

Seafood and prevention of colon cancer: potential mechanisms of action

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- **This is not the full presentation as presented at the meeting. It has been edited to preserve confidentiality of new research results, prior to their publication in a scientific journal.**

The Nature of Cancer



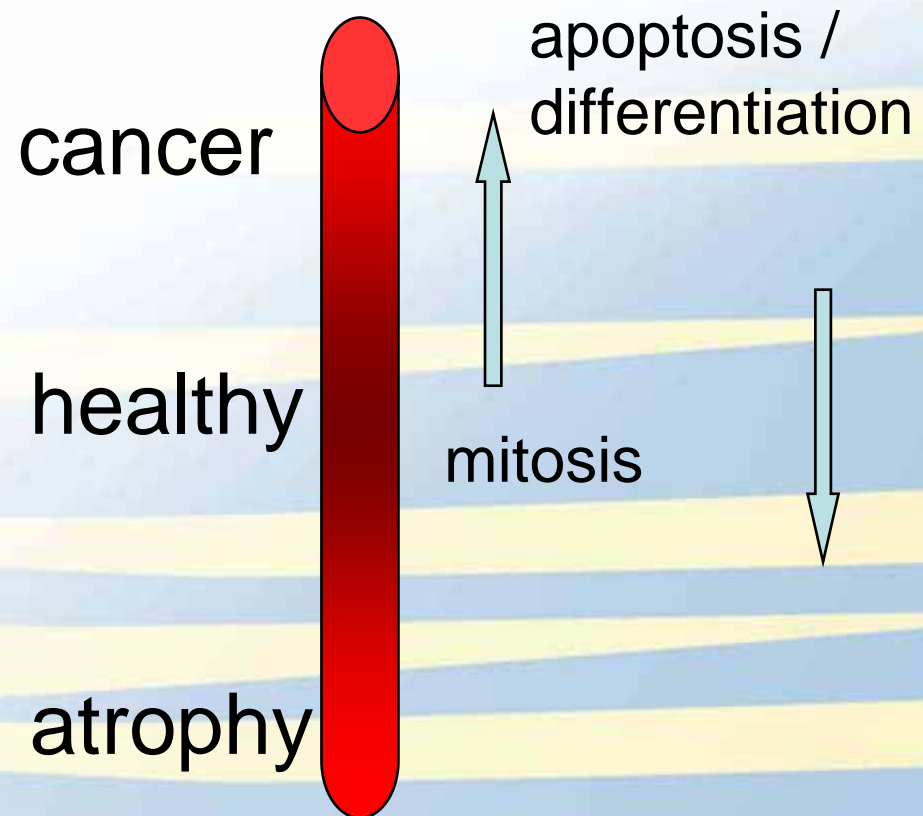
- “An inappropriate increase in the number of cells needed for growth and function of an organ...”

- **Benign tumours**

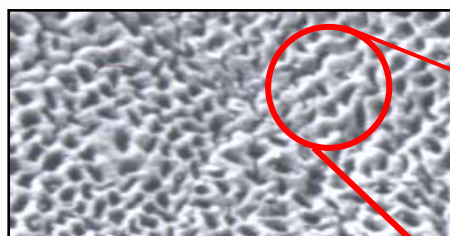
- Relatively slow
- Differentiated cells
- Non-invasive

- **Malignant tumours**

- Faster growth
- Less differentiation
- Invasive



Hypothesis: *The vulnerability of the colonic epithelium to neoplasia is shaped by decades of exposure to metabolic, dietary and microbial effects, prior to emergence of focal lesions...*



