Nutritional modulation of the immune response in dairy livestock

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dipav

cisma

An overview of innate immune defenses involved in inflammation

Time of crisis:
peripartum and related troubles

Can we modulate with specific diets immune defenses?

What should a good immune system do?

- Recognize a wide array of enemies
- Kill them ALL once recognized
- Spare the host tissues (as much as possible)

senses the presence of pathogens

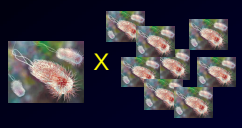
The sensing arm

modulates the response

The effector arm

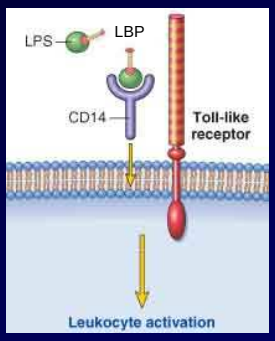
strikes back and fights

The sensing arm



serum LBP
(LPS binding protein)

LPS+LBP

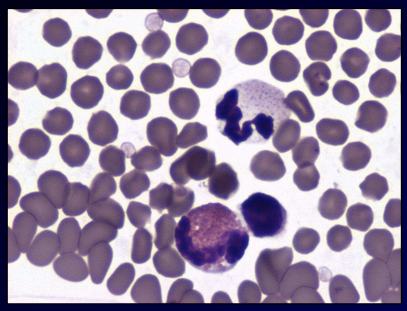


shedding of
LPS
from bacteria membranes

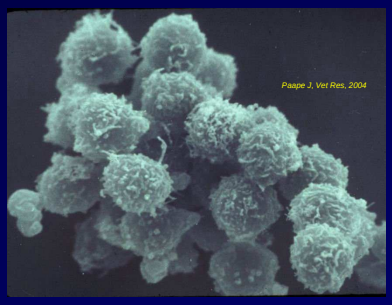
production of cytokines

direct targeting of bacteria

The effector arm



The polimorphonuclear cells

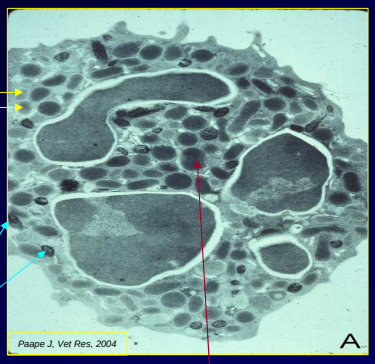


Secondary granules

Lactoferrin
Acute Phase Proteins

Primary granules

Myeloperoxidase



Peroxidase negative large granules

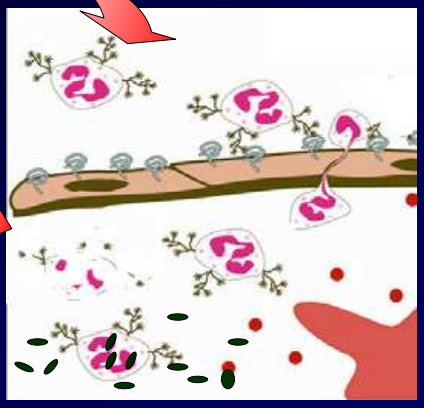
Lactoferrin
Specific antibacterial proteins

