Effects of hydrogenated fish oil in experimental colorectal carcinogenesis in A/J mice

Thesis in clinical nutrition for the degree of Candidata Scientiarum

by

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2005

Acknowledgements

The work with this thesis in clinical nutrition for the degree of *Candidata Scientiarum* was carried out from January 2003 to August 2005 at the Department of Food Toxicology, Division of Environmental Medicine, Norwegian Institute of Public Health, and was a study preliminary to a larger project in cooperation with Rikshospitalet University Hospital.

My deep gratitude goes to my main teaching supervisor Dr. Scient. Jan Erik Paulsen, Senior Scientist at MINT, for introducing me to the world of science. He has been sharing his great knowledge and deep insights through excellent teaching and advice during the experiment. Special thanks go to Dr. Philos. Kari Almendingen, my teaching supervisor on behalf of Rikshospitalet University Hospital, for her efforts to help my project go forwards right from the start by her highly skilled advice on literature, her "primus motor" theoretical and practical help, and especially I would like to thank her for her enthusiasm and friendly advice. I would also like to give warm thanks to Associate Professor Dr. Philos. Helle Margrete Meltzer, my teaching supervisor on behalf of the Department of Nutrition, University of Oslo, and also Senior Scientist at MINT, for giving me excellent help in the finishing stage of the writing process.

I am also grateful to the other members of MINT and the animal department for creating a friendly and pleasant environmental atmosphere for my work.

Butter oil was donated by Tine Fellesmeieriet BA and fish oils and soybean oils by Denofa. Thanks to Nutrition Supervisor Anne Sofie Biong at Tine for providing general information on butter oil. Thanks to Development Manager Håkon Standal and other personnel at Denofa for providing laboratory production of the totally hydrogenated fish oil, for analyzes and transport of the oils, and general presentations of product information and fat history.

Last, but not least, I thank my family and friends for their patient and never failing support.

Oslo, August 18th 2005

Preface

This thesis in clinical nutrition for the degree of *Candidata Scientiarum* is written in the form of an article, i.e., it includes a manuscript for an article, which represents the work performed for this thesis. The results are thoroughly presented and discussed in the article. A more in depth introduction than the one in the article is included in the thesis, and a detailed section on materials and methodology, as well as a presentation of the most important results and a more thorough conclusion than contained in the article.

Lisa Berg Ajmi

Summary

Background

This experiment was performed at the Norwegian Institute of Public Health as a study preliminary to a larger project in cooperation with Rikshospitalet University Hospital.

The reason for the increasing colorectal cancer (CRC) incidence trend over time in Norway compared with its neighbouring countries is obscure. Norway is, however, diverging in an important factor; we have been consuming hydrogenated fish oil and cod liver oil to a greater extent than our neighbours during the last century.

The *first aim* of this study was to examine whether hydrogenated fish oil (HFO) in the diet would increase induction and/or growth of CRC. Since it is unethical to treat humans with potentially harmful amounts of fat, the effects of HFO in the diet was tested on A/J mice treated with azoxymethane (AOM). Because of the complex composition of hydrogenated oils, the intension was to correlate potential biological effects to degree of hydrogenation rather than to specific fatty acids in the oil. This was done by using three degrees of HFO; partially hydrogenated fish oil (PHFO), highly hydrogenated fish oil (HHFO), and totally hydrogenated fish oil (THFO). As controls, fish oil (FO), butter oil (BO), soybean oil (SO), and soybean oil similarly hydrogenated as the fish oil (HSO); partially hydrogenated soybean oil (PHSO), highly hydrogenated soybean oil (HHSO), and totally hydrogenated soybean oil (THSO), were used. In this way, the study included oils exemplifying some of those used in the Norwegian household during different time periods.

The *second aim* was therefore to examine patterns of effect in the material which might be elicited through the four analyses. This was done by thorough description and discussion of the results; also including non statistically significant, in the manuscript for an article. In the thesis, mostly statistically significant results.were treated in the Results Chapter. In addition, a view to the specific oils was given.

The problem was approached by setting up three hypotheses, which were tested by the use of general working hypotheses in four series of analyses. To aid the problem solving, the data material was first checked for possible protecting properties of unhydrogenated FO.

Methods

Relations between induction and/or growth of CRC and diets with 19 w/w % (and 1 w/w % corn oil) of three different degrees of HFO; PHFO, HHFO or THFO, mixed with AIN-76M, were studied. As controls, native FO, BO exclusive water and salt, refined SO, and three degrees of HSO; PHSO, HHSO, and THSO, similarly hydrogenated as the HFO, were used. Tumors were induced by AOM-injections at age 1 and 2 weeks, and the animals were fed the diets from day 4 until killed at age 15 weeks. The surface of unsectioned methylene blue stained colon preparations of 104 female and 95 male animals was examined by transillumination in an inverse light microscope, and differences in induction (tumor incidence and number) and growth (tumor size) of CRC and in tumor load (considers both induction and growth) between the diets were studied.

Results

The words "tend" and "tendency" are used when $0.20 \geq p > 0.05$, "seem" and "seemingly" when $p > 0.20$, and the rest is statistically significant, i.e., $p \leq 0.05$.

The protecting properties of FO. In the preanalysis, unhydrogenated FO tended to reduce induction of CRC and tumor load, and seemed to increase growth of CRC in females relative to males. Compared with SO, FO protected females considerably against induction and seemed to protect them moderately against growth, but males were seemingly not protected. Compared with BO, FO seemed to protect both genders considerably against induction, but not against growth.

When fish oil was hydrogenated, no evidence was found of HFO increasing induction and/or growth of CRC regarding to unhydrogenated FO, irrespective of gender. Compared with FO; in females, all the HFO seemed to have lost some protecting properties regarding induction, increasing it considerably, but to have gained protecting properties regarding growth, reducing it considerably, and only PHFO seemed to increase tumor load compared with FO.

In males, THFO tended to have lost protective properties regarding induction, increasing it considerably, seemed to have gained protecting properties regarding growth, reducing it, and seemed to increase tumor load considerably, while HHFO seemed to have gained protecting properties regarding both induction and growth of CRC, reducing all the tumor parameters, compared with FO.

Judged by hydrogenation degree, the PH degree of fish or soybean oil seemed to be the most harmful in increasing induction of CRC, but of the two PH, only PHSO led to seemingly extremely large tumor load irrespective of gender, possibly indicating harmful substances in PHSO and protecting substances in PHFO. PHSO seemed to almost double tumor load in females relative to males, possibly indicating differential modulation by female and male sex hormones.

For males, the TH degree of fish or soybean oil seemed to be equally harmful as PHSO in induction, but only PHSO and THFO led to equally substantially large tumor load in males, possibly indicating harmful substances in THFO compared with THSO. THFO seemed substantially more harmful for males than for females regarding induction of CRC and tumor load, but substantially more harmful for females than for males regarding growth, which might indicate differential effects in the induction and growth phases of CRC by substances in this oil, also differentially modulated by female and male sex hormones. These indications also seemed to pertain to THSO, albeit to a lesser degree, and THFO seemingly increased tumor load substantially compared with THSO, but only in males.

The HH degree of both fish and soybean oil seemed to give body weight among the lowest and to protect the animals of both genders regarding induction and tumor load (together with THFO in females), while HHSO seemed to give the most extreme tumor size.

Conclusion.

Certain effect patterns in this material seemed to indicate that the effects of the experimental oils on colorectal carcinogenesis in the AOM treated A/J mice, in addition to being influenced by gender and body weight, might be different in the induction and growth phase of CRC, and dependent on type of oil in the diet and hydrogenation degree, the latter might differ between HFO and HSO. These indications were not based on statistically significant results only, so they must be taken for what they are worth. Some evidence was however found.

No statistically significant evidence was found of increasing CRC by HFO relative to unhydrogenated FO, with loss of protecting properties, nor of increasing effects with increasing hydrogenation degree; which would have indicated that the effects of HFO were due to a change in fish oil by the hydrogenation process *per se*.

That the HH degree of HSO reduced induction of CRC relative to the TH degree, indicated that the effects of HSO were dependent on specific hydrogenation degrees, i.e., were related to specific substances in these oils. Even if the null hypotheses for HFO could not be rejected, the possibility that also the effects of HFO might be dependent on specific hydrogenation degrees could not be ruled out.

No statistically significant evidence was either found of increased effects of HFO relative to corresponding hydrogenation degrees of HSO. On the contrary, when the effects were examined with a view to the specific oils, however, it seemed as HSO were more harmful than HFO; all the tumor parameters seem to be increase by PHSO relative to PHFO, and statistical evidence was found for tumor number and load in males.

In the view to the specific oils, it actually seemed as any of the experimental oils, including those that were exemplifying oils consumed in Norway over different time periods, might contain substances that are risk factors in colorectal carcinogenesis; in any way in these animals. Even if it had been possible to draw this conclusion for the A/J mice, findings in experimental animals will not necessarily apply to man. The mouse is, however, the model organism which is closest to humans.