Epithelial proliferation and apoptosis

Differentiation
Extrusion

Mitosis

Mitosis
Apoptosis

Gut bacteria

- Metabolites
- Immune interactions
- Pathogens

Nutrition

- Cellular lipid composition
- Micronutrients
- Phytochemicals

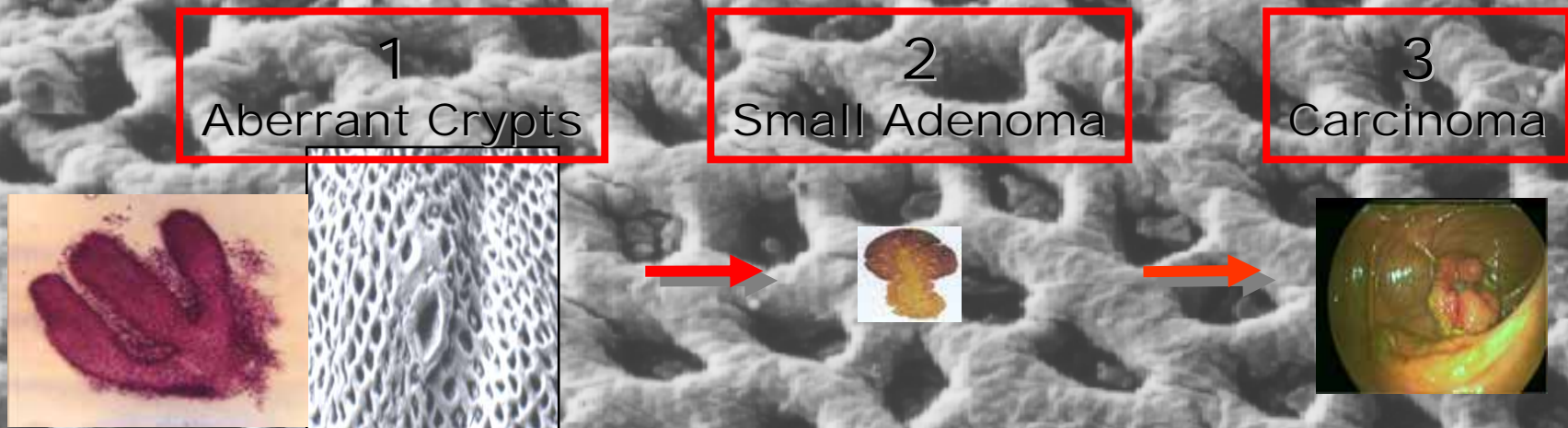
Immune system

- Lymphoid cells
- Myeloid cells

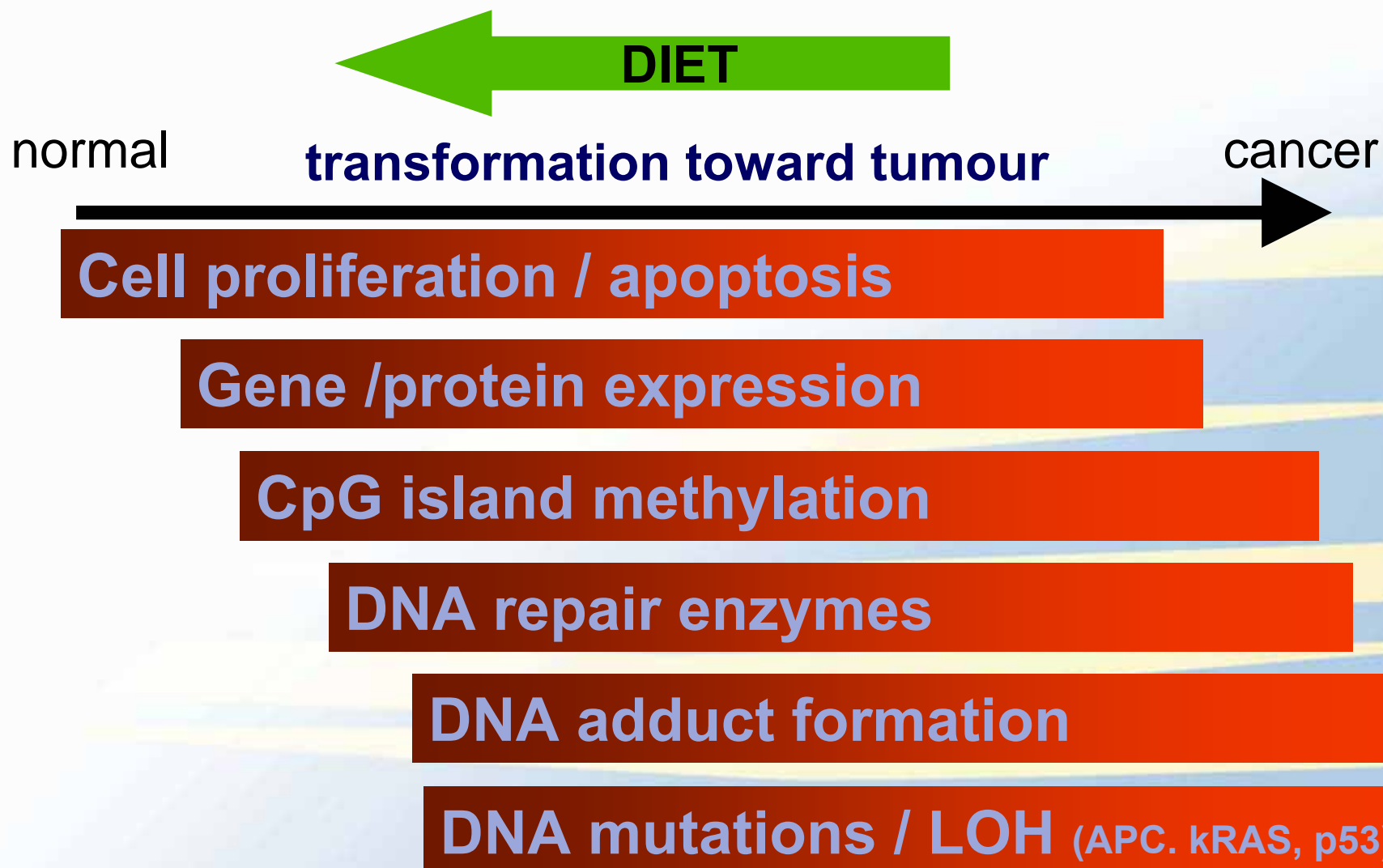
Blood-borne factors

- Hormones
- Cytokines

Colorectal Cancer Develops From Mucosal Epithelial Cells Via the Adenoma-Carcinoma Sequence



Biomarkers of Risk



Potentially protective factors in fish

Lean Fish

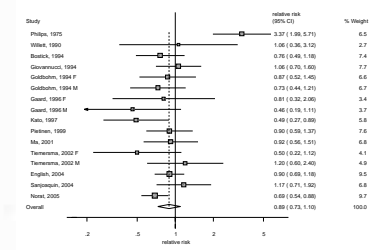
- Low energy protein source
- Bioactive peptides
- Selenium

Oil-rich Fish

- Selenium
- Bioactive peptides
- Vitamin D
- Omega-3 / n-3 fatty acids

Evidence for a protective effect of omega-3 fatty acids

- Case-control & cohort studies??
- Human intervention studies
- Animal intervention studies
- Cell culture



Human intervention studies - omega-3s

- Anti et al. Fish oil (7.7 g/d) supplementation has short- term and long-term normalizing effects on the abnormal rectal proliferation patterns associated with increased colon cancer risk. *Gastroenterology* (1994, 1997)
- Cheng et al. 3g/d fish oil increases apoptosis after 24 months *Cancer Letters* 2002

Impact of EPA supplementation on colon crypt cytokinetics in patients with polyps



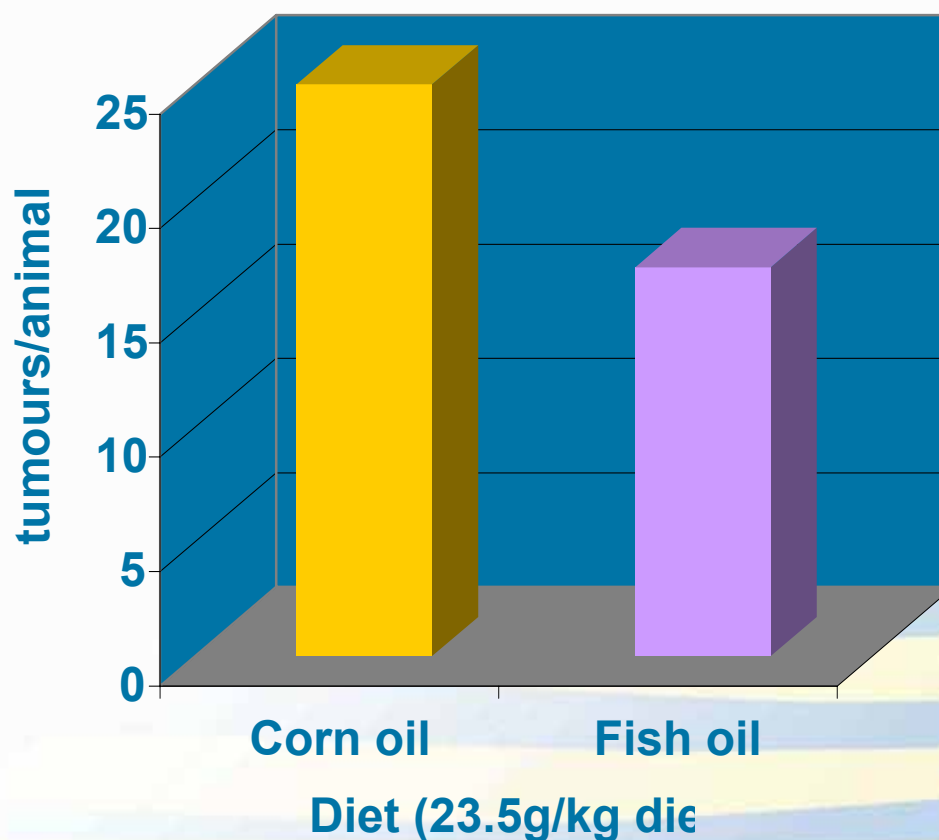
(Courtney et al. Gastroenterology - 2004/2005)