The leukocyte attack sequence

I. adhesion and chemotaxis

III. Apoptosis

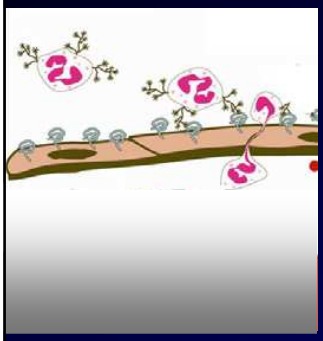
II. Phagocytosis and killing

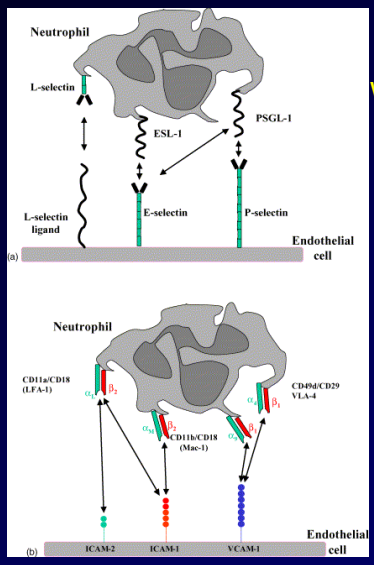


6-18 hrs

Ceciliani F, CPPS 2007

I. adhesion





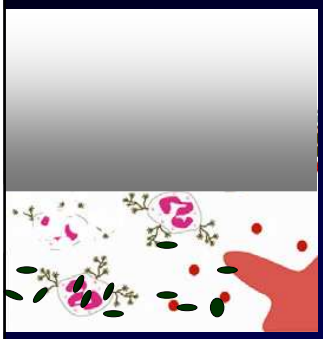
weak adhesion

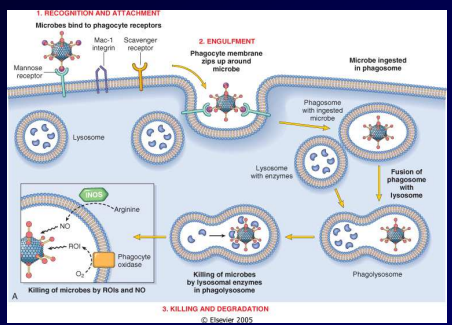
firm adhesion

Beutler, 2004

II Phagocytosis and killing

Engulfment and destruction (ROS – enzymes)





Degranulation outside the cell (ROS – enzymes)

Robbins, Pathologic basis of diseases, 2004

The Flow Chart of inflammation

Local invasion

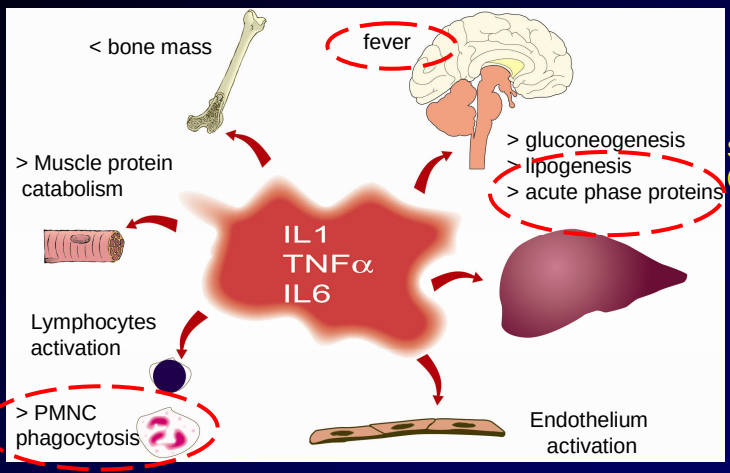
can the local defences cope with it?

NO

YES

ACUTE PHASE REACTION
(Systemic reaction)

The sistemic response to inflammation: The acute phase



SAA, AGP, Hp, CRP
(> 1000 fold increase)

Ceciliani F, The Acute Phase Protein α_1-Acid Glycoprotein: A Model for Altered Glycosylation During Diseases, CPPS, 2007

first 24 hrs

last 24 hrs

Raising of a rapid and efficient inflammatory response

Dampening the response when the menace is over

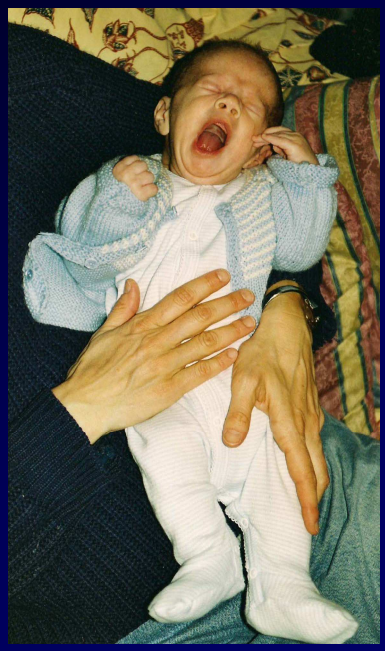
high number of PMN
very aggressive
long lifespan (low apoptosis rate)

low number of PMN
less aggressive
short lifespan (high apoptosis rate)

never too early – never too late

48-72 hours

Take home message!!



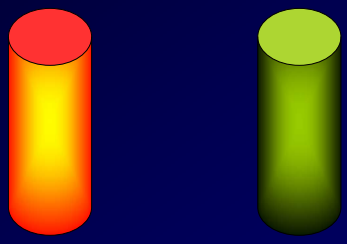
The inflammatory reaction is finely tuned by modulating

➔ the adherence and chemotaxis
(the number of cells in inflamed tissues)

➔ the destroying capability of the cell
(their activity)

➔ the apoptosis rate
(the lifespan)

Can we manipulate the timetable and the intensity of inflammation?

