

14°

CONGRESSO NAZIONALE SINut

SINut
Società Italiana di Nutraceutica

12-14 settembre 2024

Bologna



METODOLOGIA DELLA RICERCA IN NUTRACEUTICA:

IDENTIFICAZIONE DEI MECCANISMI D'AZIONE

Prof. Alma Martelli

Dipartimento di Farmacia

Università di Pisa

La sottoscritta Alma Martelli

ai sensi dell'art. 3.3 sul Conflitto di Interessi, pag. 17 del Reg. Applicativo dell'Accordo Stato-Regione del 5 novembre 2009,

dichiara

che negli ultimi due anni NON ha avuto rapporti diretti di finanziamento con soggetti portatori di interessi commerciali in campo sanitario

La scientificità di un nutraceutico non può prescindere dall'identificazione del meccanismo d'azione

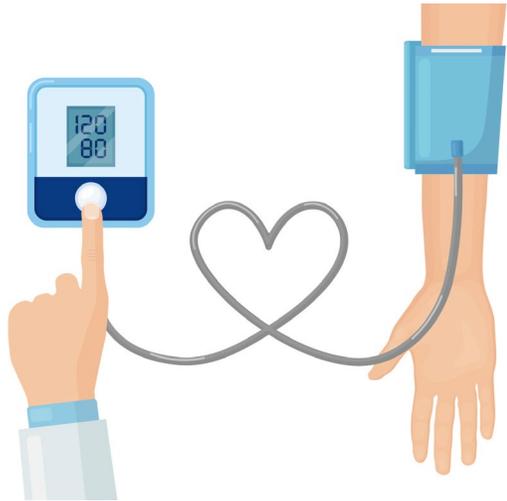
Asterix e il Druido....



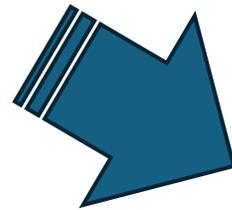
Vischio e Barbabietola....



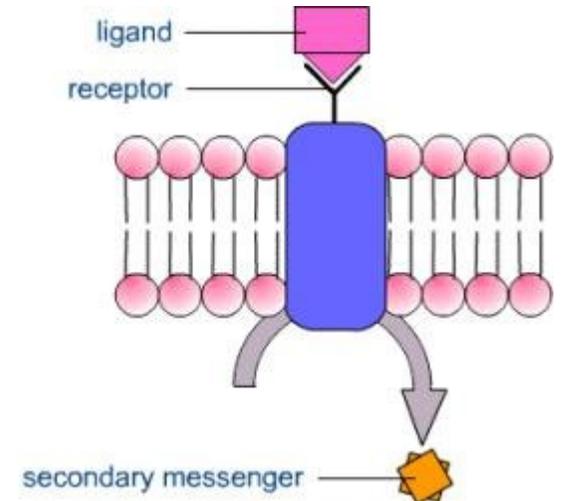
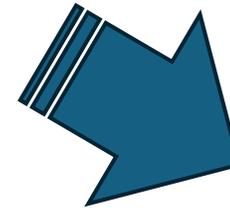
Nell'identificazione del meccanismo d'azione la sperimentazione pre-clinica ha un ruolo fondamentale



Effetto Terapeutico

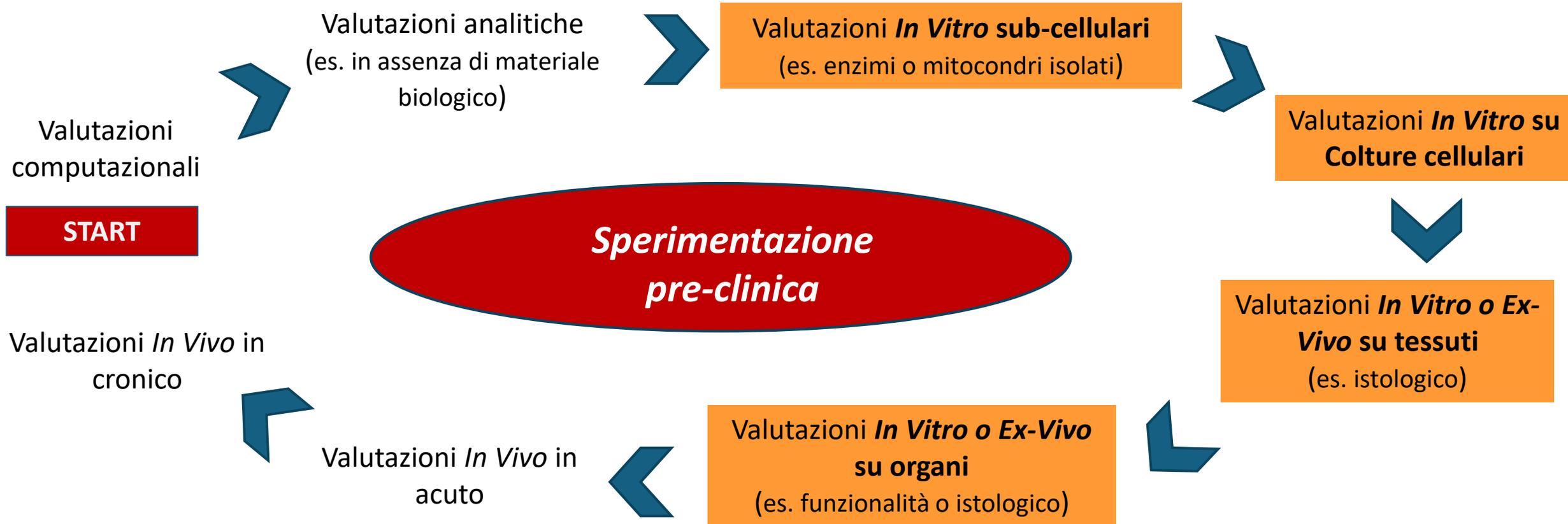


Sperimentazione Preclinica

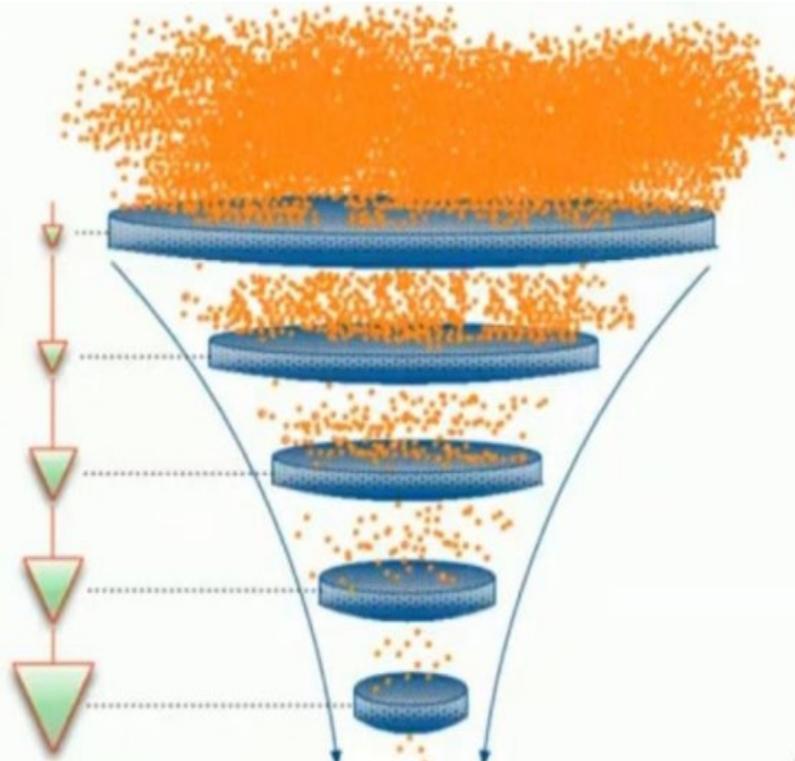


Meccanismo d'azione

Nell'ambito della sperimentazione pre-clinica, al fine d'identificare il meccanismo d'azione, la sperimentazione *in vitro* e quella *ex-vivo* sono senza dubbio più utili rispetto a quella *in vivo*



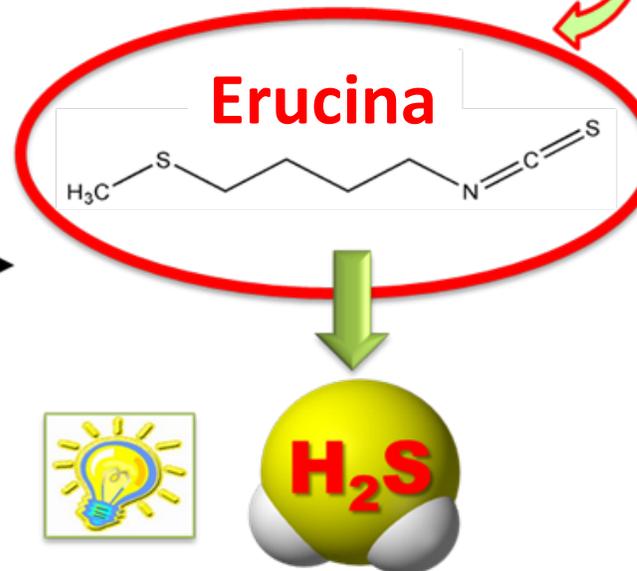
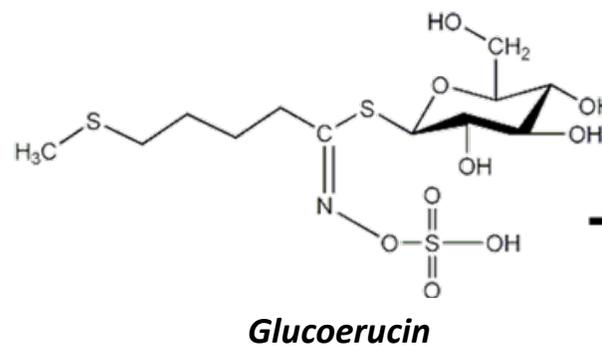
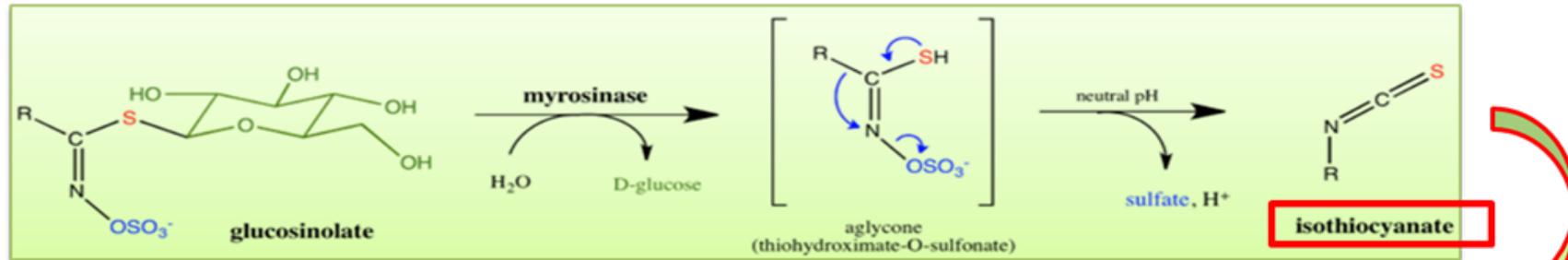
Nel *mare magnum* dei possibili targets, lo sperimentatore dovrà selezionare alcune opzioni possibili sulla base:

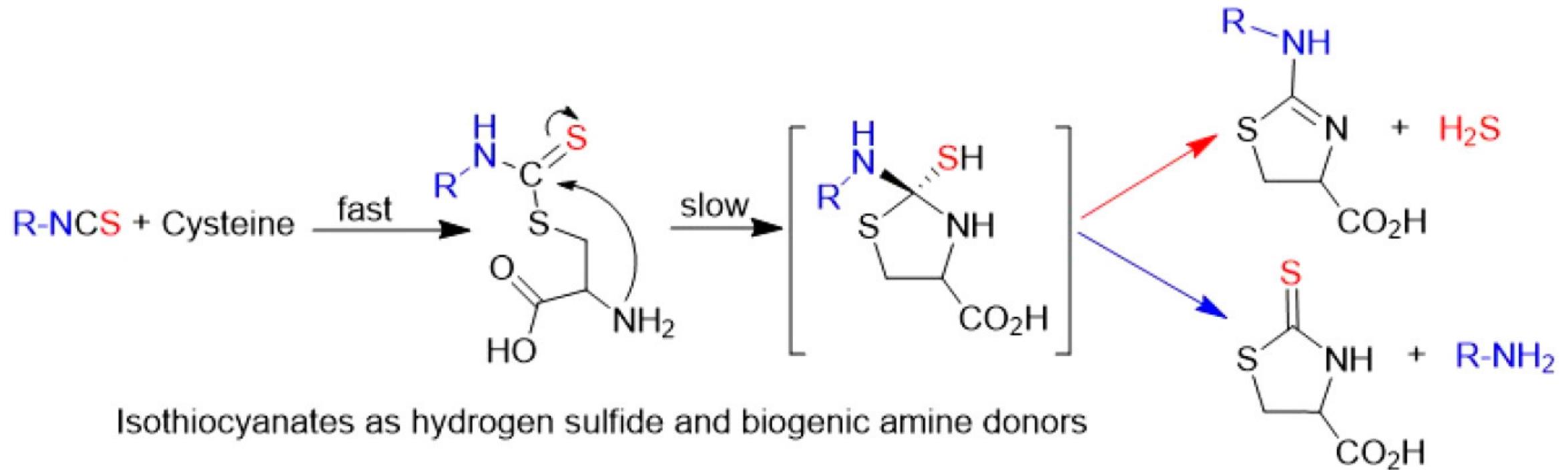


- **Della sua Esperienza**
- **Delle sue Competenze:**
 - **di Chimica**
 - **di Farmacologia**



Brassicaceae (Crucifere)

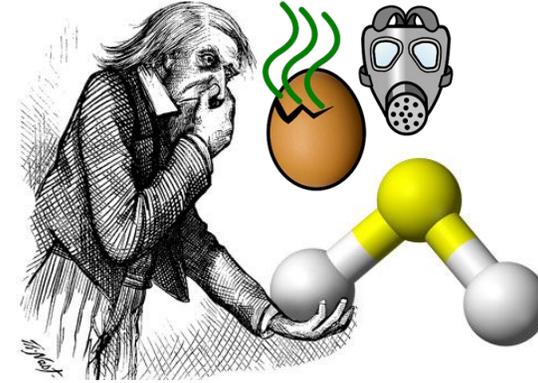
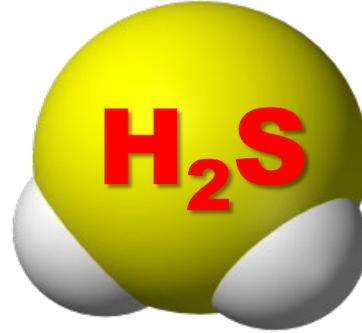




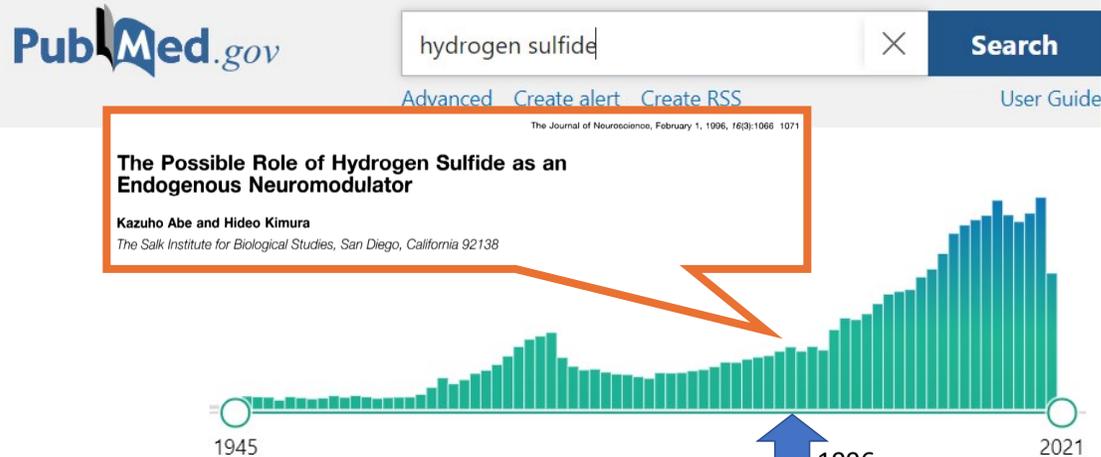
Isothiocyanates as hydrogen sulfide and biogenic amine donors

Solfuro d'idrogeno (H₂S): gas tossico e gastrasmittitore endogeno

Prima degli anni '90...



...Finchè, nel 1996, Abe e Kimura, descrissero H₂S come gastrasmittitore endogeno, identificato inizialmente nel SNC...



Hydrogen sulfide:
the «new entry» in the team of
gasotransmitters



Endothelial Dysfunction

Impaired Hydrogen Sulfide–Mediated Vasodilation Contributes to Microvascular Endothelial Dysfunction in Hypertensive Adults

Jody L. Greaney,* Jessica L. Kutz,* Sean W. Shank, Sandeep Jandu, Lakshmi Santhanam, Lacy M. Alexander

Hypertension

Volume 69, Issue 5, May 2017, Pages 902-909



Systematic Review

Circulating Levels of Hydrogen Sulfide (H₂S) in Patients with Age-Related Diseases: A Systematic Review and Meta-Analysis

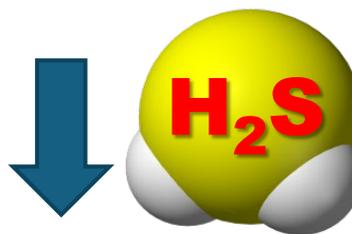
Eugenia Piragine¹, Marco Andrea Malanima², Ersilia Lucenteforte², Alma Martelli^{1,*} and Vincenzo Calderone¹

¹ Department of Pharmacy, University of Pisa, 56126 Pisa, Italy; eugenia.piragine@unipi.it (E.P.); vincenzo.calderone@unipi.it (V.C.)

² Department of Clinical and Experimental Medicine, University of Pisa, 56126 Pisa, Italy; m.malanima@studenti.unipi.it (M.A.M.); ersilia.lucenteforte@unipi.it (E.L.)

* Correspondence: alma.martelli@unipi.it

Alcune condizioni patologiche, come diabete di tipo II ed ipertensione sono caratterizzate da un decremento della biosintesi endogena di H₂S.



Study

type = HTN and vascular disease

Study	Mean	Sd	N	Control Mean	Control Sd	Control N	SMD	[95% CI]	Weight
Zheng, 2011	57.60	2.9000	62	52.60	4.6000	64	1.29	[0.90; 1.67]	5.4%
Peter, 2013	0.44	0.1800	140	0.37	0.1500	53	0.40	[0.09; 0.72]	5.5%
Xiao, 2018	1.00	0.3000	15	2.40	2.1000	22	-0.84	[-1.52; -0.15]	5.3%
Wang, 2014	34.20	7.1000	200	43.50	6.2000	100	-1.36	[-1.63; -1.10]	5.5%
Pan, 2015	42.90	4.8000	16	50.30	3.9000	16	-1.65	[-2.46; -0.83]	5.2%
Longchamp, 2021	57.00	8.4000	115	80.80	12.9000	20	-2.58	[-3.15; -2.01]	5.3%
Gao, 2015	25.60	6.1000	63	43.10	3.6000	11	-2.98	[-3.78; -2.17]	5.2%
Feng, 2017	3.50	1.5000	30	10.20	2.8000	22	-3.08	[-3.90; -2.25]	5.2%
random			641			308	-1.32	[-2.43; -0.22]	42.5%

Heterogeneity: $\chi^2_7 = 282.62$ ($P < .001$), $I^2 = 98\%$

type = T2D

Study	Mean	Sd	N	Control Mean	Control Sd	Control N	SMD	[95% CI]	Weight
Grabowska-Polanowska, 2017 CKD+T2D	39.60	24.2000	10	22.50	6.6000	5	0.79	[-0.34; 1.91]	4.9%
Ran, 2014 T2D	52.20	9.5000	75	49.70	9.7000	30	0.26	[-0.17; 0.68]	5.4%
Bahadoran, 2022 T2D	36.50	38.7000	111	42.90	43.6000	511	-0.15	[-0.35; 0.06]	5.5%
Qiu, 2018 T2D	4.30	3.5000	101	5.20	0.9000	20	-0.28	[-0.76; 0.20]	5.4%
Suzuki, 2017 T2D	45.10	15.5000	154	54.00	26.4000	66	-0.46	[-0.75; -0.17]	5.5%
Whiteman, 2010 MetS+T2D	10.50	12.7000	11	38.90	11.4000	10	-2.25	[-3.39; -1.11]	4.9%
Li, 2014, CHD+T2D	22.90	8.3000	36	56.50	6.9000	15	-4.17	[-5.21; -3.14]	5.0%
random			498			657	-0.86	[-2.09; 0.37]	36.6%