- **Patients**
 - number = 14 test & 14 control
 - treatment 2x 1g EPA ffa capsules/d
 - time 3 months
- **Measured**
 - cell proliferation *by Ki67*
 - Apoptosis *by M30 stain*

Animal Studies



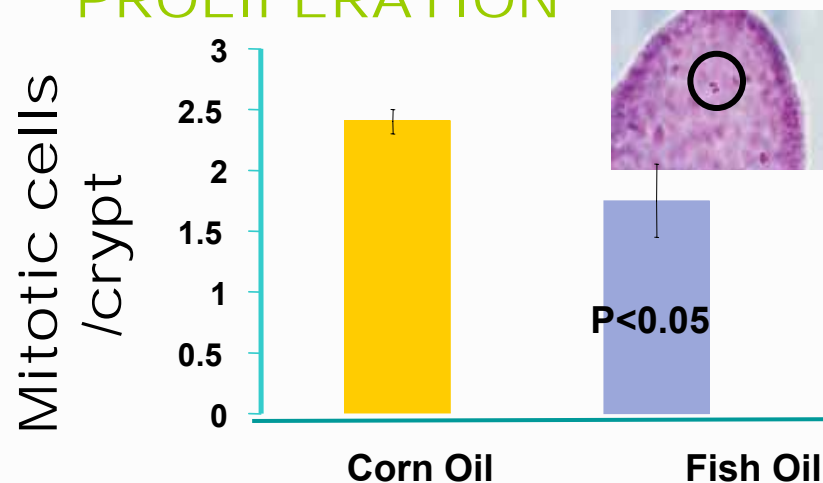
Fish oil reduces tumour number in a rat model of colorectal cancer



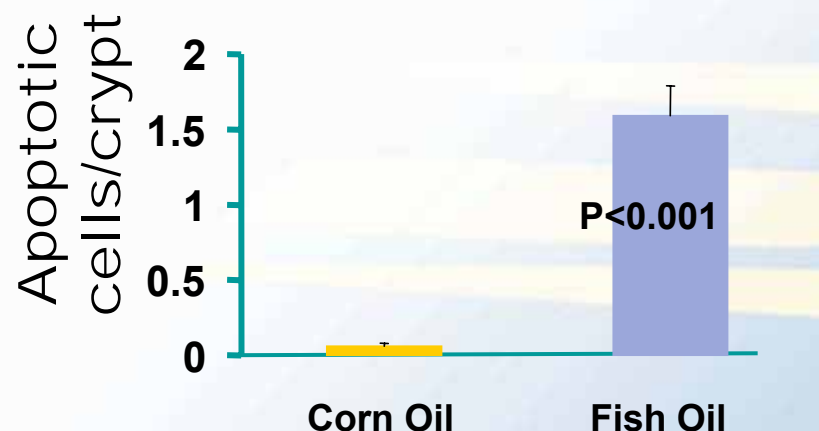
Reddy et al. *Cancer Research* 1986

Fish oil reduces mitosis & increases apoptosis

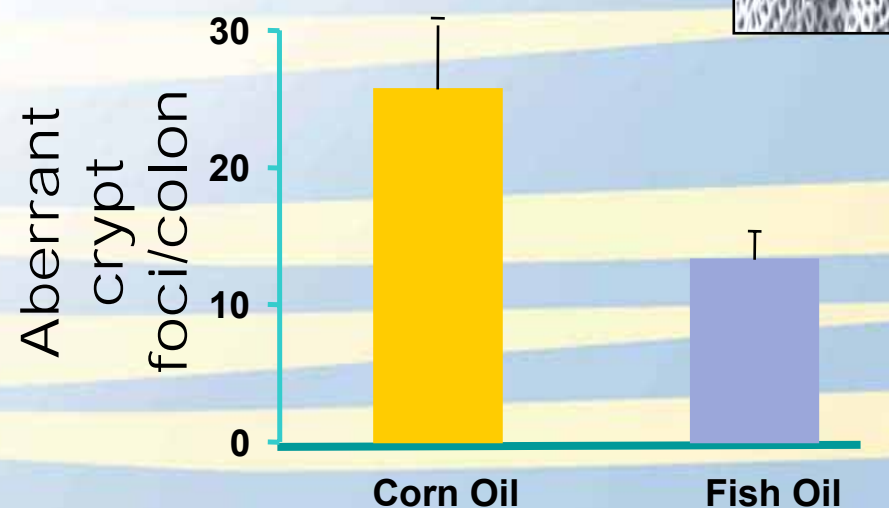
CELL PROLIFERATION



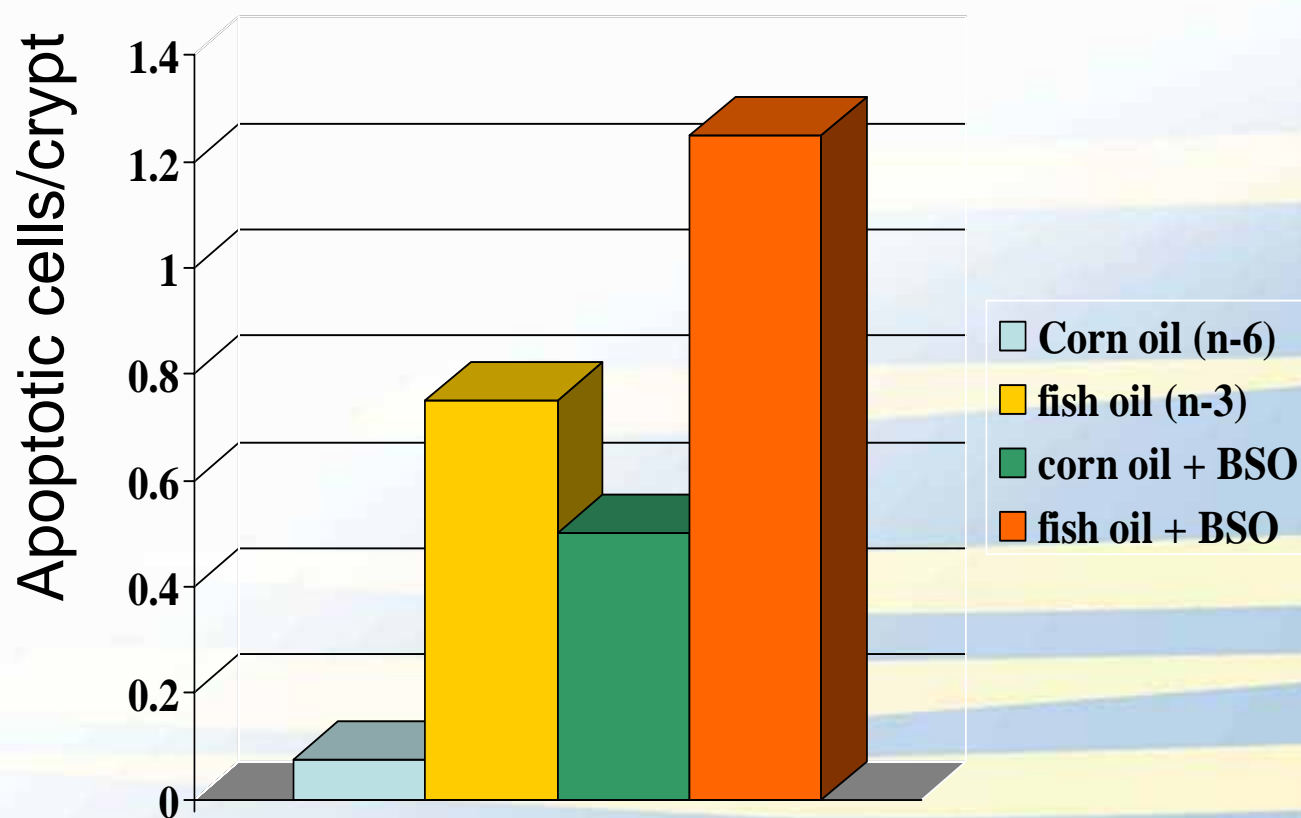
APOPTOSIS (CELL SUICIDE)



