Effetti nutrigenomici dell'acido grasso Omega-3 docosaesaenoico (DHA) e la salute cardiovascolare

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Acidi grassi Omega-3 e malattie infiammatorie

The NEW ENGLAND JOURNAL of MEDICINE
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REVIEW ARTICLE

DRUG THERAPY

n–3 Fatty Acids in Cardiovascular Disease

Raffaele De Caterina, M.D., Ph.D.

CARDIOVASCULAR DISEASE IS THE LEADING CAUSE OF DEATH WORLDWIDE, and preventive approaches, particularly achievable dietary changes, have major public health implications. An increased dietary intake of n–3 (polyunsaturated) fatty acids is one such dietary approach. This review discusses advances since the topic was last reviewed in the *Journal*[1] and highlights current gaps in knowledge.

HISTORICAL PERSPECTIVE

In response to anecdotal reports of a low prevalence of coronary heart disease among Greenland Eskimos (Inuits), Bang and Dyerberg undertook six expeditions to Greenland starting in the late 1960s. They confirmed a very low incidence of myocardial infarction and reported an antiatherogenic blood lipid pattern, as well as markedly reduced platelet reactivity, in this population as compared with Danish controls.[2,3] These findings were attributed to the Inuit diet, which was composed mainly of seal and whale and was extremely rich in marine n–3 fatty acids. The prevalence of inflammatory and immune diseases among the Inuits was also reported to be very low.[4] In a seminal article in 1978, Dyerberg and colleagues presented the hypothesis that marine n–3 fatty acids might provide protection against atherosclerosis and thrombosis,[5] and they began research on the potential effects of n–3 fatty acids in the prevention and treatment of vascular disease.

MECCANISMI d'azione degli Omega-3

Non-genomici: agiscono sul tono vascolare e sull'aggregazione piastrinica diminuendo la produzione di trombossani

Genomici: modulano l'espressione genica

Effetti nutrigenomici del DHA in modelli vascolari umani in vitro

PLOS ONE

RESEARCH ARTICLE

Transcriptome-based identification of new anti-anti-inflammatory and vasodilating properties of the n-3 fatty acid docosahexaenoic acid in vascular endothelial cell under proinflammatory conditions

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1 National Research Council (CNR), Institute of Clinical Physiology, Lecce, Italy, **2** CEINGE Biotecnologie Avanzate, Naples, Italy, **3** Department of Medicine and Surgery of Salerno University, Salerno, Italy, **4** "Gabriele d'Annunzio" University and Center of Excellence on Aging, Chieti, Italy, **5** Department of Biological and Environmental Science and Technology (Disteba), University of Salento, Lecce, Italy, **6** Fondazione Toscana "Gabriele Monasterio", Pisa, Italy

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OPEN ACCESS

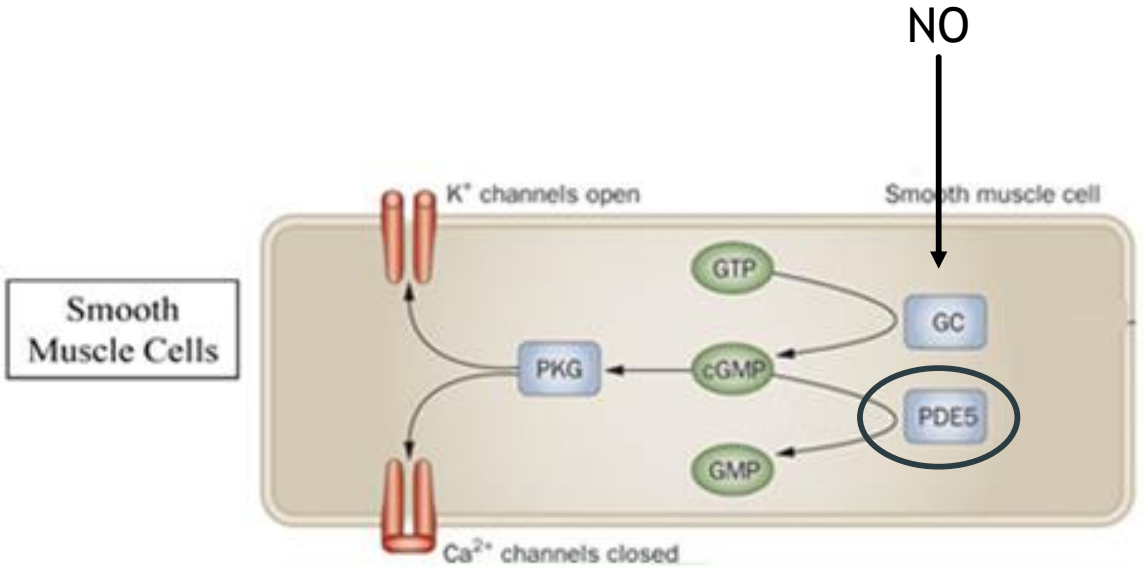
Citation: Massaro M, Martinelli R, Gatta V, Scoditti E,

Geni la cui espressione è regolata dal DHA

- Citocromo CYP4F2 (↑)
- Fattore di cerscita trasformante TGF-β2 (↓)
- Molecola di adesione giunzionale F11R (↓)
- Fosfodiesterasi 5 alfa PDE5a (↓)

PLoS One. 2015 Jun 26;10(6):e0129652. doi: 10.1371/journal.pone.0129652. eCollection 2015.
Transcriptome-based identification of new anti-inflammatory and vasodilating properties of the n-3 fatty acid docosahexaenoic acid in vascular endothelial cell under proinflammatory conditions [corrected].
Massaro M[1], Martinelli R[2], Gatta V[3], Scoditti E[1], Pellegrino M[4], Carluccio MA[1], Calabriso N[1], Buonomo T[5], Stuppia L[3], Storelli C[6], De Caterina R[7].

La PDE5a nella parete vasale

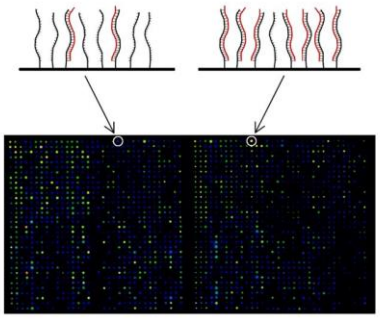


From Khazaei et al. Pathophysiology 15 (2008) 49–67 modified

NO attiva la guanilato ciclasi citosolica (GC) a generare cGMP che attiva la protein chinasi G (PK)G che innesca l'apertura dei canali del potassio con seguente iperpolarizzazione della membrana plasmatica e inibizione dei canali del calcio con diminuzione della contrattilità cellulare e, quindi, vasodilatazione.

