Omega-3 fatty acids in inflammation: Actions & impact on rheumatoid arthritis, inflammatory bowel disease and asthma

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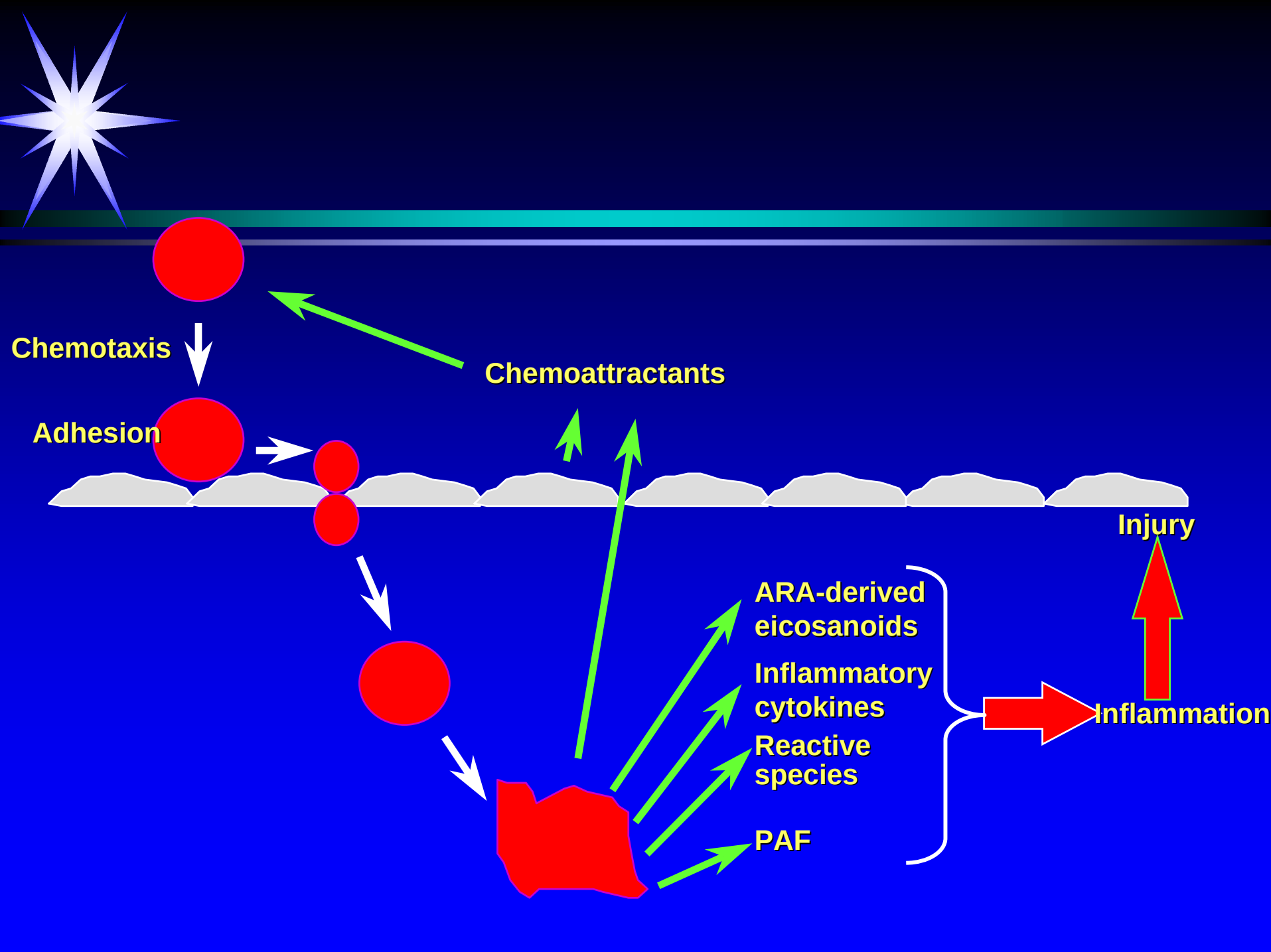
What is inflammation?

- Inflammation is a NORMAL response to infection, injury and trauma
- Typified by redness, swelling, heat and pain
- Normally it is protective (and so beneficial)
- Can be acute (i.e. short lived) or chronic (i.e. long term)
- Involves various cells including granulocytes (e.g. neutrophils), macrophages and lymphocytes
- Involves mediators


Chemotaxis
Chemoattractants
Adhesion
Injury
ARA-derived eicosanoids
Inflammatory cytokines
Reactive species
PAF
Inflammation

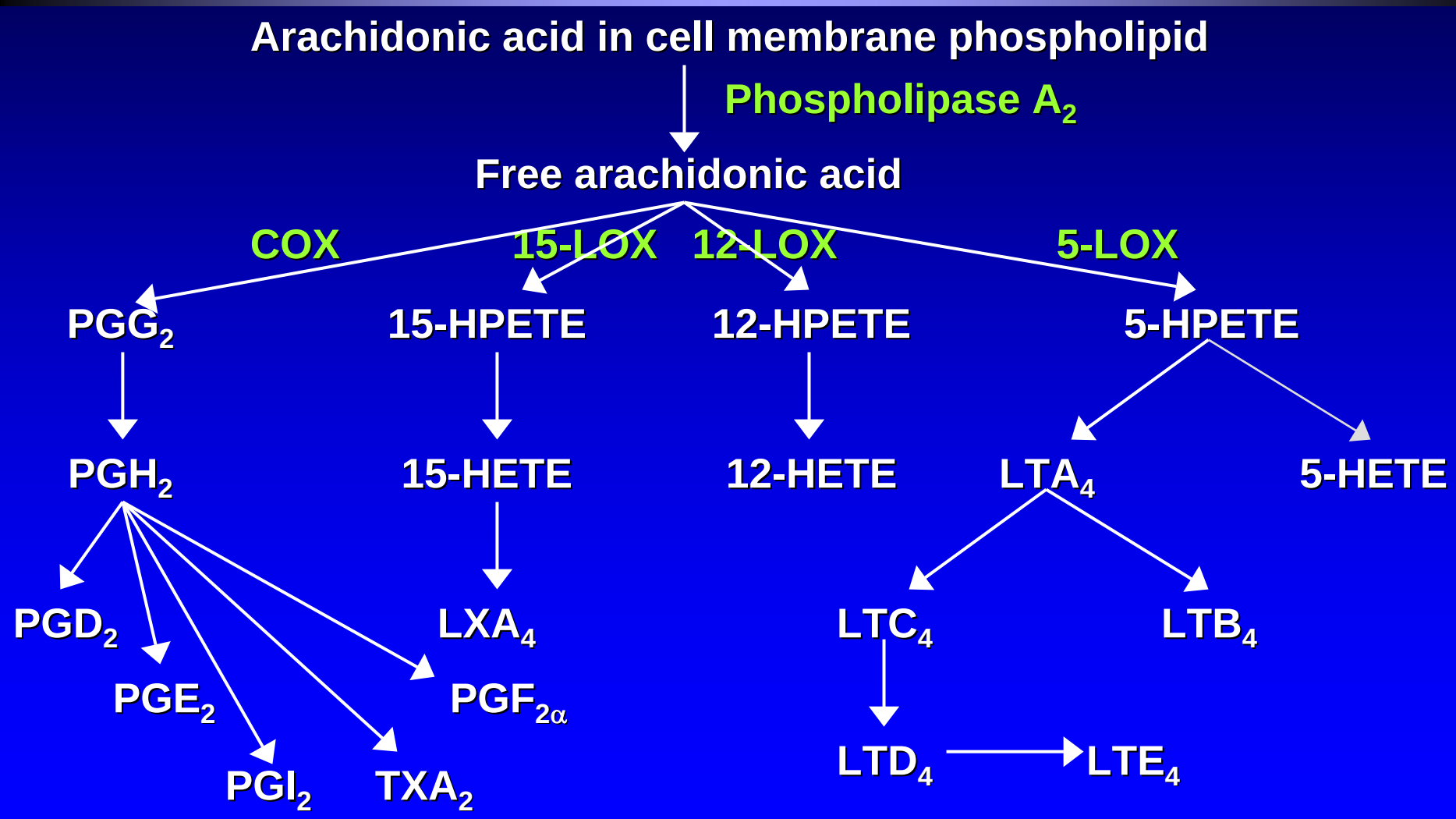
Diseases or conditions that involve inflammation

Rheumatoid arthritis
Crohn's disease
Ulcerative colitis
Cystic fibrosis
Psoriasis
Lupus
Type-1 diabetes
Childhood asthma
Adult asthma
Allergic diseases
Atherosclerosis
Acute cardiovascular events
Post-surgery
Trauma & sepsis
Obesity …….

ω-6 PUFA content of human mononuclear cells

	% of total fatty acids
Linoleic acid (18:2ω-6)	10
DGLA (20:3ω-6)	1.5
Arachidonic acid (20:4ω-6)	20

A major role of arachidonic acid is as a precursor for eicosanoids



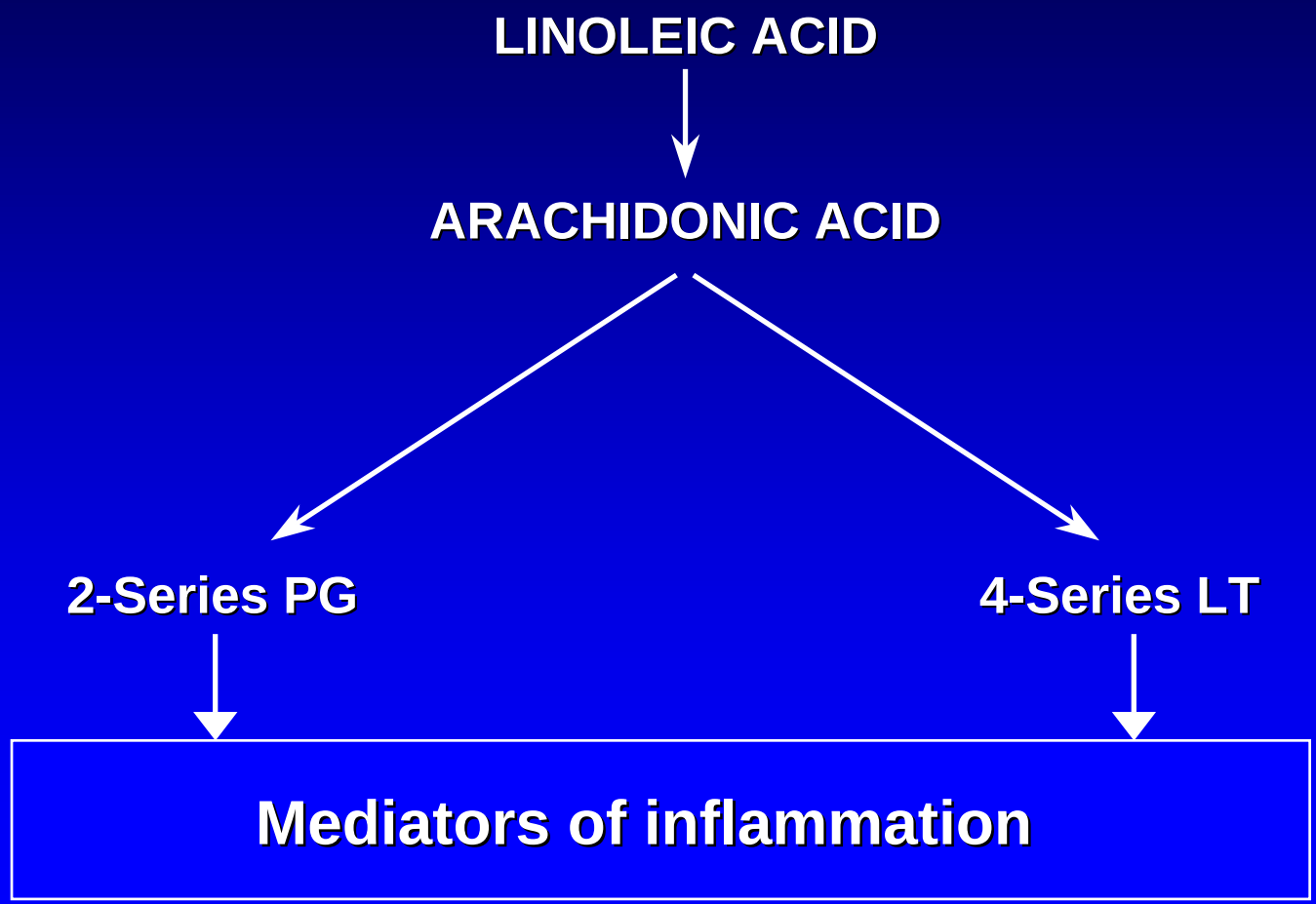
Some pro-inflammatory effects of PGE_2

Induces fever
Increases vascular permeability
Increases vasodilation
Causes pain
Enhances pain caused by other agents

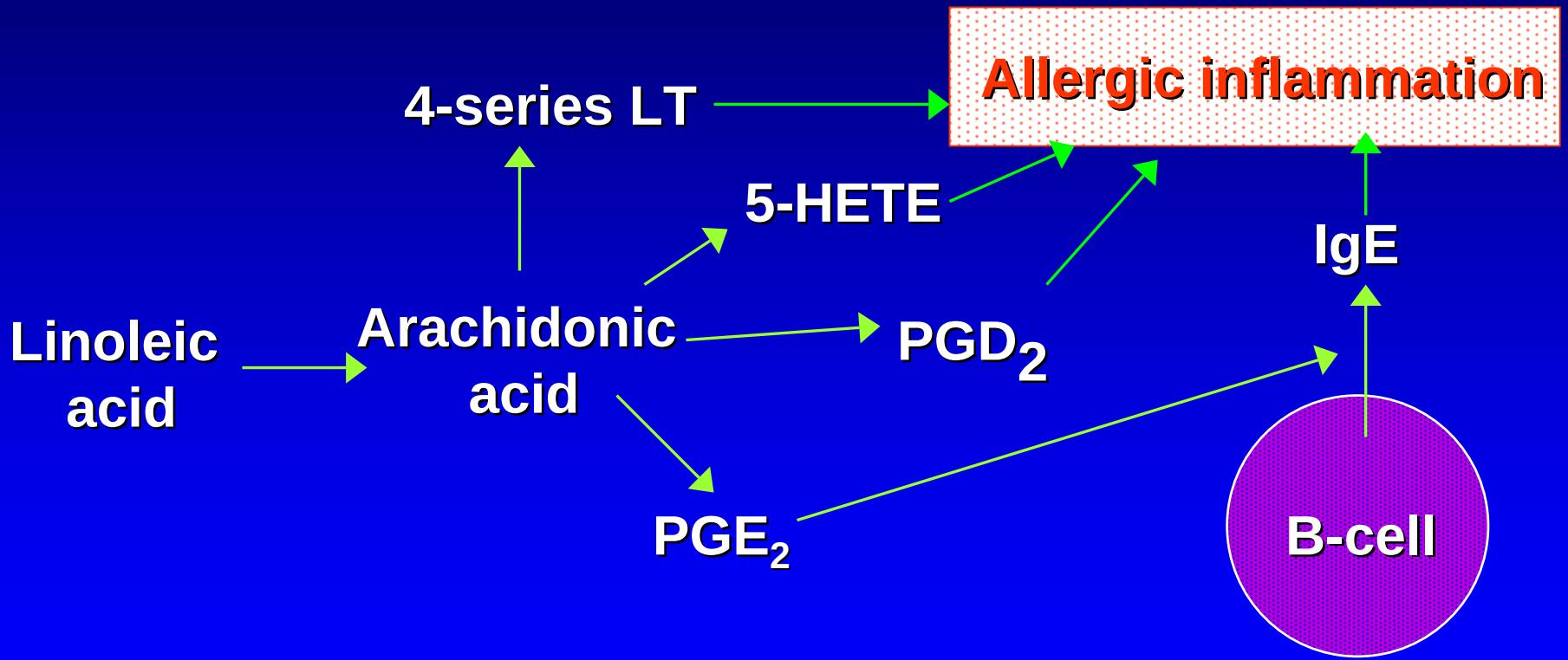
Induces its own production
Induces production of IL-6 (a pro-inflammatory cytokine)