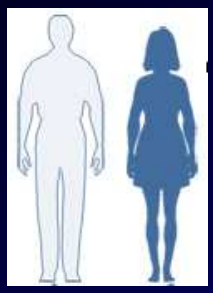


pro-inflammatory effects

anti-inflammatory effects

IN HUMANS

many diseases are caused by
unbalanced inflammatory responses



and we can manage
the primary cause of disease

The issues in dairy livestock

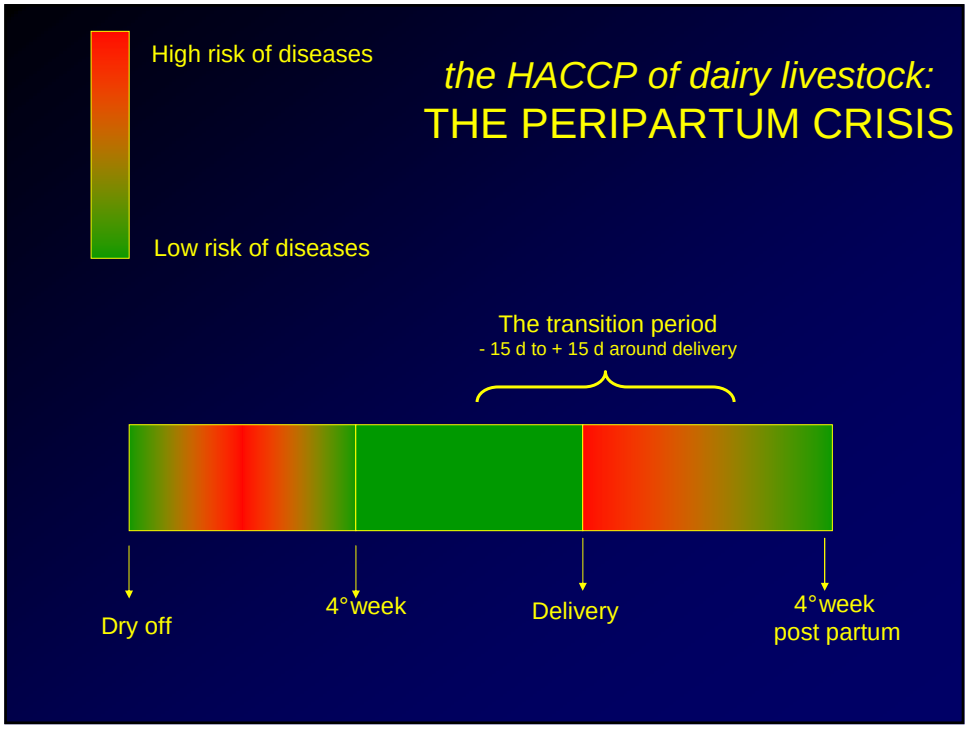
✓inflammation is basically a protective reaction

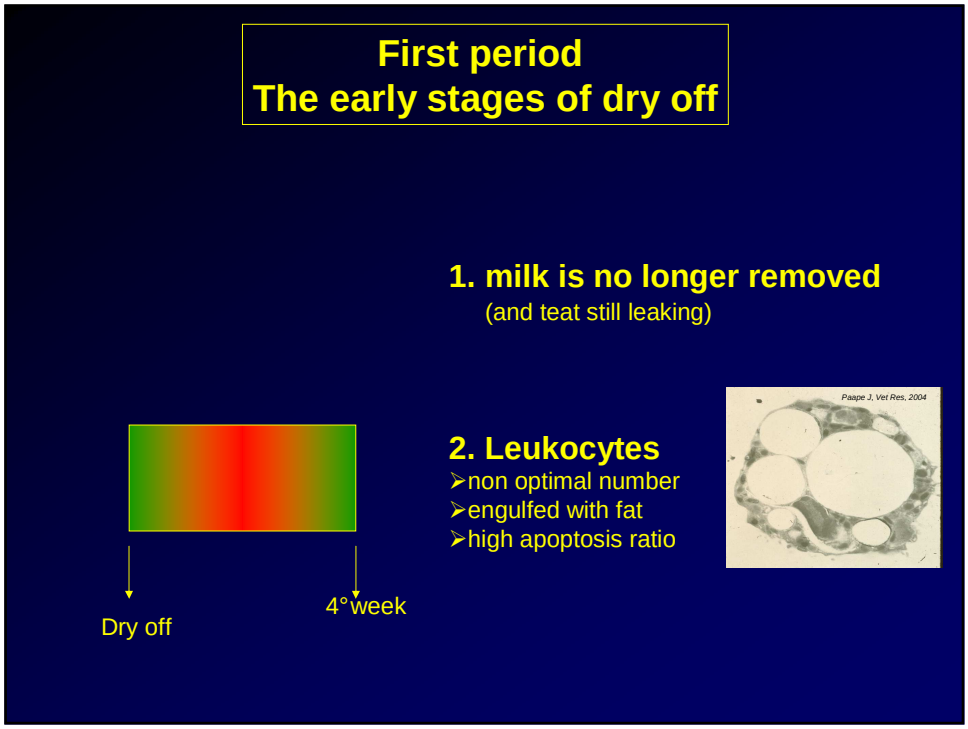
✓we can not so easily manage the primary cause of disease

✓yet, we have to avoid an excessive response (mastitis!)