La PDE5a catalizza l'idrolisi del cGMP a GMP, limitandone la biodisponibilità

Trascrittomica

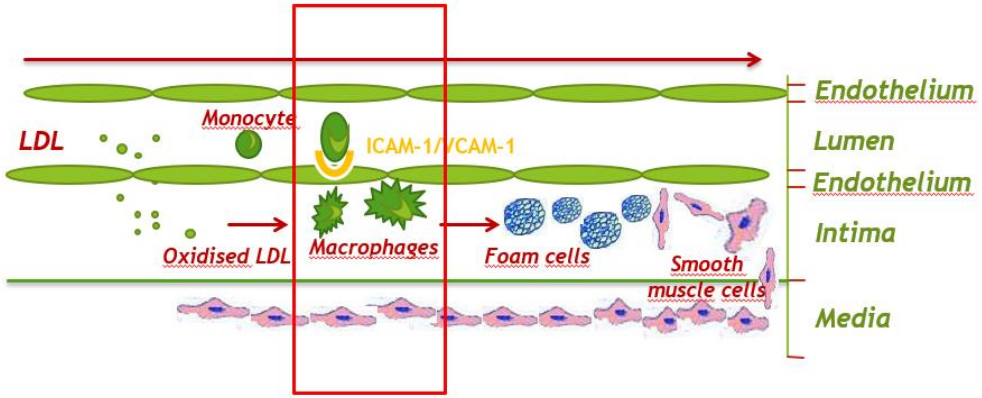


HUVEC

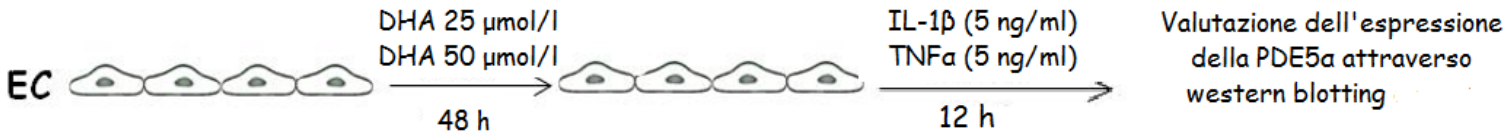
Esplorare effetti cellulari

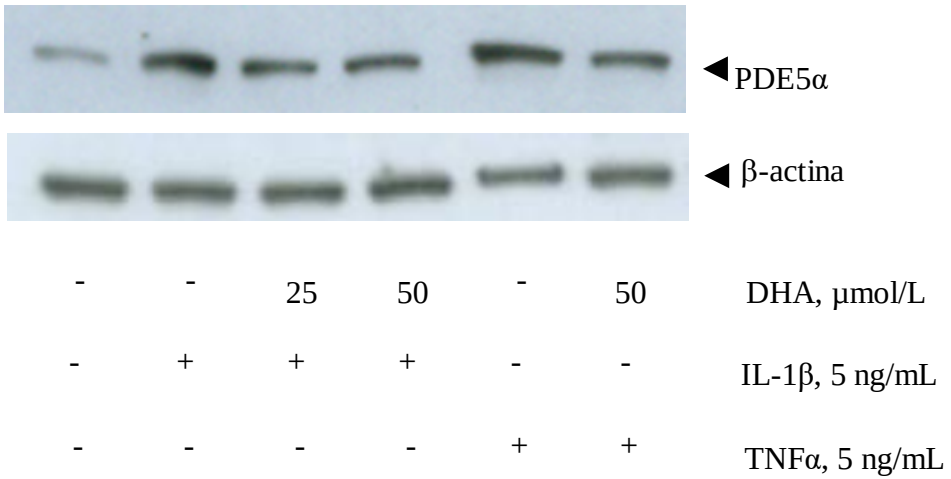
ADESIVITA' ENDOTELIO vs MONOCITI

DOSAGGIO cGMP

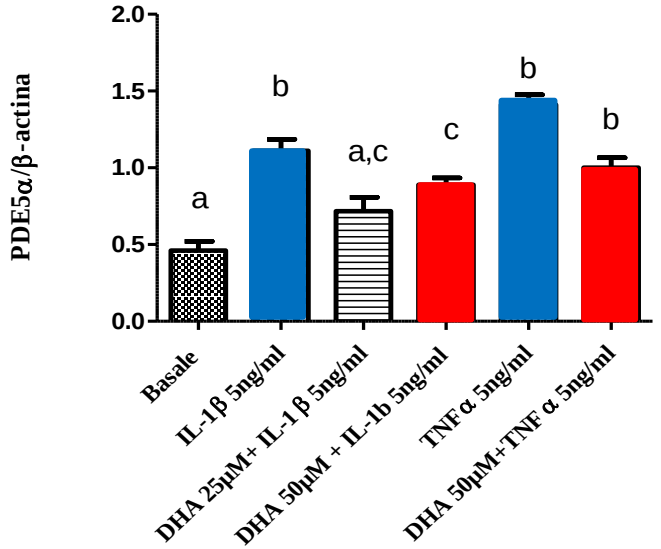


Effetto del DHA sull'espressione endoteliale della PDE5a mediata da stimoli infiammatori





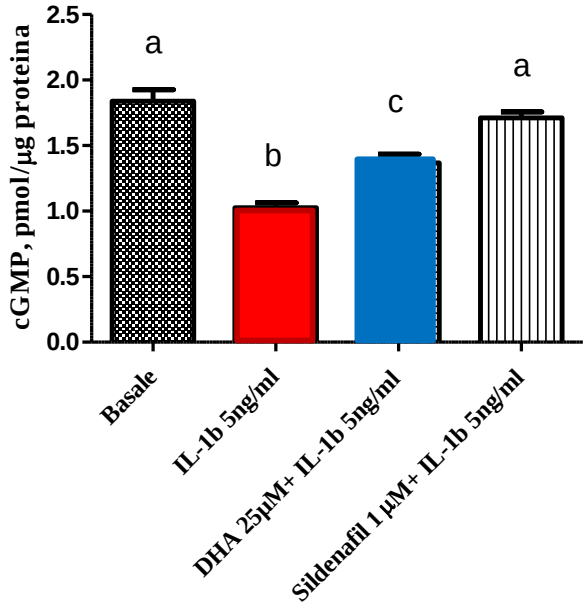
A



B

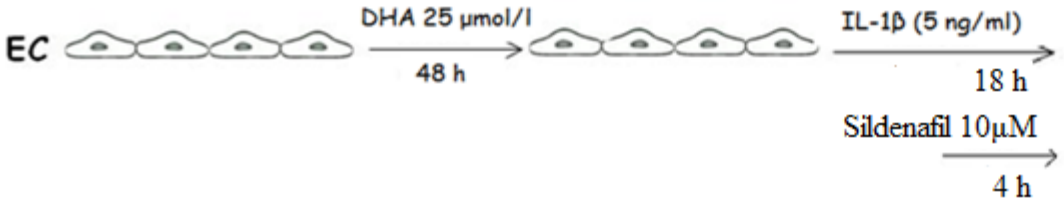
In HUVEC IL-1β e TNF inducono l'espressione della PDE5a di due volte circa rispetto al controllo. Il trattamento con DHA riduce di circa il 30% l'espressione della PDE5a mediata da agenti infiammatori