Heterogeneity: $\chi^2_4 = 78.54$ ($P < .001$), $I^2 = 92\%$

type = CKD/CHD

Study	Mean	Sd	N	Control Mean	Control Sd	Control N	SMD	[95% CI]	Weight
Grabowska-Polanowska, 2017 CKD and CKD+T2D	35.20	19.0000	20	22.50	6.6000	5	0.70	[-0.30; 1.70]	5.0%
Kuang, 2018	7.30	3.1000	157	14.10	5.2000	37	-1.89	[-2.29; -1.48]	5.4%
Perna, 2009	10.90	0.7000	65	14.80	1.5000	31	-3.77	[-4.46; -3.08]	5.3%
Li, 2014 CHD and CHD+T2D	25.80	7.8000	68	56.50	6.9000	15	-3.97	[-4.81; -3.14]	5.2%
random			310			88	-2.25	[-4.34; -0.15]	20.9%

Heterogeneity: $\chi^2_3 = 71.3$ ($P < .001$), $I^2 = 96\%$

Random effects model

Study	Mean	Sd	N	Control Mean	Control Sd	Control N	SMD	[95% CI]	Weight
random			1449			1053	-1.35	[-2.12; -0.58]	100.0%

Heterogeneity: $\chi^2_{18} = 567.64$ ($P < .001$), $I^2 = 97\%$

Test for overall effect: $z = -3.43$ ($P < .001$)

Test for subgroup differences: $\chi^2_2 = 1.27$ ($P = .53$)

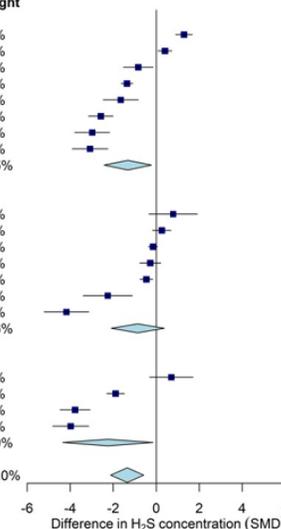


Figure 3. A forest plot with standardized mean difference (SMD) of circulating levels of H₂S in patients with CVDs (hypertension and vascular disease) (first group), T2D (second group), or CKD/CHD (third group) compared with subjects without disease (control group). Abbreviations:

British Journal of Pharmacology (2008) 155, 617–619
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www.bjpharmacol.org

COMMENTARY

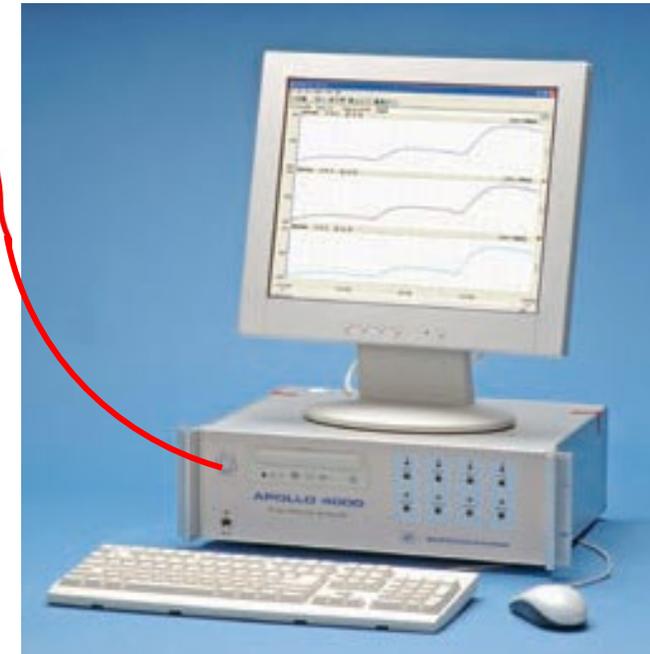
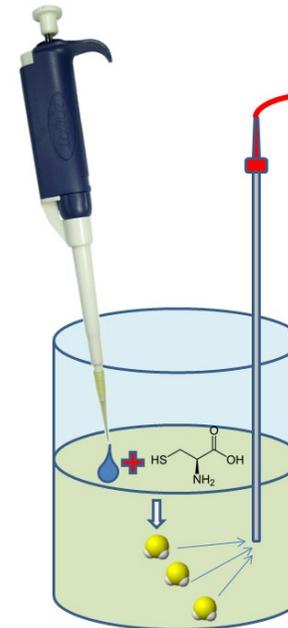
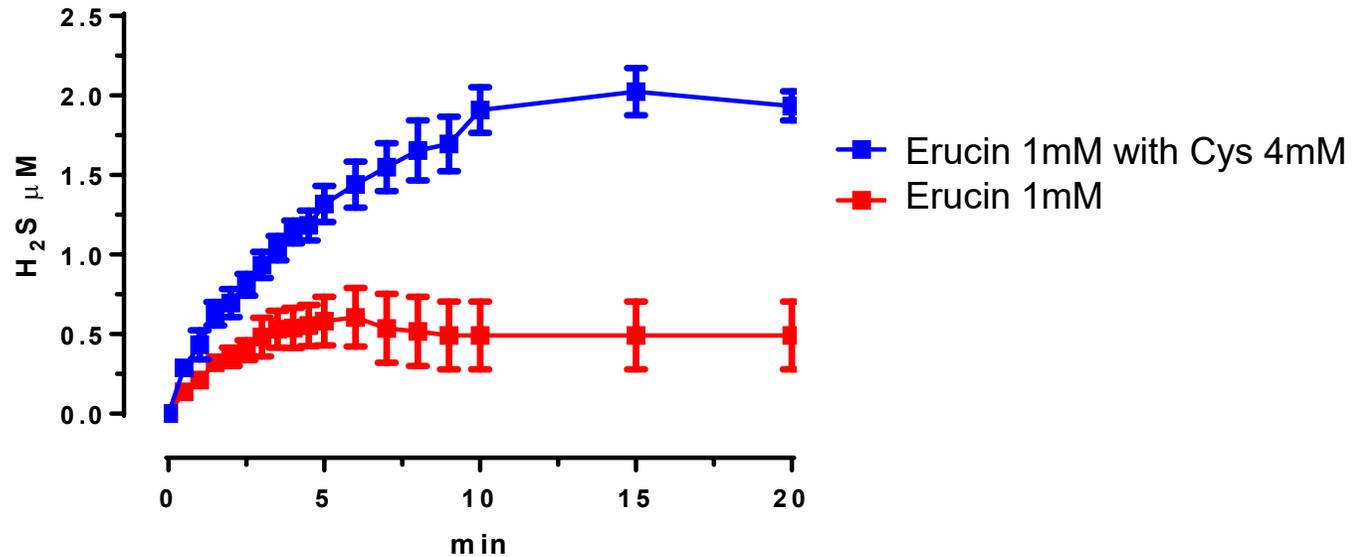
Potential importance of alterations in hydrogen sulphide (H₂S) bioavailability in diabetes

DJ Lefer

Division of Cardiothoracic Surgery, Department of Surgery, Emory University School of Medicine, Atlanta, Georgia, USA



Erucina è...uno *smart* H₂S-donor



Compound	Cmax (μM)	Cmax+ L-Cys (μM)
Erucin	0.5 ± 0.2	1.9 ± 0.1
NaHS	>200	>200



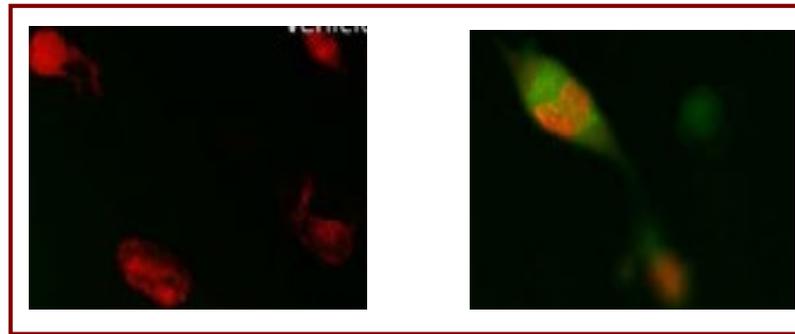
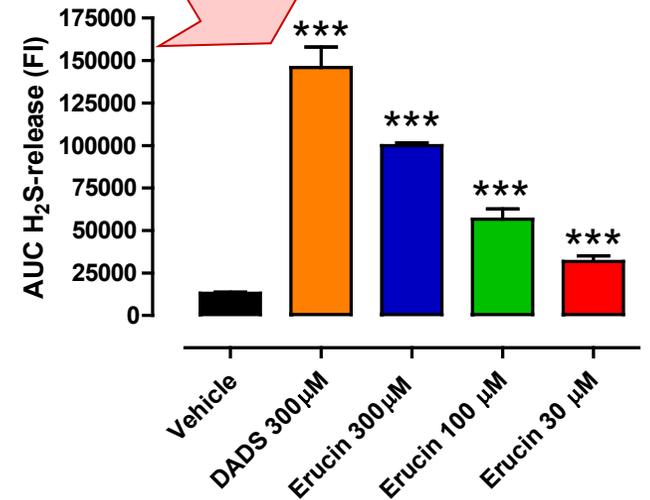
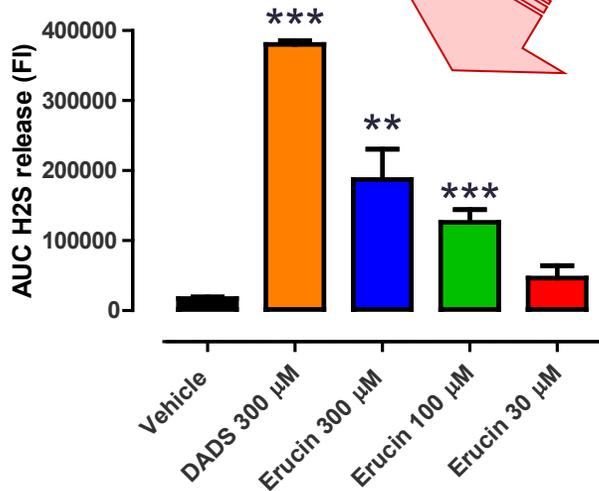
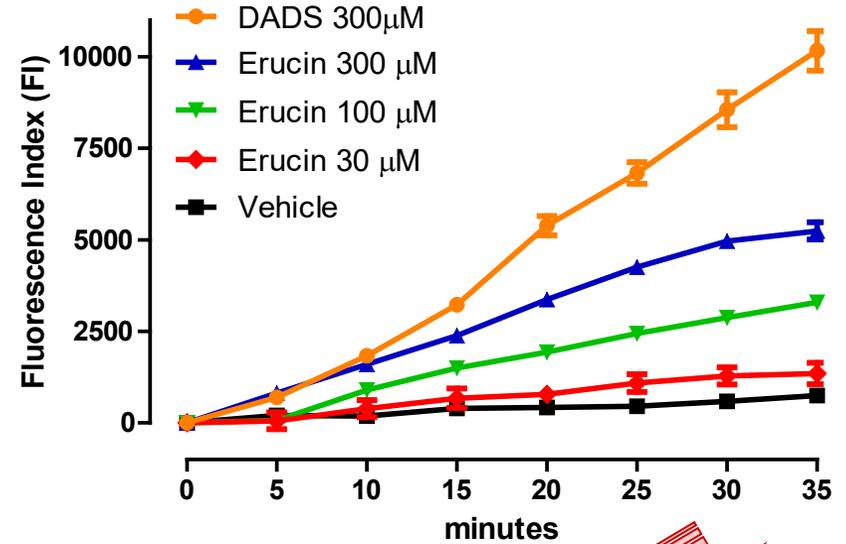
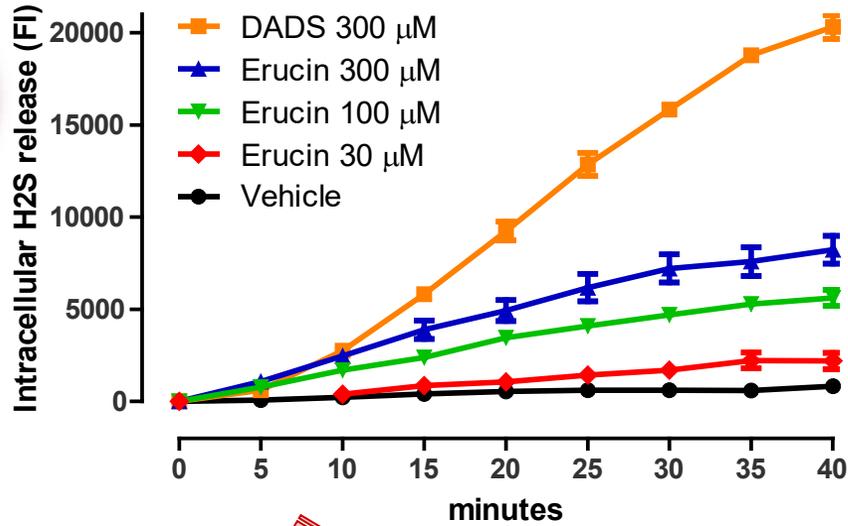
RILASCIO INTRACELLULARE di H₂S indotto da ERUCINA