If the oils in this experiment really may exemplify oils used in Norway, and if the results of this study might apply to humans; the results would have indicated that of the examined oils, HHFO might not be the most likely fat to play a role in the increasing CRC trend in Norway compared with its neighbouring countries. More likely candidates would be PHFO or FO (as cod liver oil), which besides HHFO were the only of the experimental oils with increased consumption relative to the other Nordic countries, and which were used to a great degree long enough and early enough to have affected the rising trend in Norway, which started at least as late as in the 1950s, when the registering of the CRC incidence started.

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ABBREVIATIONS

AA	Arachidonic acid
AC	Aberrant crypts
ACF	Aberrant crypt foci
ACF_{Min}	Flat ACF demonstrated in Min mice
AICR	American Institute for Cancer Research
A/J mice	The A inbred strain of JAX mice susceptible to colon cancer
AKR/J mice	JAX mice resistant to colon cancer
ALA	α-linolenic acid
ALDH	Aldehyde dehydrogenase
AOM	Azoxymethane, genotoxic substance
APC	*Adenomatous polyposis coli* (the gene, man)
APC	Adenomatous polyposis coli (the protein, man)
BO	Butter oil, without water and salt
c	Double bond in *cis* configuration: the hydrogen atoms are placed on the same side of the bond
CFA	Changed fatty acids
CI	Confidence interval
CIN	Chromosomal instability, the accelerated rate of chromosomal gains and losses
CLA	Conjugated linoleic acids, a collective term for positional and geometric isomers of linoleic acid
CM	Chylomicrons
CMR	Chylomicron remnants
COX	Cyclooxygenase (prostaglandin H synthase)
COX-1	Constitutive-type cyclooxygenase
COX-2	Inducible-type cyclooxygenase
$cPLA_2$	Cytosolic phospholipase A_2
CR	Caloric restriction
CRC	Colorectal cancer
CYP450	Cytochrome P450
DAG = DG	Diacylglycerols, also referred to as diglycerides

DGLA	Dihomo-γ-linolenic acid
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acids, make up the genes
EFA	Essential fatty acids
EPA	Eicosapentaenoic acid
ER	Endoplasmatic reticulum
ER	Estrogen receptor
FAP	Familial adenomatous polyposis
FDA	The Food and Drug Administration
FFA	Free fatty acids, equivalent to non-esterified fatty acids
FO	Fish oil, native raw
FE	Feed expenditure
FW	Final weight
GLA	γ-linolenic acid
GRAS	Generally regarded as safe
GST	Glutathione serum transferase
HDL	High density lipoproteins
HFO	Hydrogenated fish oils: PHFO, HHFO and THFO
HH	High hydrogenation
HHFO	Highly hydrogenated fish oil
HHSO	Highly hydrogenated soybean oil
HNPCC	Hereditary non-polyposis colorectal cancer
HSO	Hydrogenated soybean oils: PHSO, HHSO and THSO
IDL	Intermediate density lipoproteins
IV	Iodine value
K-ras	Kirsten rat sarcoma oncogene
LA	Linoleic acid
LCFA	Long chain fatty acids
LCFFA	Long chain free fatty acids
LCPUFA	Long chain polyunsaturated fatty acids
LDL	Low-density lipoproteins
LM	Light microscope

LOH	Loss of heterozygosity, i.e. loss of one allele in a tumor cell from a chromosomal region for which the individual's normal cells are heterozygous
LOX	Lipoxygenase
LP	Lipoproteins
Lp(a)	Lipoprotein (a)
LPL	Lipoprotein lipase
LT	Leukotrienes
MCFA	Medium chain fatty acids
md	Estimated median difference
MG	Monoacylglycerols, monoglycerides
2-MG	Glycerol with a fatty acid esterified at carbon 2
MIN	Microsatellite instability
MMR	Mismatch repair
MP	Melting point
MTHFR	Methylene tetrahydrofolate reductase
MUFA	Monounsaturated fatty acids
n	Number of animals (or tumors) in the treatment group
N	Total number of animals (or tumors) in the comparison
NF-κB	Nuclear factor κB
NAT	N-acetyltransferase
$NO^{\bullet}$	Nitric oxide
NSAID	Nonsteroidal anti-inflammatory drugs
ODC	Ornithine decarboxylase
PG	Prostaglandins
PH	Partial hydrogenation
PHFO	Partially hydrogenated fish oil
PHSO	Partially hydrogenated soybean oil
PL	Phospholipids
PLA_2	Phospholipase A_2
PPAR	Peroxisomal proliferator-activated receptors, transcription factors that can bind to regulatory elements of DNA along with another protein and a fatty acid

P/S ratio	Ratio of PUFA:SFA
PUFA	Polyunsaturated fatty acids
Ras	Proto-oncogene
RM1 (E)	Rat & Mouse No. 1 Maintenance Diet
RM3	Rat & Mouse No. 3 Breeding Diet
RR	Relative risk
SCFA	Short chain fatty acids
SFA	Saturated fatty acids
SO	Soybean oil, refined
t	Double bond in *trans* configuration: the hydrogen atoms are placed on opposite sides of the bond
TFA	*Trans* fatty acids
TG	Triglycerids, also referred to as triacylglycerols
TGF	Transforming growth factors
TH	Total hydrogenation
THFO	Totally hydrogenated fish oil
THSO	Totally hydrogenated soybean oil
TX	Thromboxanes
UFA	Unsaturated fatty acids
VLCFA	Very long chain fatty acids
VLCSFA	Very long chain saturated fatty acids
VLCUFA	Very long chain unsaturated fatty acids
VLDL	Very low density lipoproteins
Wnt	Pronounced "wint". Wnt was introduced 20 years ago and fused the names of two orthologous genes: *Wingless* (*Wg*), a *Drosophila* gene, and *Int-1,* a mouse proto-oncogene
w/w %	% by weight

WORD EXPLANATIONS

Aberrant crypt foci	Defined as crypts that have altered luminal openings, exhibit thickened epithelia, and are larger than adjacent normal crypts
Adenocarcinoma	Malignant neoplasm, cancer
Adenoma	Gland like structure, benign neoplasm of epithelial tissue, dysplastic polyp, non-invasive tumor
Angiogenesis	The growth of new blood vessels
Apoptosis	Programmed cell death
Carcinoma	Invasive tumor, cancer
Carcinoma in situ	A lesion characterized by cytologic changes of the type associated with invasive carcinoma, but with the pathologic process limited to the lining epithelium; a localized and curable face of carcinoma; must not exceed the muscularis mucosae
Chylomicron	The primary secretory unit of the enterocyte, containing re-organized absorbed fatty acids, 2-monoglycerids, phospho-lipids, lysophospholipids, cholesterol, phytosterols and smaller amounts of glycerol
Circadian	Relating to biologic variations or rhythms with a cycle of about 24 hours
CYP-450 monooxygenase	Microsomal cytochrome P450 monooxygenase system is a hemeprotein oxidase system found in the liver
De novo fatty acid synthesis	Lipogenesis
Eicosanoids	Oxidized products of fatty acids with 20 carbon atoms
Epigenetic mechanisms	A variety of mechanisms that influence the behaviour but not the structure of DNA
Genotype	The genetic constitution of an individual or, more specifically, the alleles at specific genetic loci

Geometrical isomerism	Here: An unsaturated fatty acid in two or more forms that are identical with respect to percentage composition but differs as to the restriction of free rotation about a carbon-carbon bond in the molecules, cf. cis-, trans-, and also in physical and chemical properties
Iodine value, decreasing	Indicates a general loss of unsaturation
Leptin	Protein secreted by fat cells, acting via its receptor in hypothalamus, and regulating energy expenditure and metabolism
MIN pathway	Microsatellite instability pathway
MMR proteins	Mismatch repair proteins, take care of replication errors
Morphologic	Relating to configuration (form, shape, structure)
Mount	Preparat
Mucosa	In the GI tractus: A mucous tissue consisting of epithelium, lamina propria and a layer of smooth muscle
Oncogene	An activated proto-oncogene, which allow unchecked proliferation of the cells (the accelerator is stuck). “Gain of function”
Positional isomerism	Here: An unsaturated fatty acid in two or more forms that are identical with respect to percentage composition but differs as to the position of one or more double bonds within the molecules, and also in physical and chemical properties
Prevalence	The number of persons who at a specific point in time have a specific diagnosis
Prostanoids	Prostaglandins and thromboxanes
Proto-oncogene	A gene which produces growth-controlling proteins, only one genetic event is required to turn it into an oncogene
Tumor	Swelling, benign or malign neoplasm
Tumor suppressor gene	Gene producing proteins that prevent the cell from reproducing at inappropriate times, or when the DNA is extensively damaged; requires two genetic events, one in each allele, for its inactivation; “Loss of function”

Wnts	A large family of secreted glycoproteins with at least 19 known human members, expressed in species ranging from Drosophila to man, the most upstream ligands of the Wnt signalling pathway

ORGANIZATIONS OF CURRENT INTEREST

Directorate for Health and Social Affairs

Norwegian Ministry for Agriculture

Norwegian National Council of Nutrition

Norwegian Food Safety Authority

The Norwegian Scientific Committee for Food Safety

The Animal Welfare Act

A Regulation in a special provision of the Act

The Norwegian Animal Research Authority

European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes issued by

Council of Europe

European Union (EU)

The EOES agreement

Codex Alimentarius

1 INTRODUCTION

1.1 COLORECTAL CANCER

1.1.1 COLORECTAL CANCER IN THE NORDIC COUNTRIES

Colorectal cancer (CRC) is the most common cause of cancer related death in the Western world (1). For the Nordic countries, CRC in 1993-1997 comprised 12.8% and 12.9% of all male and female cancer cases, respectively (2). The risk of CRC increases with increasing age; in this period, more than 90% of the cases were diagnosed among people aged 55 years or more (3). The trends in the incidence rates for males and females have been similar in each of the Nordic countries but greatly different between the countries (Figure 1). Since the 1970s, the Norwegian rates have overtaken both the Swedish and the Danish rates, and are now the highest in the Nordic countries (2).