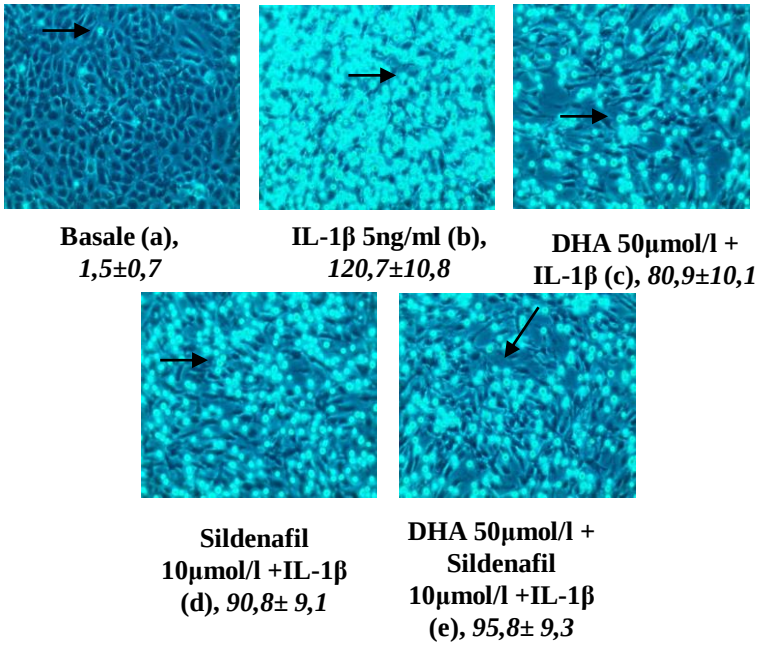
Il DHA e Sildenafil aumentano la concentrazione endoteliale di cGMP ridotta da IL-1β



In HUVEC l'IL-1β riduce a circa la metà la concentrazione di cGMP rispetto al controllo, ed il pretrattamento con DHA riduce l'abbassamento del 28% circa

Valutazione adesività delle HUVEC vs THP1





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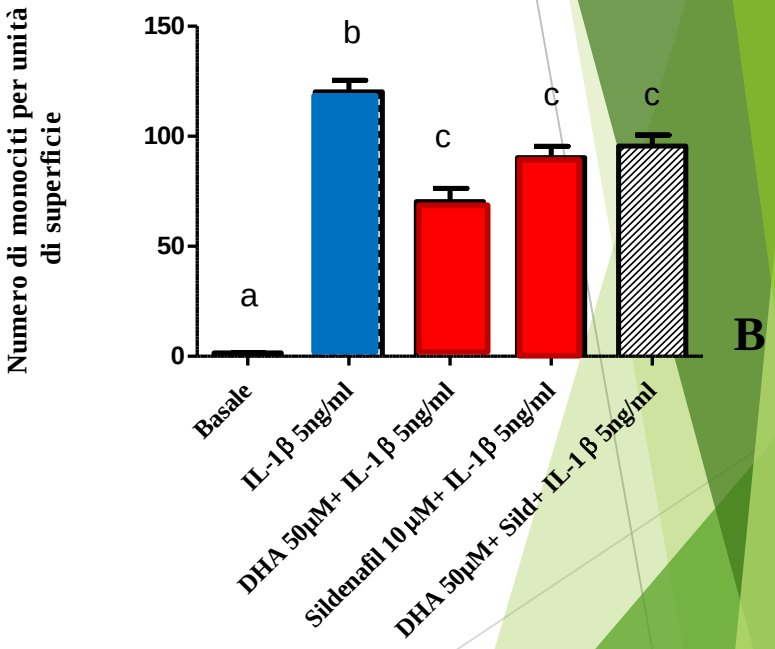

B

Sildenafil e DHA riducono l'adesione monociti-endotelio indotta da stimolo infiammatorio

Valutazione adesività delle HUVEC alle THP1

Antiinflammatory activity of soluble guanylate cyclase: cGMP-dependent down-regulation of P-selectin expression and leukocyte recruitment

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*William Harvey Research Institute, Barts and The London, Charterhouse Square, London EC1M 6BQ, United Kingdom; and †Wolfson Institute for Biomedical Research, University College London, Cruciform Building, Gower Street, London WC1E 6AE, United Kingdom

Edited by Louis J. Ignarro, University of California School of Medicine, Los Angeles, CA, and approved December 5, 2003 (received for review July 9, 2003)

Nitric oxide (NO) production by the vascular endothelium maintains an essential antiinflammatory, cytoprotective influence on the blood vessel wall. A key component of this activity is attributed to prevention of leukocyte–endothelial cell interactions, yet the underlying mechanisms remain unclear. The NO receptor, soluble guanylate cyclase (sGC), is expressed in endothelial cells but fulfils an unknown function. Therefore, we used intravital microscopy in mesenteric postcapillary venules from WT and endothelial nitric oxide synthase (eNOS) knockout ($eNOS^{-/-}$) mice, and an sGC activator (BAY 41–2272), to investigate a potential role for sGC in the regulation of adhesion molecule expression and leukocyte recruitment. Leukocyte rolling and adhesion was 6-fold greater in $eNOS^{-/-}$ than WT animals. BAY 41–2272 and the NO-donor, diethylamine-NONOate, reduced leukocyte rolling and adhesion in $eNOS^{-/-}$ mice to levels observed in WT animals. These effects were blocked by the sGC inhibitor ODQ [1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one], which itself caused a 6-fold increase in leukocyte rolling and adhesion in WT mice. Increased leukocyte rolling and adhesion in IL-1β-treated mice was also inhibited by BAY 41–2272. Fluorescence-activated cell sorting analysis *in vitro* and a specific P-selectin neutralizing antibody *in vivo* revealed that selective down-regulation of P-selectin expression accounted for the antiadhesive effects of sGC activation. These data demonstrate that sGC plays a key antiinflammatory role by inhibiting P-selectin expression and leukocyte recruitment.