(but we also to take great care to balance the inflammation not toward the antinflammatory side)

✓livestock is basically an unbalanced machine

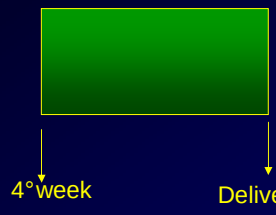

High risk of diseases
Low risk of diseases
the HACCP of dairy livestock:
THE PERIPARTUM CRISIS
The transition period
- 15 d to + 15 d around delivery
Dry off
4°week
Delivery
4°week
post partum


First period
The early stages of dry off
1. milk is no longer removed
(and teat still leaking)
2. Leukocytes
➢non optimal number
➢engulfed with fat
➢high apoptosis ratio
Paape J, Vet Res, 2004
Dry off
4°week

The second period: the mammary steady-state involution

1. the teat is sealed

2. optimal concentration of leukocytes
   (and few milk available)

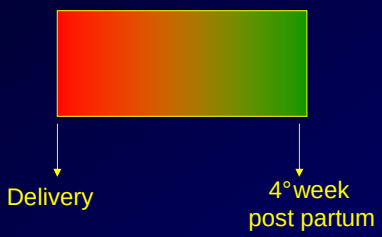


The third period: the second half of the transition period

highest percentage of diseases:

- metabolic disorders
- abomasal displacement
- subacute ruminal acidosis

1. Presence of milk

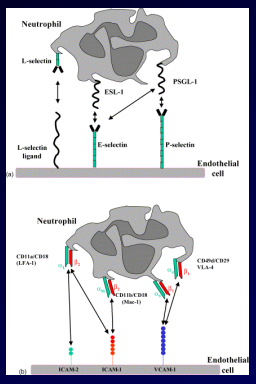


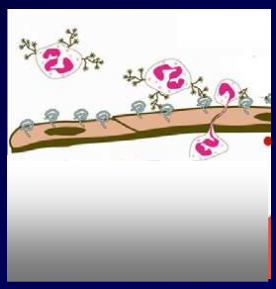
2. Leukocyte functions are heavily compromised

Reduced mobility

> β-estradiol
< progesteron
> Acute Phase Proteins

< expression adhesion molecules





Delay in PMN recruitment

Reduced PMN activity

> β-estradiol
< progesteron
> Acute Phase Proteins

- phagocytosis
- degranulation
- ROS production

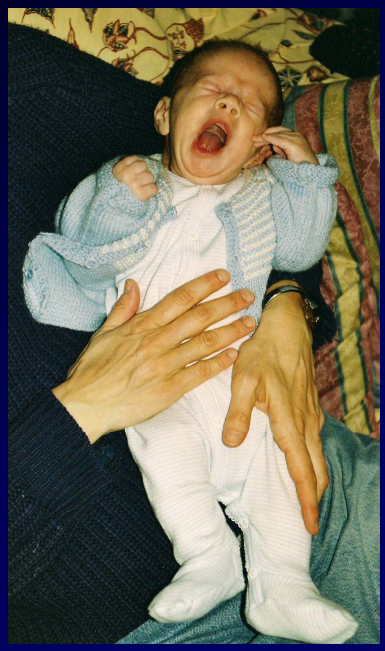
Reduced PMN lifespan

> β-estradiol
< progesteron
> Acute Phase Proteins

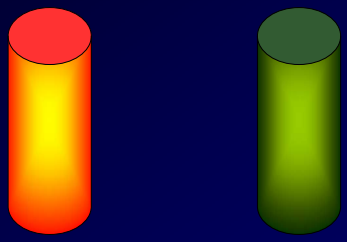
↑ PMN apoptosis

↓ monocyte apoptosis

Take home message!!



During peripartum the inflammatory process is already strongly unbalanced



pro-inflammatory effects

anti-inflammatory effects

Can we manage peripartum immunodeficiencies with nutrition?

HOW?

What can we work with?