Some pro-inflammatory effects of 4-series leukotrienes

	LTB_4	LTC_4, D_4, E_4
Produced by	Neutrophils, macrophages	Mast cells, basophils, eosinophils
Actions	Leukocyte chemotaxis Vascular permeability Epidermal proliferation Leukocyte degranulation Leukocyte adhesion Inflammatory mediator production (Superoxide; Inflammatory cytokines) Pain	Bronchoconstriction Vascular permeability Mucus secretion Hypersensitivity Skin vasodilation Arteriole constriction

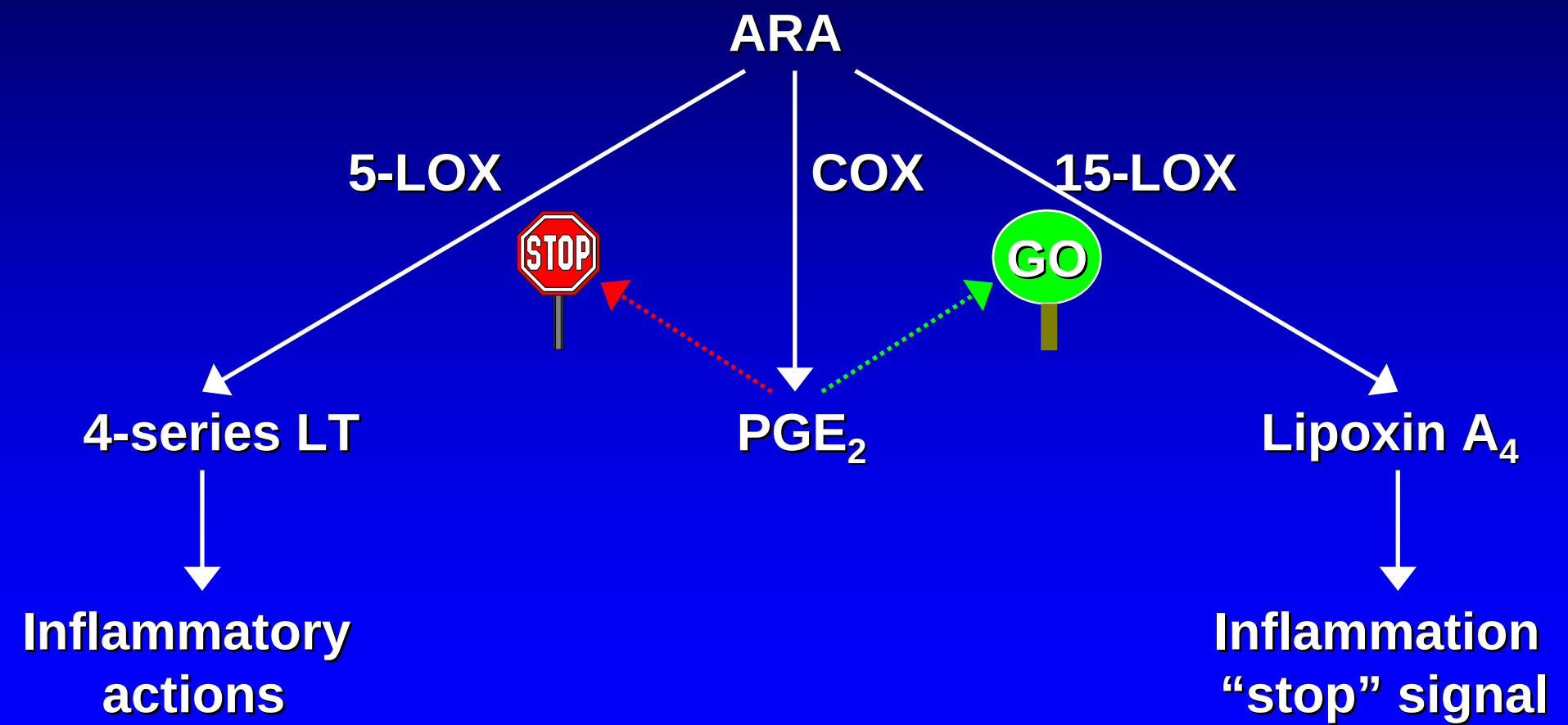

LINOLEIC ACID
ARACHIDONIC ACID
2-Series PG
4-Series LT
Mediators of inflammation

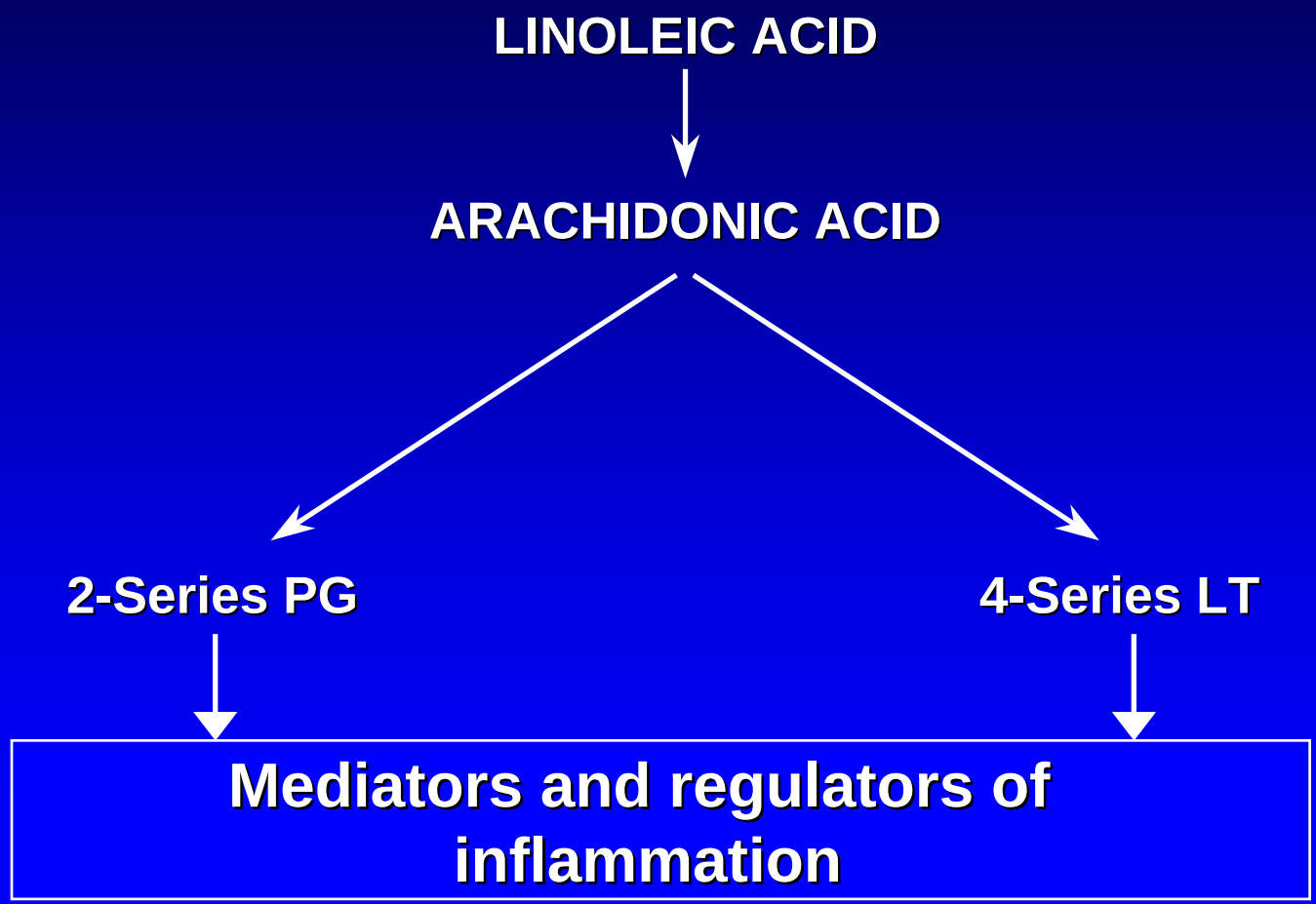
Allergic inflammation
- role of arachidonic acid-derived mediators



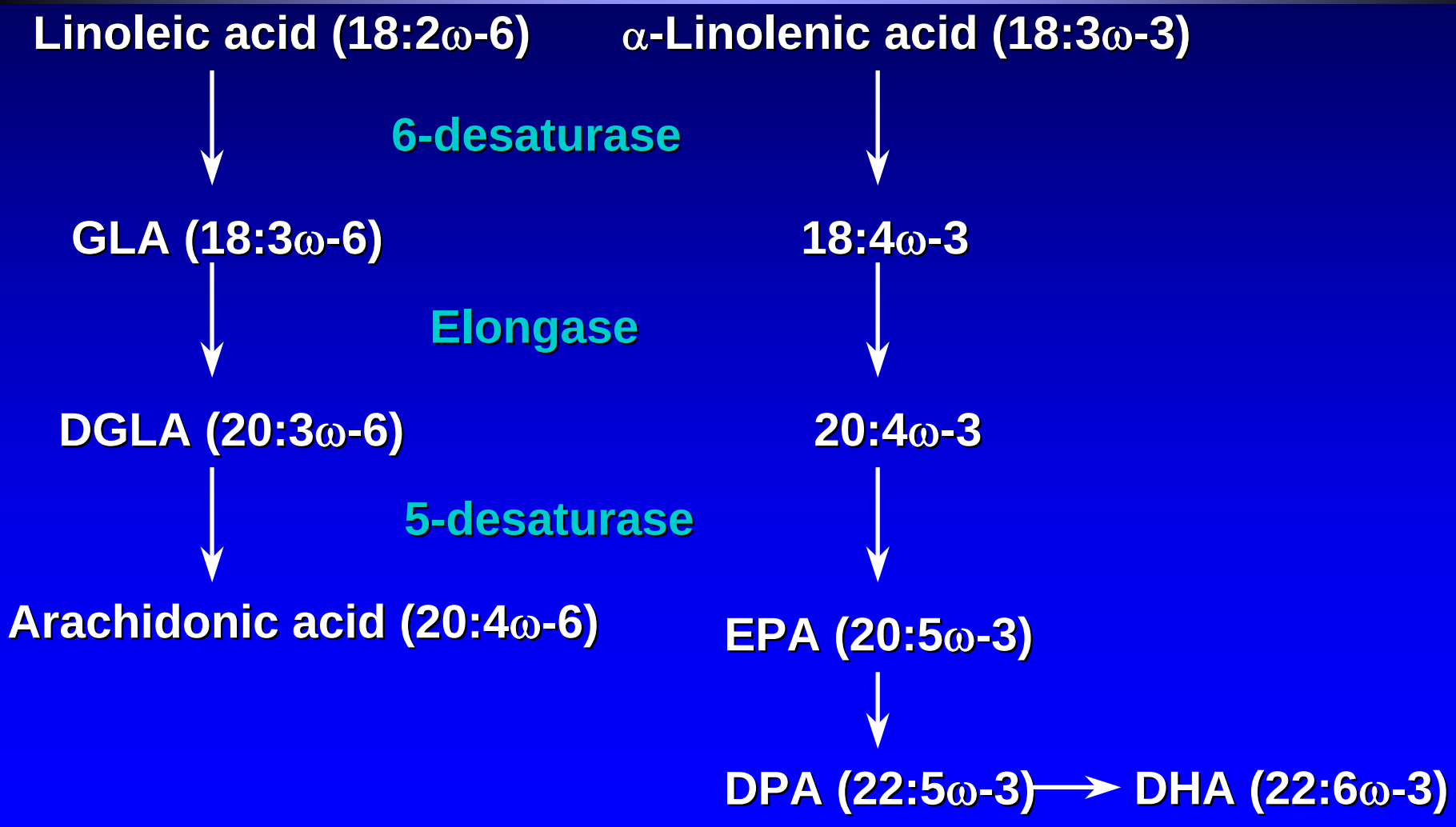
A new anti-inflammatory role for PGE_2?

Levy et al. (2001) Nature Immunol. 2, 612-619




LINOLEIC ACID
ARACHIDONIC ACID
2-Series PG
4-Series LT
Mediators and regulators of inflammation