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Bologna

HUVEC Human Umbilical Vein Endothelial Cells

HASMC Human Aortic Smooth Muscle Cells



* significance vs Vehicle ANOVA one-way post test Bonferroni

PROTEZIONE della PARETE VASCOLARE contro un DANNO di tipo OSSIDATIVO

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CONG

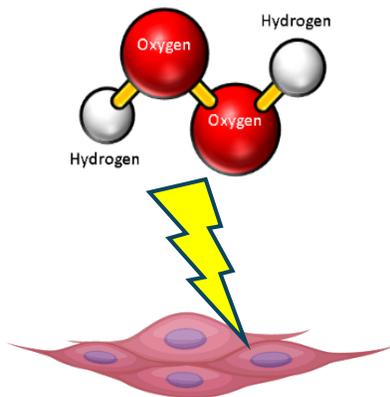
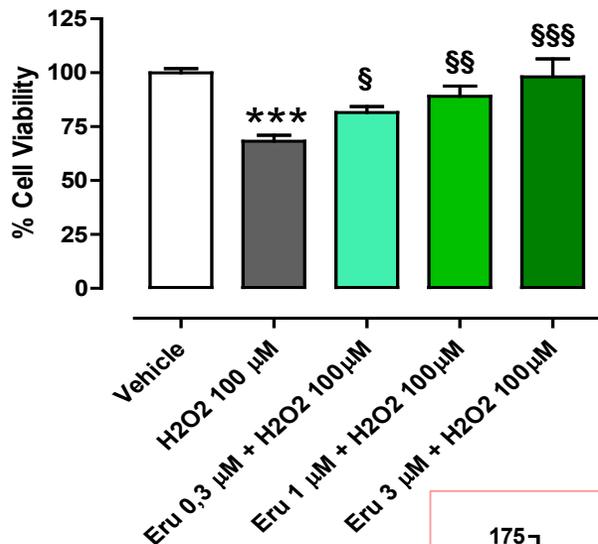
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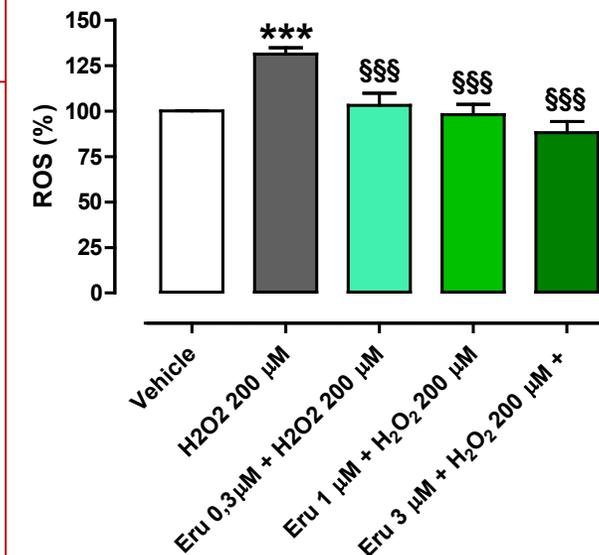
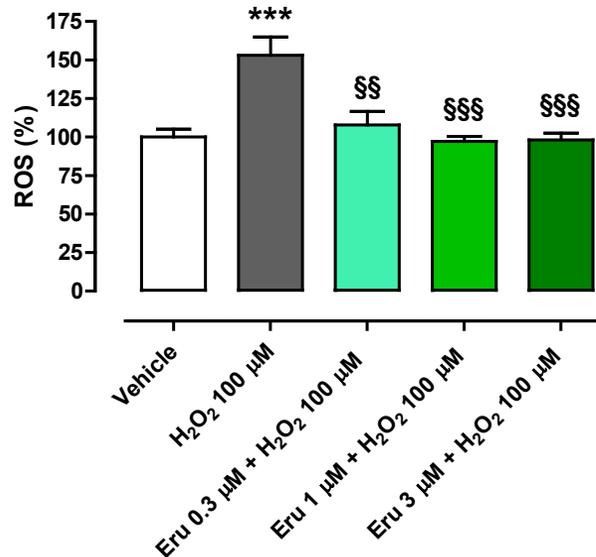
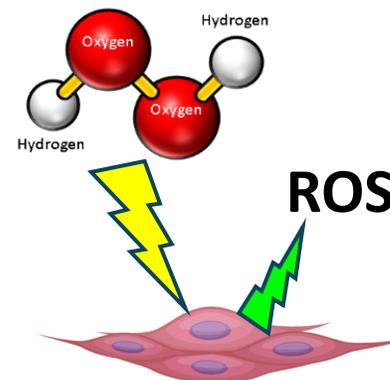
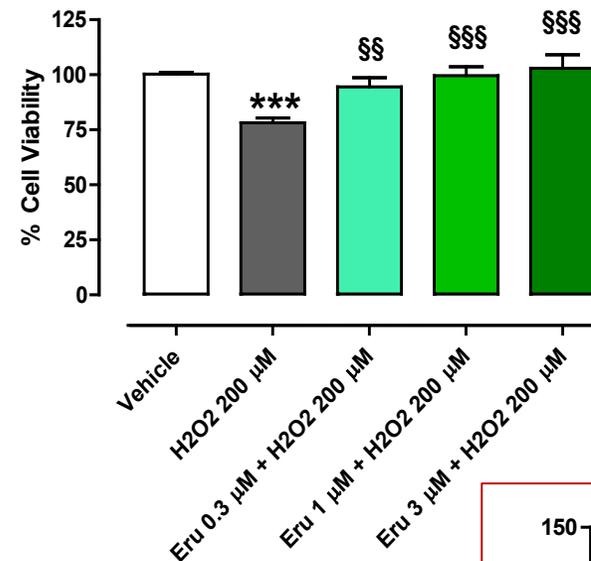
HUVEC