- proteins
- minerals
- lipids

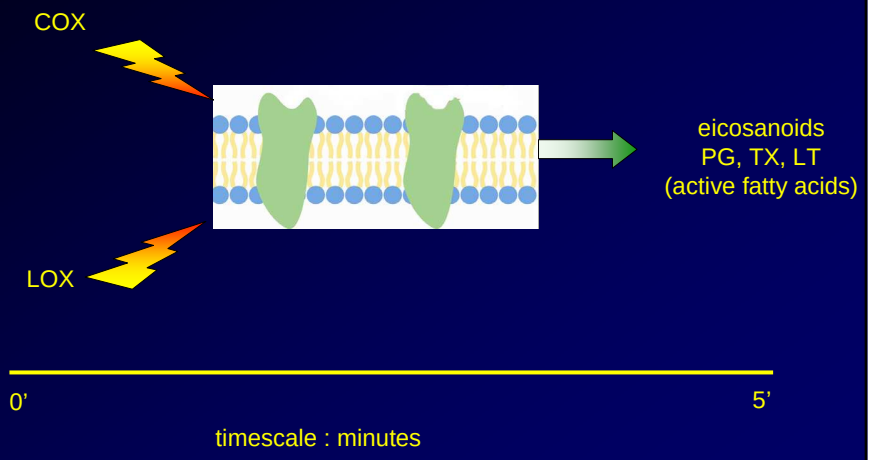
ω-3 polyunsaturated fatty acids are believed to reduce the unwanted inflammatory effects (Atherosclerosis, inflammatory diseases …..)

animal diets are often integrated with ω-3 as energy source to
- enhance their content in milk
- because they improve the fertility

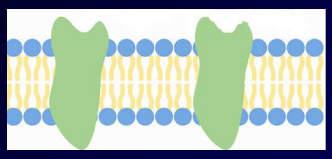
The theory

Immune activity of ω-3 in humans and rodents

The first wave of inflammatory mediators comes from the cell membranes



The composition of inflammatory cell membrane phospholipids



6-10% linoleic acid
15-20% arachidonic acid
} ω-6 PUFA

0.1-0.8% EPA
2-4% DHA
} ω-3 PUFA

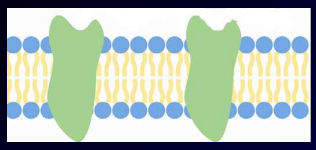
Pro-inflammatory mediators come from ω-6 fatty acids

HOW CAN ω-3 PUFA MODULATE INFLAMMATION?

a) by modifying the composition of cell membranes
(30-40 days)

b) by directly interacting with specific receptors
(hours)

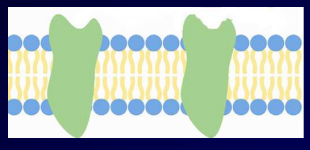
The effects of ω-3 PUFA-rich membranes on immune cell activity



arachidonic acid rich membranes

pro-inflammatory eicosanoids (LTB4, PgE_2)

➔ reduced availability of pro-inflammatory eicosanoids



ω-3 rich membranes

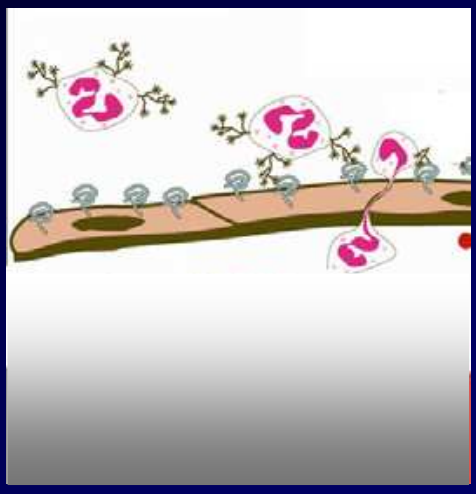
➔ **ω-3** PUFA may act as substrate for COX/LOX BUT give raise to lower activity eicosanoids (PgE_3 and LTB_5)

➔ **ω-3** PUFA generate "anti-inflammatory" eicosanoids (resolvins, lipoxins etc. etc.)

The direct effects of ω-3 PUFA on leukocyte attack sequence:

I. adherence

↓ adhesion protein expression (chemotaxis)



The direct effects of ω-3 PUFA on leukocyte attack sequence:

II. phagocytosis and killing

↓ - phagocytosis
↓ - ROS production

but these effects are debated

The practice

Immune activity of **ω-3** PUFA in farm animals

EX VIVO

ω-6 ↓ lymphocyte proliferation (but not ω-3)

no effect on cytokines

ω-6 ↓ LPS-stimulated cytokine production

IN VIVO

↓ of Acute Reaction (Fever)

IN VITRO

ω-6 ↓ lymphocyte proliferation (but not ω -3)

↓ PgE_2 production

EX VIVO

ω-6 ↓ adhesion molecules expression

no effect on proliferation

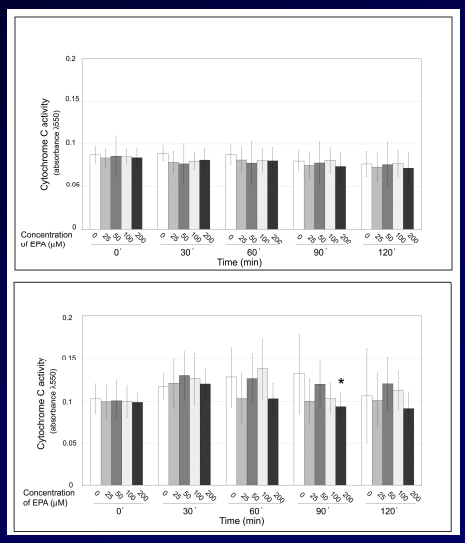
Do EPA and DHA modulate leukocyte (monocytes, PMN) functions?