In Norway, colorectal cancer is the most common form of cancer men and women seen together, in males secondary to prostate cancer and in females secondary to breast cancer (4). In 2001 there were 1,059 cases of colon cancer and 570 cases of rectal cancer in men, compared to 1,178 and 471 cases in women. Prevalence of diagnosed colon cancer 31.12.2001 was estimated to 13,576 and of rectal cancer 7,169. Because of a decline in the risk for younger cohorts, the Norwegian incidence rates are predicted to peak 2003-2007, and decrease towards 2018-2022 for both sexes (5).

1.1.2 CARCINOGENESIS

Carcinogenesis results from interplay between environmental factors and susceptibility genes that sets off a complex series of neoplastic events. Hanahan and Weinberg suggested that most if not all cancers have acquired the same set of functional capabilities during their development, albeit through various mechanistic strategies (Figure 2) (6).

Important genes in carcinogenesis

The gut epithelium is a highly proliferative organ, with high rates of cell loss that need to be continually replaced. Homeostasis is achieved by a fine balance between cellular proliferation, differentiation and cell death (7). Genes that are involved in this delicate balance

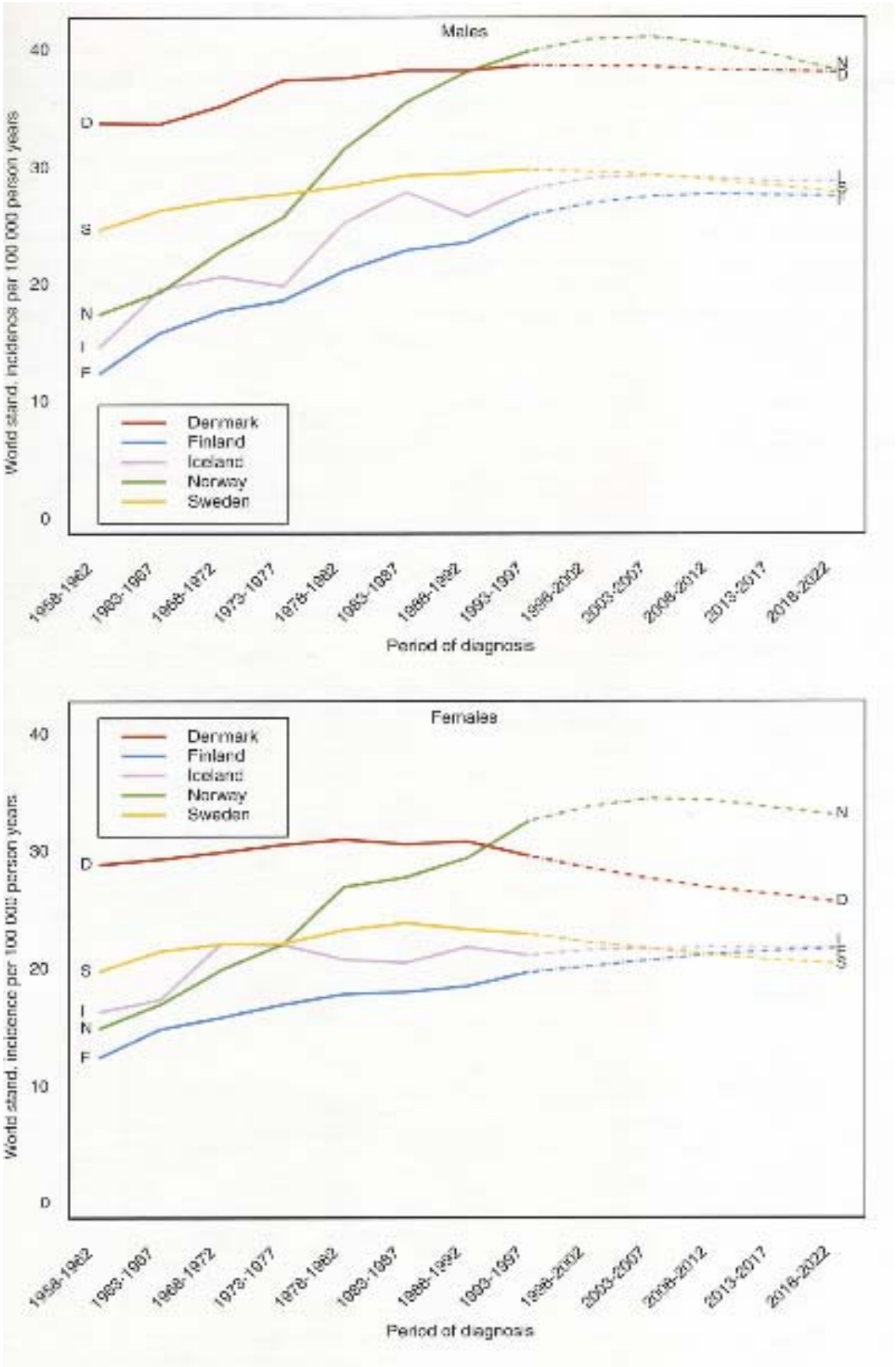

Figure 1 Observed and predicted age-adjusted incidence rates in the five Nordic countries: colorectal cancer. Møller B. (2).

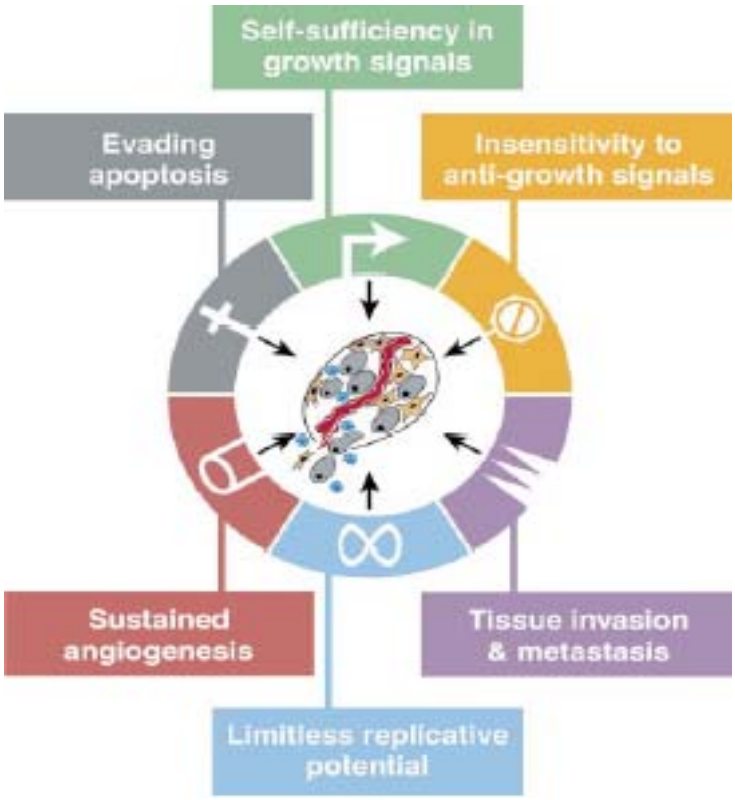

Figure 2 Essential alterations inn cell physiology which collectively dictate malignant growth. Hanahan D. (6).

and also involved in genetic alteration, may be classified into *proto-oncogenes*, *tumor suppressor genes* and *DNA repair genes* (8). Proto-oncogenes and tumor suppressor genes are genes controlling the cell cycle (9), while DNA repair genes are involved in controlling the rate of mutation of other genes (10). One genetic event is enough to activate proto-oncogenes to oncogenes, which result in "gain of function", causing cells to continue to grow in the absence of growth signals, an example is *K-RAS*. Tumor suppressor genes require two genetic events, one in each allele, for their inactivation, which results in "loss of function" (10;11), examples are *APC*, and *p53*. DNA repair genes survey newly replicated DNA for errors and repair mismatched bases in the molecule (12), an example is *SMAD4*.

The adenoma-carcinoma sequence

Fearon and Vogelstein proposed a multistep adenoma-to-carcinoma model for colorectal tumorigenesis (13), based on the assumption that a cell must accumulate four or five defects in order to undergo full malignant transformation (Figure 3) (14).