Human Umbilical Vein Endothelial Cells



HASMC

Human Aortic Smooth Muscle Cells

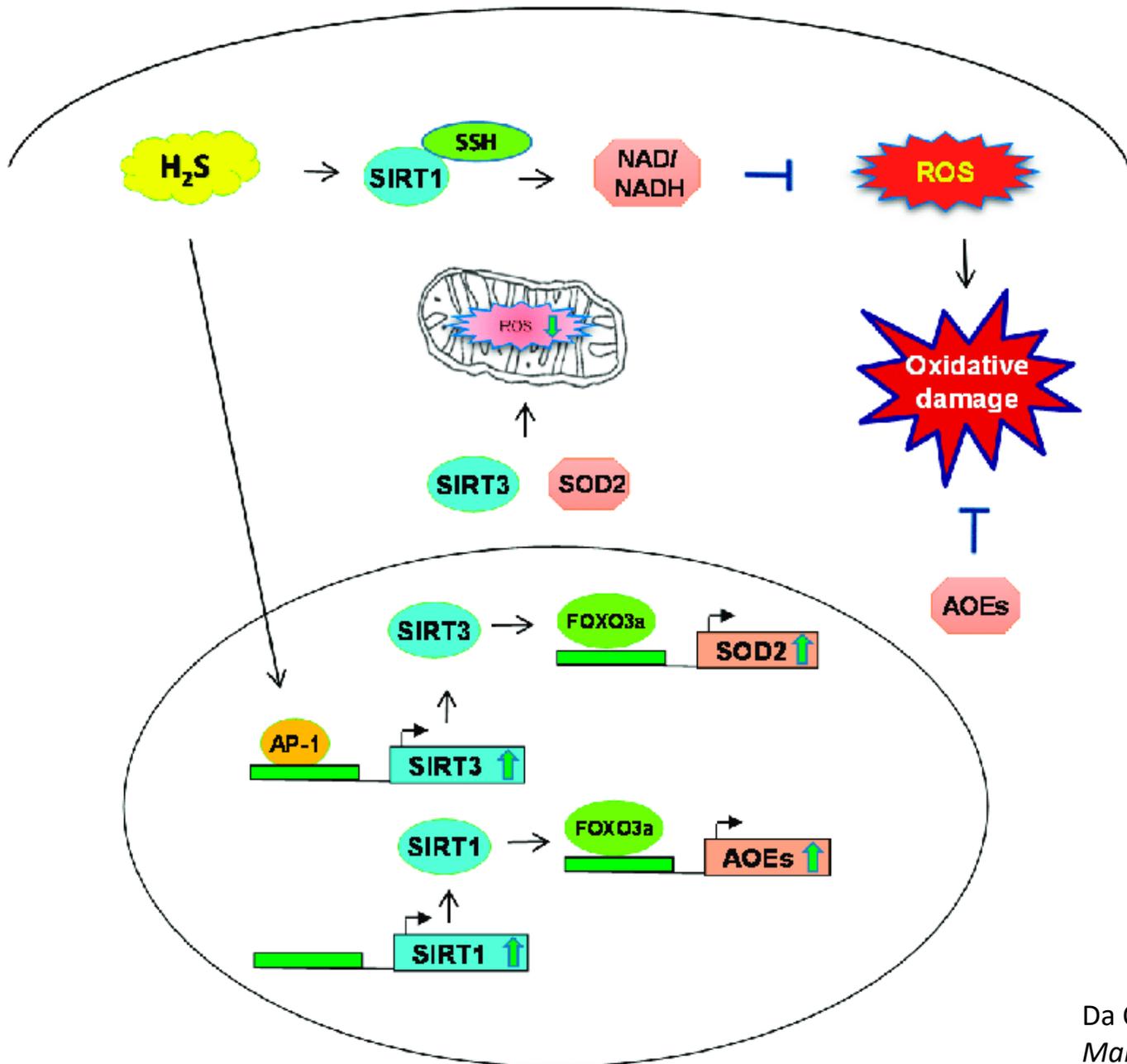


* significance vs Vehicle

§ significance vs H₂O₂

ANOVA one-way post test
Bonferroni

TARGET COINVOLTO nella PROTEZIONE esercitata da ERUCINA contro un DANNO di tipo OSSIDATIVO



H₂S

È un attivatore di SIRT1

SIRT1

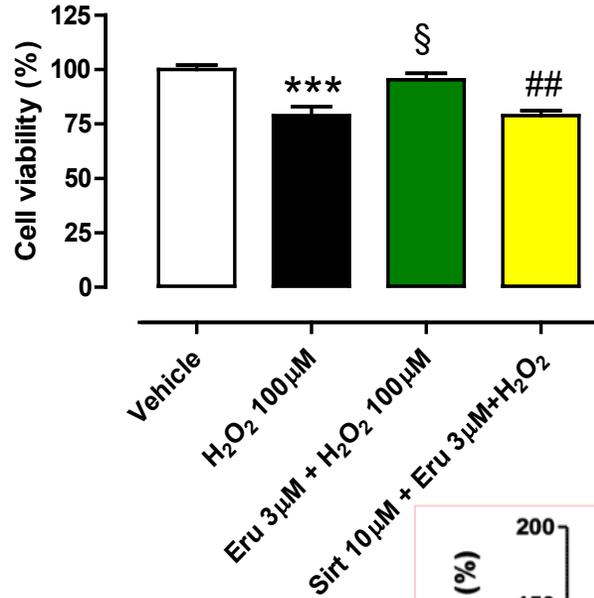
Enzima appartenente alla famiglia delle SIRTUINE. Le Sirtuine sono enzimi coinvolti in processi anti-ossidanti e anti-ageing

SIRTUINE: TARGET della PROTEZIONE indotta da ERUCINA nei confronti di STIMOLI PRO-OSSIDANTI

COI

HUVEC

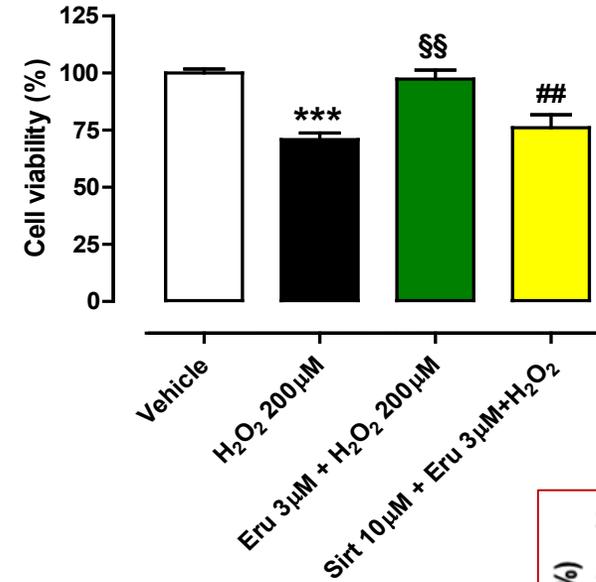
Human Umbilical Vein Endothelial Cells



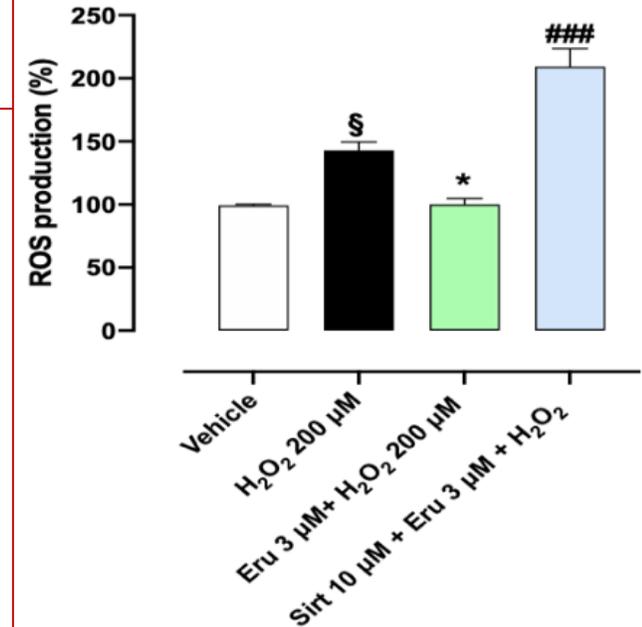
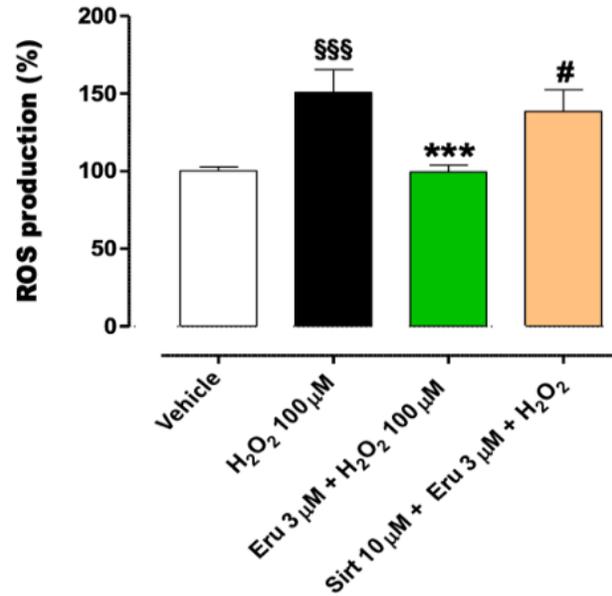
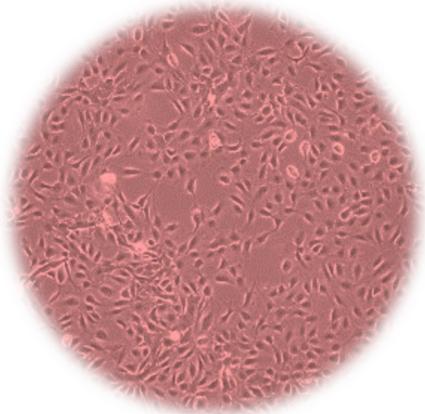
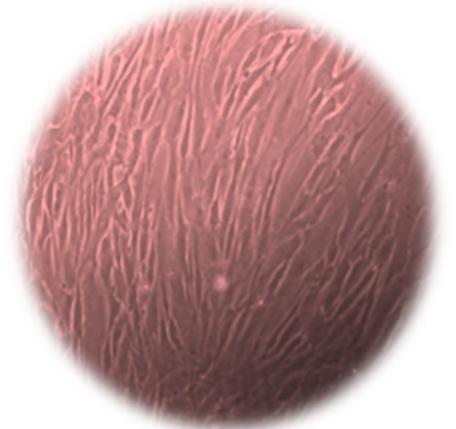
* significance vs Vehicle
§ significance vs H₂O₂
significance vs Eru+H₂O₂
ANOVA one-way post test
Bonferroni

HASMC

Human Aortic Smooth Muscle Cells



§ significance vs Vehicle
* significance vs H₂O₂
significance vs Eru+H₂O₂
ANOVA one-way post test
Bonferroni

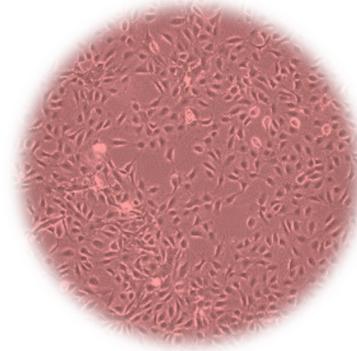


EFFETTO di ERUCINA sull'ESPRESSIONE GENICA di *SIRT1* e sui LIVELLI di PROTEINA SIRT1 in cellule HUVEC

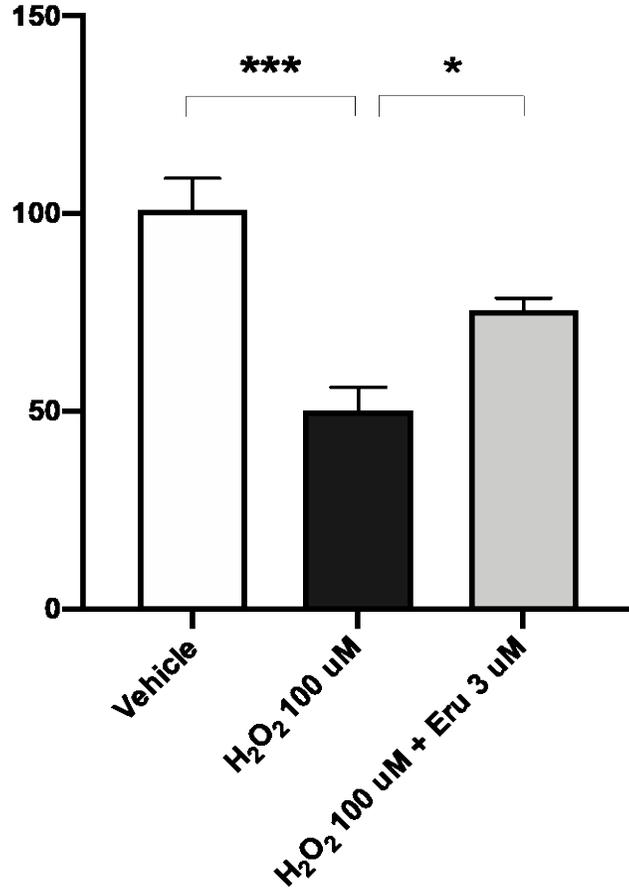
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CON