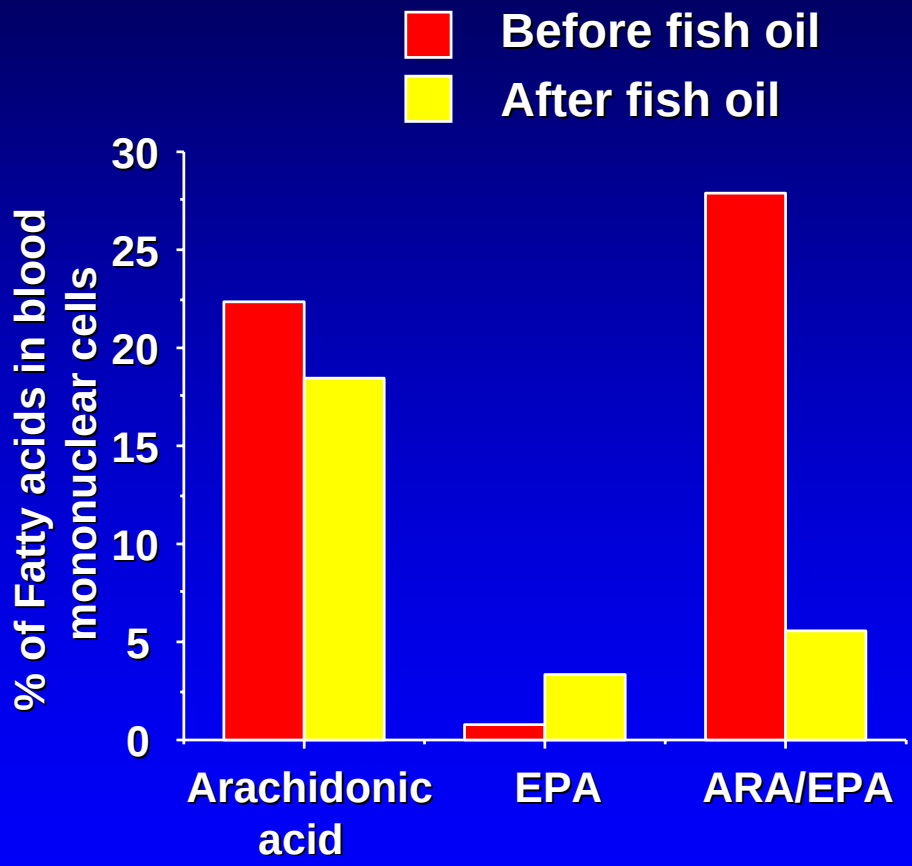
Metabolism of ω-6 and ω-3 PUFA



ω-6 and ω-3 PUFA contents of human mononuclear cells

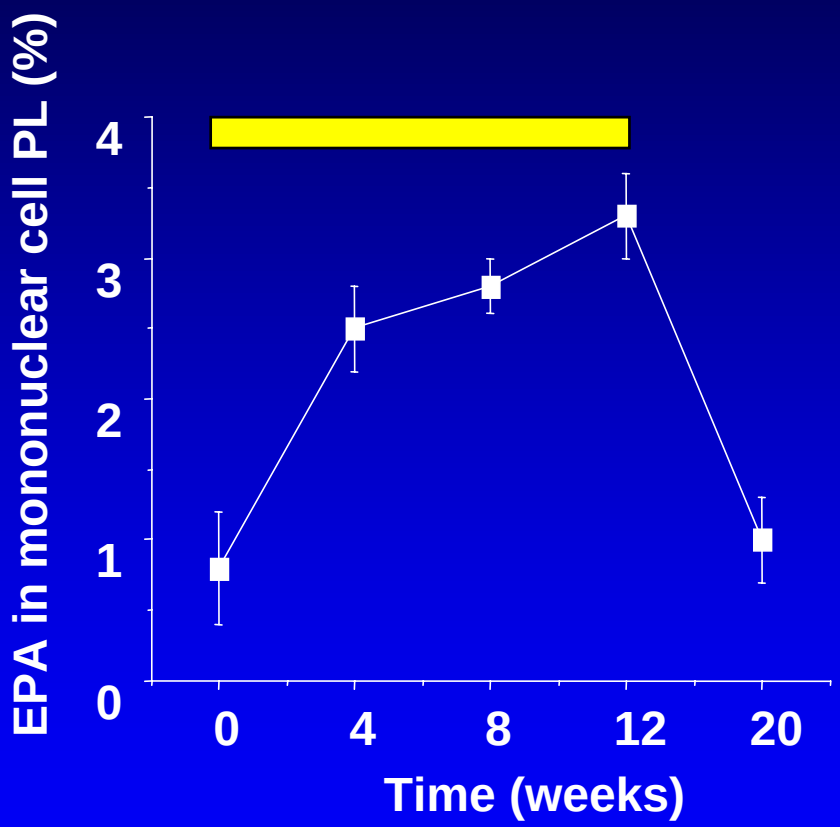
	% of total fatty acids
Linoleic acid (18:2ω-6)	10
DGLA (20:3ω-6)	1.5
Arachidonic acid (20:4ω-6)	20
α-Linolenic acid (18:3ω-3)	< 0.5
EPA	1.0
DHA	2.5

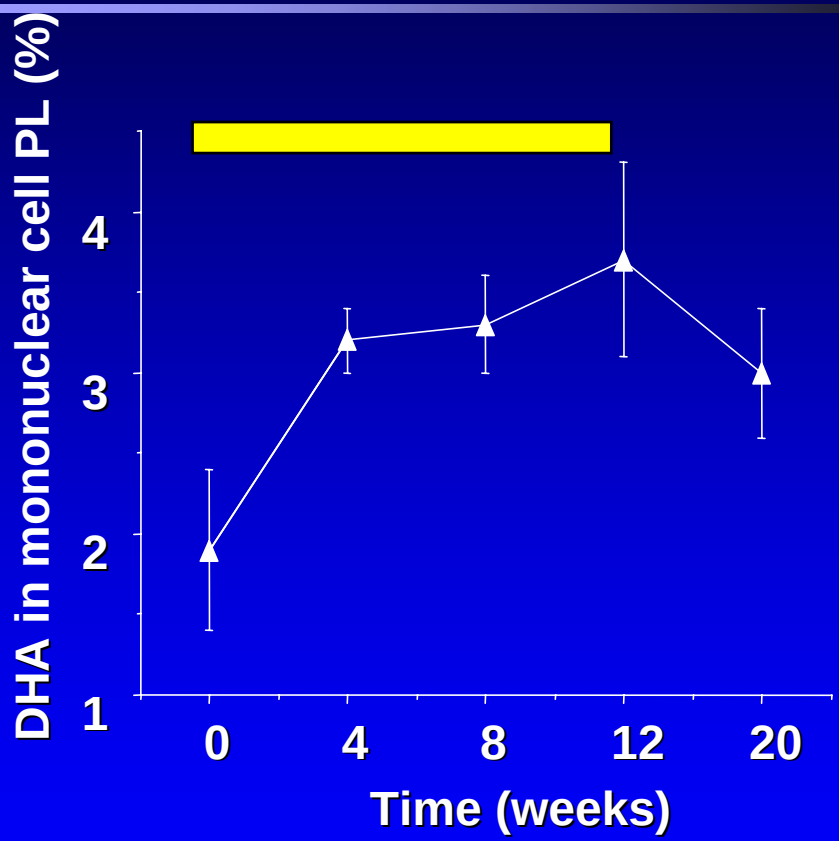
Feeding fish oil decreases the amount of arachidonic acid in human mononuclear cells



Healthy subjects given fish oil providing 2.1 g EPA + 1.1 g DHA/day for 12 weeks
Yaqoob et al. (2000) Eur. J. Clin. Invest. 30, 260-274

Time course of incorporation of EPA and DHA into human mononuclear cell phospholipids

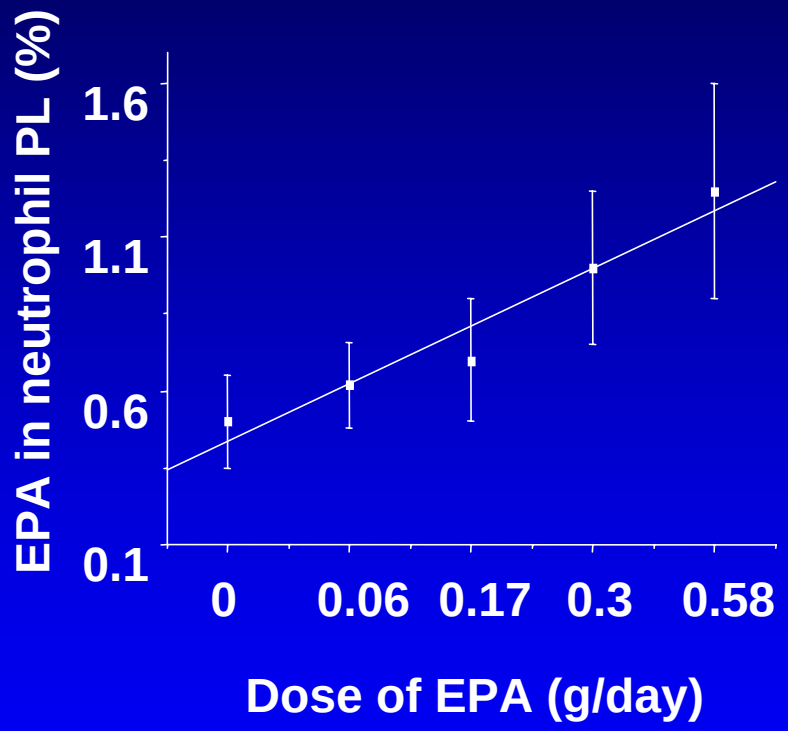


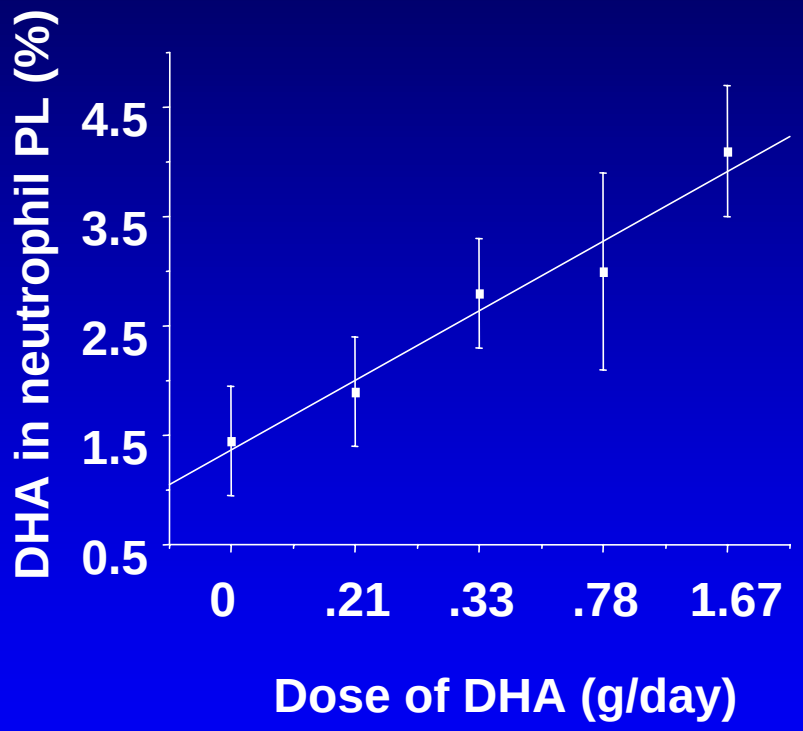


Healthy volunteers given fish oil (2.1 g EPA and 1.1 g DHA/day) for 12 weeks

Yaqoob et al. (2000) Eur. J. Clin. Invest. 30, 260-274

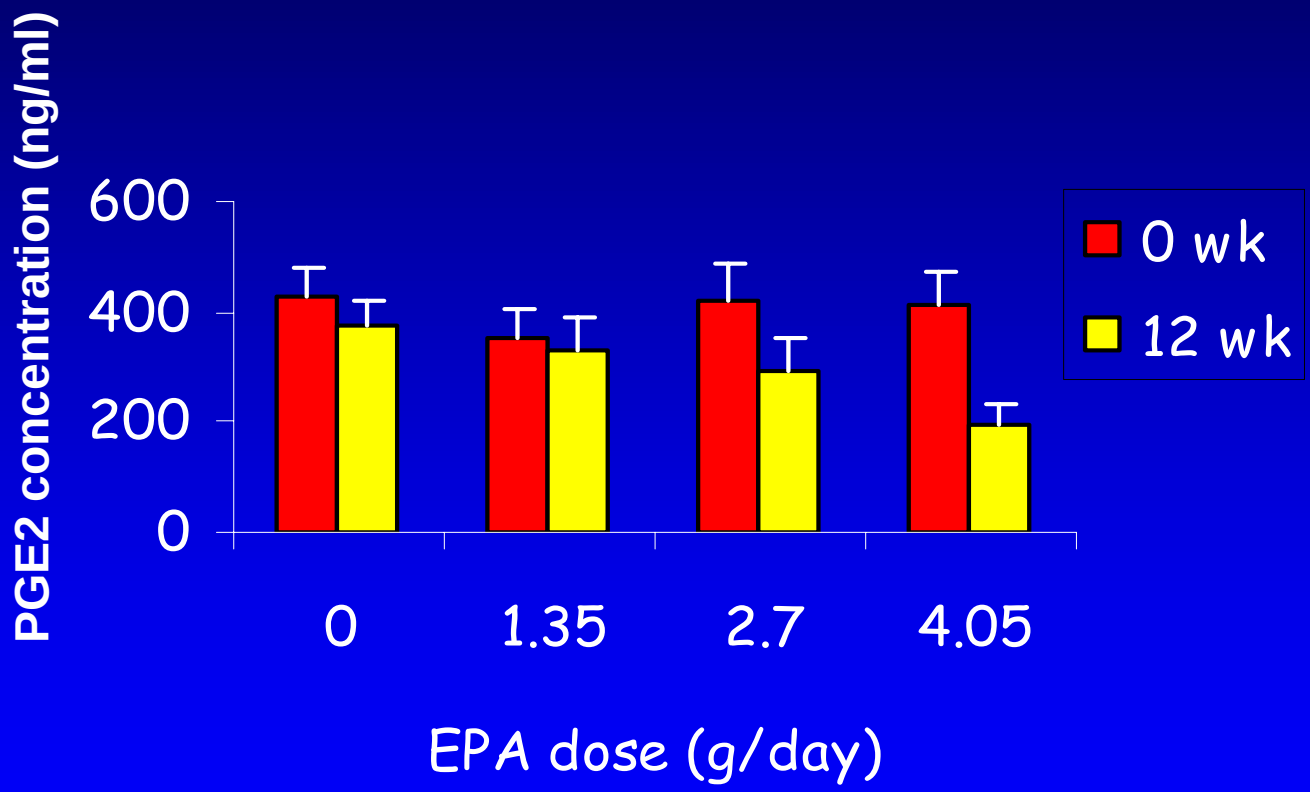
Dose response of incorporation of EPA and DHA into human neutrophil phospholipids





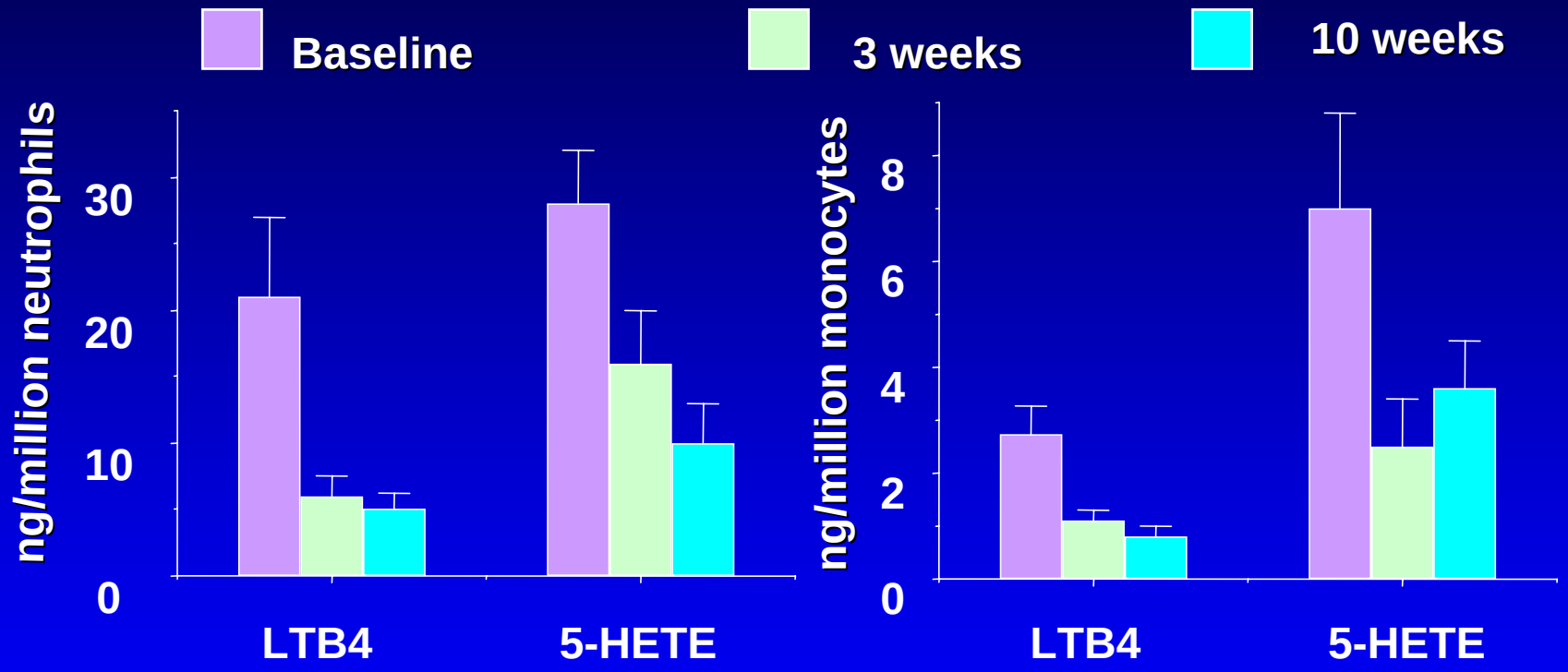
Healthy volunteers given fish oil (0 to 9 g/day) for 12 weeks
Healy et al. (2000) Lipids 35, 763-768