ABERRANT CRYPT FOCI



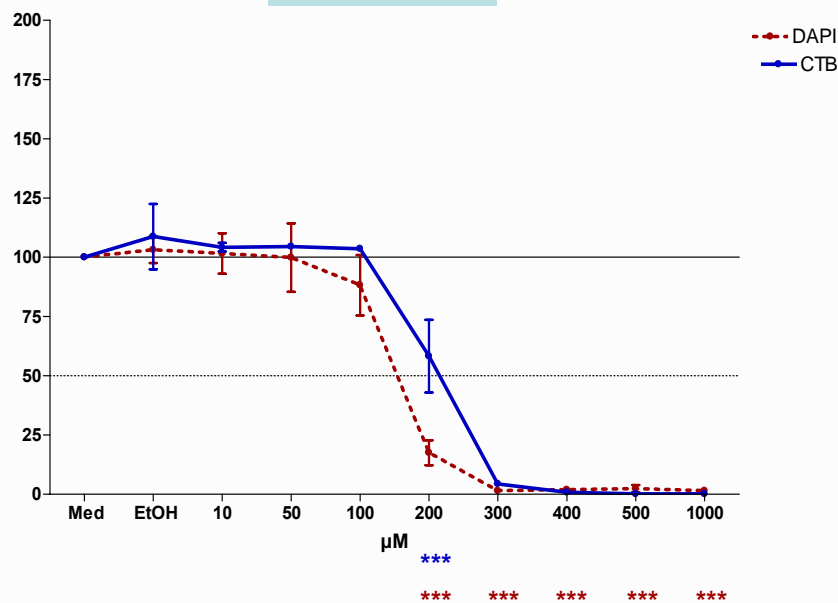
Depletion of endogenous glutathione exacerbates the effect of fish oil



Cell culture studies

- EPA is rapidly taken up into cancer cells in culture
- This is associated with a change in redox state (more oxidised)
- Cells commit suicide (apoptosis)
- Cell death prevented by anti-oxidants!

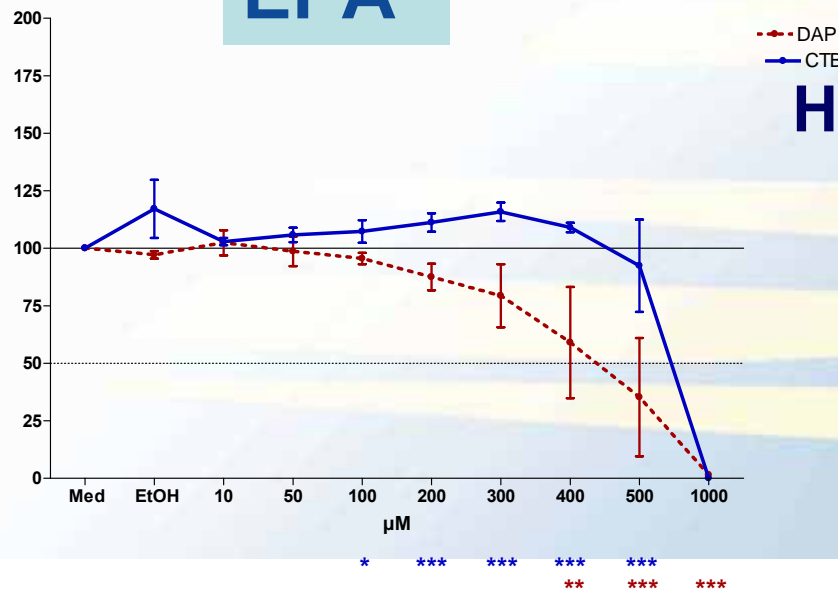
DHA



α-LA



EPA



EPA or DMH ?

Habermann et al. (unpublished)