SIRT1 espressione genica

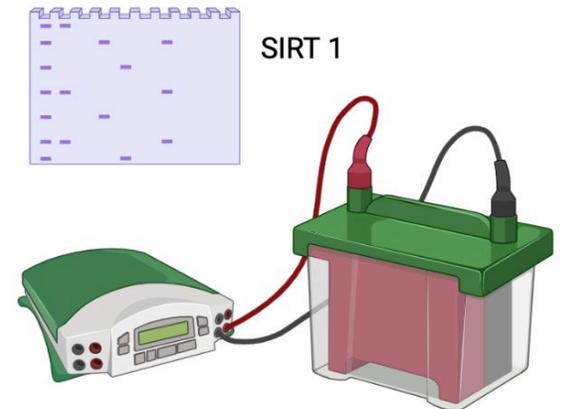
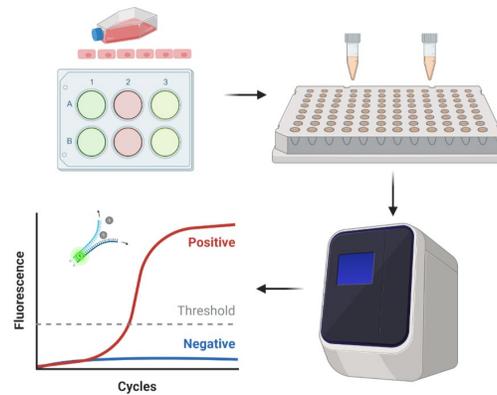
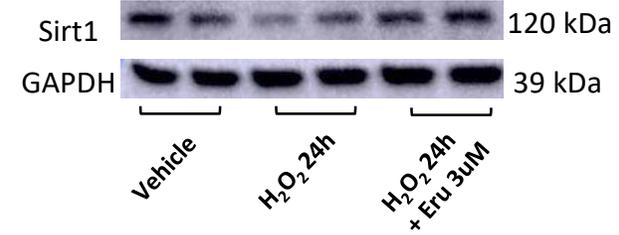
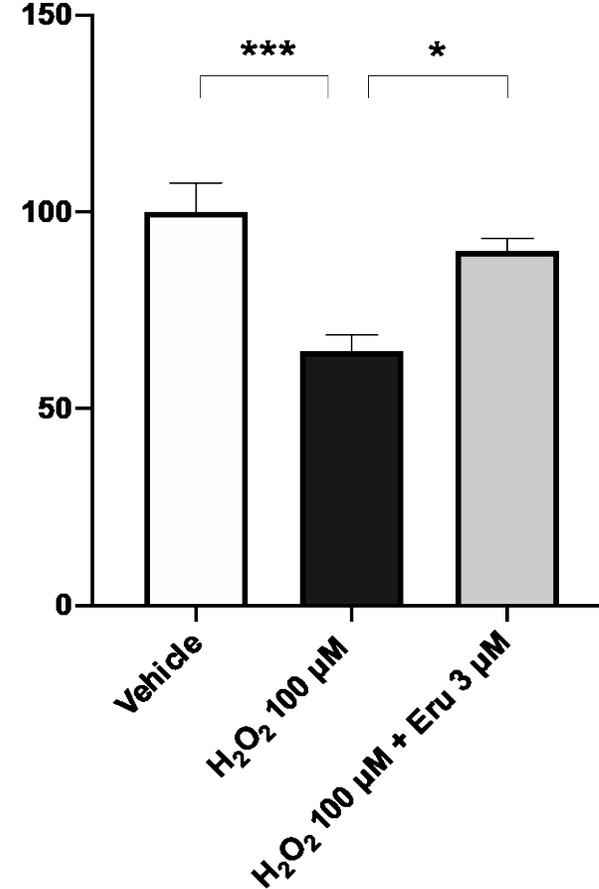
HUVECs



SIRT1/GAPDH
(% of vehicle)



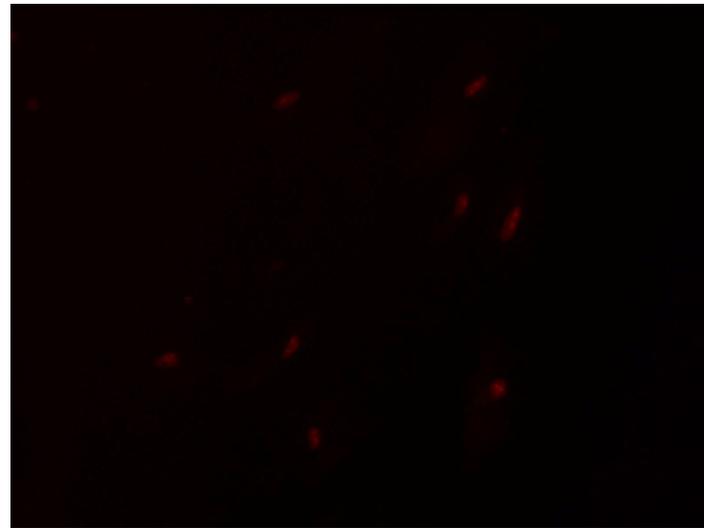
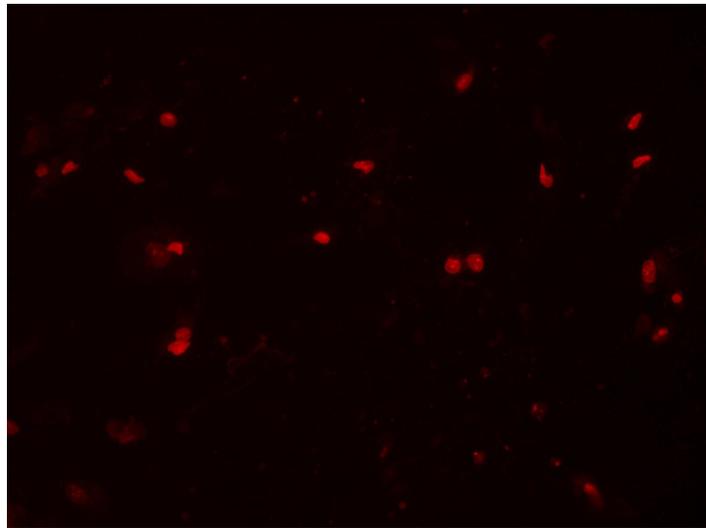
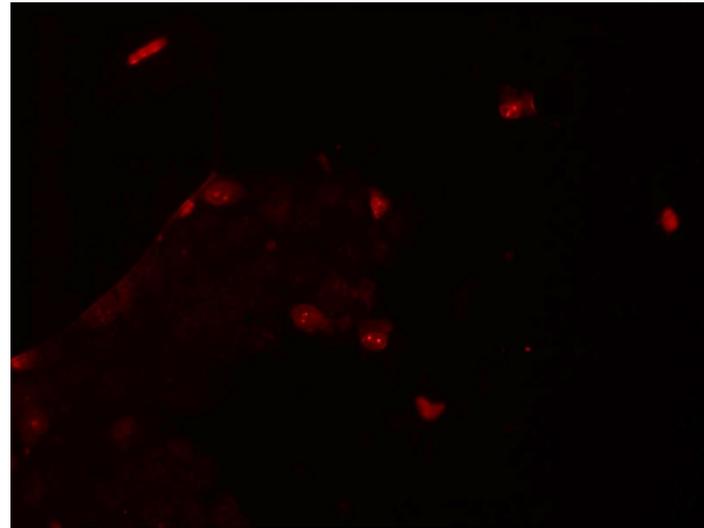
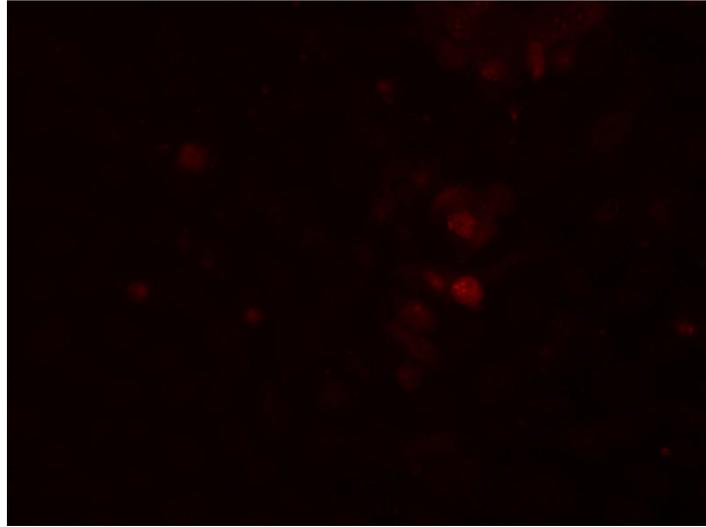
SIRT1 livelli di proteina



GENE	FORWARD	REVERSE
Human		
<i>SIRT1</i>	5'-GAAAAACCTCCACGAACAA-3'	5'-ATTACCATCAAGCCGCTACT-3'
<i>TBP</i>	5'-CGTGGCTCTTATCCTC-3'	5'-GCCCGAACGCCGAATAT A-3'