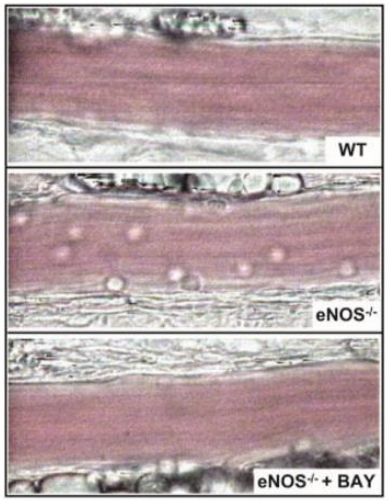
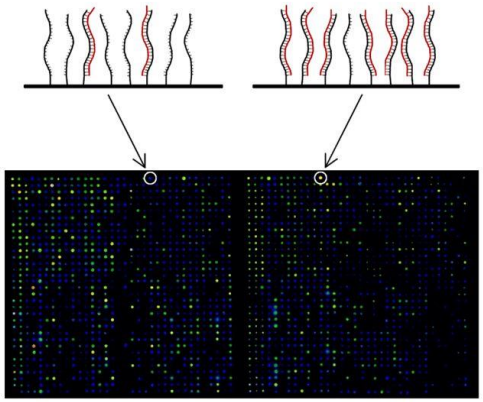


Fig. 1. Video-microscopy images of leukocyte trafficking responses in mouse mesenteric postcapillary venules *in vivo* under basal conditions in WT mice (*Top*), $eNOS^{-/-}$ mice (*Middle*), and $eNOS^{-/-}$ mice in the presence of BAY 41–2272 (1 μM) (*Bottom*). The images are representative of at least five separate experiments.

In the presence of BAY 41–2272 the expression of P-selectin was dramatically attenuated

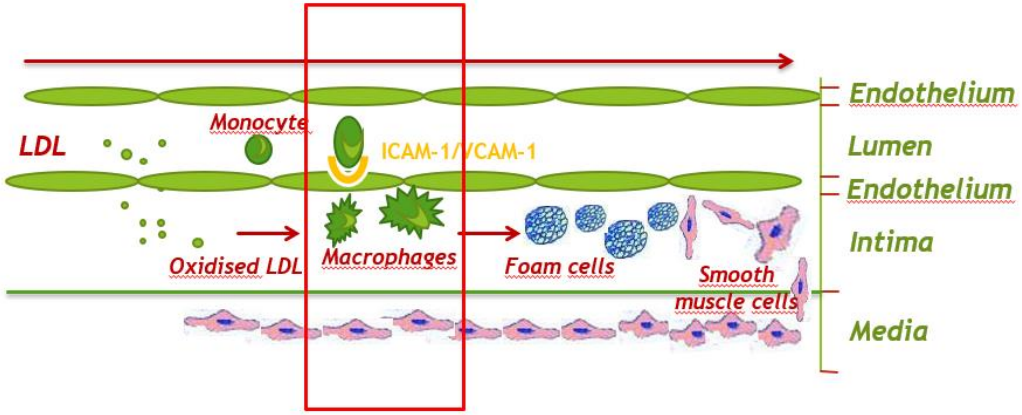
Trascrittomica



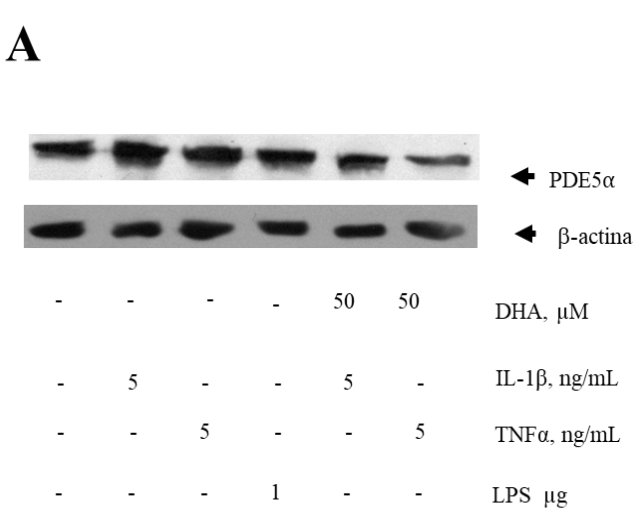
THP1

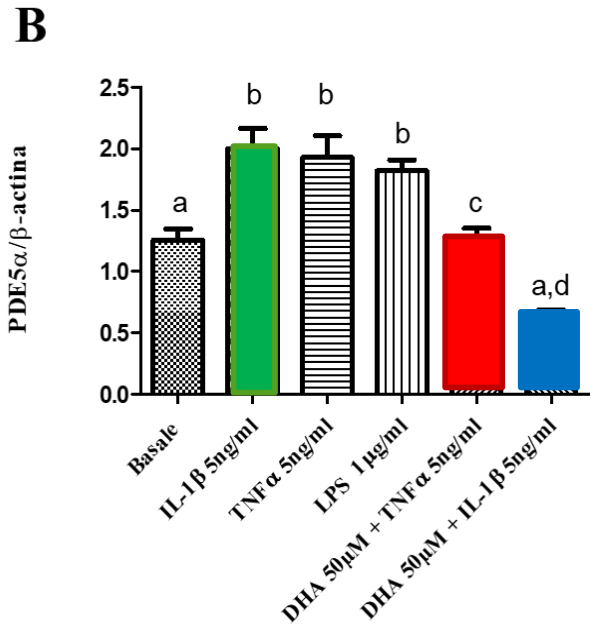
Esplorare effetti cellulari

ADESIVITA' MONOCITI
vs ENDOTELIO



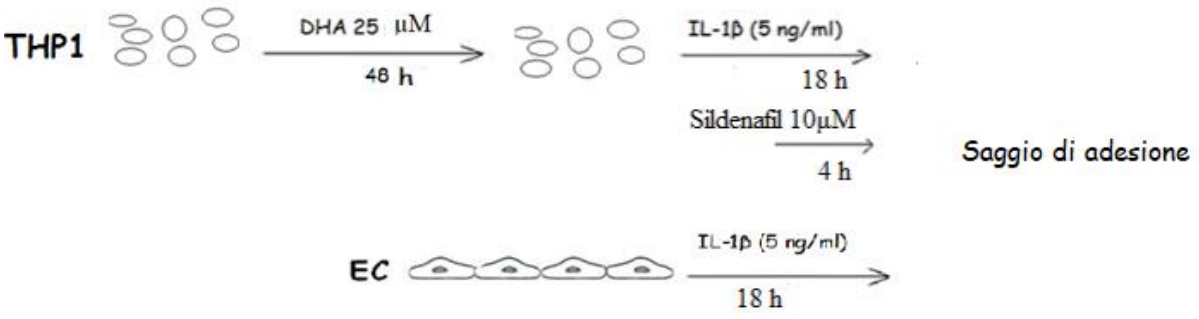
Effetto del DHA sull'espressione della PDE5a indotta da stimolo infiammatorio nelle THP1