48h incubation –HT29 cells

Cell titer blue - metabolic activity

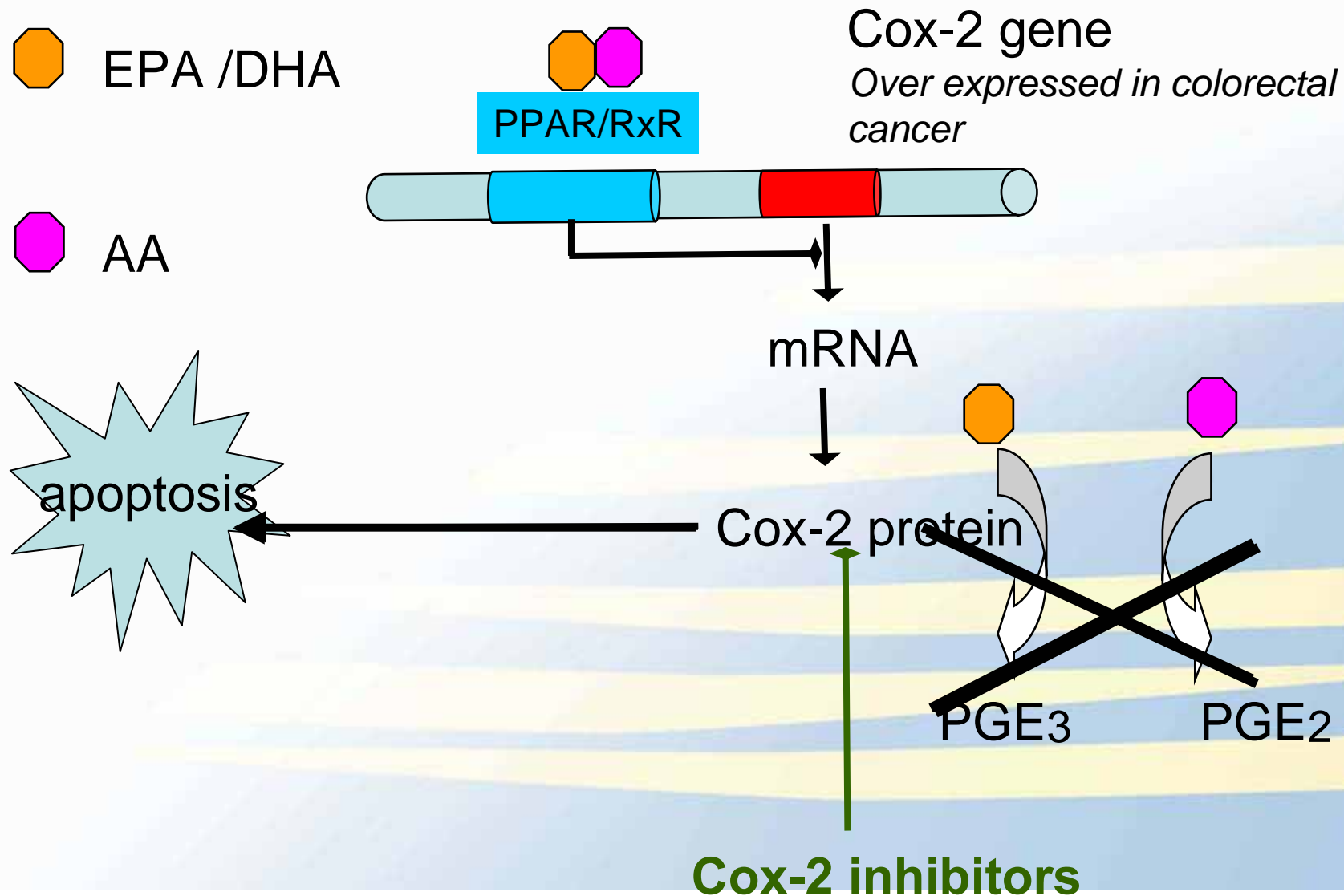
DAPI – DNA content - cell number

How do omega-3s increase apoptosis and decrease mitosis?



- Redox signalling
- **PPAR / RxR signalling**
- Reduced Cox-2 expression
- **Production of less inflammatory eicosanoids**
- Less responsive to pro-inflammatory cytokines
- **One mechanism for all n-3s?**

Omega-3 fatty acids and Cox-2



Evidence for a protective effect of vitamin D

Human studies – vitamin D

- **Miller et al. *Cancer epidemiol biomarkers*. 2005**
 - Low vitamin D status associated with reduced apoptosis
- **Recommended intake values (5-10 ug/d)**
 - Salmon contains 8ug/100 fish

Animal intervention studies – vitamin D

- **Sitrin et al. *Cancer Research* 1991**
 - Calcium plus vitamin D had no effect on tumour incidence but reduced tumour number and size
- **Mokady et al. *Nutrition & Cancer* 2000**
 - Vitamin D3 normalises aberrant cell proliferation in DMH treated rats and reduced tumour number.

Cell culture studies – vitamin D

- **Diaz et al. Cancer Research 2000**

- **Vitamin D3 can:**

- **block cell division,**
 - **induce cell differentiation**
 - **followed by apoptosis**

Selenium & colorectal cancer



- Reasonably good evidence for a protective effect of selenium based on human and animal intervention studies
- Selenium is approx 1.5 times as bioavailable from fish as from yeast or selenite (Fox et al. EJCN 2004)
- The UK reference nutrient intake (RNI) for selenium is 75 and 60 ug/day for adult males and females respectively
 - tuna contains approximately 80ug/100g fish
 - salmon contains approximately 24ug/100g fish

But does fish consumption actually protect us?



The FishGastro Study

Human Intervention Study

Hypothesis

The consumption of two portions of fish (300g) per week provides protection against colorectal disease, especially colorectal cancer.

Does it matter whether it is oil-rich fish or not?

FISHGASTRO INTERVENTION STUDY



Recruit patients undergoing colonoscopy /sigmoidoscopy

- Polyps/resection
 - Ulcerative colitis
 - Controls (IDA/IBS)
- 90 patients/group
1-2 patients per week

Dietary advice /6 month intervention



fruit & veg advice
'5-a day'

White fish
& '5-a day' advice



Oil-rich fish
& '5-a day' advice



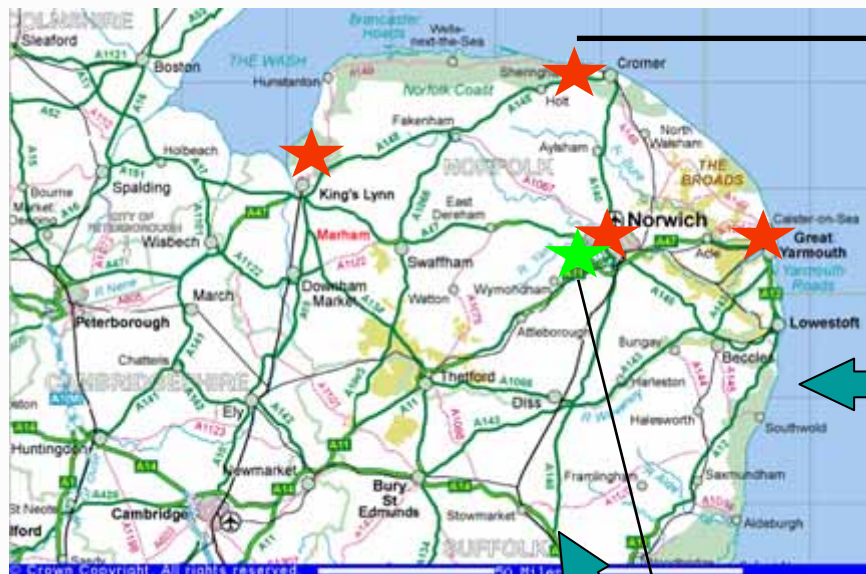
Collect:

- blood samples
- biopsies

Dietary
monitoring

Markers of:

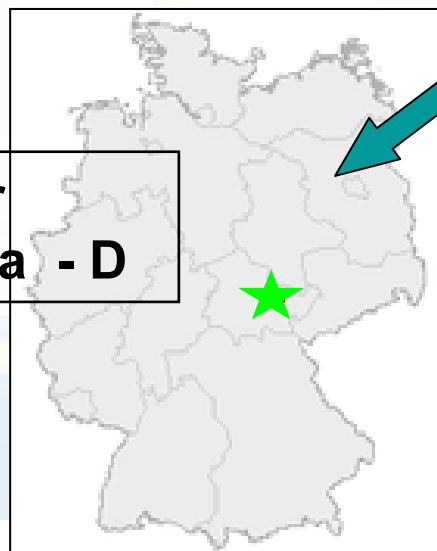
- cancer risk
- inflammation



Institute of Food Research - UK



Friedrich Schiller University of Jena - D



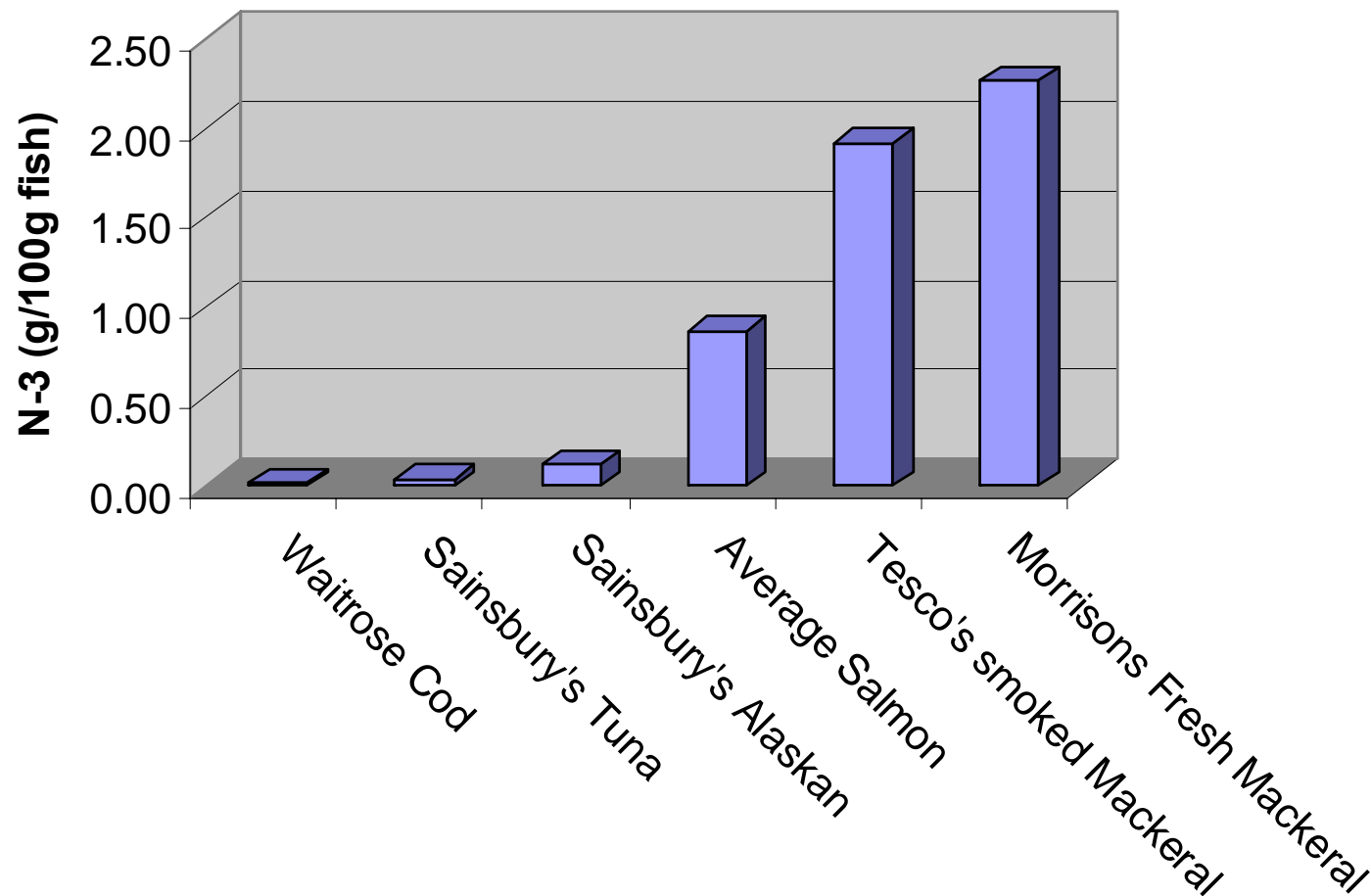
TNO Zeist



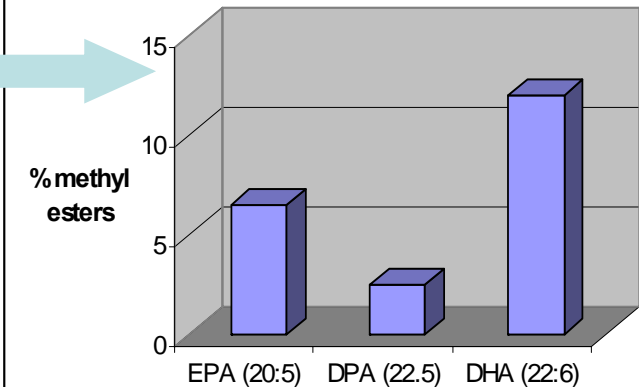
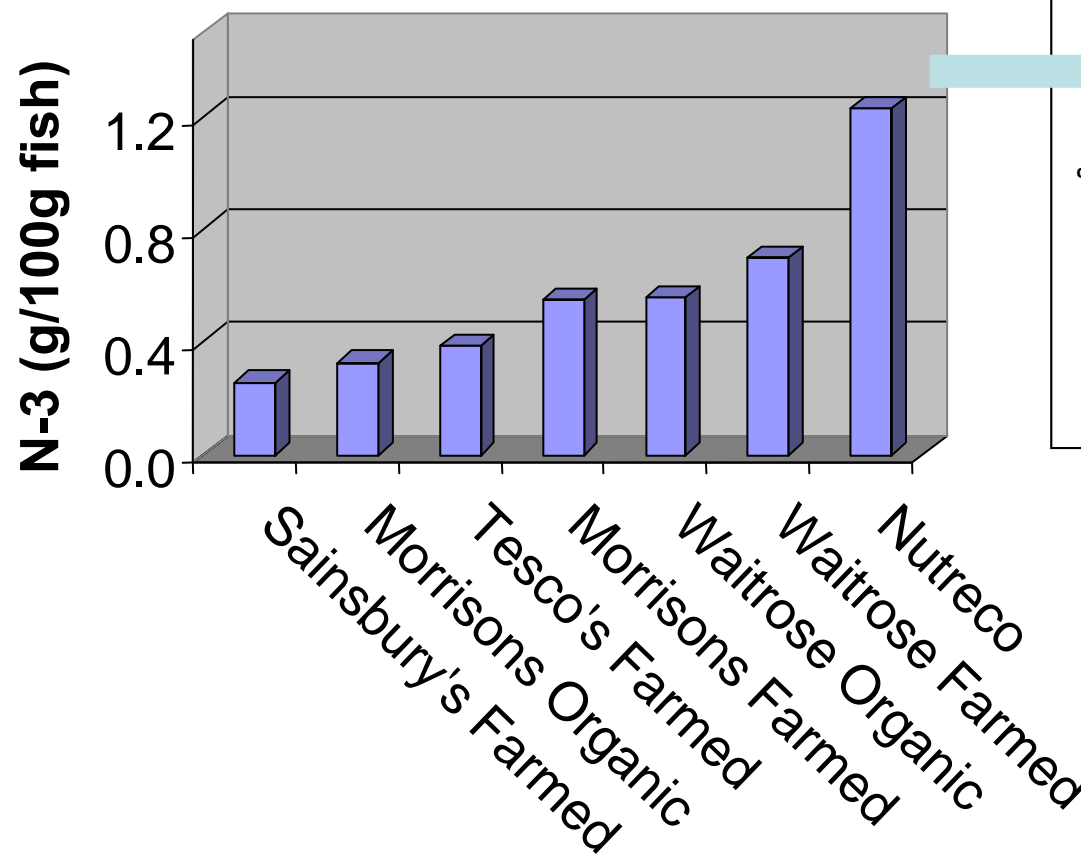
Wageningen University - NL