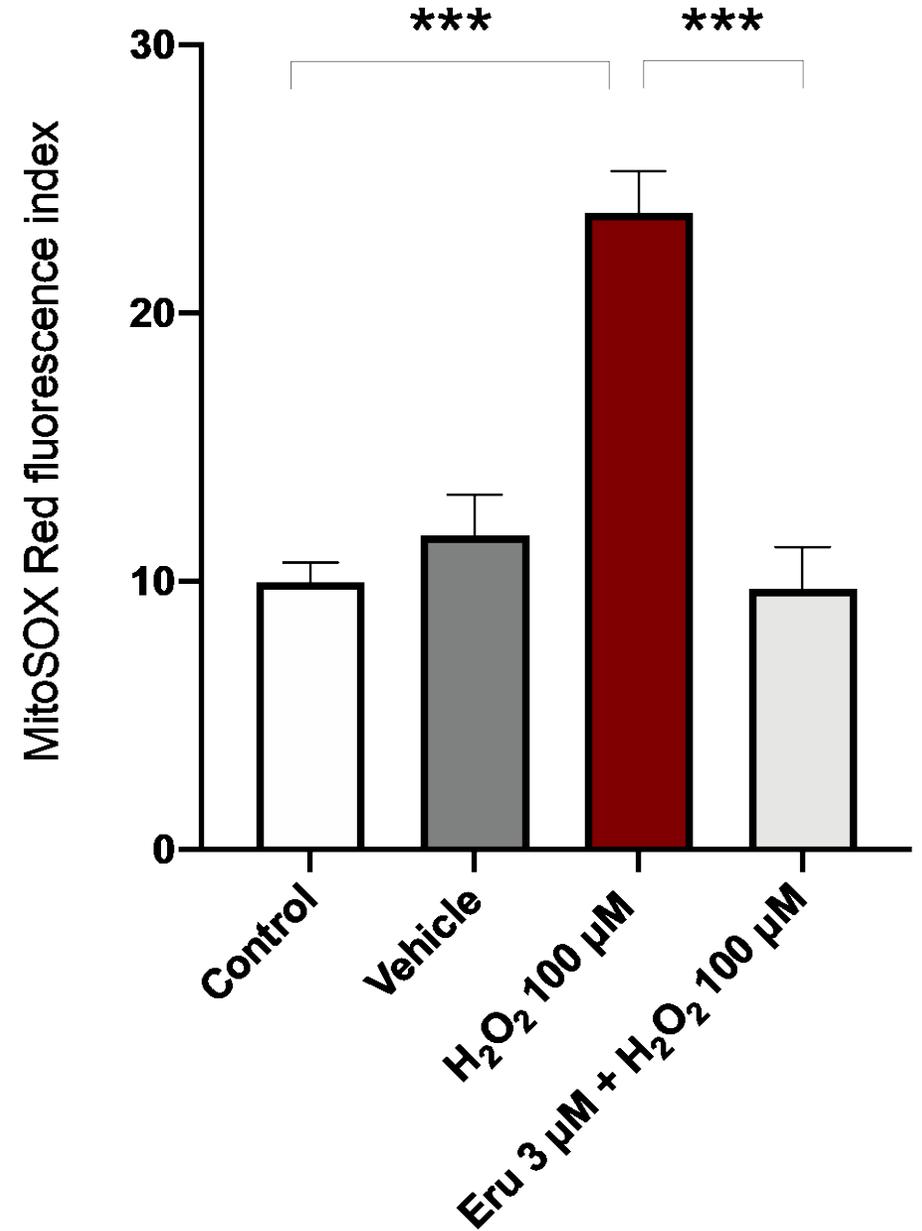
Controllo

Veicolo (0.01% DMSO)



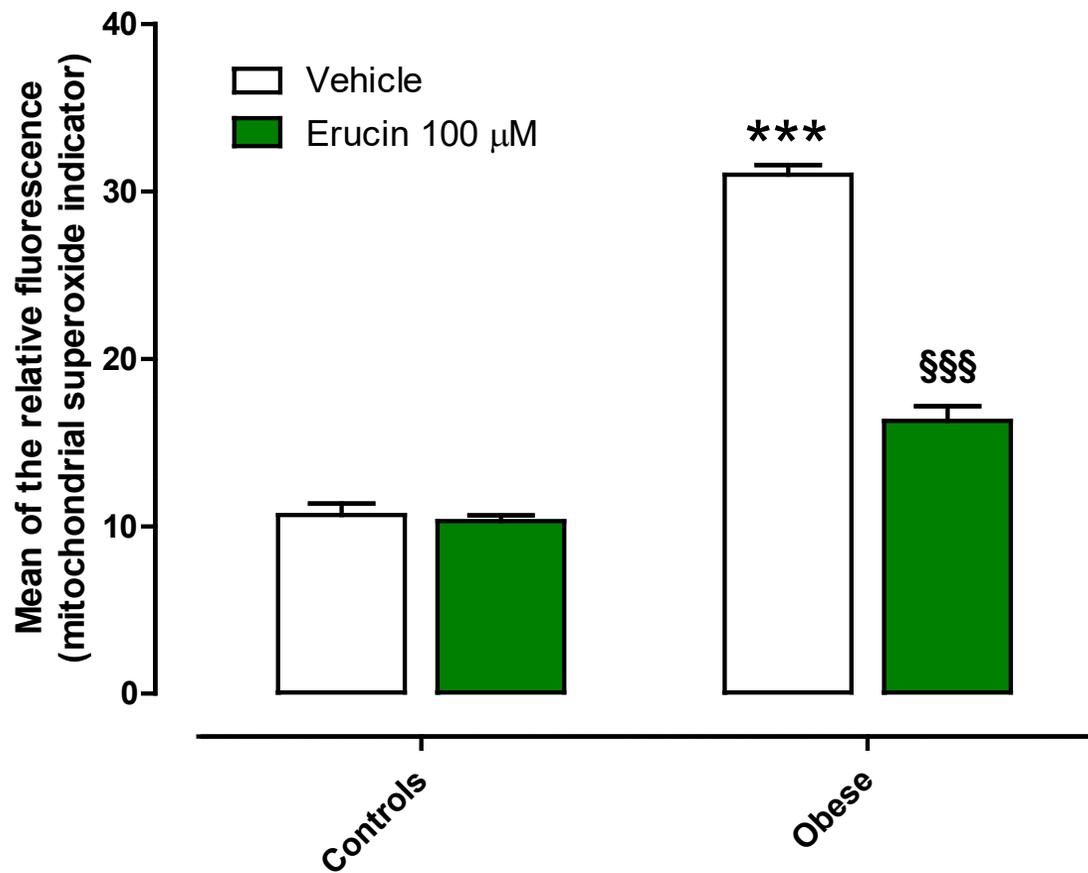
H₂O₂ 100 μM

H₂O₂ 100 uM + Erucin 3 μM

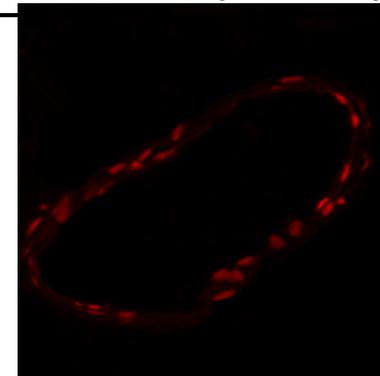


Arteriole di resistenza isolate dal tessuto adiposo periadventiziale di pazienti obesi

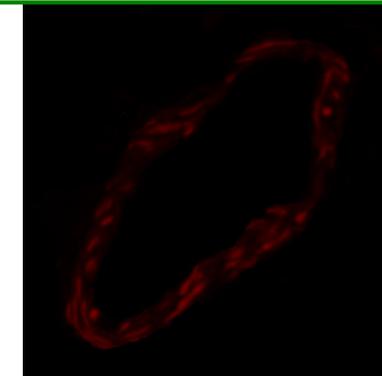
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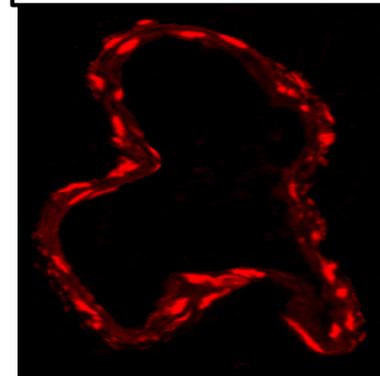
Controlli (Veicolo)



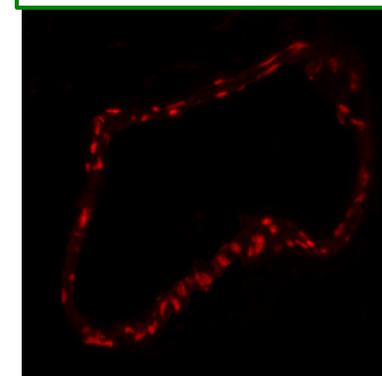
Controlli + Erucina



Obesi (Veicolo)



