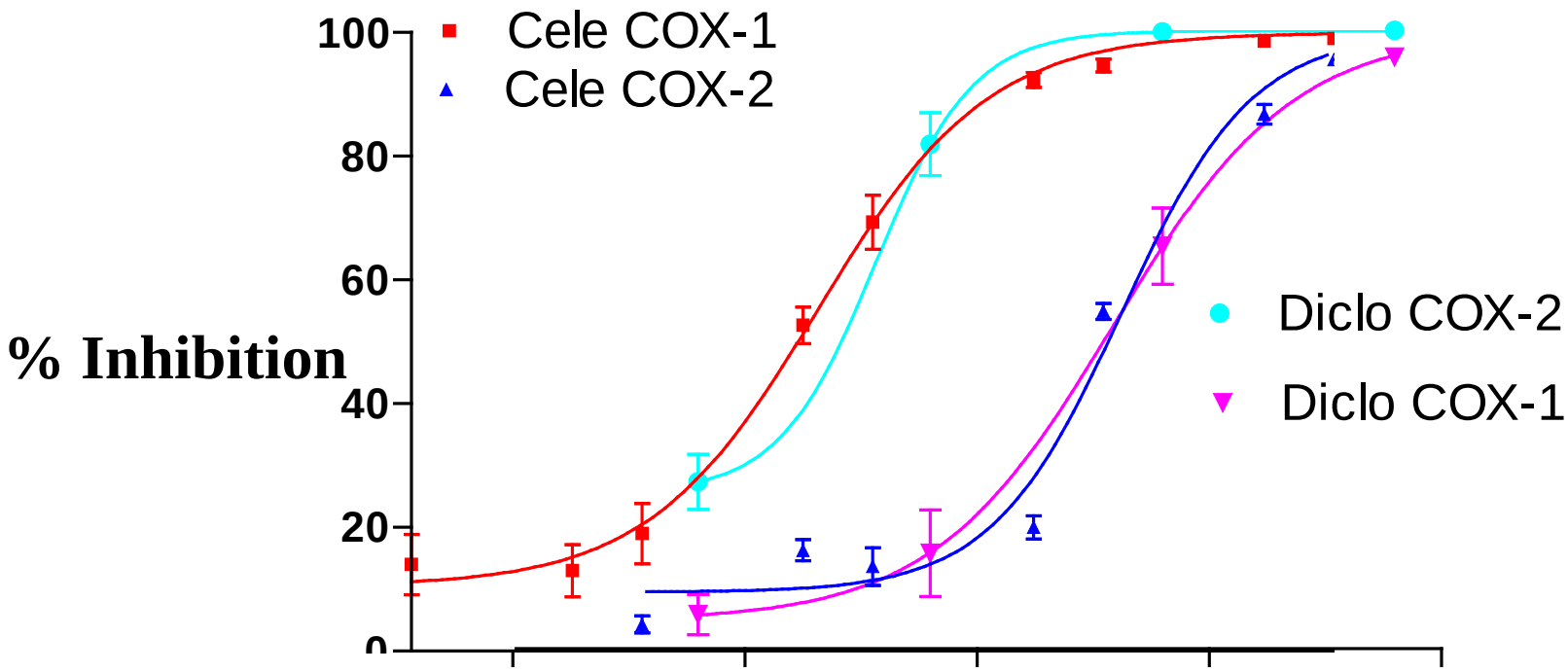
Effects of Aspirin or Ibuprofen Alone on COX-1