Omega-3 content of salmon and other commonly consumed fish

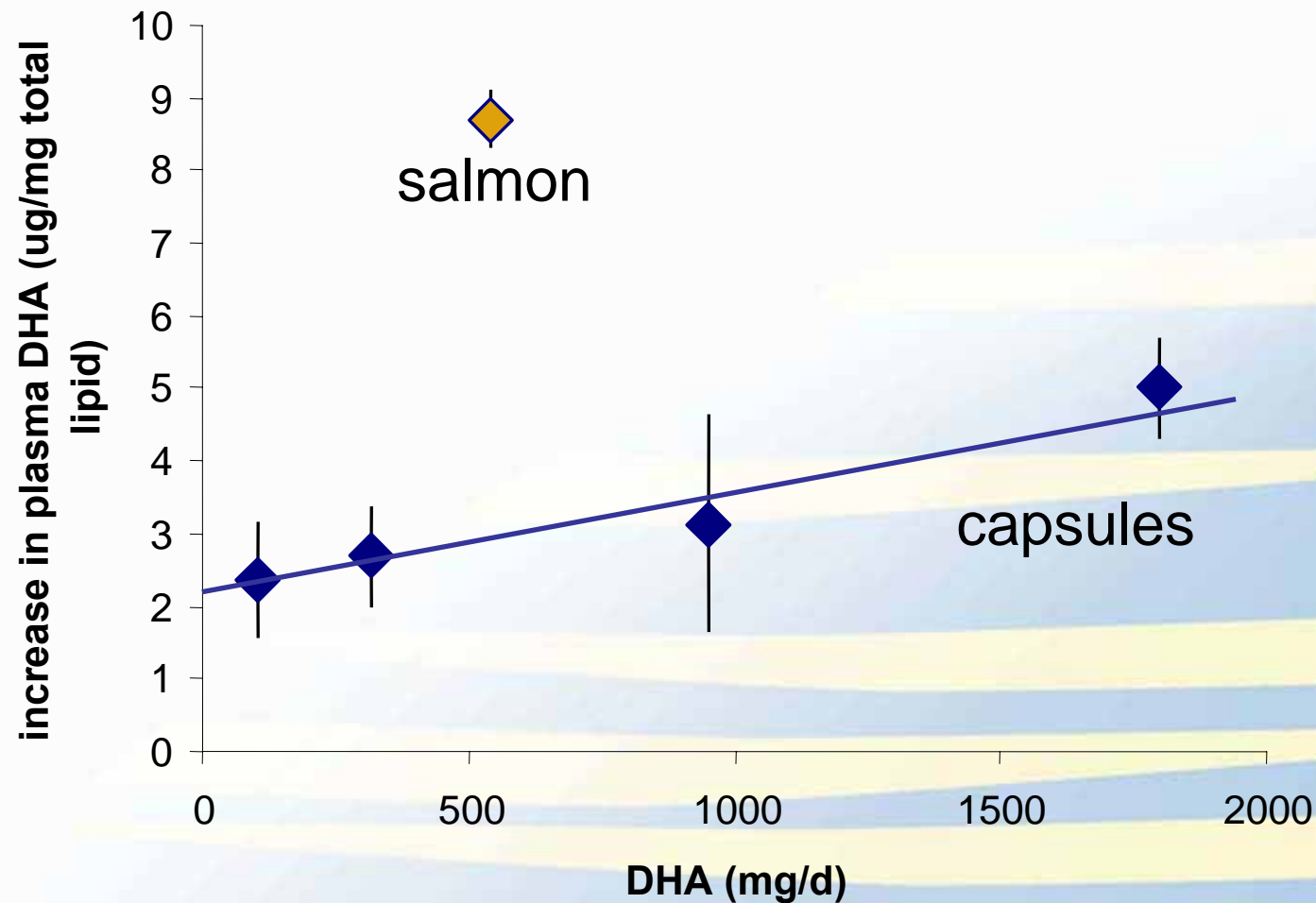


Omega-3 fatty acid content of salmon purchased in Norwich (Jan 05)