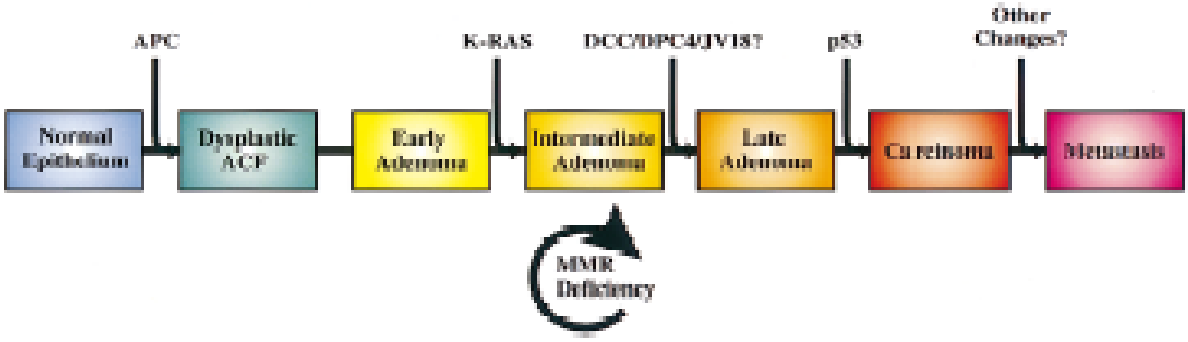

Figure 3 Genetic changes associated with colorectal tumorigenesis. A variety of other genetic alterations have each been described in a small fraction of advanced colorectal cancers. These may be responsible for the heterogeneity of biologic and clinical properties observed among different cases. Kinzler K. W. (15).

Genetic events interact with epigenetic mechanisms, i.e. heritable traits mediated by DNA changes other than nucleotide sequence; the pathways of chromosomal instability (CIN) and microsatellite instability (MIN) (Figure 4), ensure fast accumulation of mutations allowing loss of tumor suppressor genes, activation of proto-oncogenes, and dysregulated expression of various molecules (16).

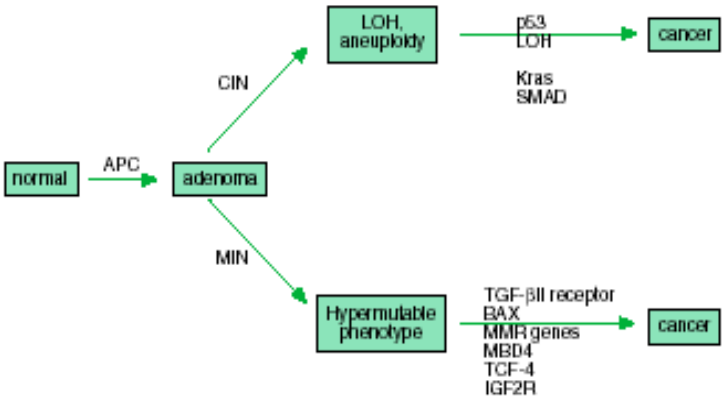

Figure 4 Two independent pathogenic pathways for colorectal cancer. They are supposed to diverge after initial inactivation of both alleles of the gatekeeper *APC*. Tejpar S. (17).

Aberrant crypt foci

Normally, most new intestinal crypts arise in a short postnatal period by crypt fission, and only gradually with age (18-23). Elevated rates of crypt fission is the major defect in preneoplastic intestine and the mode by which microadenomas enlarge (24), common both in human hyperplastic polyps and sporadic colorectal adenomas (25). Aberrant crypt foci (ACF), detected by R. Bird in 1987 on the surface of intestinal mouse mucosa as elevated lesions (26), was later found by Paulsen *et al* to include flat lesions with dysplastic crypts similar to

those found in adenomas (27). As putative adenoma precursors, ACF are used as biomarkers to evaluate agents influencing colon carcinogenesis (28).

1.1.3 HEREDITARY COLORECTAL CANCER

Hereditary CRC is defined by an inherited predisposition (29). Epidemiological studies have suggested that approximately 15% of CRC occur in dominantly inherited patterns (30;31). Examples are familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC).

Adenomatous polyposis was first observed in the mid 18th century, the inherited nature of adenomatous polyposis coli (APC) was recognized by 1900, and *APC* was identified and proved to cause FAP in 1991 (32;33). The rate limiting step in tumor initiation is a somatic mutation or loss of the wildtype *APC* allele inherited from the unaffected parent (34;35), involving the CIN pathway (17). FAP patients typically develop hundreds to thousands of benign colorectal tumors during their second and third decades of life, of which some are virtually guaranteed to progress to carcinomas. FAP affects about 1 in 7000 individuals (15).

HNPCC is characterized by an early onset of CRC at a median age of 42, usually in the proximal colon (36). The diagnosis is based mainly on family history. The patient has multiple primary tumors (12). The defect in HNPCC largely targets the genome guardian function of DNA mismatch repair involving the MIN pathway (17). HNPCC is thought to account for 5-10% of all CRCs (15).

These syndromes have provided unique insights into the process of colorectal carcinogenesis, also at work in sporadic forms of human tumors (15).

1.1.4 SPORADIC COLORECTAL CANCER

CRC not associated with hereditary cancer syndromes are defined as sporadic, but the distinction between inherited and spontaneous CRC is vague. Sporadic cancers depend on interaction between host genetic background of polymorphisms in genes controlling many aspects of tumor development and environmental carcinogens (1;37-41). A colorectal epithelial cell generally requires 20-40 years to transform into a metastatic tumor cell (42). Average lifetime risk of developing sporadic CRC after age 50 is approximately 5%, while the likelihood is doubled in persons with one affected first degree relative (14). Tumors can

arise anywhere in the large bowel, although the majority of sporadic cancers are distal to the splenic flexure (1). For human carcinogenesis, particularly in relation to the interactions with diet, the process of carcinogenesis can be envisaged as a series of events including the three stages of *initiation*, *promotion* and *progression*, originally identified in animals (11).

APC can arise spontaneously (43), and adenomatous polyps are found sporadically in approximately 33% of the general population by the age of 50 and in approximately 50% by the age of 70 (14). 10-20% of these polyps progress to cancer (13;44). The National Polyp Study shows a definite relationship between colorectal adenomas and carcinomas (14).

The *APC* gene is regarded as *the* gene for CRC (15), mutated in approximately 85% of cases (45;46). *APC* mutations are sufficient to initiate growth of small benign tumors, but not to make them progress to more advanced forms (47). APC interacts with numerous molecules (48), and one of its functions is control of the WNT signal transduction pathway (48-51) (Figure 5). Somatic *APC* mutations lead to upregulation of β-catenin levels and activity (52). Activation of the β-catenin signalling pathway is not only an ***initiating*** event, but also plays a

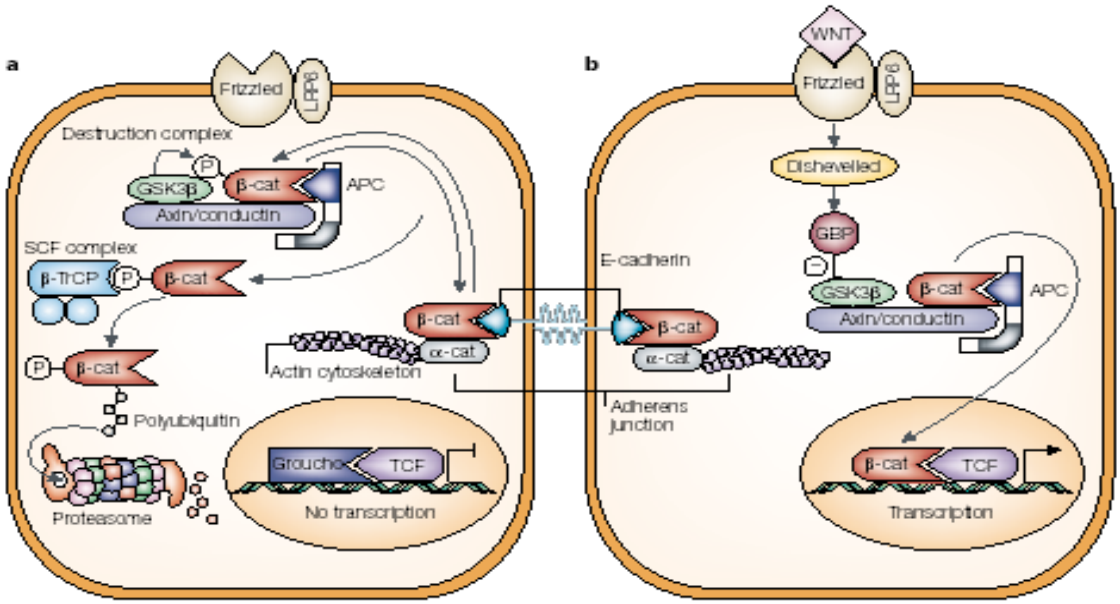

Figure 5 The WNT signalling pathway. **a**. In the absence of a WNT signal, the level of free intracellular β-catenin, which is in equilibrium with β-catenin at adherens junctions, is minimized by sending it for degradation in the destruction complex. Consequently, β-catenin cannot reach the nucleus. **b**. In the presence of WNT, its receptor is activated. This leads to a signalling cascade which inhibits the regulating destruction of β-catenin and keeps is free to diffuse into the nucleus, where it acts as a co-activator for TCF-responsive genes. Fodde R. (49).

pivotal role in the ***promotion*** stage of colorectal carcinogenesis (53). In the few CRC without mutations in *APC* or *β-catenin*, other genes in the same pathway are likely to blame (42).