PGE_2 production by human mononuclear cells before and after fish oil supplementation



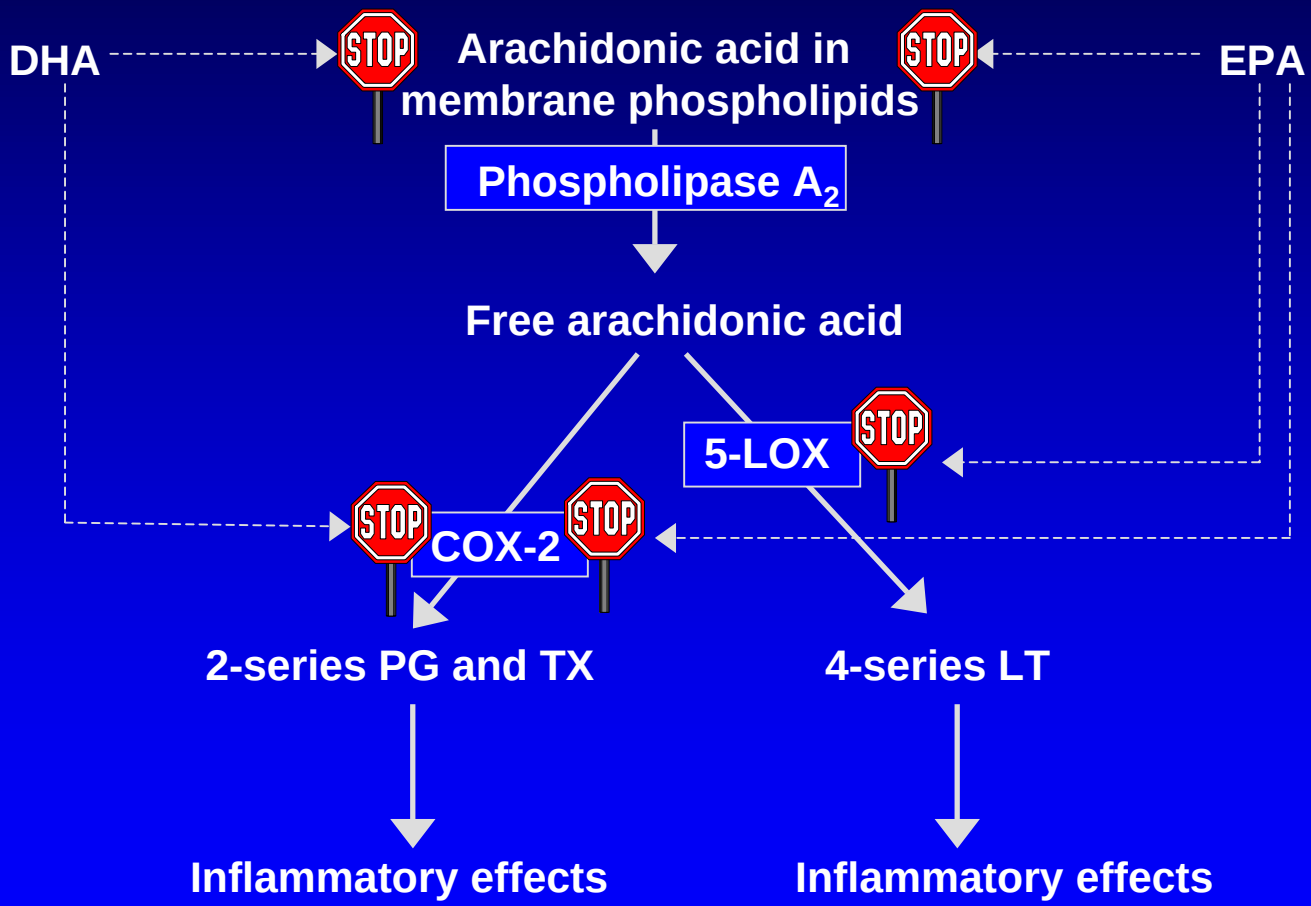
Miles & Calder, unpublished

Effect of fish oil on 5-LOX metabolite production by human inflammatory cells

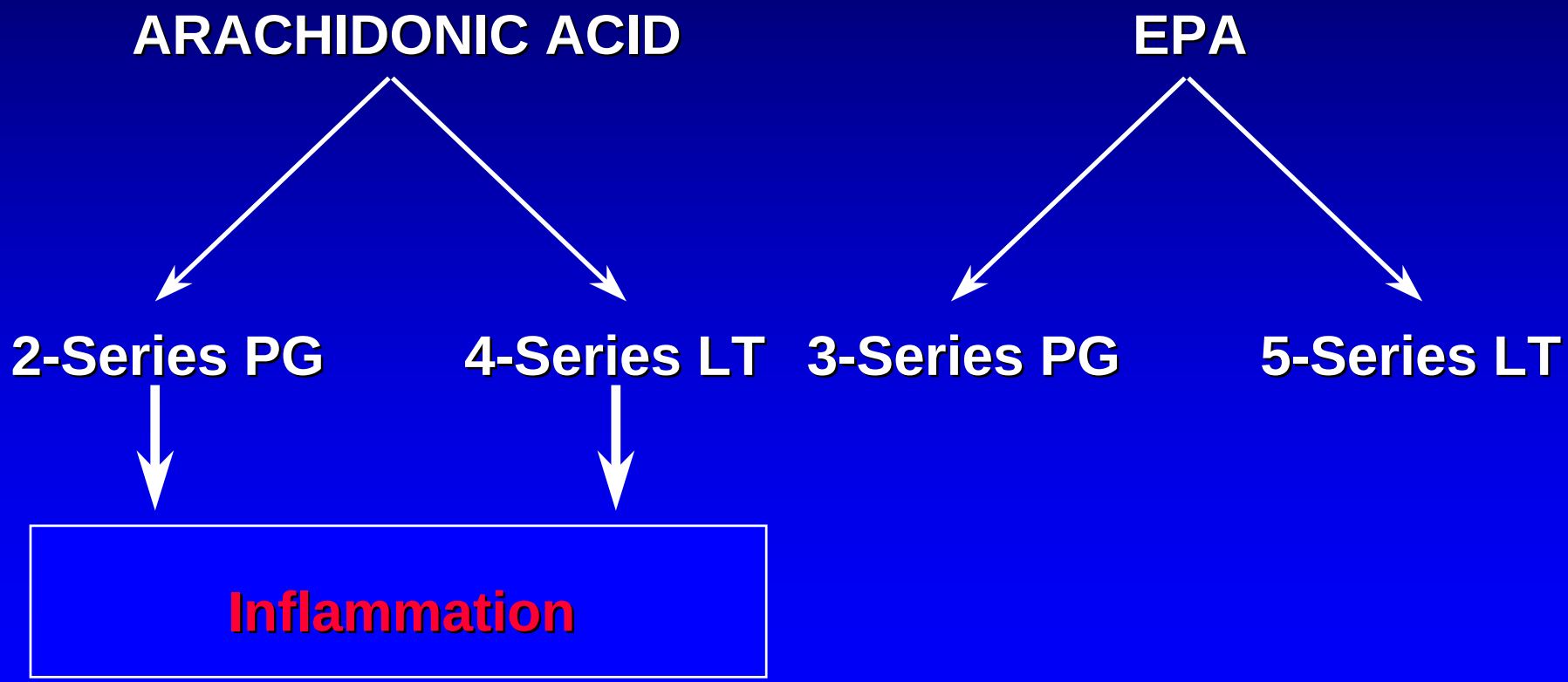


Healthy volunteers given fish oil (9.4 g EPA + 5 g DHA/day) for 10 weeks
Sperling et al. (1993) J. Clin. Invest. 91, 651-660

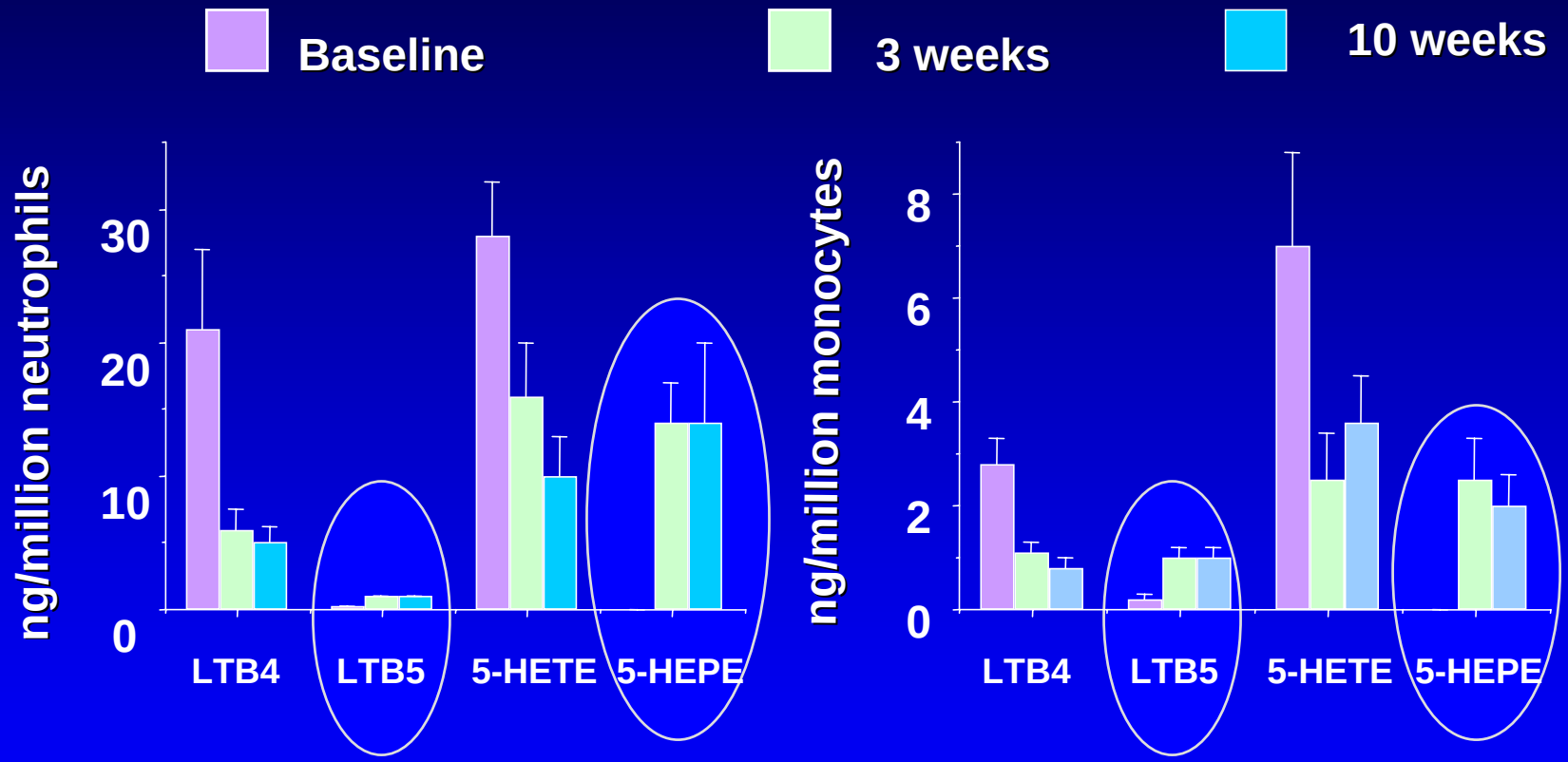
Classic view of the anti-inflammatory action of long chain ω-3 PUFA