Salmon
volunteers
consuming
0.5g EPA+DHA
per day

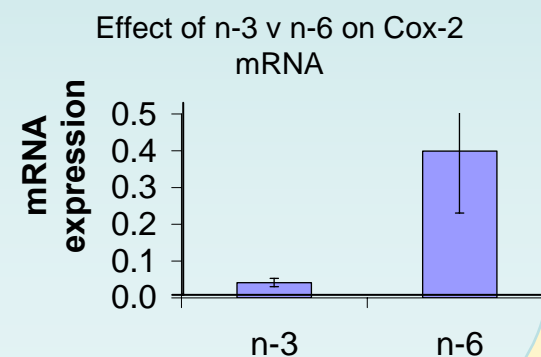
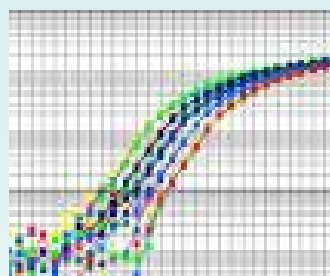
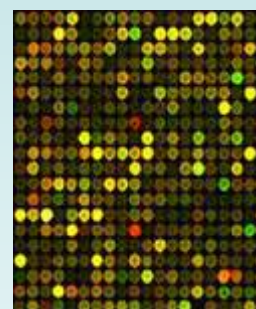
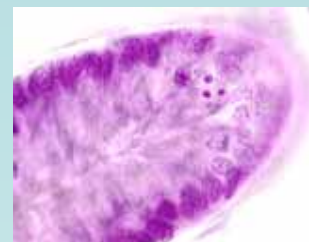
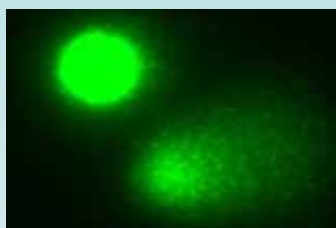
Bioavailability of omega-3 from fish is much greater than from fish oil



Visioli et al. Lipids 03

End-points 1: Biopsies

- Apoptosis and cell proliferation
- COMET formation
- Gene expression patterns (arrays)
- Taqman



End-points:2

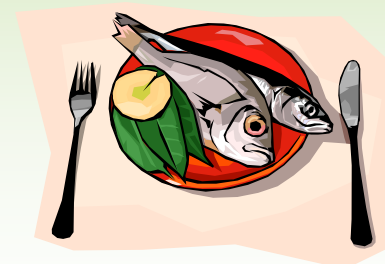


Blood

- n-3: n-6 fatty acid ratio
- Markers of inflammation
- Vitamin D
- Selenium

Fish consumed

- n-3 content
- Selenium content
- Vitamin D



End-points 3: faeces

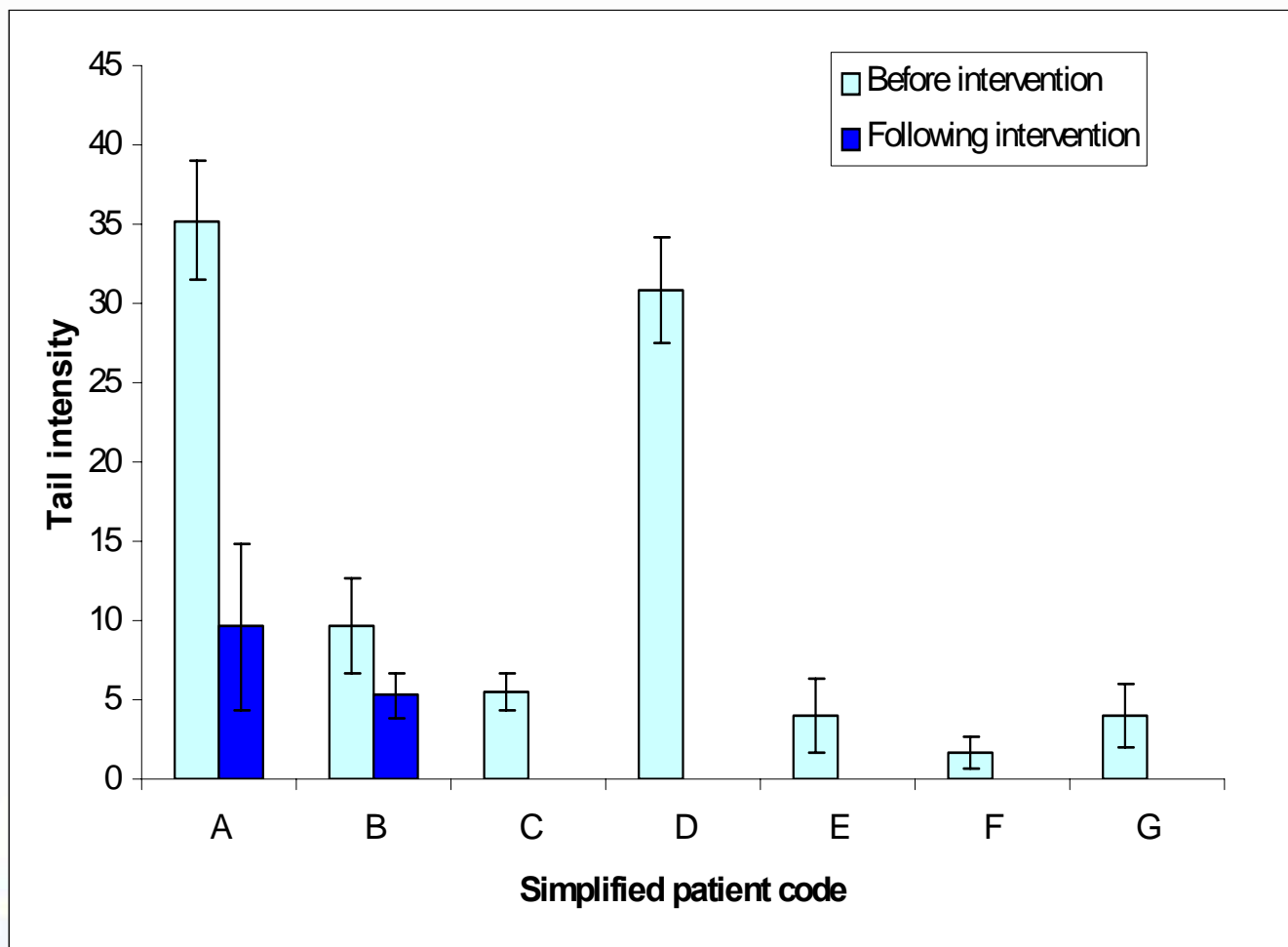
- Faeces In vitro faecal water studies
 - Cytotoxicity
 - COMETs
 - Gene expression (*FSA BIOMICS project*)
- Faeces CpG island methylation
(*FSA - FishMet Project*)

Expected outcomes 1

A comparison between cod, salmon & standard dietary advice for a number of colonic health end points:

- **Induction of apoptosis**
- **Reduction in**
 - **mitosis**
 - **inflammatory signalling molecules**
 - **DNA methylation**
 - **DNA damage**

COMET Assay on Primary



Expected Outcomes 2

- **Transcriptomic analysis of gene expression in:**
 - biopsy samples
 - colorectal cell lines exposed to faecal water
- **Development of a panel of genes in which expression is relevant to early stage cancer development and can be manipulated by diet**

Overall Outcome

An assessment of:

**THE IMPACT OF FISH
CONSUMPTION ON COLORECTAL
TUMOUR RISK**

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Jan Uil -NL

Paul van de Meeberg -NL

Robin Timmer -NL

Peter Wahab -NL

Adriaan Tan -NL

Ellen Witteman -NL

A better life with seafood...



www.seafoodplus.org