Experimental design

- Isolation of cells (**PMN**) from goat blood
- co-incubation with different concentrations of EPA and DHA
- determination of
  - ✓phagocytosis
  - ✓extracellular ROS production

Does EPA modulate extracellular ROS production in PMN?

No it does not



resting PMN

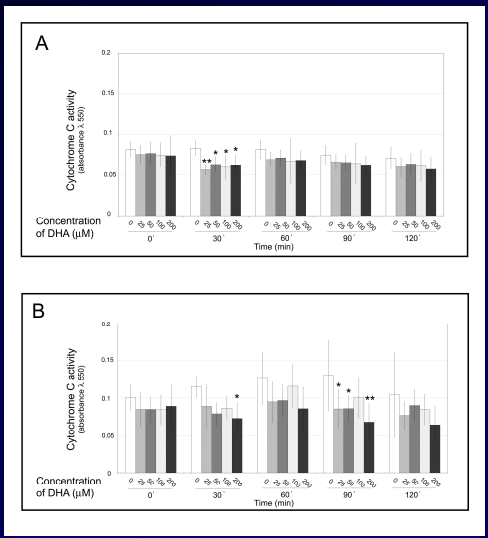
activated PMN

Pisani et al. 2009

Does DHA modulate extracellular ROS production in PMN?

YES it does

but the results are not evident



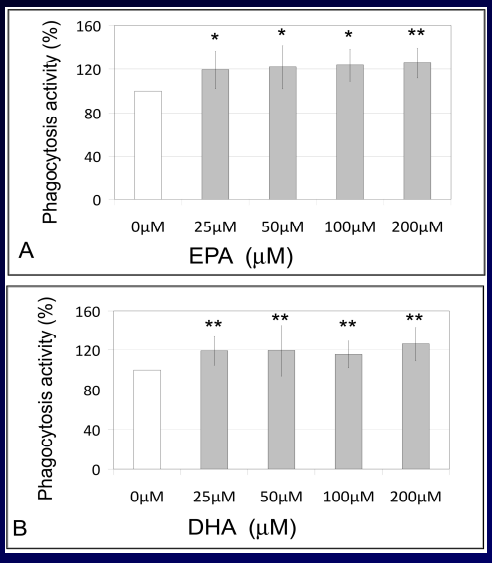
resting PMN

activated PMN

Pisani et al. 2009

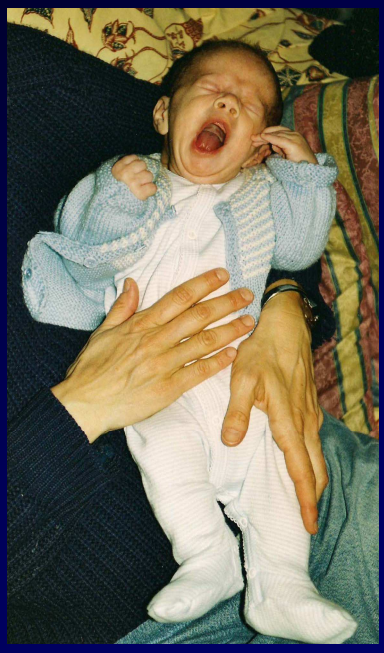
Do EPA and DHA modulate PMN phagocytosis

YES, they do



Pisani et al. 2009

Take home message!!