EPA is also a precursor of eicosanoids



Effect of dietary fish oil on eicosanoid production by human inflammatory cells

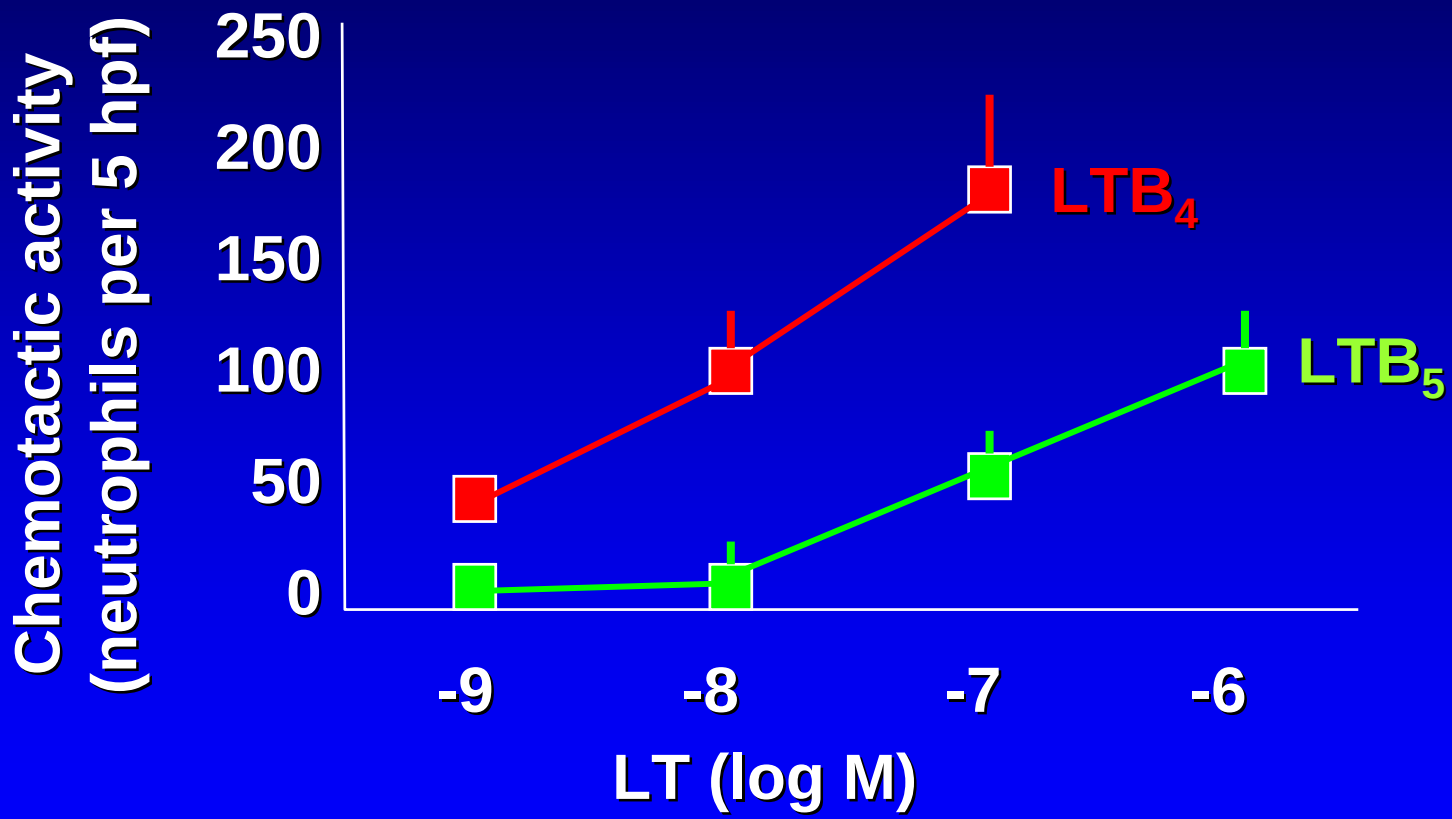


Healthy volunteers given fish oil (9.4 g EPA + 5 g DHA/day) for 10 weeks

Sperling et al. (1993) J. Clin. Invest. 91, 651-660

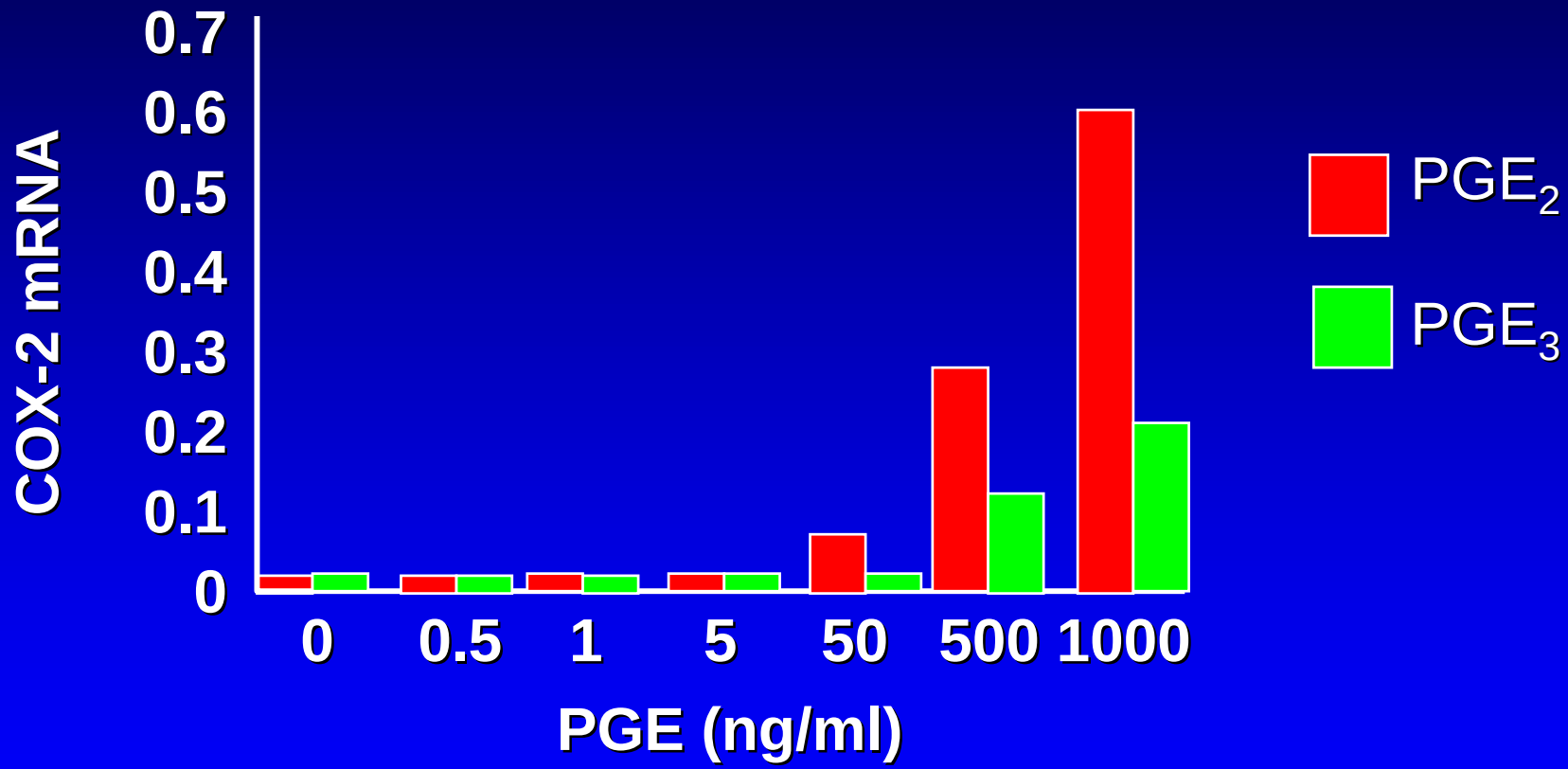
Mediators formed from EPA often have different biological potencies than those formed from arachidonic acid

Neutrophil chemotaxis: LTB_4 vs. LTB_5



Lee et al. (1988) Clin. Sci. 74, 467-475

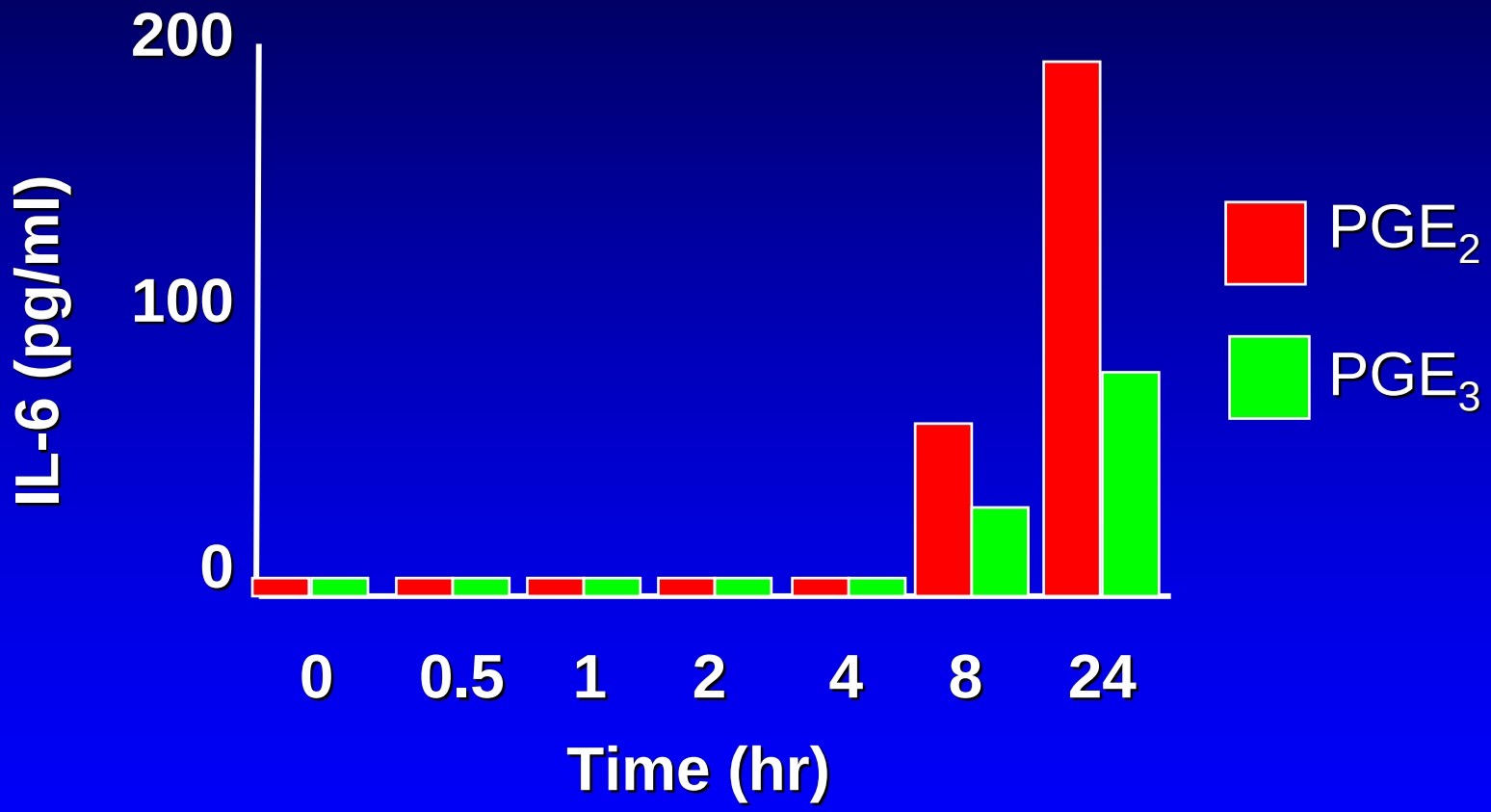
PGE_2 vs. PGE_3 and induction of COX-2



NIH3T3 fibroblasts at one hour

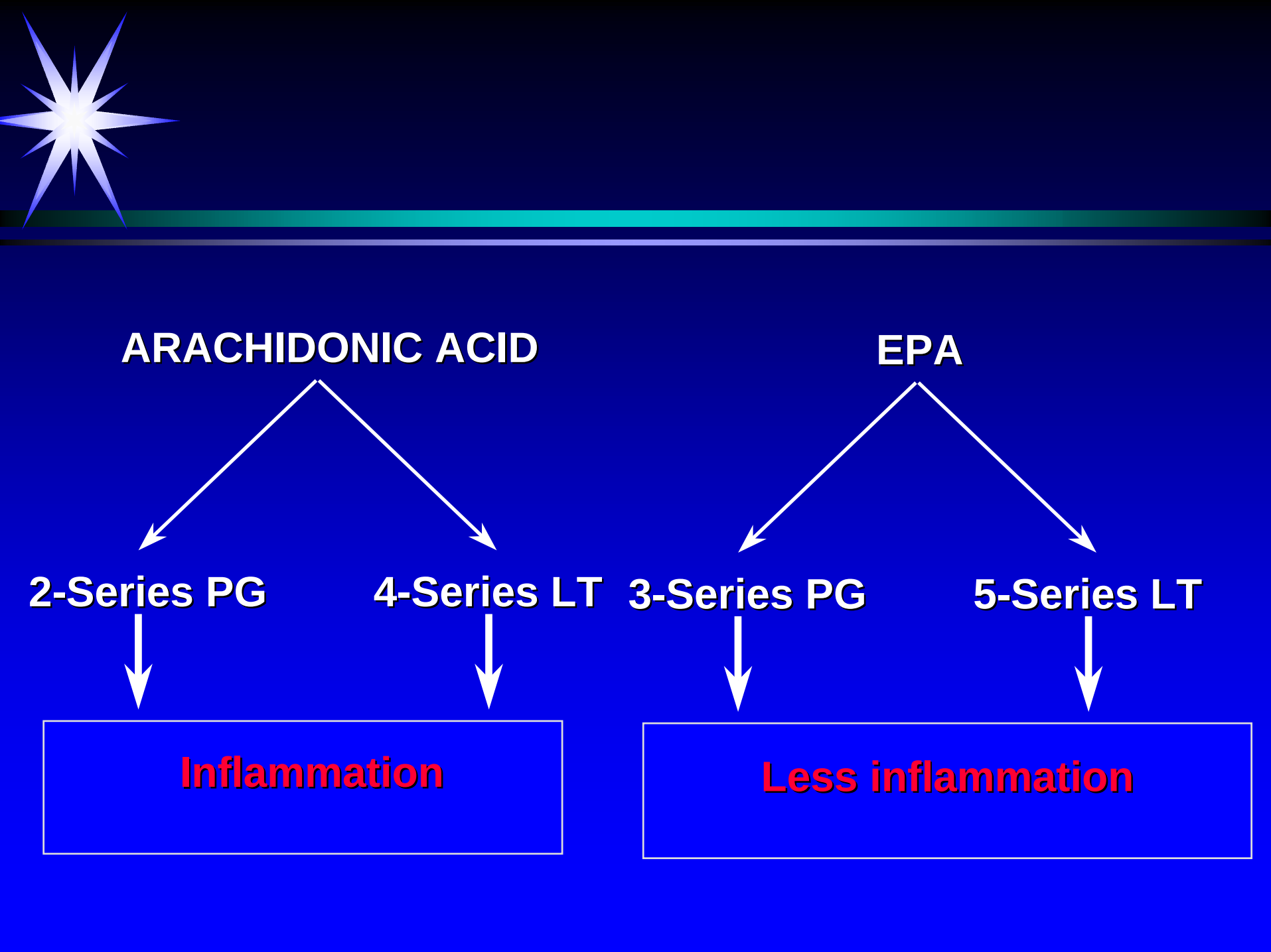
Bagga et al. (2003) PNAS 100, 1751-1756

PGE_2 vs PGE_3 and IL-6 production

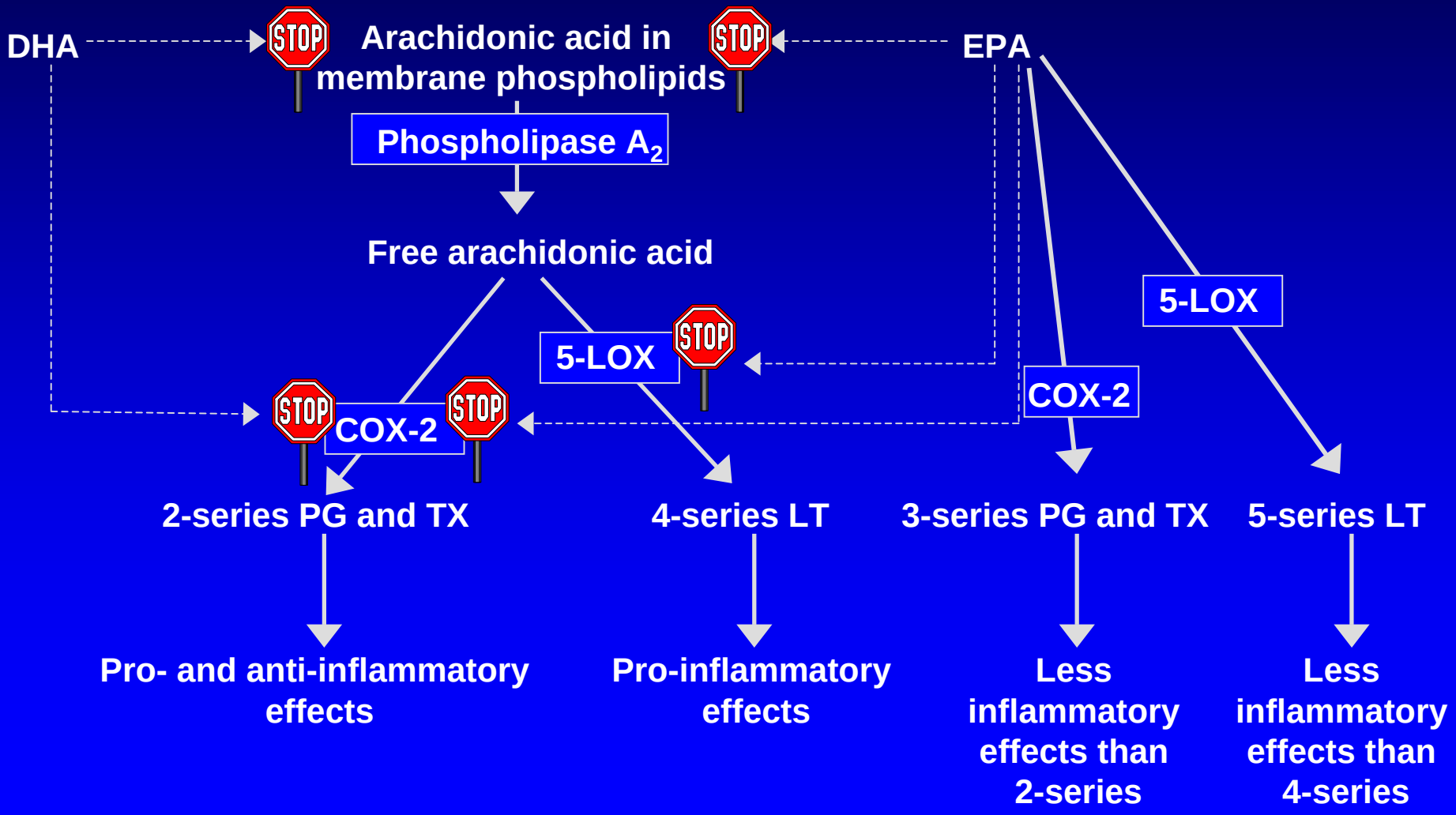


RAW 264.7 macrophages with 50 ng/ml PGE

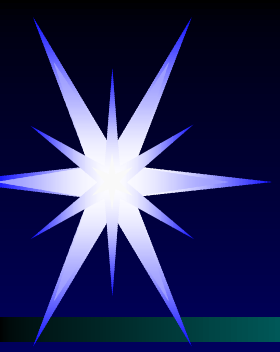
Bagga et al. (2003) PNAS 100, 1751-1756


ARACHIDONIC ACID
EPA
2-Series PG
4-Series LT
3-Series PG
5-Series LT
Inflammation
Less inflammation