Mutations in members of the transforming growth factor-β (TGF-β) signalling pathway are thought to have a rate limiting role in CRC (Figure 6). In the colorectum, TGF-β inhibits cell growth (54). Binding of TGE-β to its receptor leads to translocation of SMAD to the nucleus, where it co-activates or represses transcription of specific target genes (49). Mutations can inhibit TGF-β signalling and overcome its growth inhibition (54). In general, tumors acquire TGF-β resistance ***at later stages*** of malignancy. Loss of the *SMAD4* wild-type allele was detected in 95% of invasive and metastatic cancers (55).

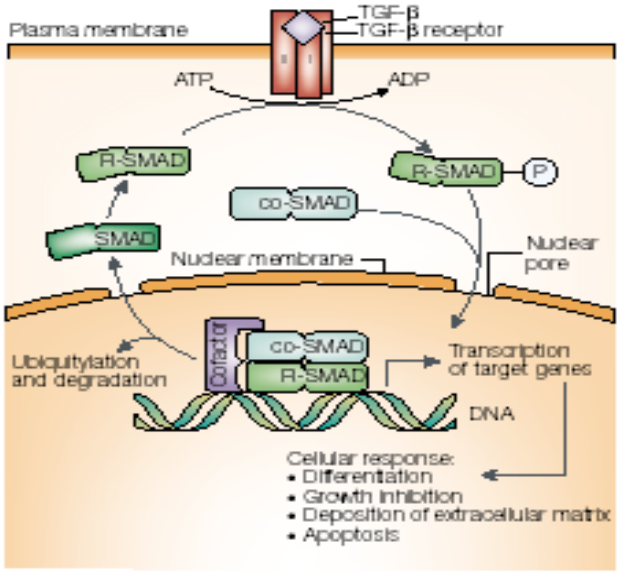

Figure 6 The transforming growth factor-β (TGF-β) signalling pathway. Fodde R. (49).

Cancer ***metastasis*** is a highly complex process that involves angiogenesis and invasion, dissemination, survival in the circulation, and subsequent attachment and growth of cancer cells in the metastasis organ (56). Metastases are the cause of 90 % of cancer deaths (6).

1.2 ANIMAL EXPERIMENTS AND RELATION TO HUMANS

Within cancer research experimental animals are used to elicit preventing or predisposing factors and to develop methods for diagnostic and treatment of this disease (57). In experimental systems it is possible to ensure the stages of initiation, promotion and progression as consequences of exposure to specific, sequential, ordered and non-overlapping agents. For humans or other freeliving animal, none of these conditions is likely to occur (11).

Every animal model has its strengths and weaknesses; however, some of these models have proven useful in evaluating hereditary factors, whereas other models were found to be useful in understanding relations between nutritional factors and colon cancer (58).

Colorectal tumors seldom arise in animal populations, but since rodents easily develop adenomas and adenocarcinomas when exposed to synthetical or naturally occurring chemical carcinogens, effects of various factors on colorectal carcinogenesis can be studied (59).

1.2.1 FROM RODENTS TO HUMANS

Similarities between humans and rodents are expected because many of the control systems preventing the unregulated cell division that leads to cancer must predate the split of rodents and primates (60). Analyses have shown that most of the proto-oncogenes and tumor suppressor genes found to be altered in humans also were altered in rodents. Even the molecular pathways involved in carcinogenesis appear to be similar in some organs (61). The genomes of mice and humans are equal in size, and 90 % identity is found in sequence (62).

1.2.2 ANIMAL EXPERIMENTS AND ETHICS

The close relationship between animals and humans and a feeling of solidarity should lead to respect for the animals for what they are. They do not only have an instrumental value, they have intrinsic value. The fact that this limits our use of animals, is clearly expressed in laws of animal protection (62). Indeed the "Three Rs", that is reduction, refinement and replacement (Table 1) should constantly be borne in mind by all users of experimental animals (63).

Table 1 Guidance to caretaking of the interests of experimental animals. The three Rs scheduled in "The principles of humane experimental technique" by Russel and Burch in 1959, reprinted 1992.

Reduction	Reduction means a decrease in the number of animals used previously with no loss of useful information. This may be achieved by reducing the number of variables through good experimental design, by using genetically homogeneous animals or by ensuring that the conditions of the experiment are rigorously controlled.
Refinement	Refinement means a change in some aspect of the experiment that results in a reduction or replacement of animals or in a reduction of any pain, stress or distress that animals may experience. The establishment of early endpoints for intervention in a study that has the potential to cause pain or distress is an example of refinement.
Replacement	Replacement often means the use of an inanimate system as an alternative (e.g., a computer model or program, a mannequin). It can also mean the replacement of sentient animals (usually vertebrates) with less sentient animals (usually invertebrates such as worms, bacteria, etc). It also includes the use of cell and tissue cultures. The cells must come from somewhere and often this means animals.

Animal experiments are in Norway regulated by the Animal Welfare Act given by the Norwegian Ministry for Agriculture and an additional new Regulation in a special provision of the Act. This law states that animal experimentation may not be carried out without special permission from the Norwegian Animal Research Authority or persons with authority

delegated by this committee (64). Permission can be given when the aim is to collect knowledge, examine a hypothesis, produce or control a product or register the effect of a certain procedure (62). At the Norwegian Institute of Public Health, the criteria for this permission are fulfilled; approved laboratory animal units, animal species, competent persons in charge, and research protocols (65).

1.3 LIPID RELATED RISK FACTORS FOR COLORECTAL CANCER

Epidemiologic studies strongly suggest that the diet can influence CRC incidence. However, human diets are so complex that it has been difficult to determine which dietary components are responsible for this modulation (66).

Two examples of carcinogens produced endogenously through physiological processes such as inflammation, oxidative stress, repetitive tissue injury, and hormonal or nutritional imbalances (11;67), are given here. The first is the radical species nitric oxide ($NO^{\bullet}$) which **reacts readily with lipids** to form products with biochemical actions (68) and is associated with human CRC (69). The second is modified bile acids. A greater excretion of these were found in patients with colon cancer compared to healthy subjects (70), and the secondary bile acid deoxycholate, a promoter of colon cancer, is present at high levels in the colonic lumen of individuals on **a high fat diet** (71).

There is at present a justifiable debate as to the optimum intake level of dietary fat (72). When 87 colorectal adenoma cases were compared with healthy controls, an increased risk of adenomas was found related to **a high intake of total fat** (73). In a population based case control study among five ethnic groups at different risks of CRC in Hawaii, intakes of total, saturated (S) and polyunsaturated (P) fat were not related to the risk of CRC, but an inverse association was found for the **P/S ratio** for both genders (74).

The World Health Organization (WHO) have put forward a classification terminology for the evidence of causal relationship between dietary factors and cancer (75) (Appendix I). The following underlined judgements of causal relationships were taken from the report of 1997 by the World Cancer Research Fund/American Institute for Cancer Research (11). Among dietary factors for which there is convincing evidence for an increase in risk of CRC are **overweight and obesity**. Among dietary factors which possibly increase the risk of CRC are

animal fats (75). The mentioned report is, however, somewhat disputed, which is in line with the difficulties on the area in singling out causal factors due to complicated interactions of dietary, genetic and other bodily factors (76).

Epidemiologic data suggest that particularly the intake of **lipids early in life** influences later cancer incidence (77;78).

1.3.1 CHEMOPREVENTION

Chemoprevention is defined as the employment of natural compounds or drugs to prevent the development of tumors (79). More than 50 pharmacologic agents, singly or in combination, have been evaluated as chemoprotective agents in preclinical models of colorectal carcinogenesis, including antioxidants such as **vitamin E** and **carotenes** (80;81).

Nonsteroidal anti inflammatory drugs (NSAID) are the best known chemopreventive drugs (Figure 7) (14). The non selective NSAID inhibit the constitutive as well as the inducible cyclooxygenases (COX), resulting in decreased prostaglandin production and a build up of intracellular **arachidonic acid (AA)**, promoting apoptosis, and also promote apoptosis via other pathways. NSAID may inhibit angiogenesis, and deprive tumors of necessary nutrients for growth (82). In an NSAID study, higher levels of apoptosis in endoscopy patients were associated with **relatively low BMI** and **relatively low fat intakes** as a proportion of energy (83). NSAID also affect leukotrienes via the 5-lipoxygenase (5-LOX) pathway. Selective NSAID inhibit only inducible COX-2 (84).