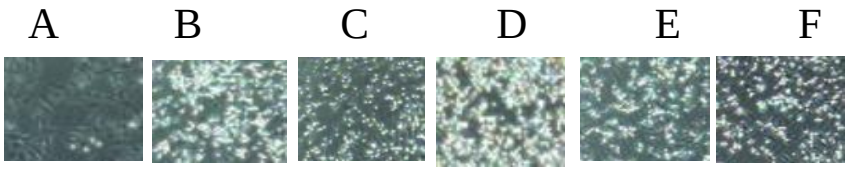


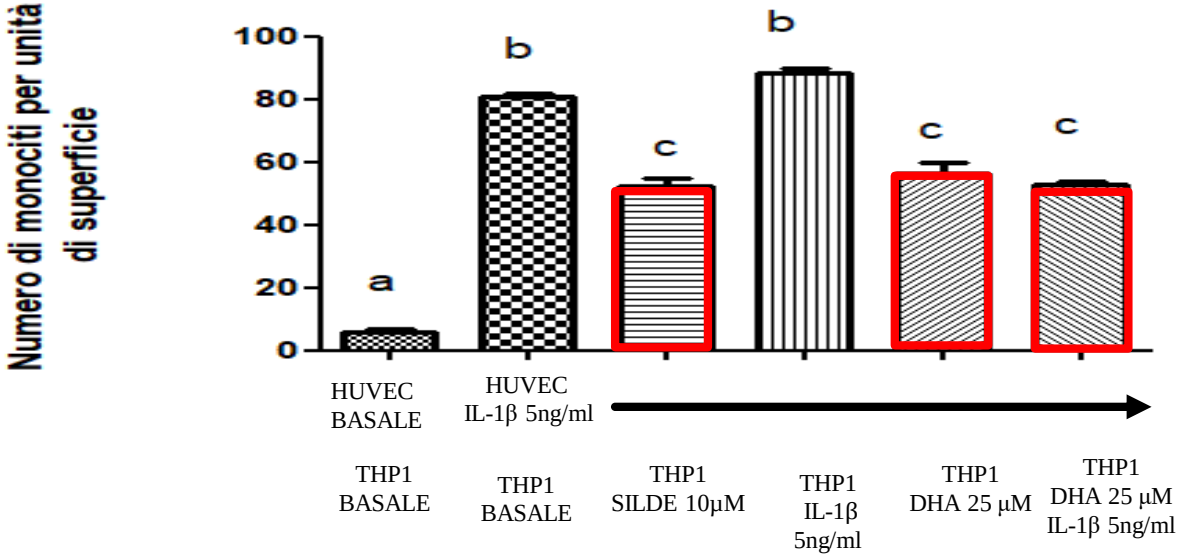


DHA riduce del 50% circa l'espressione proteica della PDE5a indotta dalla stimolazione con IL-1β e del 30% quella indotta da stimolazione con TNFa

Valutazione adesività delle THP1 vs HUVEC

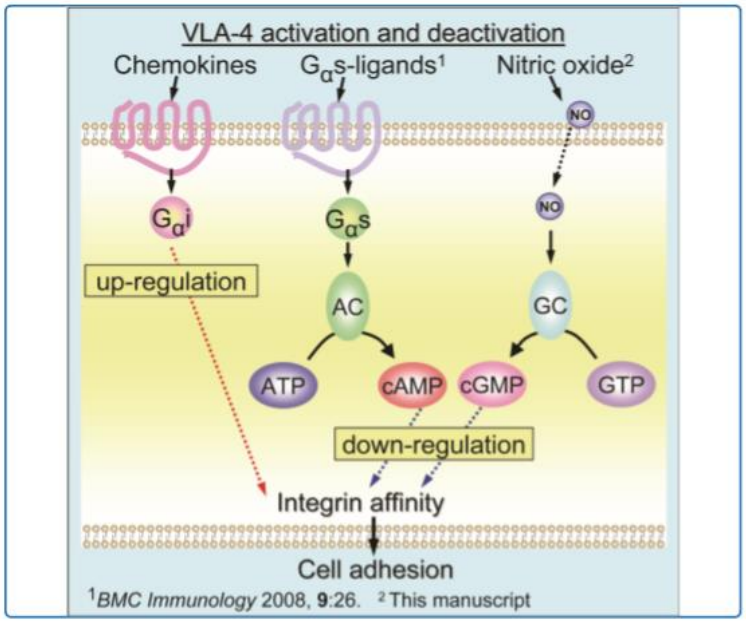






DHA e Sildenafil riducono l'adesione dei monociti all'endotelio attivato

Valutazione adesività delle THP1 alle HUVEC



Nitric oxide/cGMP pathway signaling actively down-regulates α4β1-integrin affinity: an unexpected mechanism for inducing cell de-adhesion

Chigaev *et al.*

BioMed Central

Chigaev et al. BMC Immunology 2011, 12:28
http://www.biomedcentral.com/1471-2172/12/28 (17 May 2011)

La PDE5a nei pazienti con ipertensione polmonare

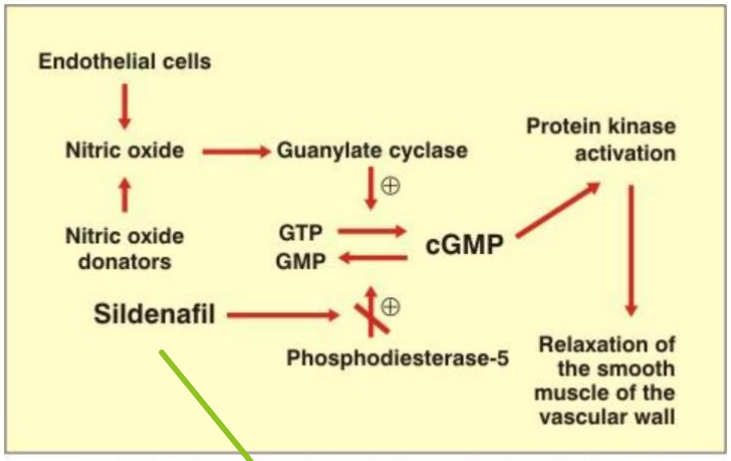


Figure 1 - Sildenafil action mechanism algorithm, modified by Abrams et al.[6]

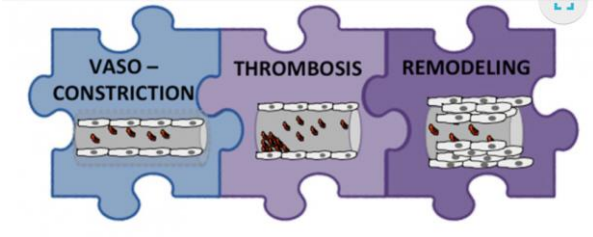


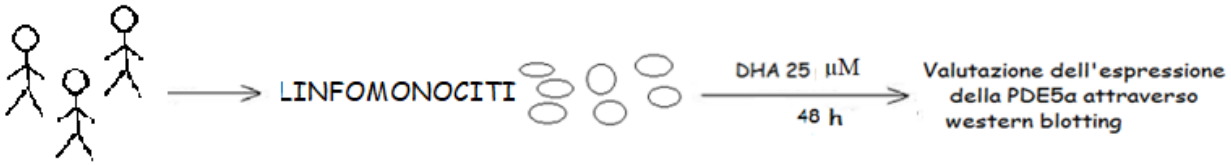
Figure 1

The three pieces in the pathogenesis of pulmonary hypertension. Pure vasoconstriction occurs in early disease; microthrombotic events are observed at increasing frequency during evolution of the disease; remodelling of the small pulmonary arteries is arguably the most important factor.