Modified view of the anti-inflammatory action of long chain ω-3 PUFA



Resolvins & related compounds



Less arachidonic acid

↓

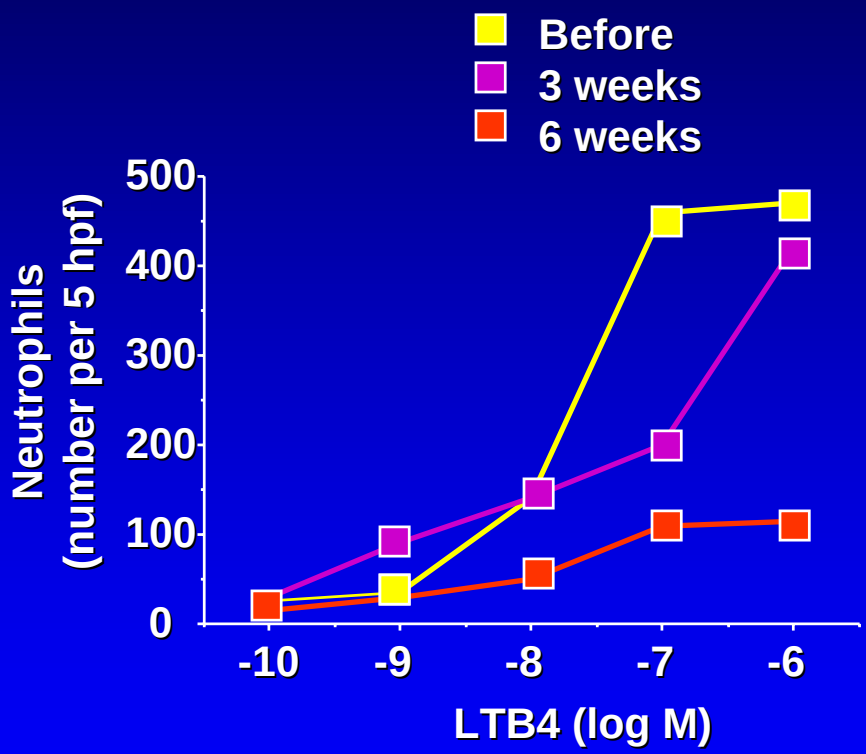
Lower concentration of 2-series PG and 4-series LT

↙ ↘

Cytokines and other mediators → Cellular responses

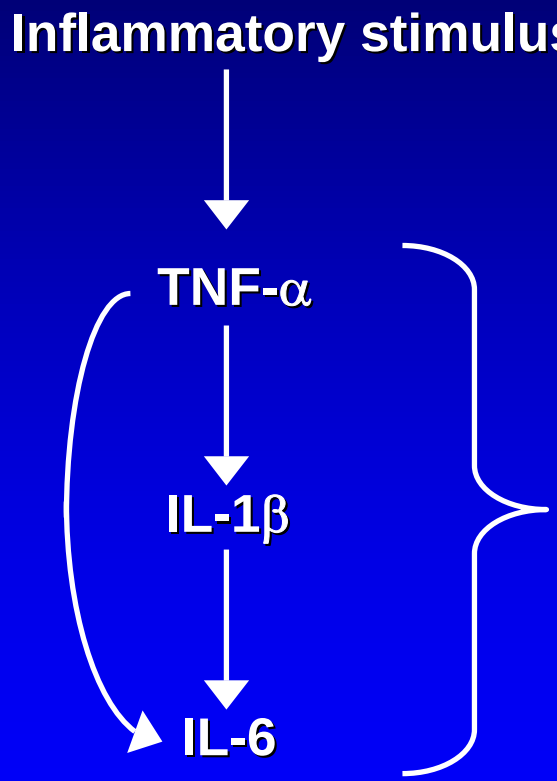
Leukocyte chemotaxis

- n = 7 (male; 22 to 53 years)
- 5.4 g EPA + DHA/ day for 6 weeks
- Neutrophil chemotaxis to LTB_4 decreased



Lee et al. (1985) N. Engl. J. Med. 312, 1217

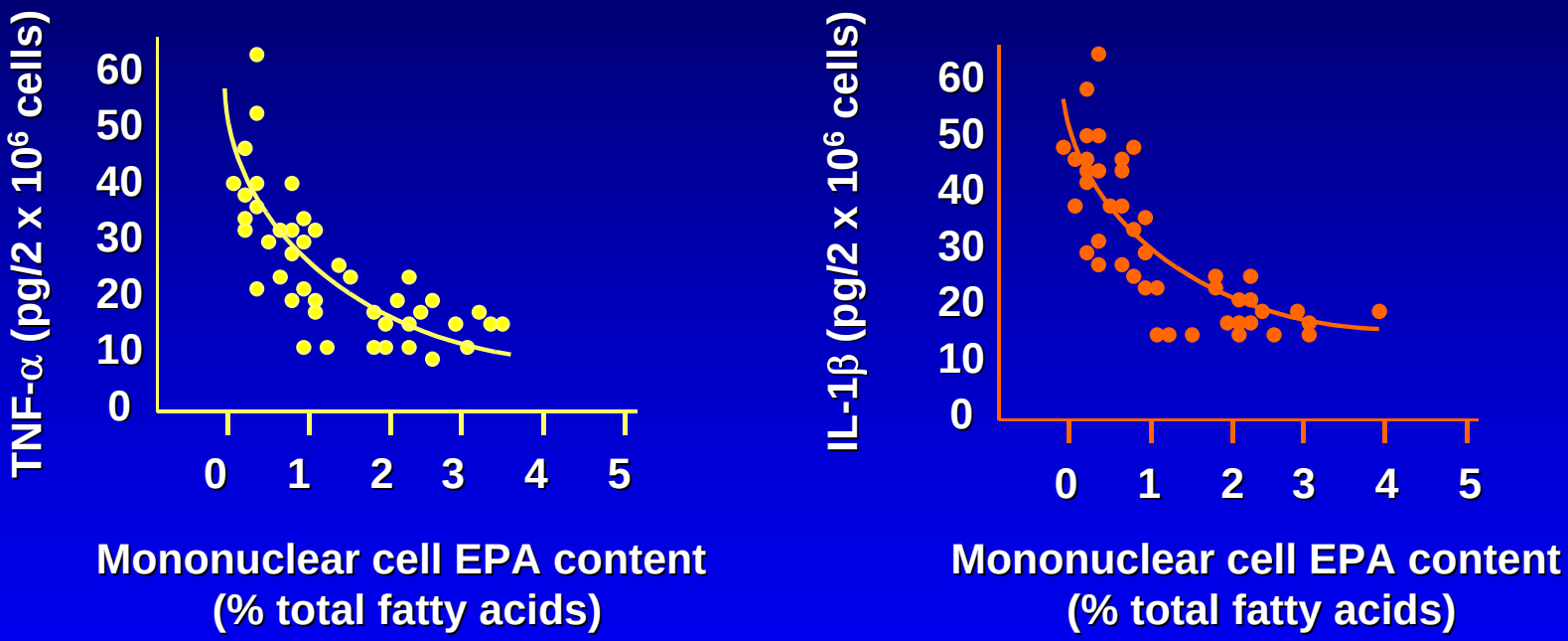
Pro-inflammatory cytokines



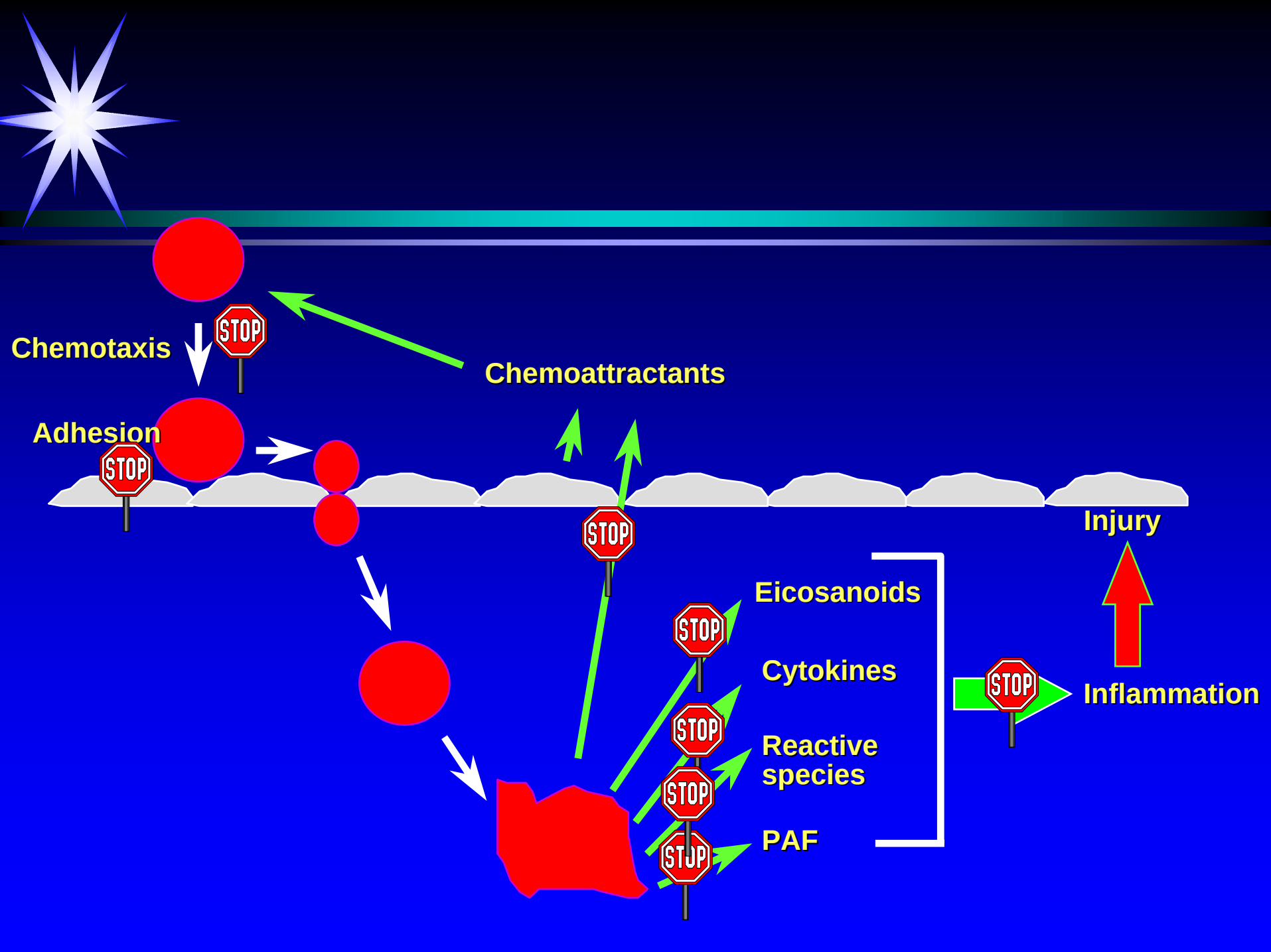
Cause:

- Local inflammation
- Fever
- Activation of T and B cells
- Acute phase protein synthesis
- Hypotension
- Coagulation
- Body wasting
- Bone loss

Inverse relationship between ω-3 fatty acid status and human cytokine production



Caughey et al. (1996) Am. J. Clin. Nutr. 63, 116-122


Chemotaxis
STOP
Chemoattractants
Adhesion
STOP
STOP
Injury
Eicosanoids
STOP
Cytokines
STOP
Reactive species
STOP
PAF
STOP
STOP
Inflammation

EPA or DHA?