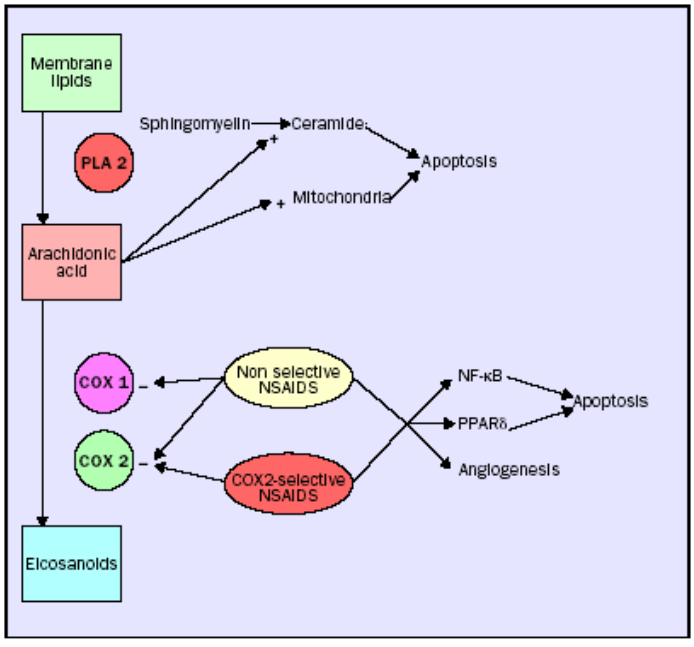

Figure 7 Mechanisms of NSAID-mediated apoptosis. Chan T. A. (82).

A diet rich in fruits and vegetables, which according to AICR probably reduces the risk of CRC, nuts and grains, with a **reduction in fat intake** seems to protect against cancer (85). The diet should include fish, since fish consumption was reported inversely associated with

the risk of CRC in several case-control and cohort studies (86-90), and especially **fat fish**, since Eskimos on Greenland had lower incidence of several diseases than those who lived on a traditional Western diet (91). Doll and Peto estimated 90% of colon cancers as avoidable by dietary change, largely on the basis of international comparisons. This estimate should be reduced to 50% or less because of the clear evidence that physical activity almost surely explains some of the international variation (92).

1.4 FAT

1.4.1 LIPIDS

Lipids are heterogeneous hydrophobic organic molecules that can be extracted from tissues by nonpolar solvents (Figure 8) (93).

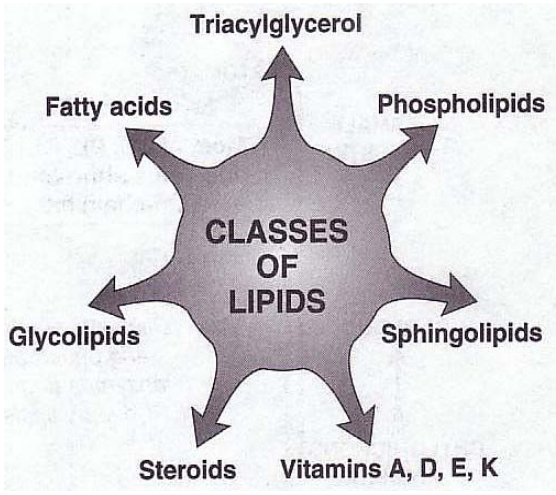

Figure 8 Classes of lipids. Lippincott (93).

Dietary lipids

More than 90% of our dietary lipids consist of triacylglycerols (TG). The remainder is made up of cholesterol, cholesteryl esters (CE), phospholipids (PL), and free fatty acids (FFA) (93). Fats contain much less oxygen, i.e. they are more reduced than carbohydrates or proteins, and therefore yield more energy when oxidized. The complete oxidation of 1 g TG to CO_2 and H_2O in the body produces approximately 9 kcal, while the oxidation of 1 g carbohydrate or protein produces 4 kcal. A TG molecule contains 3 fatty acids esterified to one glycerol moiety (94).

Fat degeneration

When water is present, *hydrolytic rancidity* of the fat may be the result. FFA are released from the glycerol molecule, and the fat pH is decreasing (95). FFA are even more prone to oxidation than TG (96). When oxygen is present, *oxidative rancidity* may be the result, and the double bonds of unsaturated fatty acids are easily broken. Rancidity is accelerated by high temperature, enzymes and other catalysts such as iron and copper ions and metalloproteins, pH, light, and oxidation products such as peroxides and hydroperoxides (97). The oxidation of

fat produces free radicals and volatile substances with intensive tastes (96). Fat degeneration may be reduced by antioxidants or by hydrogenation of the fat (95;97).

Bodily functions of lipids

Important bodily functions of lipids are thermic and electric isolation, energy source, transport and storing of cholesterol and fat soluble vitamins (98). Lipids also provide the hydrophobic barrier that permits partitioning of the aqueous contents of cells and subcellular structures. Some fat soluble vitamins have regulatory or coenzyme functions, and the prostaglandins and steroid hormones play major roles in the control of the body's homeostasis (93). Lipids also function as a preface for bile acids and signalling molecules, and as ligands in the regulation of gene expression (91;99).

1.4.2 FATTY ACIDS

Fatty acids are basically hydrocarbon chains with a carboxylic acid head group at one end that can combine with another molecule. Each carbon (C) in the chain has four binding sites. In a saturated fatty acid (SFA), all binding sites not linked to carbon are "saturated" with hydrogen. In an unsaturated fatty acid (UFA), one or more pairs of hydrogens have been removed and double bonds formed between adjacent carbons (100). The structure of some important fatty acids is shown in Figure 9.

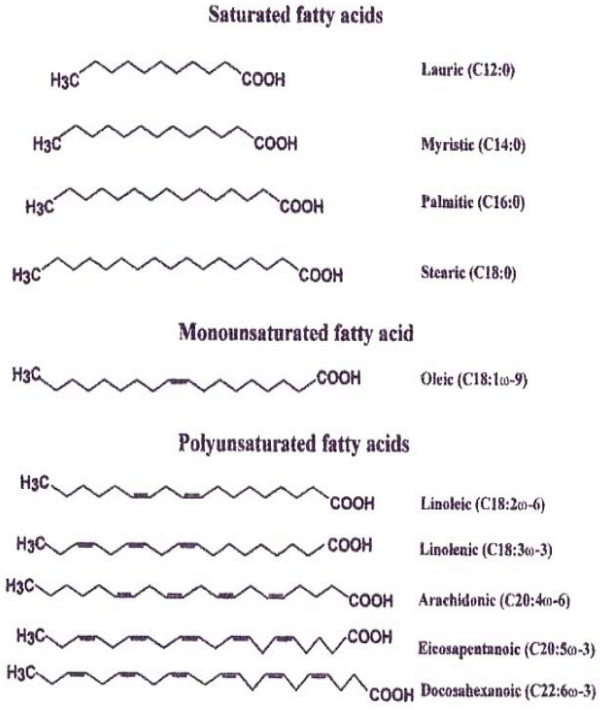

Figure 9 Formulae and structures of some important fatty acids. Bartsch H. (101).

The physical and chemical properties of fatty acids are dependent on the length of the carbon chain, the saturation degree, and the placing of double bonds in the chain (positional isomerism) and hydrogen atoms around the double bond (geometrical isomerism) in UFA. Both chain length and saturation contribute to the melting temperature of a fat. In general, fats with shorter fatty acid chains or with more double bonds are liquid at room temperature. Saturated fats, especially those with long chain fatty acids, as in beef tallow (18C), are solid at room temperature (102).

Milk fat has a relatively high percentage of short (C2-6) chain fatty acids (SCFA) and medium (C6-12) chain fatty acids (MCFA), and fat from land animals of long (C12-18) chain fatty acids (LCFA), largely saturated. Marine derived fat contain very long (C≥20) chain fatty acids (VLCFA), but highly unsaturated (100).

Fatty acids are classified according to the number of carbons in the chain, the number of double bonds, and the position of the first double bond (100). Systematic and notional names of some fatty acids are given in Table 2 by two different conventions for characterization.

Digestion, absorption, and transport

In the intestinal lumen, the TG are emulsified by bile salts (94), and pancreatic lipase cleaves the *sn*-1 and *sn*-3 position of TG yielding 2-monoacylglycerols (2-MG) and FFA (103).

Fatty acids are differently absorbed and undergo different metabolic fates depending on their chain length and degree of saturation. Fatty acids containing less than 14 carbons and fatty acids containing several double bonds can, to a variable degree, be absorbed in the stomach by diffusion through the PL bilayer and undergo direct internal transport to the liver via the portal circulation (103).

Generally long chain fatty acids (LCFA) are almost completely absorbed by enterocytes in the jejunum and ileum (104;105). Along with some other substances, LCFA interact with bile salts to form tiny microdroplets called micelles, which help apolar lipids to go through the unstirred water layer and reach the microvillous membrane where they are absorbed into the intestinal epithelial cells (94).

In the intestinal epithelial cells, the fatty acids and 2-MG are resynthesized into TG and PL, which are packaged into chylomicrons (CM) travelling through the lymphatic system and the left subclavian vein to the liver (94;104).

The positional distribution of fatty acids in dietary TG determines whether fatty acids are absorbed as 2-MG or FFA, and hence, influences the composition of CM. Generally, the absorption of fatty acids in the *sn*-2 position is favoured, whereas no specificity has been found for the fatty acids in the *sn*-1 or *sn*-3 positions (106). Eicosapentaenoic acid (EPA) and

Table 2 Some naturally occurring fatty acids.