Catella et al NEJM 345:1809 -1817,2001

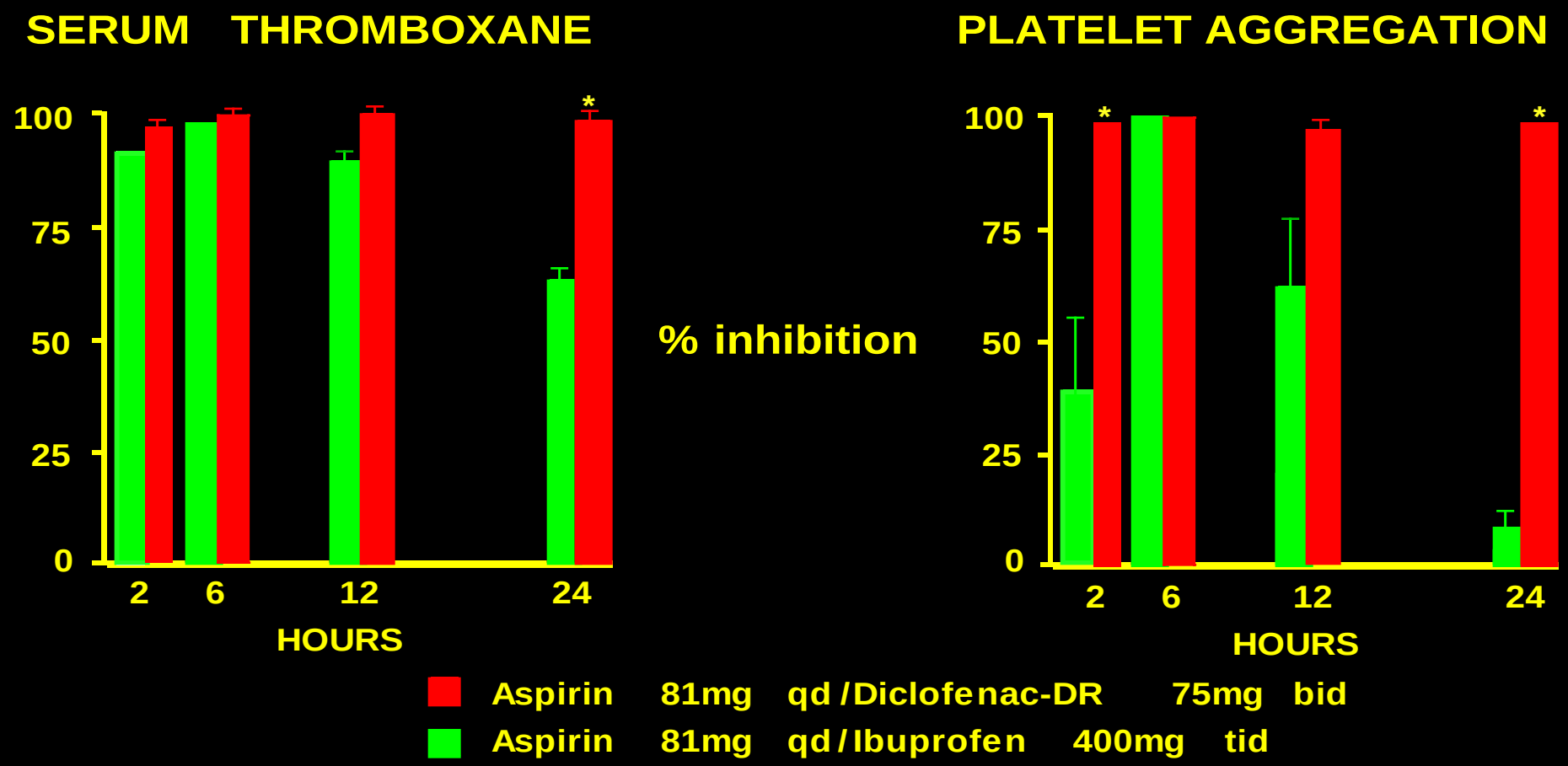

tNSAID
% Cox Inhibition
Cox-1
Cox-2
0
8
16
24
Hours
% Inhibition of Function
Cox-1
Cox-2
0
8
16
24
Hours