Study of the effect of DHA alone on inflammatory mediator production

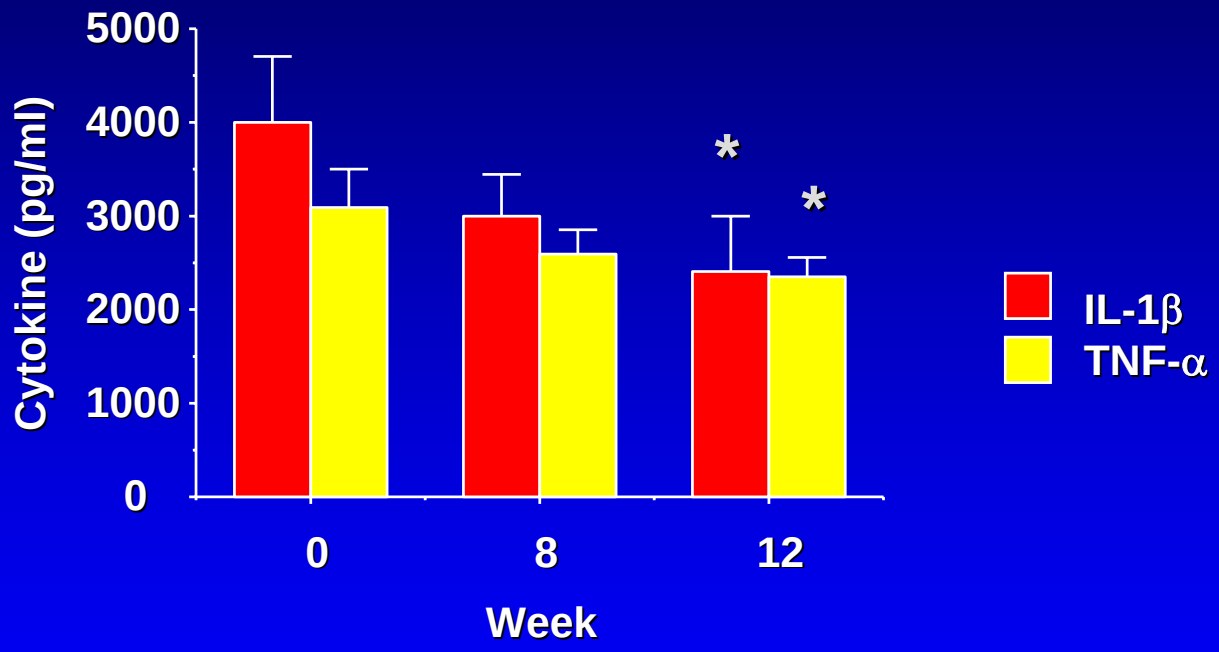
Kelley et al. ((1999) Lipids 34, 317-324

Healthy men mean age 33 years
N = 7
Consumed 6 g DHA/day (DHASCO) for 12 weeks
Fairly low fat background diet (30% en from fat; DHA < 0.1 g/day)

Inflammatory eicosanoids (LPS-stimulated PBMCs)

	Before	After
PGE_2 (ng/million cells)	13.1 +/- 2.0	5.0 +/- 1.0*
LTB_4 (pg/million cells)	140 +/- 30	34 +/- 10*

Inflammatory cytokines (pg/ml) (LPS-stimulated PBMCs)



Kelley et al. (1999) Lipids 34, 317-324

Study of EPA vs. DHA

Mori et al. (2003) Free Rad. Biol. Med. 35, 772-781

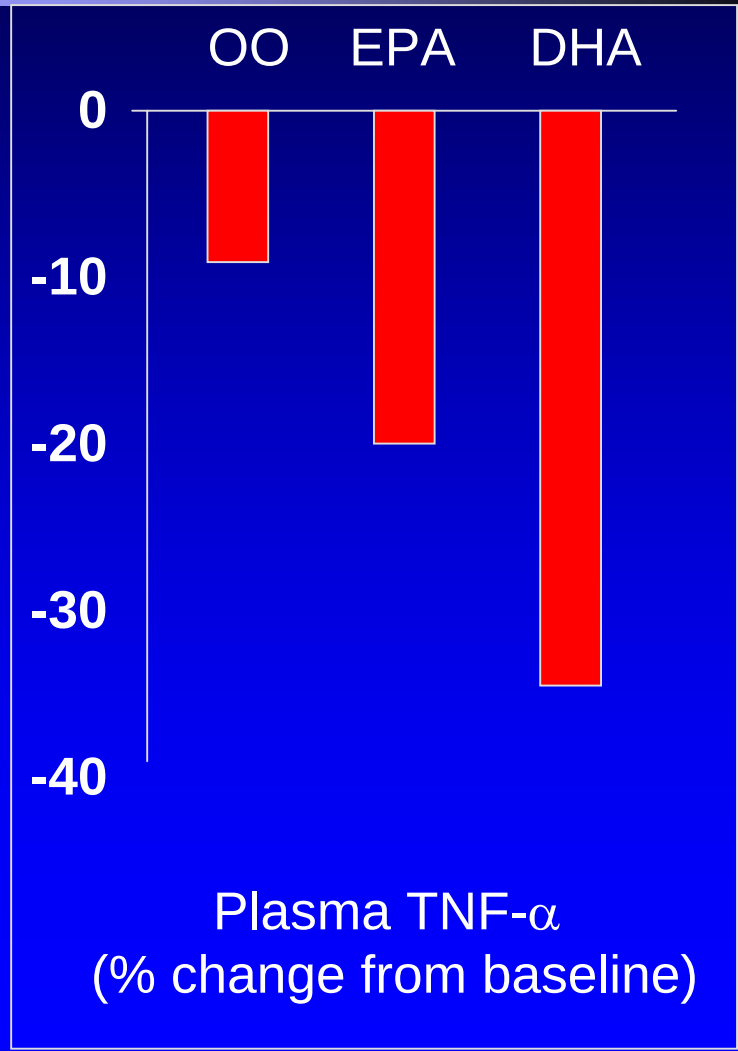
Hypertensive type 2 diabetics; both male & female; non-smokers; aged 40 to 75 years

4 g/d EPA vs. 4 g/d DHA vs placebo (olive oil)

6 weeks

Plasma TNF-α

Some other plasma markers of inflammation (CRP, IL-6) did not change



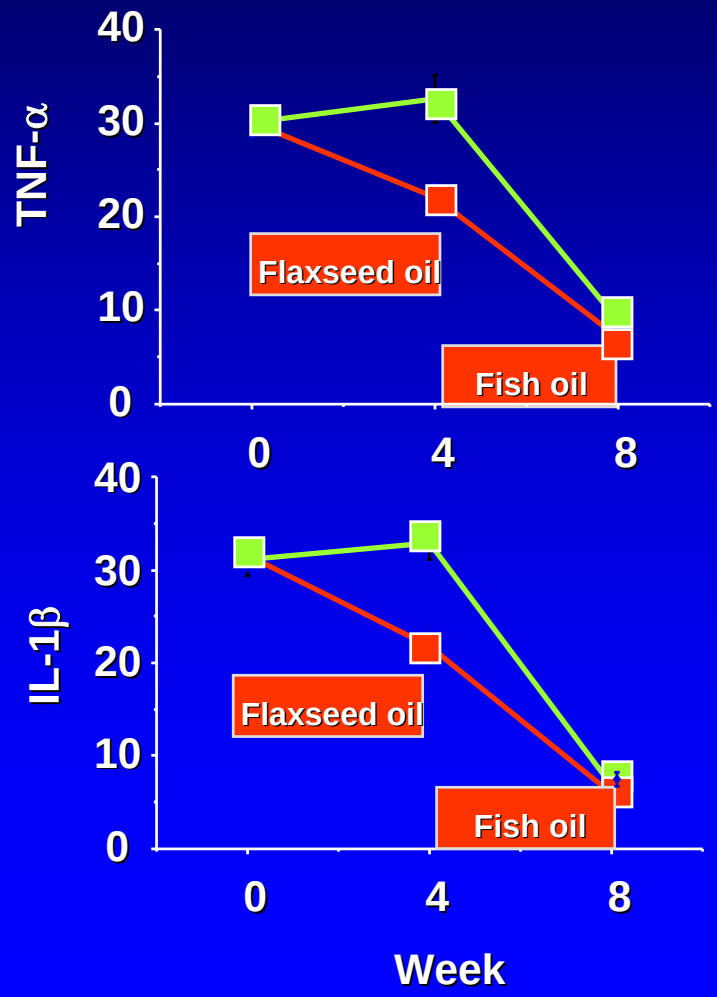
What about α-linolenic acid?

Inflammatory cytokines

- Males aged 22 to 44 years
- Sunflower oil-based diet

  vs. Flaxseed oil-based diet (13.7 g αLNA/day) for 4 weeks

  Then + 2.9 g EPA + DHA/day 4 weeks
- IL-1β and TNF-α production in response to LPS decreased but decrease greater with long chain n-3 fatty acids



Caughey et al. (1996)
Am. J. Clin. Nutr. 63, 116-122

Many studies report no effect of α-linolenic acid:

Intake (g/d)	Outcome	Reference
2.0	TNF, IL-1 & IL-6 production Monocyte respratory burst Neutrophil respiratory burst sCAM concentration	Thies et al. (2001)
4.1	Neutrophil chemotaxis Neutrophil respiratory burst	Healy et al. (2000)
4.1	TNF, IL-1, IL-6 production	Wallace et al. (2003)
4.5, 9.0	TNF, IL-1, IL-6 production Neutrophil respiratory burst Monocyte respiratory burst ICAM-1 expression on monocytes	Kew et al. (2003)

These studies suggest that an intake of α-linolenic acid of at least 10 g/d is required to see anti-inflammatory effects

and

even then these effects will be weaker than those exerted by long chain ω-3 PUFA

Potential clinical benefits of the anti-inflammatory effects of long chain ω-3 PUFA

Rheumatoid arthritis
Crohn's disease
Ulcerative colitis
Cystic fibrosis
Psoriasis
Lupus
Type-1 diabetes
Childhood asthma
Adult asthma
Allergic diseases
Atherosclerosis
Acute cardiovascular events
Post-surgery
Trauma & sepsis
……

Fish oil and RA

- Fish oil exerts anti-inflammatory actions in RA patients (e.g. decreased LTB_4 production)
- There have been 17 placebo-controlled, double-blind, clinical trails of fish oil in RA
- First was reported in 1985; most recent in 2005
- Used 1 to 7.1 g EPA plus DHA per day (most used about 3.3 g per day)
- Duration of 4 to 52 weeks
- Two trials used more than one dose of fish oil
- Several trials report outcomes at more than one time point
- Report a variety of clinical outcomes

Fish oil and RA

- 16/17 studies report improvement in at least two clinical outcomes
- 6/17 studies report improvement in at least four clinical outcomes
- Studies report decreased number of swollen and tender joints, decreased joint pain, increased grip strength, decreased duration of morning stiffness
- All studies which monitored NSAID use reported a significant reduction
- Two other studies required cessation of NSAID use - patients could endure this

Editorial by Cleland and James (2000) J. Rheumatol. 27, 2305-2307

"One can conclude that the findings of benefit from dietary fish oil in RA treatment are robust"

"Thus, dietary fish oil supplements in RA have treatment efficacy"

"Why are fish oil supplements not used more widely in RA?"

"…. dietary fish oil supplements should now be regarded as part of the standard therapy for RA"

Meta-analysis of 10 trials (1985-1992)

Fortin et al. (1995) J. Clin. Epidemiol. 48, 1379-1390

“Dietary fish oil supplementation for three months significantly reduced tender joint count (mean difference -2.9; P = 0.001) and morning stiffness (mean difference -25.9 minutes; P = 0.01)”

AHRQ Report 2004
Reviewed 10 trials 1985-2002

“no effect on patient report of pain, swollen joint count, disease activity of patient’s global assessment”

“of seven studies that assessed the effect on anti-inflammatory drug or corticosteroid requirement, six demonstrated reduced requirement for these drugs” ……. “n-3 fatty acids may reduce requirements for corticosteroids”

Did not assess tender joint count but reiterated “n-3 fatty acids reduce tender joint counts”

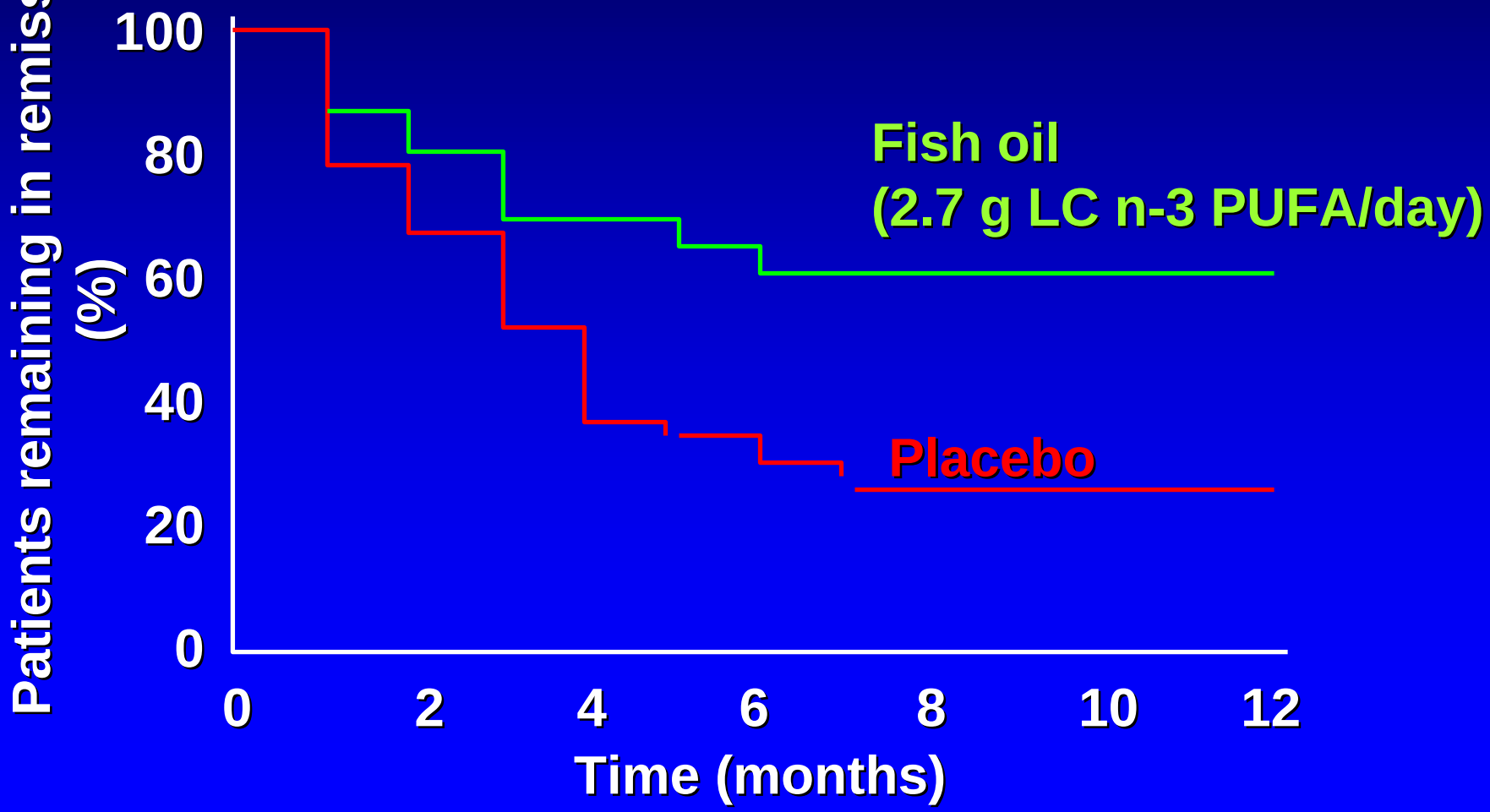
Fish oil and IBD (UC & CD)

- Fish oil exerts anti-inflammatory actions in IBD patients (e.g. decreased LTB_4 production by gut mucosa and by leukocytes)
- Uncontrolled or open trials report benefit from fish oil
- There have been 12 placebo-controlled, double-blind, clinical trails of fish oil in IBD (8 in UC; 3 in CD; 1 in UC+CD)
- First was reported in 1989; most recent in 2005
- Used 2.7 to 5.8 g EPA plus DHA per day (most used about 4.5 to 5 g per day)
- Duration of 12 to 104 weeks
- Report a variety of clinical outcomes

Fish oil and IBD

- 7/12 studies report improvement in at least two clinical outcomes
- Studies report improved gut histology, decreased use of corticosteroids, decreased disease activity
- 5/12 studies report no improvement in any clinical outcome

Patients with Crohn's Disease (in remission) given fish oil
Belluzzi et al. (1996) N. Eng. J. Med. 334, 1557-1616



AHRQ Report 2004

Reviewed 13 trials published 1989 to 2002

Looked at clinical score, sigmoidoscope score, gut mucosal histology score, induced remission and relapse

Concluded that sufficient data to meta-analyse only relapse and only in UC patients - 5 studies considered and 3 used - “n-3 fatty acids have no effect on relative risk of relapse in ulcerative colitis” …. “there was a statistically non-significant reduction in requirement for corticosteroids for n-3 fatty acids relative to placebo in two studies”

Fish oil and asthma

- Fish oil exerts anti-inflammatory actions in asthma patients (e.g. decreased LTB_4 production and decreased leukocyte chemotaxis)
- Epidemiological evidence of a protective effect on long chain ω-3 PUFA on adult and childhood asthma
- Uncontrolled or open trials report benefit from fish oil in adult asthma
- There have been 9 placebo-controlled, double-blind, clinical trails of fish oil in asthma (7 in adults, 2 in children)
- First was reported in 1988; most recent in 2000
- Studies in adults used 1.0 to 6.0 g EPA plus DHA per day
- Duration of 4 to 52 weeks
- Report a variety of clinical outcomes related to lung function, disease severity etc.