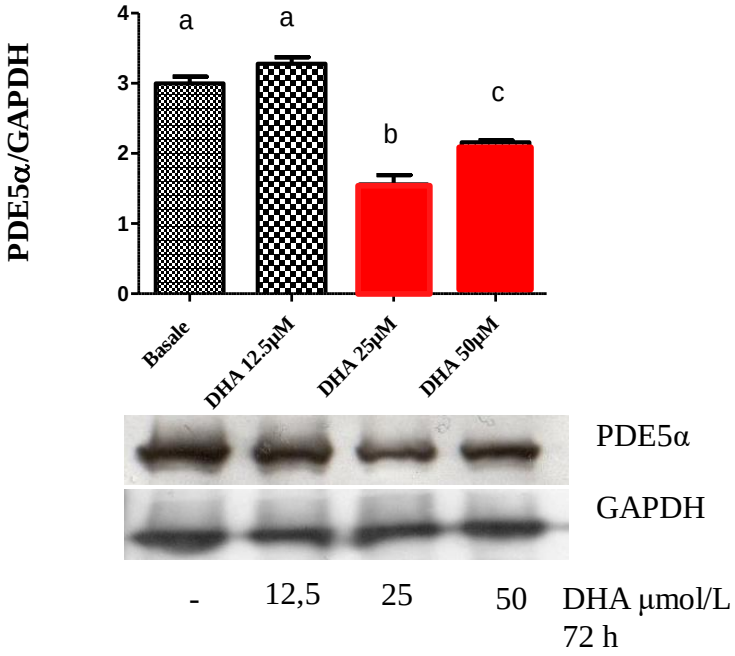
The pathogenesis of pulmonary hypertension – an update
Lars C. Huber, Hannah Bye, Matthias Brock)

Viagra

Effetto del DHA sull'espressione della PDE5a in linfomonociti di pazienti con ipertensione polmonare

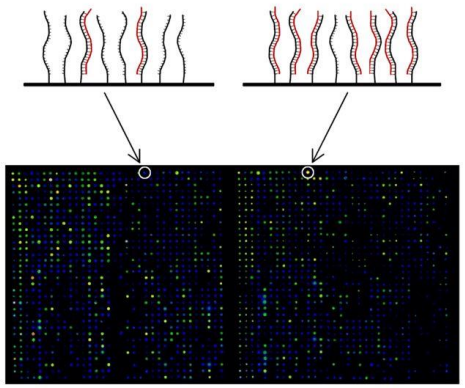


n=3



Il DHA 25µM e 50µM per 72h riduce l'espressione della PDE5a del 50% e del 30% circa, rispettivamente

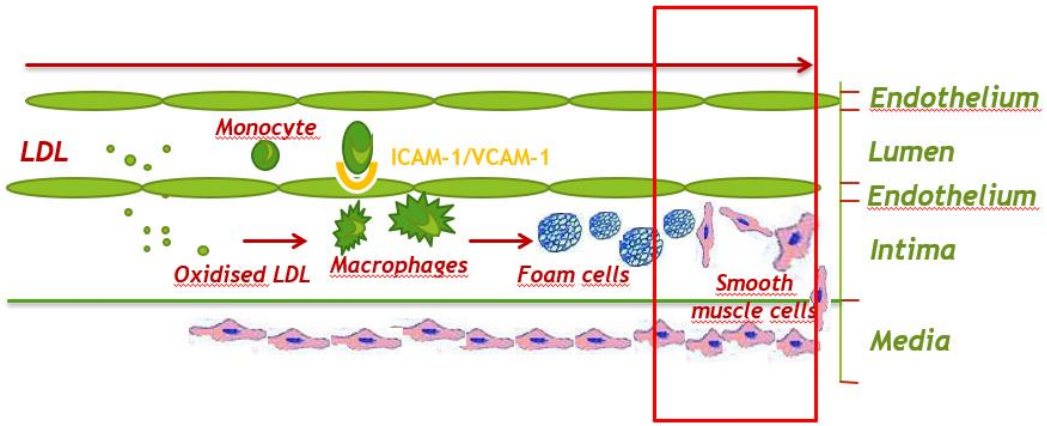
Trascrittomica



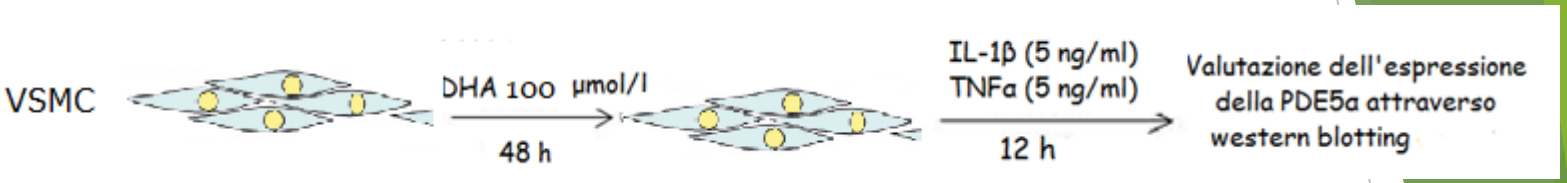
VSMC

Esplorare effetti cellulari

CAPACITA' MIGRATORIA



Effetto del DHA sull'espressione della PDE5a indotta da stimolo infiammatorio nelle cellule muscolari lisce



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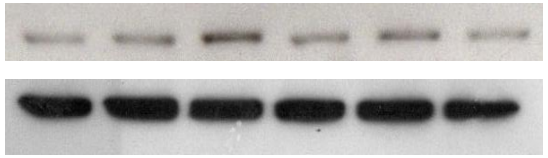
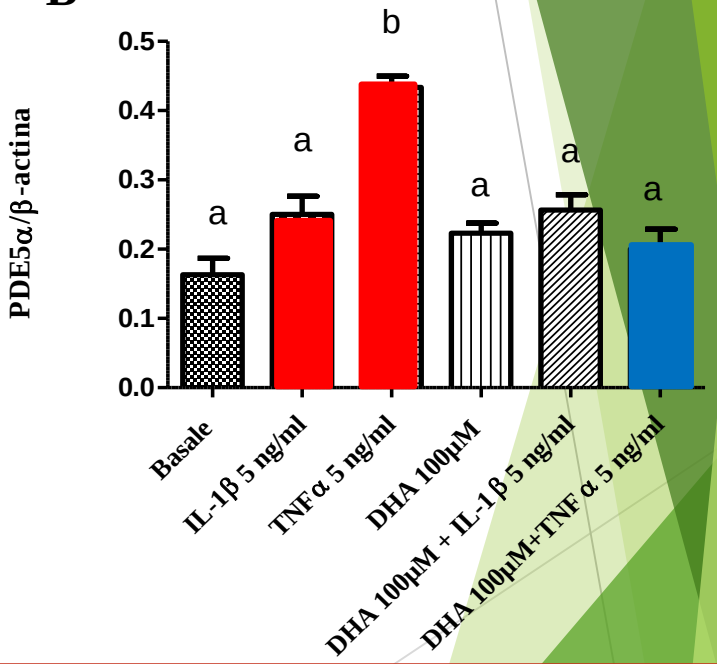