Obesi + Erucina



* significance vs Controls Vehicle

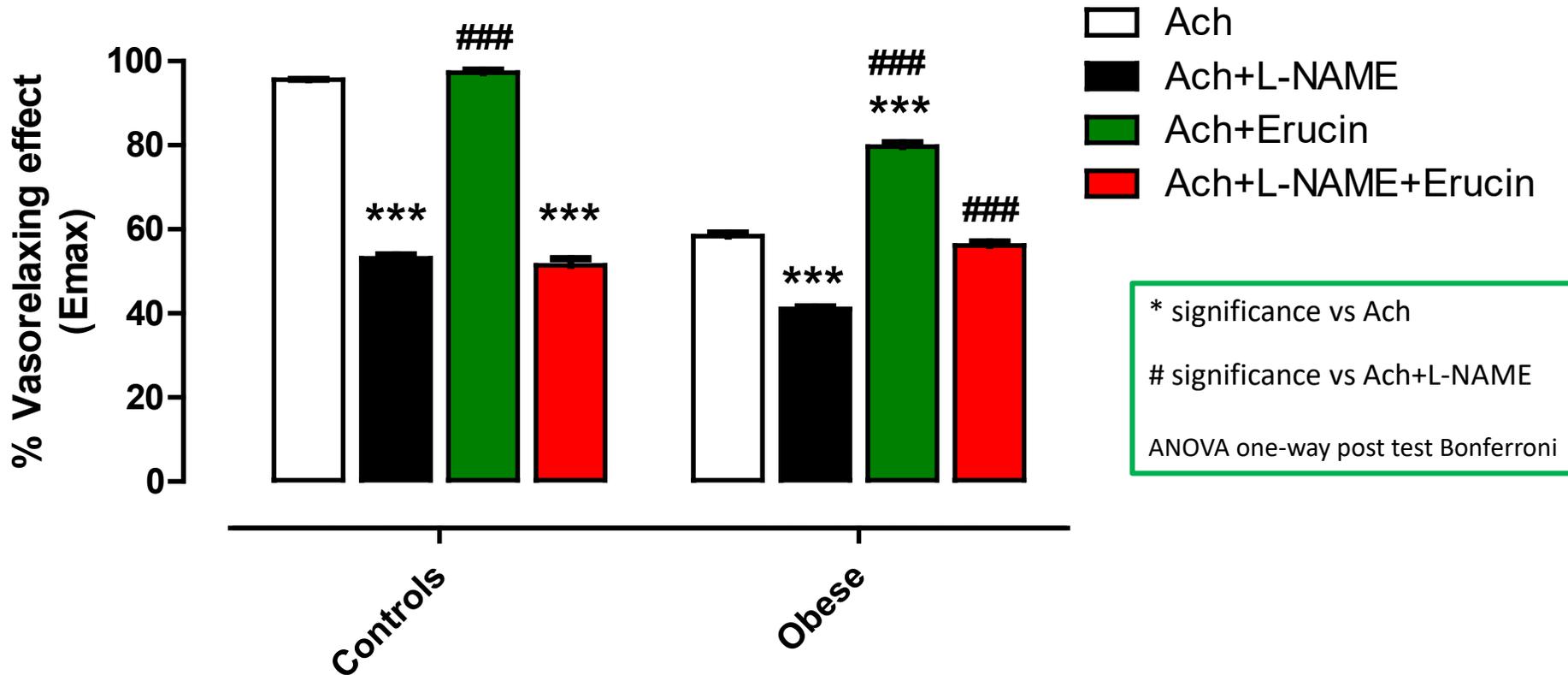
§ significance vs Obese Vehicle

ANOVA one-way post test Bonferroni

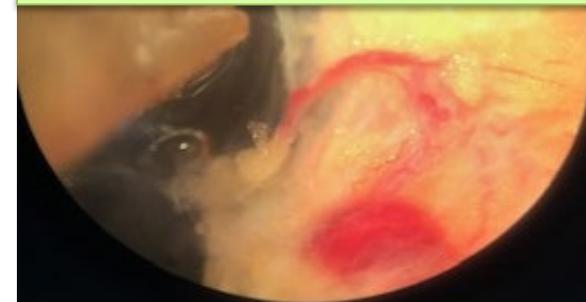
Immagini acquisite al microscopio confocale mediante l'impiego della sonda fluorescente MitoSOX

EFFETTO di ERUCINA sulla VASODILATAZIONE ACETILCOLINA-INDOTTA in ARTERIOLE di PAZIENTI OBESI

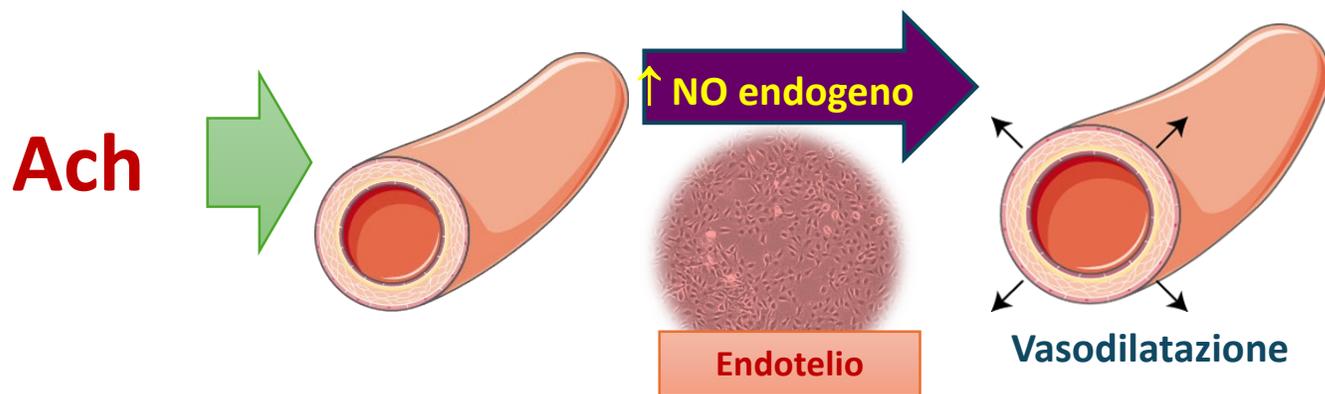
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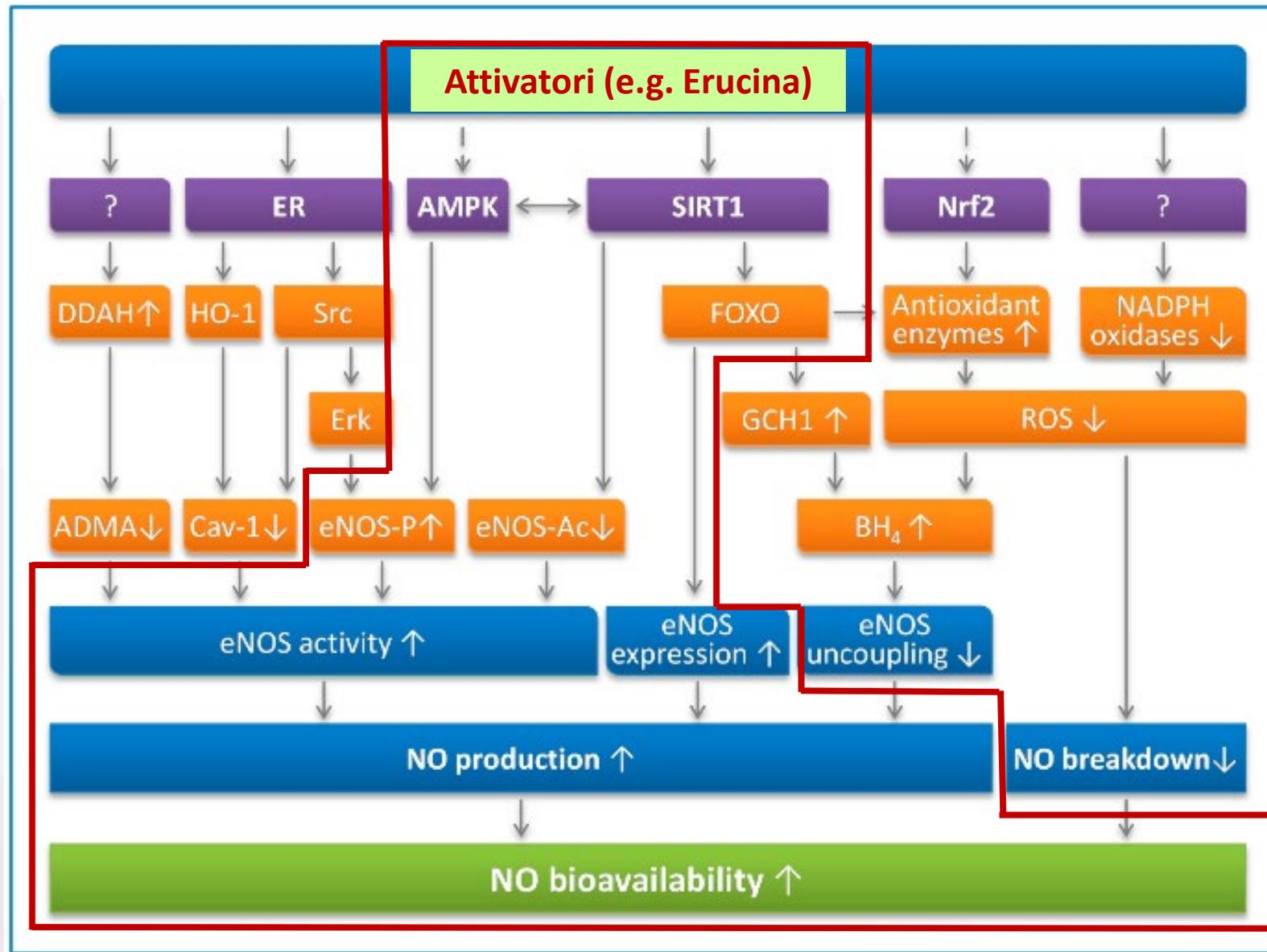
Arteriole di resistenza isolate dal tessuto adipose periadventiziale e montate su un micromiografo



Nelle arteriole di pazienti obesi
ERUCINA
 Induce una protezione significativa della funzione endoteliale

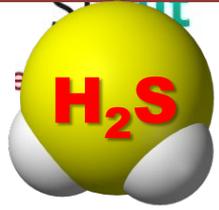


ATTIVAZIONE di SIRT1 e AUMENTO della VASODILATAZIONE NO-DIPENDENTE





Erucina



l'isotiocianato H_2S -donor che deriva dalla rucola:

☪ Protegge le **cellule della parete vascolare** dal danno ossidativo
Tale protezione:

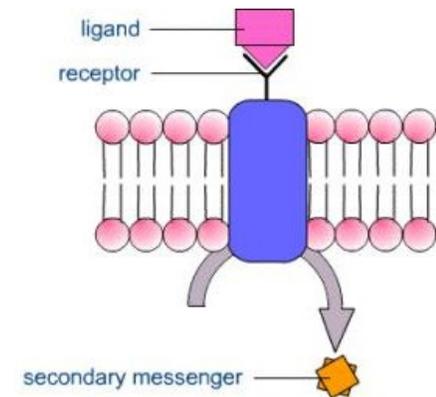
☪ coinvolge **SIRT1** un enzima appartenente alla
famiglia delle Sirtuine

☪ **riduce lo stress ossidativo mitocondriale** sia in cellule endoteliali umane che
in arteriole di pazienti obesi

☪ determina parziale ma significativa **protezione della funzione vascolare**
nelle arteriole di resistenza isolate dal tessuto periadventiziale
di pazienti obesi

Take Home Messages

- *La scientificità di un nutraceutico non può prescindere dall'**identificazione del meccanismo d'azione** mediante il quale esso svolge l'azione salutistica/terapeutica riscontrata.*
- *Nell'identificazione del meccanismo d'azione, spesso metodologie pre-cliniche in vitro o ex-vivo sono più utili perché permettono l'evidenza **dell'interazione fra il principio attivo ed il target**.*
- *Per selezionare i target da testare sono utili **competenze chimiche e farmacologiche**.*



Ringraziamenti:



**Department of
Pharmacy**

**Pharmacology
Group**



UNIVERSITÀ DI PISA

**Department of Clinical and
Experimental Medicine**

**Internal Medicine
Group**

- **Prof Vincenzo Calderone**
- **Prof Alma Martelli**
- **Prof Lara Testai**
- **Prof Simone Brogi**
- **Dr Valentina Citi**
- **Dr Eugenia Piragine**
- **Dr Era Gorica**
- **Dr Lorenzo Flori**
- **Dr Jacopo Spezzini**



- **Prof Agostino Viridis**
- **Prof Stefano Masi**
- **Dr Alessandro Mengozzi**
- **Dr Emiliano Duranti**

CREA of Bologna



**Dr Eleonora Pagnotta
Dr Luisa Ugolini
Dr Roberto Matteo**



**University of
Zurich^{UZH}**

**Prof Francesco Paneni
Dr Sarah Constantino**

Center for Molecular Cardiology

Grazie per l'attenzione!