EPA and DHA differentially increase phagocytosis

AND

reduce extracellular ROS production

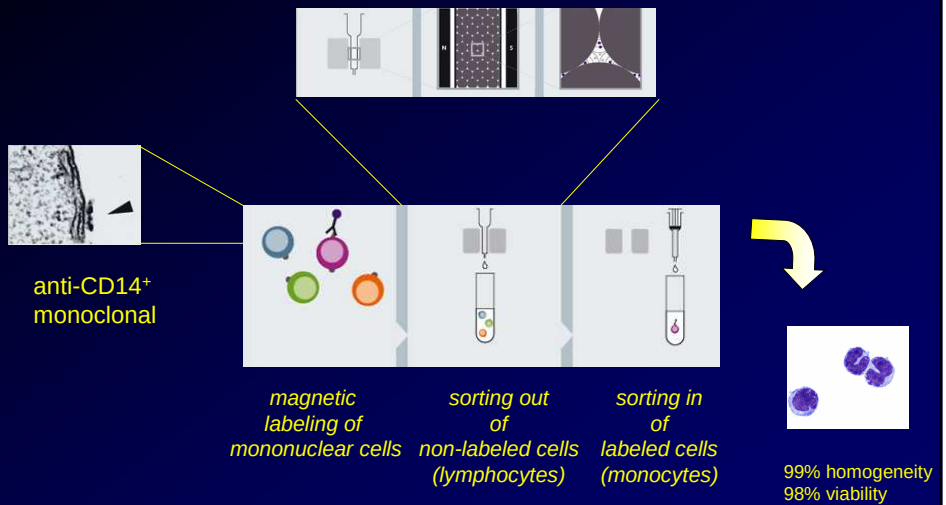
in goat PMN cells

(possibly reducing cell damages)

Experimental design

- Isolation from goat blood of cells (**monocytes**)
- co-incubation with different concentrations of EPA and DHA
- determination of
  - ✓phagocytosis
  - ✓ROS production
  - ✓apoptosis level
  - ✓cytokine production
  - ✓formation of lipid droplets

Sorting of the cells: the magnetic activated cell sorting technique

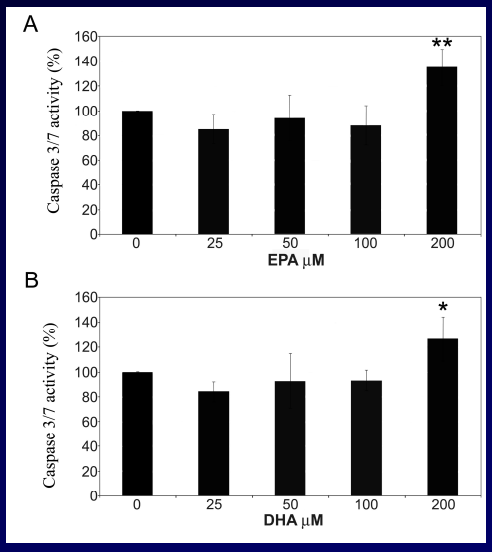


Do EPA and DHA cause apoptosis in monocytes?

YES they do

(but only at very high concentration)

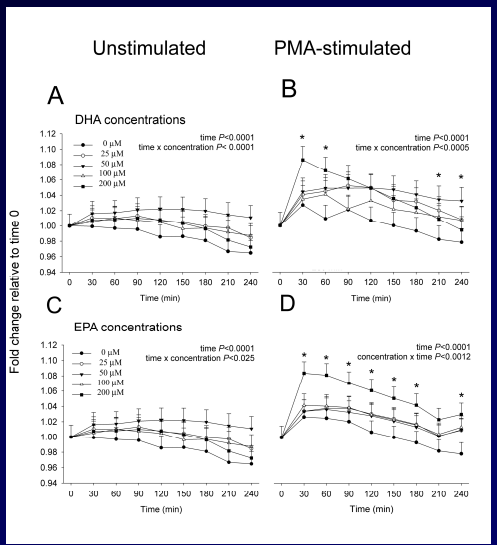
in humans the same effect is obtained at 50 μM



Do EPA and DHA modulate ROS production?

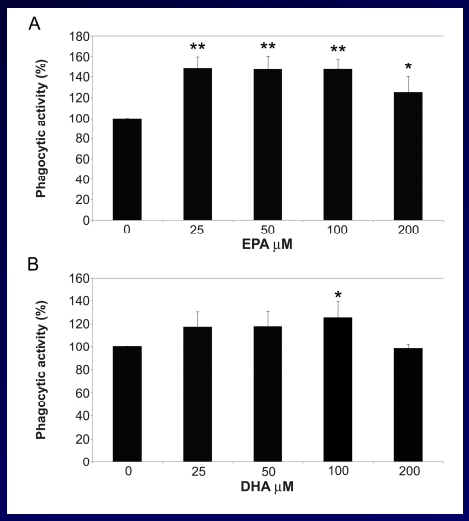
No they don't
(except when used at 200μM)

and probably due to apoptosis!!



Do EPA and DHA modulate phagocytosis?

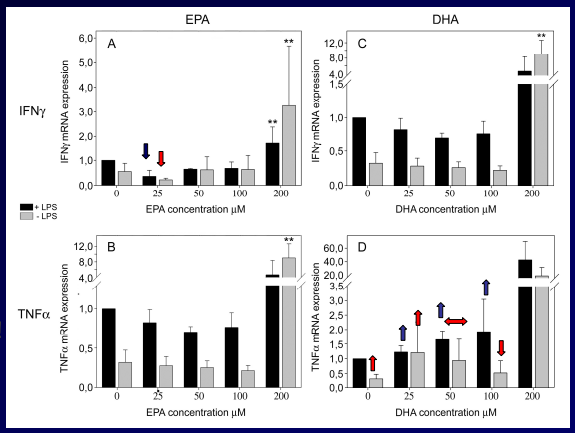
Only EPA does



Do EPA and DHA modulate cytokine gene expression?