Diclofenac = Celebrex in whole human blood in vitro



Patrignani et al 2005

Aspirin / Ibuprofen vs Aspirin / Diclofenac

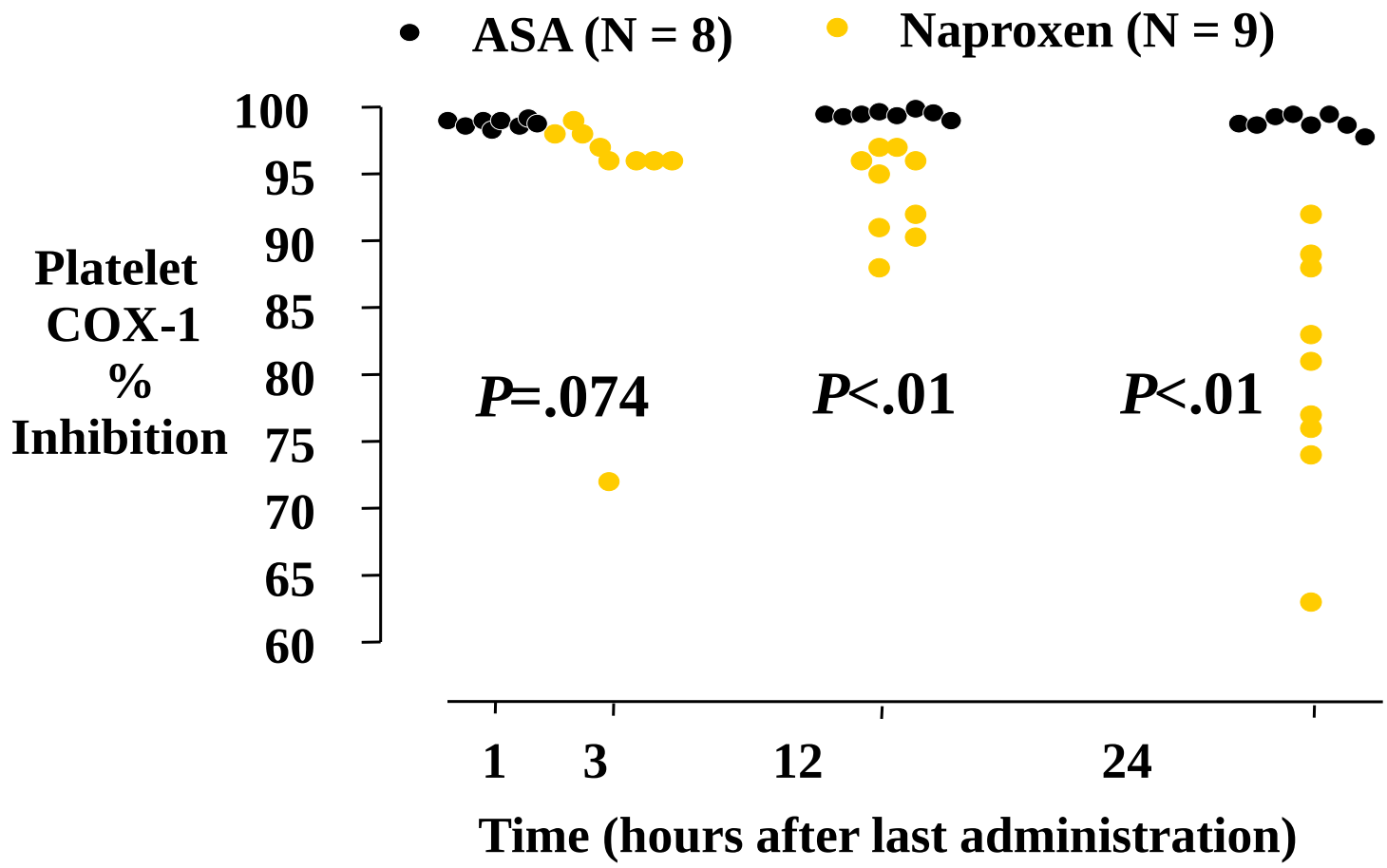


Missed message

"Thus, the inhibitory effects of daily low-dose aspirin on platelets are competitively inhibited by the prolonged use of multiple daily doses of ibuprofen, even when aspirin is administered before the first dose of the NSAID".