TRIVIAL NAME	SYSTEMATIC NAME*	NOTIONAL NAME**	TYPICAL FAT SOURCE
Saturated			
Butyric	Butanoic	C4:0	Butterfat
Caproic	Hexanoic	C6:0	Butterfat
Caprylic	Octanoic	C8:0	Coconut oil
Capric	Decanoic	C10:0	Coconut oil
Lauric	Dodecanoic	C12:0	Coconut and palm kernel oils
Myristic	Tetradecanoic	C14:0	Butterfat, coconut oil
Palmitic	Hexadecanoic	C16:0	Palm oil, animal fat
Stearic	Octadecanoic	C18:0	Cocoa butter, animal fat
Arachidic	Eicosanoic	C20:0	Peanut oil
Behenic	Docosanoic	C22:0	Peanut oil
Lignoceric	Tetracosanoic	C24:0	Beech-wood tare
Cerotic	Hexacosanoic	C26:0	Wax
Montanic	Octacosanoic	C28:0	Montan wax (extracted from lignite)
Unsaturated	**Δ**		
Caproleic	9-Decenoic	C10:1 ω-1	Butterfat
Lauroleic	9-Dodecenoic	C12:1 ω-3	Butterfat
Myristoleic	9-Tetradecenoic	C14:1 ω-5	Butterfat
Crotonic	*Trans*-2-tetraenoic	C14:1	Castor-oil plant
Palmitoleic	9-Hexadecenoic	C16:1 ω-7	Some fish oils, beef fat
Oleic	9-Octadecenoic	C18:1 ω-9	Olive oil, canola oil
Elaidic	*Trans*-9-octadecenoic	C18:1	Butterfat
Cis-vaccenic	11-Octadecenoic	C18:1 ω-7	Beef fat
Trans-vaccenic	*Trans*-11-octadecenoic	C18:1	Butterfat
Taxoleic	5,9-Octadecadienoic	C18:2 ω-9	Conifer seed oil
Linoleic	9,12-Octadecadienoic	C18:2 ω-6	Safflower, corn and soybean oils
Pinolenic	5,9,12-Octadecatrienoic	C18:3 ω-6	Conifer seed oil
γ-Linolenic (GLA)	6,9,12-Octadecatrienoic	C18:3 ω-6	Evening primrose oil
α-Linolenic	9,12,15-Octadecatrienoic	C18:3 ω-3	Soybean oil, canola oil, walnuts
Gadoleic	9-Eicosenoic	C20:1 ω-11	Some fish oils
Gondoic	11-Eicosenoic	C20:1 ω-9	Redfish oil
Dihomolinoleic	11,14-Eicosadienoic	C20:2 ω-6	Conifer seed oil
Sciadonic	5,11,14-Eicosatrienoic	C20:3 ω-6	Conifer seed oil
Dihomo-α-linolenic	11,14,17-Eicosatrienoic	C20:3 ω-3	Conifer seed oil
Arachidonic	5,8,11,14-Eicosatetraenoic	C20:4 ω-6	Lard, meats
Juniperonic	5,11,14,17-Eicosatetraenoic	C20:4 ω-3	Conifer seed oil
Timnodonic (EPA)	5,8,11,14,17-Eicosapentaenoic	C20:5 ω-3	Some fish oils, shellfish
Erucic	13-Docosenoic	C22:1 ω-9	Crusiferae seed oil: mustard, rape
Cetoleic	13-Docosenoic	C22:1 ω-11	Redfish oil
Clupanodonic (DPA)	7,10,13,16,19-docosapentaenoic	C22:5 ω-3	Herring, fish oil
Cervonic (DHA)	4,7,10,13,16,19-Docosahexaenoic	C22:6 ω-3	Some fish oils, shellfish
Nervonic	15-Tetracosaenoic	C24:1 ω-9	

*Systematic names according to the IUPAC nomenclature: the Greek capital letter delta (Δ) refers to the carbon preceding the double bond, counted from the carboxyl end. **Lower case Greek letters; alpha (α) refers to the first carbon adjacent to the carboxyl group, beta (β) to the second carbon, and omega (ω) to the last carbon in the chain. Double bonds labelled with ω, are counted from the terminal methyl carbon. Adapted from the Institute of Shortening and Edible Oils. Food, fats and oils. 6th ed. Washington, DC, 1988 (100) and Metabolism at a Glance (107).

and docosahexaenoic acid (DHA) are supposed mainly to be esterified to and absorbed from the *sn*-2 position in the TG of fish oil (108).

In the circulation, hydrolysis of TG within the core of the CM results in movement of fatty acids into tissues and the subsequent production of TG-depleted chylomicrons remnants (CMR) which pick up cholesterol esters from high density lipoproteins (HDL) and are rapidly taken up by the liver (103).

The endogenous shuttle for lipids and their metabolites, consisting of very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), and low density lipoproteins (LDL), coordinate movement of lipids from the liver to peripheral tissues, while high density lipoproteins (HDL) returns lipids from peripheral tissues to the liver. In addition, FFA are transferred bound to albumin from storage reservoirs to metabolizing organs (103).

1.4.3 ESSENTIAL FATTY ACIDS

Fatty acids which the body needs but cannot synthesize are called essential fatty acids (EFA). Two long chain polyunsaturated fatty acids (LCPUFA) are essential in humans; the ω-6 linoleic acid (LA) and the ω-3 α-linolenic acid (ALA) (Table 2). Arachidonic acid (AA) becomes essential if its precursor LA is missing in the diet (93). In the case of polyunsaturated fatty acids (PUFA) that cannot be synthesized in the body, the levels in tissues rise and fall with the levels in the diet (109;110). In EFA deficiency, oleic acid and palmitoleic acid undergo the same reactions to form polyunsaturated fatty acids of other structures (111).

1.4.4 BIOSYNTHESIS OF HIGHLY UNSATURATED VLC FATTY ACIDS

Once consumed in the diet, LA can be converted via γ-linolenic (GLA) and dihomo-γ-linolenic acids (DGLA) to arachidonic acid (AA) by the pathway outlined in Figure 10. Using the same pathway, dietary ALA can be converted into EPA, docosapentaenoic acid (DPA) and DHA. Thus, the ω-6 and ω-3 fatty acids compete for these enzymes (112).

PUFA can be used for PL biosynthesis via a number of pathways in the endoplasmatic reticulum (ER). The types and amounts of PUFA found in membrane bilayer PL are controlled by the fatty acid composition of dietary fats, the intracellular metabolism of fatty acids, and specificities for esterifying fatty acids into PL (113). Animal biosynthesis of highly polyunsaturated fatty acids from LA and ALA is mainly modulated by the Δ-6 and Δ-5 desaturases through hormonal and dietary stimulated mechanisms and influenced by circadian changes. Insulin activates the enzymes, while all the other hormones tested depress them, or

at least are inactive (114). The extent to which bio-conversion occurs is influenced by the proportion and quality of dietary carbohydrates, fatty acids, and proteins (115).

Peroxisomal β-oxidation is the last step in the biosynthesis of DHA (113). But conversion of ALA to EPA is limited in men, and further transformation to DHA is very low (116). Evidence is growing for deficiencies of ω-3 fatty acids in humans under stress conditions and for the essentiality of ω-3 PUFA (117).

Some fatty acids generated in this pathway and found in the plasma membrane PL of different cell types serve as substrates for formation of eicosanoids (103;118;119).

Dietary fatty acids		**Dietary fatty acids**
ω-6 series	**Enzymes**	**ω-3 series**
C18:2		**C18:3**
Linoleic acid (LA)		**α-Linolenic acid (ALA)**
(Δ 9, 12)		**(Δ 9, 12, 15)**
↓	Δ-6 desaturase	↓
C18:3		C18:4
γ-Linolenic acid (GLA)		Stearidonic acid
(Δ 6, 9, 12)		(Δ 6, 9, 12, 15)
↓	Elongase	↓
C20:3		C20:4
Dihomo-γ-linolenic acid (DGLA)		Eicosatetraenoic acid
(Δ 8, 11, 14)		(Δ 8, 11, 14, 17)
↓	Δ-5 desaturase	↓
C20:4		**C20:5**
Arachidonic acid (AA)		**Timnodonic acid (EPA)**
(Δ 5, 8, 11, 14)		**(Δ 5, 8, 11, 14, 17)**
↓	Elongase	↓
C22:4		C22:5
Adrenic acid		Clupanodonic acid (DPA)
(Δ 7, 10, 13, 16)		(Δ 7, 10, 13, 16, 19)
↓	Elongase	↓
C24:4		C24:5
Tetracosatetraenoic acid		Tetracosapentaenoic acid
(Δ 9, 12, 15, 18)		(Δ 9, 12, 15, 18, 21)
↓	Δ-6 desaturase	↓
C24:5		C24:6
Tetracosapentaenoic acid		Tetracosahexaenoic acid
(Δ 6, 9, 12, 15, 18)		(Δ 6, 9, 12, 15, 18, 21)
↓	β-oxidation	↓
C22:5		**C22:6**
Osbond acid		**Cervonic acid (DHA)**
(Δ 4,7,10,13,16)		**(Δ 4,7,10,13,16,19)**

Figure 10 Parallel pathways for conversion of the essential ω-6 and ω-3 fatty acids.