PDE5α

B-actina

-	-	-	100	100	100	DHA, µmol/L
-	5	-	-	5	-	IL-1β, ng/mL
-	-	5	-	-	5	TNFα, ng/mL

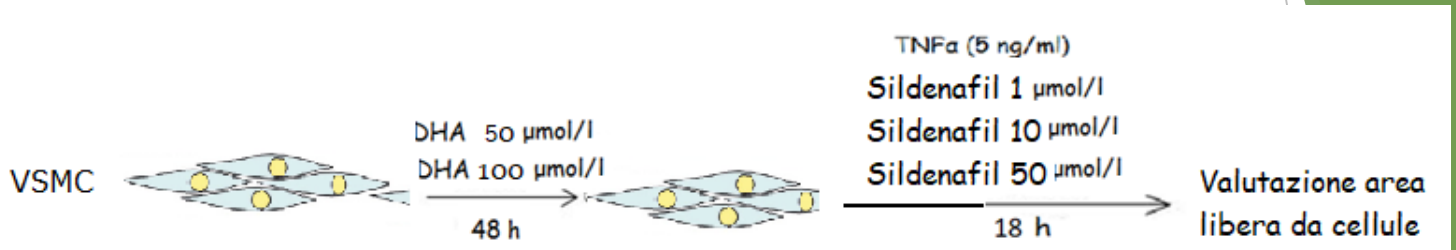
B



Stimoli infiammatori inducono l'espressione della PDE5a

Il DHA riduce l'induzione da parte del TNFa del 50% circa

Effetto dell'inibizione della PDE5a sulla migrazione delle cellule muscolari lisce indotta da stimolo infiammatorio



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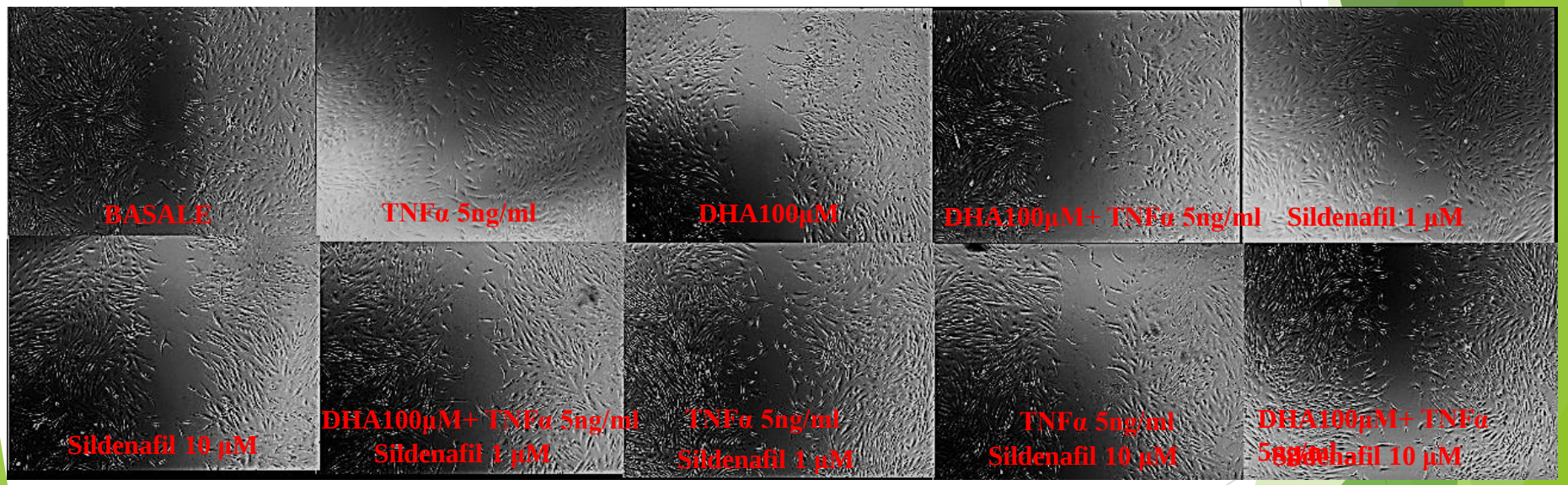
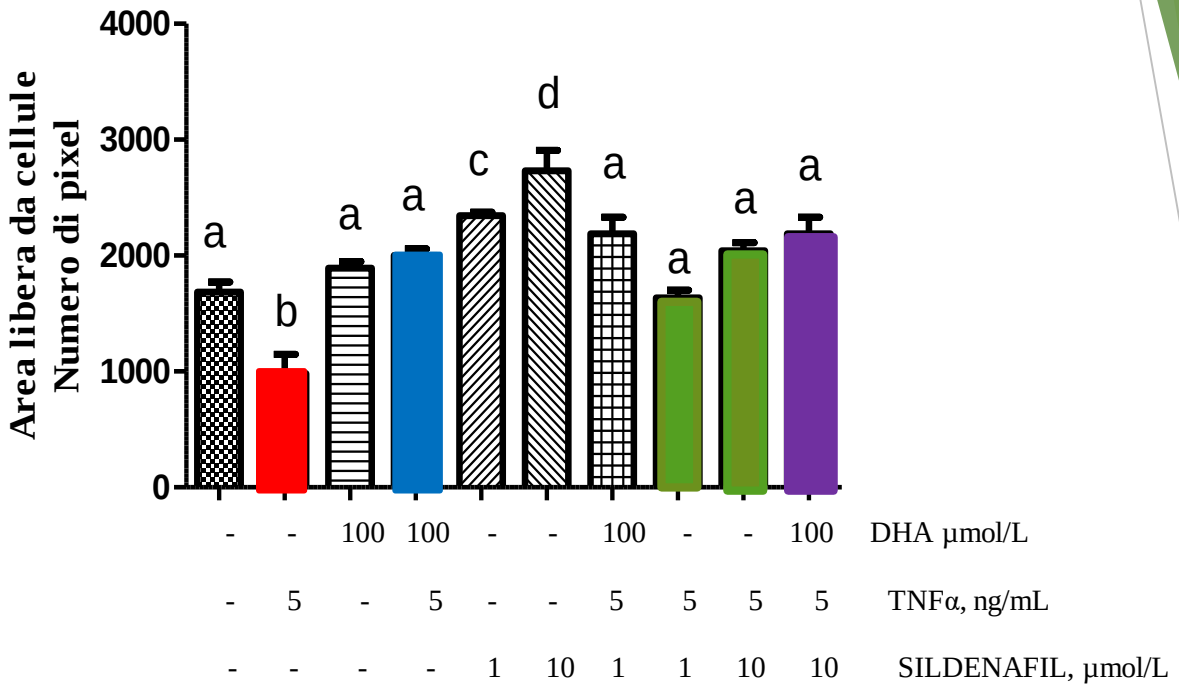