Meta-analysis of 8 trials (1988-2000)
Thien et al. (2002) Cochrane Library

"no consistent effect on FEV_1, PEF, asthma symptoms, asthma medication use or bronchial hyper-reactivity" but "one study in children showed improved peak flow and reduced asthma medication use"

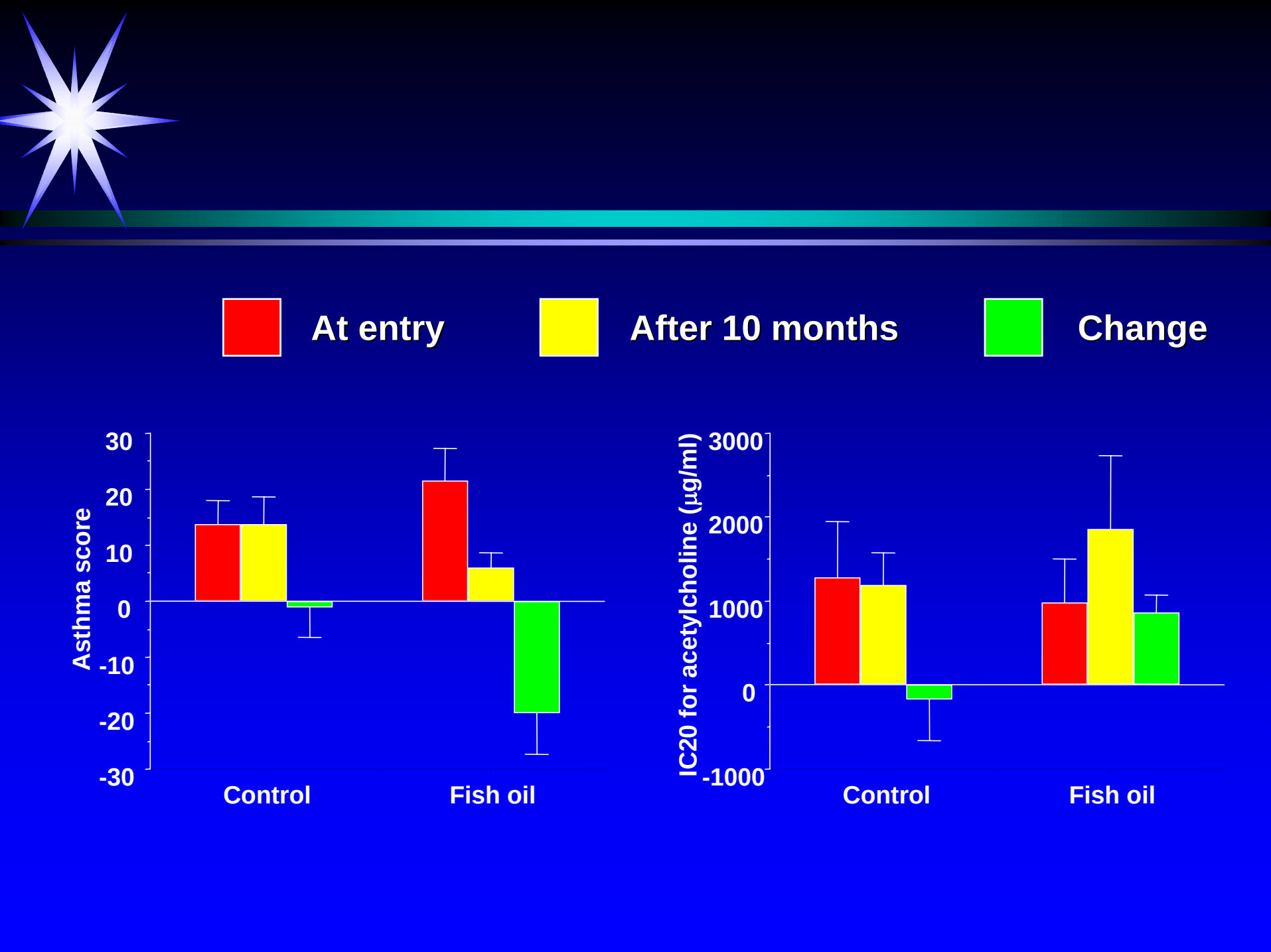
AHRQ Report 2004

Covered 26 trials (both placebo, controlled, randomized and others)

“no definitive conclusion can yet be drawn regarding the efficacy of n-3 fatty acid supplementation as a treatment for asthma in children and adults”

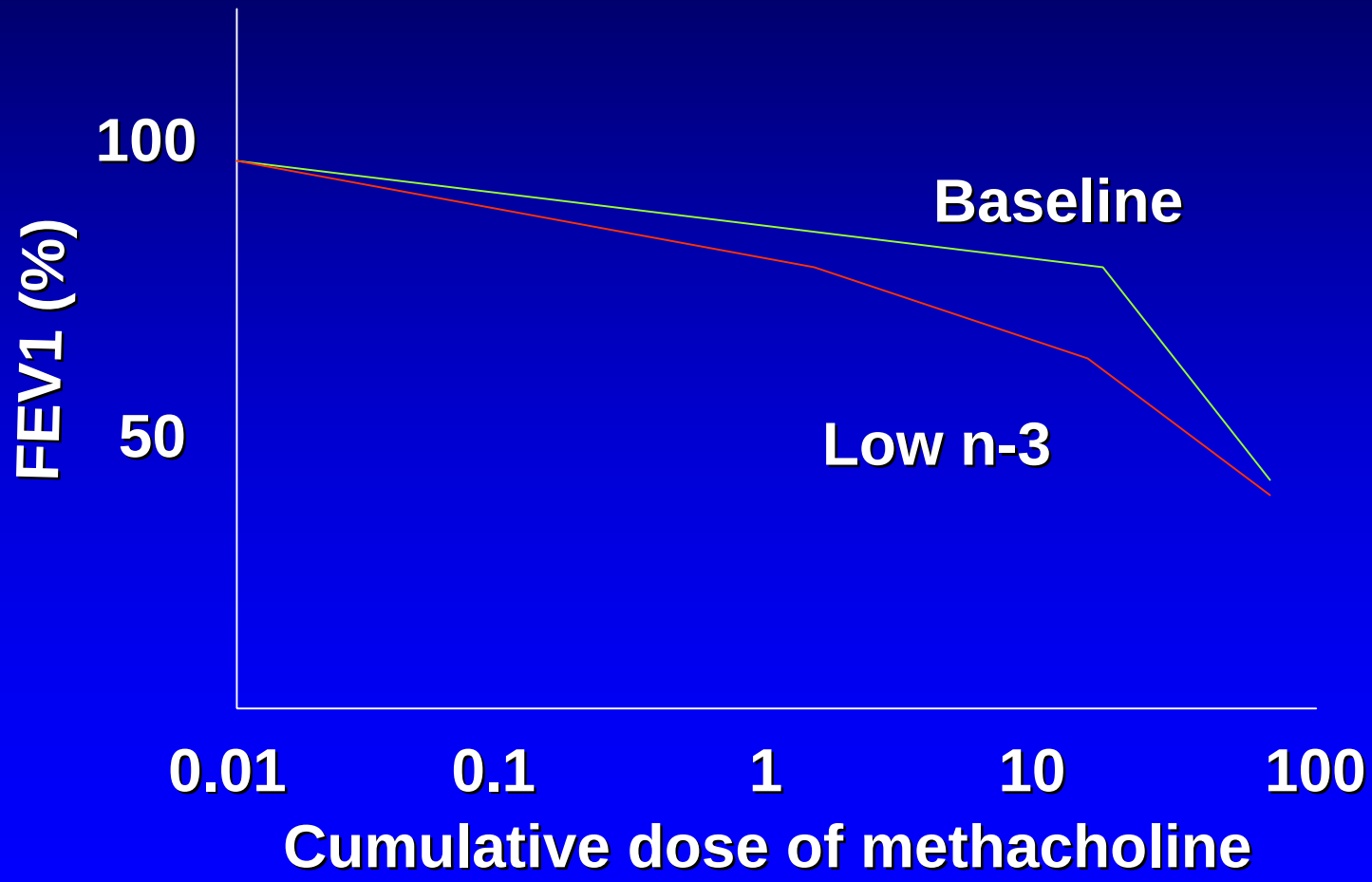
Design of study of Nagakura et al. (2000) Eur. Resp. J. 16, 861-865

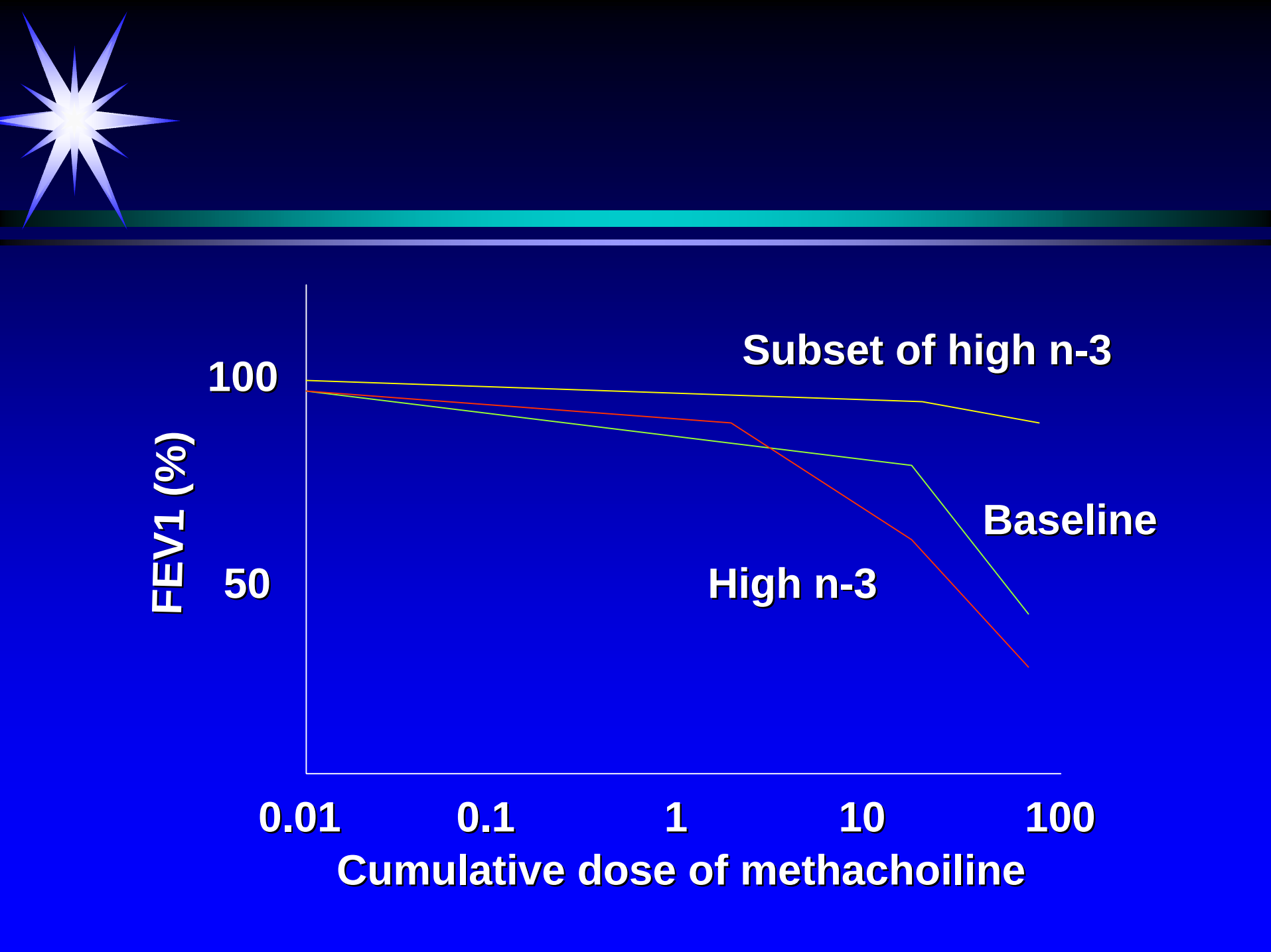
- 29 children (mean age 10 y) with bronchial asthma
- Fish oil capsules provided for 10 months
- Olive oil placebo
- Asthma score evaluated 4 times daily and totalled for each day
- Each month subjects were challenged with increasing doses of acetylcholine
- FEV_1 measured


At entry
After 10 months
Change
Asthma score
30
20
10
0
-10
-20
-30
Control
Fish oil
IC20 for acetylcholine (µg/ml)
3000
2000
1000
0
-1000
Control
Fish oil

Design of study of Broughton et al. (1997) Am. J. Clin. Nutr. 65, 1011-1017

- 26 non-smoking atopic asthmatics
- Detailed dietary assessment to determine accurately each individuals ω-6 PUFA intake
- Fish oil capsules provided on an individual basis to achieve ω-6:ω-3 PUFA ratios of 10 (low FO) and 2 (high FO); each treatment period lasted 4 weeks
- At baseline and after each treatment period subjects challenged with increasing doses of methacholine
- FVC, FEV_1, PEF and FEF_{25-75} measured
- Urinary 4- and 5-series LT measured


100
50
FEV1 (%)
Baseline
Low n-3
0.01
0.1
1
10
100
Cumulative dose of methacholine


Subset of high n-3
100
FEV1 (%)
Baseline
50
High n-3
0.01
0.1
1
10
100
Cumulative dose of methachoiline

Summary of the results of Broughton et al. (1997)

	Cumulative dose of methacholine to cause a 20% decline (units)			
	Baseline	Low dose fish oil	High dose fish oil	
			Responders	Non-responders
FVC	24.1	11.8	> 67	3.7
PEF	17.1	5.9	> 67	3.5
FEV	16.9	1.9	> 67	4.9
FEF	9.0	0.7	> 67	9.9

Summary of clinical benefits of long chain ω-3 PUFA in human inflammatory diseases

Evidence in favour of benefit:
Rheumatoid arthritis – therapeutic

Weaker evidence:
Crohn's disease – prolongs remission
Psoriasis - therapeutic

Some evidence:
Childhood asthma – therapeutic
Cystic fibrosis

Contradictory or no evidence:
Ulcerative colitis
Lupus
Type-1 diabetes
Adult asthma

Summary

- Eicosanoids derived from arachidonic acid are involved as mediators and regulators of inflammation
- EPA and DHA from oily fish/fish oil can partially replace arachidonic acid in membrane phospholipids
- ω-3 fatty acids (especially EPA) lead to decreased production of eicosanoids from arachidonic acid
- EPA and DHA give rise to anti-inflammatory resolvins (cell culture & animal work)
- ω-3 fatty acids lead to decreased production of inflammatory cytokines
- Through these effects ω-3 fatty acids act to decrease inflammation
- ω-3 fatty acids may protect against and provide therapy for diseases with an overt or covert inflammatory component
- Evidence for therapeutic benefit from ω-3 fatty acids is reasonably strong in RA but is weaker elsewhere – doses used are quite high (approx. 3.5 g/day)
- α-Linolenic acid is not anti-inflammatory at intakes < 10 g/d