No they don't
(except when used at 200µM)

and probably due to apoptosis!!

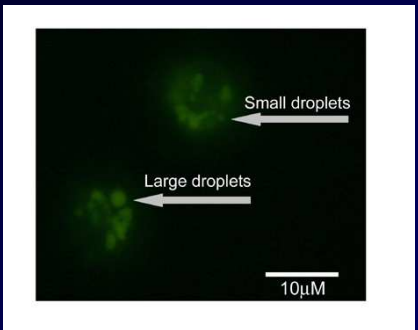


But we have to carry out more experiments

The lipid droplet formation

- lipid-rich organelles

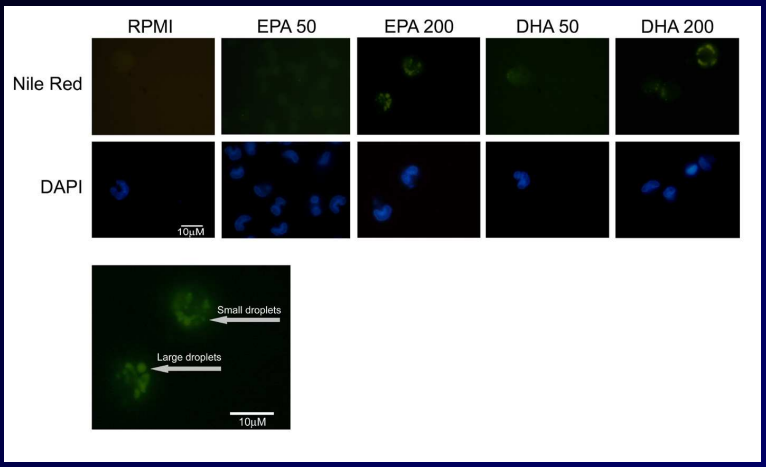
- common feature of innate immunity reactions and inflammatory conditions



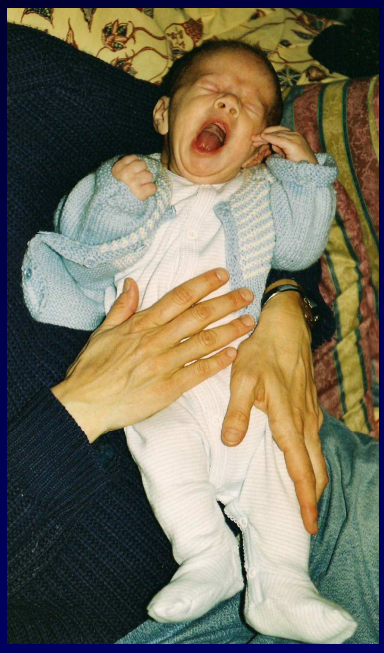
regulators of leukocyte activation rate during inflammatory reaction

number increased during inflammatory response

Do EPA and DHA modulate lipid droplet formation?



Take home message!!



EPA and DHA differentially affect goat leukocytes

1. by increasing phagocytosis rate

2. by modulating some cytokines ?

3. by modulating ROS production (PMN)

4. by modulating lipid droplet metabolism

(but we have to collect some more data)

Can we modulate immune system with PUFA in dairy livestock?

YES
probably we can

Should we?

??????????

other frontiers and new worlds to explore

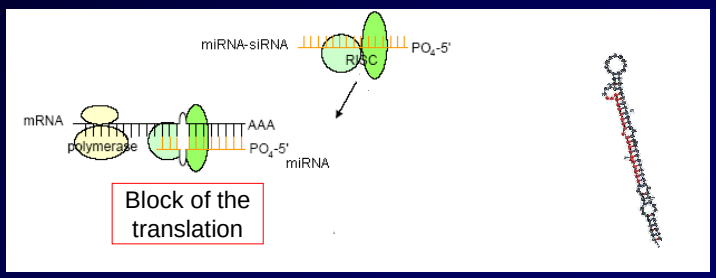
explore EPA and DHA

(but also other PUFA and saturated, and combination of them)

activities on other species

the new world of miRNA interference

- Non coding endogens RNA (21-23 nucleotides)
- Modulation of post-trascriptional DNA synthesis



Leung and Whittaker, *Pharmacology and Terapeutics* 107; (2005). (modified)

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cisma

Thank you all for your attention