Effetto dell'inibizione della PDE5a, DHA e Sildenafil mediata, sulla migrazione delle cellule muscolari lisce indotta da stimolo infiammatorio



TNFa riduce l'area libera da cellule del 40%. Il pretrattamento con il DHA, seguito da stimolo infiammatorio, aumenta di 2 volte circa l'area libera da cellule rispetto al solo stimolo. Anche il trattamento con Sildenafil seguito dallo stimolo infiammatorio aumenta l'area libera da cellule del 50% circa, rispetto al solo stimolo infiammatorio, ma non è statisticamente differente dall'aumento generato dal cotrattamento con DHA e Sildenafil

Valutazione migrazione cellule muscolari lisce

Soluble guanylyl cyclase-activated cyclic GMP-dependent protein kinase inhibits arterial smooth muscle cell migration independent of VASP-Serine 239 phosphorylation

Andrew W. Holt[1], **Danielle N. Martin**[1], **Patti R. Shaver**[2], **Shaquria P. Adderley**[1], **Joshua D. Stone**[1], **Chintamani N. Joshi**[1], **Jake T. Francisco**[1], **Robert M. Lust**[1], **Douglas A. Weidner**[3], **Brian M. Shewchuk**[2], and **David A. Tulis**[1,*]

[1]Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC

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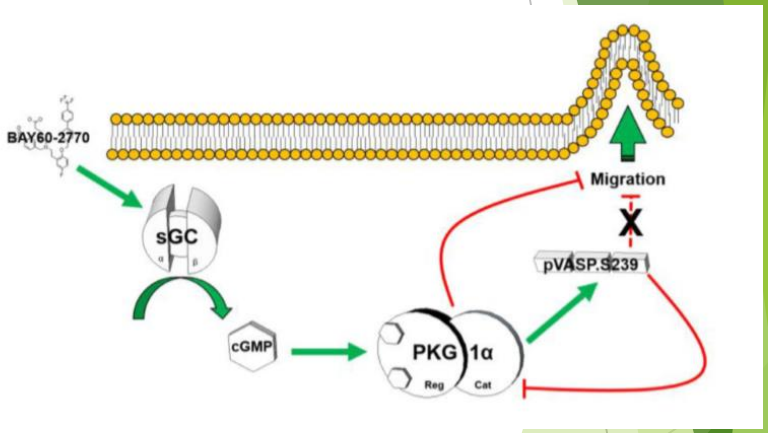
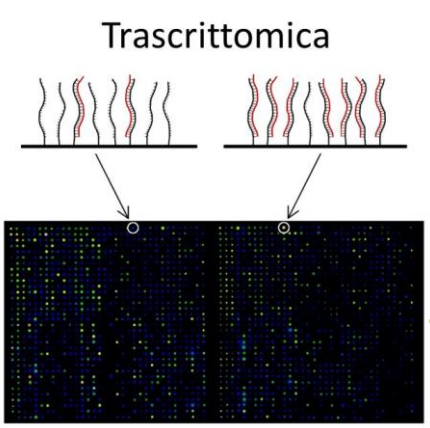
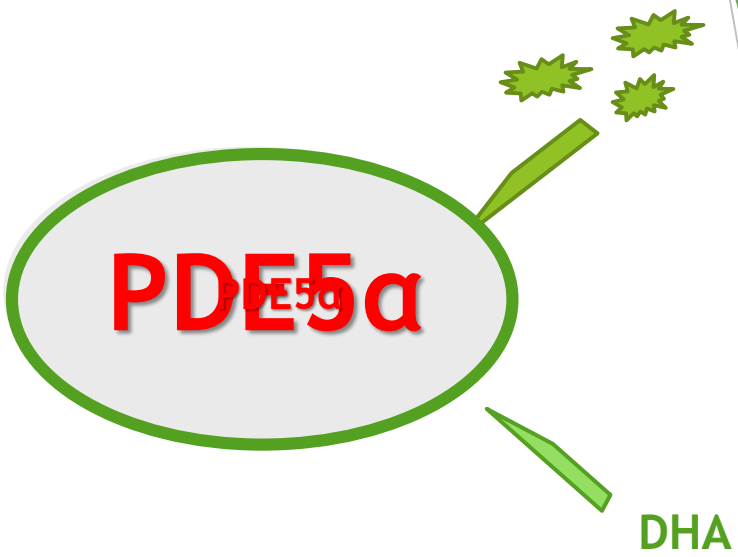
Abstract

Coronary artery disease (CAD) accounts for over half of all cardiovascular disease-related deaths. Uncontrolled arterial smooth muscle (ASM) cell migration is a major component of CAD pathogenesis and efforts aimed at attenuating its progression are clinically essential. Cyclic nucleotide signaling has long been studied for its growth-mitigating properties in the setting of CAD and other vascular disorders. Heme-containing soluble guanylyl cyclase (sGC) synthesizes cyclic guanosine monophosphate (cGMP) and maintains vascular homeostasis predominantly through cGMP-dependent protein kinase (PKG) signaling. Considering that reactive oxygen species (ROS) can interfere with appropriate sGC signaling by oxidizing the cyclase heme moiety and so are associated with several CVD pathologies, the current study was designed to test the hypothesis that heme-independent sGC activation by BAY60-2770 (BAY60) maintains cGMP levels despite heme oxidation and inhibits ASM cell migration through phosphorylation of the PKG target and actin-binding vasodilator-stimulated phosphoprotein (VASP). First, using the heme oxidant ODQ, cGMP content was potentiated in the presence of BAY60. Using a rat model of arterial growth, BAY60 significantly reduced neointima formation and luminal narrowing compared to vehicle (VEH)-treated controls. In rat ASM cells BAY60 significantly attenuated cell migration, reduced G:F actin, and increased PKG activity and VASP Ser239 phosphorylation



Conclusioni





- MONOCITI LINEA THP1
- CELLULE ENDOTELIALI

→ RIDOTTA ADESIONE MONOCITI ENDOTELIO

- CELLULE MUSCOLARI LISCE → RIDOTTA MIGRAZIONE CELLULARE
- LINFOMONOCITI DI SOGGETTI CON IPERTENSIONE POLMONARE

Inserimento del **DHA** come nutraceutico nella dieta di pazienti affetti da patologie PDE5α dipendenti

GRAZIE

STUDIO EFFETTUATO IN COLLABORAZIONE CON LA SEZIONE DI BIOLOGIA VASCOLARE DEL CNR DI LECCE