Catella-Lawson et al NEJM 2001

Differential Recovery From Steady-State Inhibition of Platelet COX-1 By ASA 100mg/d and Naproxen 500mg bid

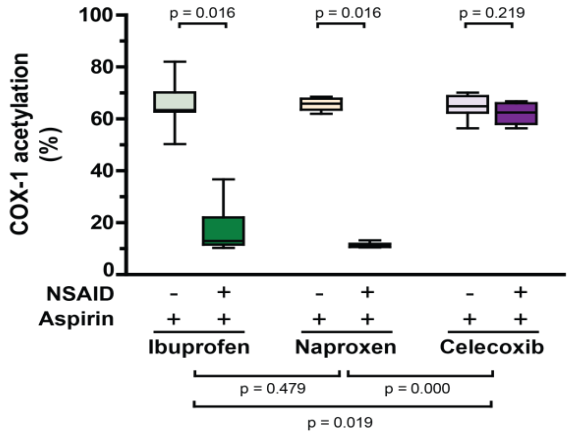


ASA = aspirin; COX = cyclooxygenase.

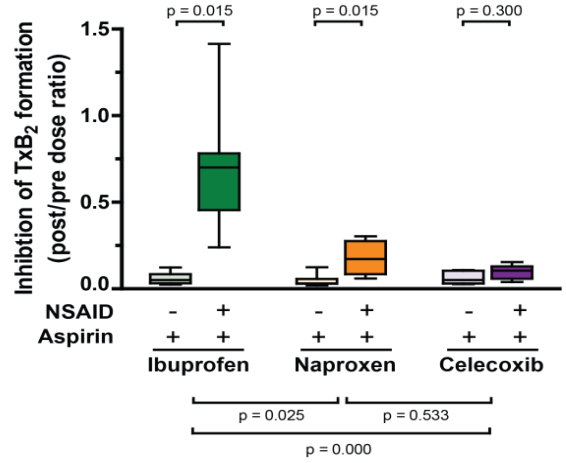
Capone et al 2003

NSAID – aspirin drug-drug interaction

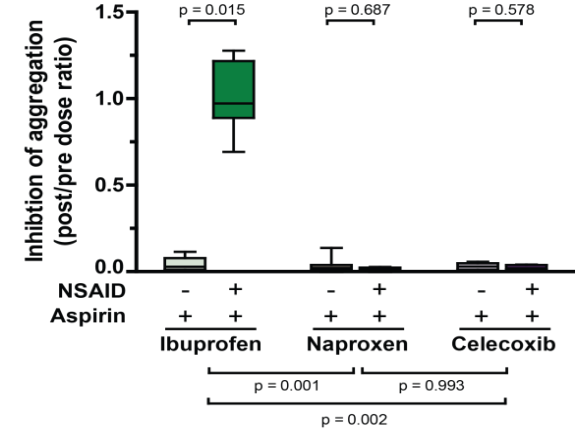
COX-1 Acetylation



Thromboxane formation



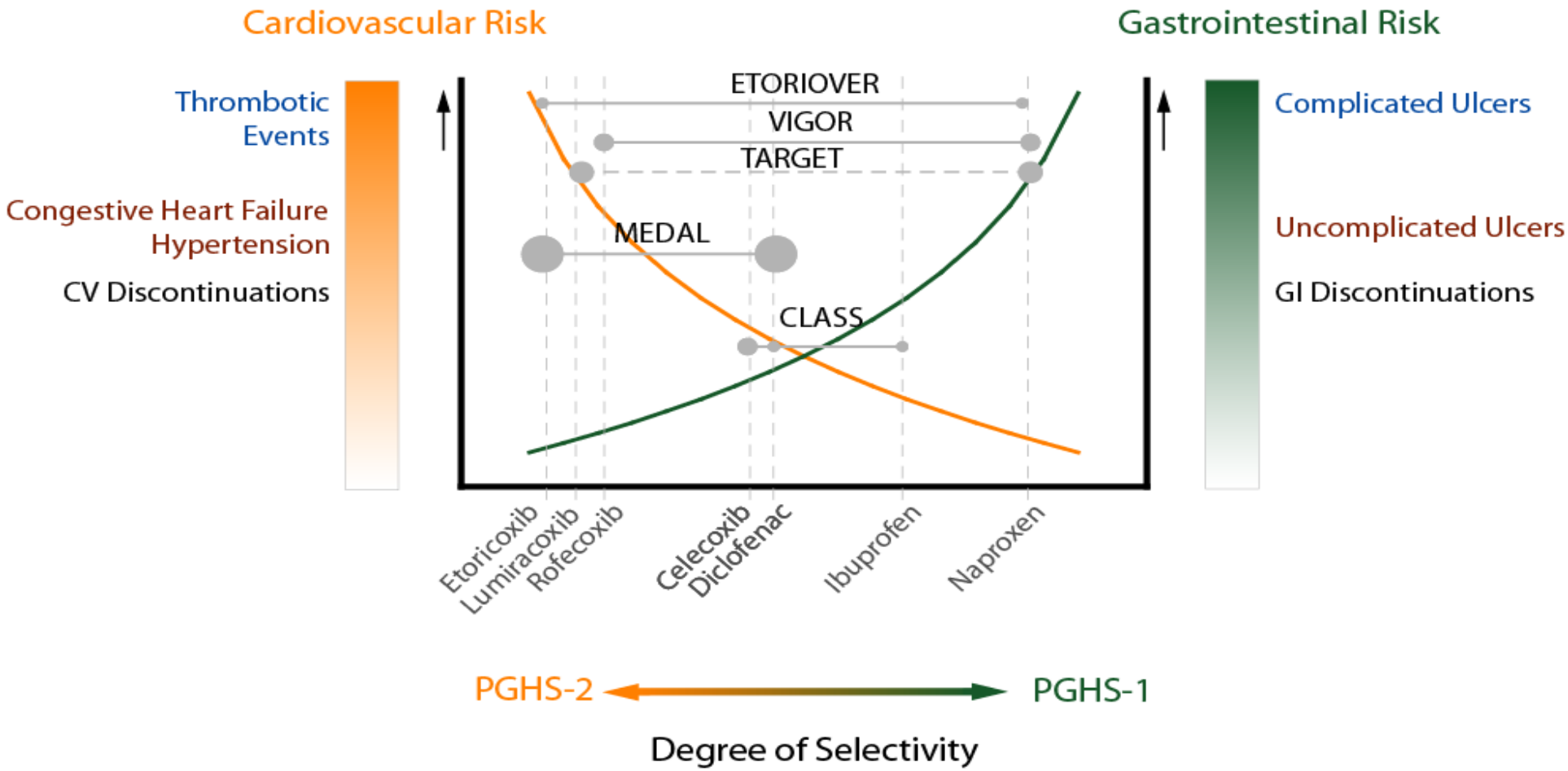
Platelet aggregation



Ibuprofen 600mg
Naproxen 500mg
Celecoxib 200mg

Relabel Naproxen?

- Extended kinetics mean that in some patients naproxen will be cardioprotective like aspirin. This may relate to dose and/or genotype.
- Naproxen may interact to undermine cardioprotection from low dose aspirin, increasing risk in patients with shorter naproxen half lives.
- It is unknown how these effects would offset each other in patients on ASA with long naproxen half lives.
- *Overall this is consistent with a "weak" aspirin signal from naproxen with wide variance around the estimate*
- Relevant discriminant information not available at the patient level



FitzGerald GA TiPS 2007.

ImPRECISION

- Comparison of celecoxib vs ibuprofen vs naproxen in patients with CVD. Noninferiority, only 80% power, upper bound 1.4. Biased to null
- Not powered for separate analysis of those on and off aspirin (50:50!) – dose timing irrelevant
- No biochemical documentation of aspirin status
- Ibuprofen and naproxen but not celecoxib may interact to undermine the platelet inhibitory effects of low dose aspirin
- The result will be uninterpretable (Science. 2005 Dec 23;310(5756):1890-1)

Mechanism Based Predictions

- *Diclofenac would confer a coxib – like hazard.* No ASA interaction.
- *Naproxen would be modestly cardioprotective. (Due to kinetics in some patients although benefit may be attenuated to some degree by an ASA interaction)*
- Ibuprofen may confer a small (relative to dicofenac) intrinsic risk and/or a risk consequent to undermining cardio-protection from ASA

Conclusions

- Selective inhibition of COX-2 dependent PGI_2 results in a risk of thrombosis, hypertension, cardiac failure and sudden cardiac death.
- Diclofenac would be expected to confer a similar hazard.
- Naproxen may be cardioprotective in some people – a weak “aspirin effect”.
- Ibuprofen may confer some risk.
- All NSAIDs would be expected to confer some risk of heart failure.