1.4.5 BIOSYNTHESIS OF EICOSANOIDS

Their enzymatic conversion into eicosanoids is one of the more important functions of PUFA, since eicosanoids modulate inflammatory and immune responses and play a critical role in platelet aggregation, cellular growth and differentiation (120).

Eicosanoids are biologically potent short lived, hormone like lipids with chain lengths of 20 carbon atoms (eicosa = 20), consisting of prostaglandins (PG) and thromboxanes (TX) (prostanoids) produced by COXs, and leukotrienes (LT) produced by LOXs. Precursor fatty acids are DGLA (leading to series 1 PG), AA (leading to series 2 PG and 4 LT), and EPA (leading to series 3 PG and 5 LT) (120).

The production of eicosanoids begins with the hydrolytic release of PUFA from membrane PL by various phospholipases (PLA) and appears to occur indiscriminately with ω-3 and ω-6 PUFA (112;120;121). The relative proportions of PUFA in cell membranes, as well as cell type, are the primary factors in regulating which eicosanoids will be generated (122).

1.5 HYDROGENATED FAT

As a substitute for butter, a new product called "oleomargarine" based on tallow, milk, and salt was invented in France in 1868 (123). Eilert Sundt and August Pellerin started the first Scandinavian margarine factory in Christiania in 1876 (97). As raw material for margarine production, marine oils were hydrogenated partially in Norway from 1912 by Denofa (124).

Liquid plant and marine oils are hydrogenated to achieve the desired consistence for use in foods, to increase stability by avoiding degeneration of fatty acids (95), and to give the products features such as spreadability, texture/"mouth feel" (the fat is supposed to melt at mouth temperature, but not in the fridge), and a longer shelf life (125).

Before the oil is hydrogenated, the raw oil is refined by neutralizing/washing and removal of FFA. The hydrogenation is performed by adding H_2 under high pressure and temperature to saturate double bonds in UFA, in the presence of a catalyst (95;126).

1.5.1 *TRANS* FATTY ACIDS

During the hydrogenation process, double bonds in UFA are changed from the *cis* configuration (the hydrogen atoms placed on the same side of the double bond) most frequently found in nature, to *trans* (the hydrogen atoms placed on opposite sides of the double bond), see Figure 12, and their position in the carbon chain may shift (110). *Trans* fatty acids (TFA) contain at least one *trans* double bond with changed geometry compared with the *cis* double bonds (127).

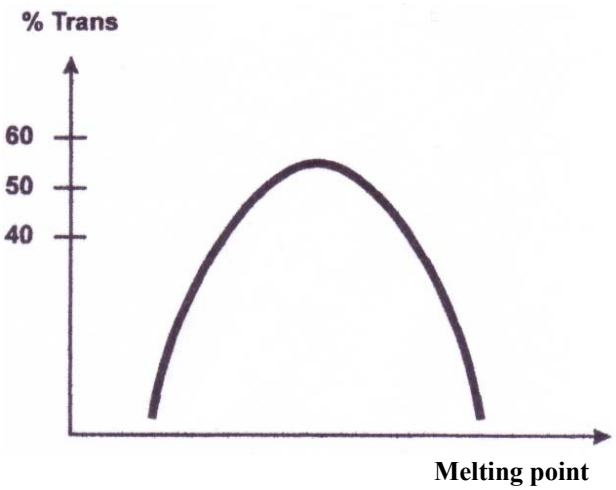

Figure 11 *Trans* fatty acids in hydrogenation. Denofa (102).

During the hydrogenation process, the content of TFA increases until the melting point (MP) is 30-40 °C (Figure 11), where after it decreases to 0 at MP 50-70 °C when the oil is totally hydrogenated. In the hydrogenation practice of 1998, the hydrogenated fats produced had concentrations of TFA in the range of 10-60% (128;129).

Besides being formed during industrial hydrogenation, TFA are naturally formed by the anaerobe bacterial flora in the intestine of most animals, especially ruminants (130), and also humans (131). The algae chloroplast membranes contain some fatty acids with a *trans* double bond (132). TFA can therefore also be detected in minor amounts in marine animals (133).

Considerable differences in the quantity of individual TFA in industrially produced *trans* fat and *trans* fat from ruminants has been found (127). The major fatty acids formed by partial industrial hydrogenation are oleic (9*c*-18:1), elaidic (9*t*-18:1), and stearic (18:0) acids (Figure 12), elaidic acid being predominant (110;134). The *trans* isomers in ruminant milk and meat are mainly monoenes with *trans* vaccenic acid (11*t*-18:1) being the most dominant (135).

Food sources of *trans* fatty acids

Industrially formed TFA fats may be present in substantial amounts in foods, especially in baking fat (136;137). In 1993, the percentage of TFA of total fat content was 37% in baked goods such as doughnuts and Danish pastry, 38% in imitation cheese, 11-49% in margarines, 27% in confectionary fats, and up to 36% in deep-fried foods such as fried chicken and French-fried potatoes (138). In 2000-2001, partially hydrogenated fat was found varying from 1 to 33.5 gram per 100 gram product in an analysis of snacks, cakes and sweets. A high content of TFA was also found in biscuits, dry food like soups and sauce basis, and in fast food, especially in pommes frites and popcorn (127).

Naturally formed TFA are present in milk and flesh of ruminants by 4-11%, tallow contains 3-5%, pork fat less than 0.5%, and fish, shellfish and sea mammals contain minor amounts

(130;133;139). Plants also contain minor amounts of TFA (132), and up to 1% TFA is formed in the processing (other than hydrogenation) of almost all edible plant oils. Refined oils have higher TFA contents than unrefined, according to the duration and temperature of refining. The edible fat sources on a world basis include 20% animal and 80% vegetable oils/fats (128).

Differences between saturated, *trans* and *cis* unsaturated fatty acids

Saturated and *trans* unsaturated fatty acids are straight, while *cis* unsaturated fatty acids make

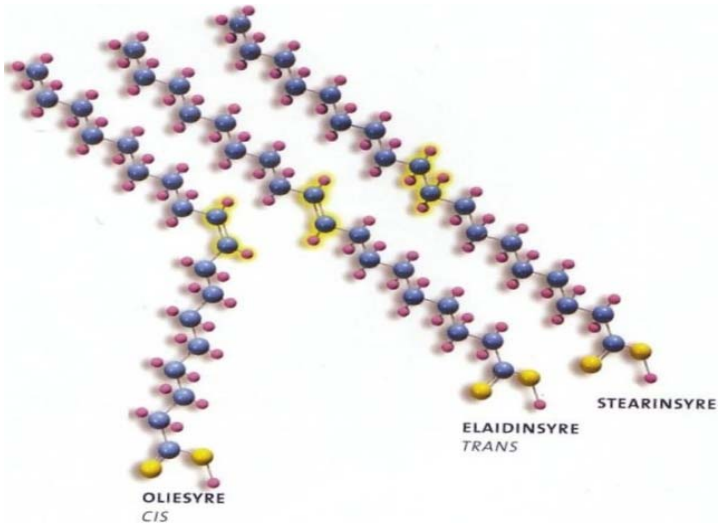

Figure 12 The chemical structure of the *cis* monounsaturated oleic acid, the *trans* monounsaturated elaidic acid and the saturated stearic acid. Stender S. (127).

a turn at the double bond (96). TFA resemble SFA in that they have some of the physical packing properties of SFA, but they are shaped differently in space (125). Geometrical and structural differences between the *cis* monounsaturated oleic acid (MP 11-13 °C), the *trans* monounsaturated elaidic acid (MP 44-45 °C) and the saturated stearic acid (MP 70-72 °C), all 18 carbons long, are shown in Figure 12. While the double bond angle of a TFA is smaller in contrast to the corresponding *cis* isomeric configuration, it is larger than that of the corresponding saturated configuration (Figure 13). The question is whether such differences may provide TFA with different biological effects from SFA (127;133).

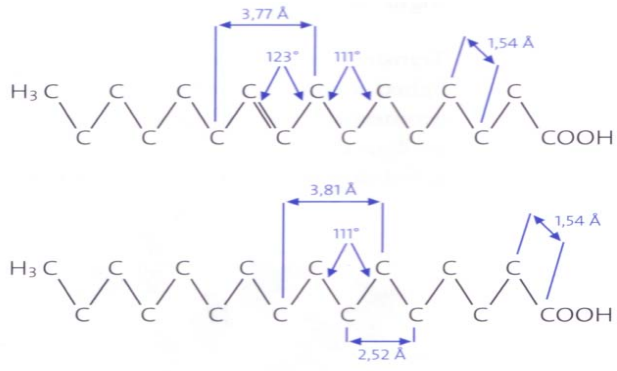

Figure 13 The molecular structure of a *trans* fatty acid (top) and the corresponding saturated fatty acid. Stender S. (127).

1.5.2 UNUSUAL FATTY ACIDS IN HYDROGENATED FATS

TFA may be common in the human body. However, fatty acids in hydrogenated fats may contain *trans* bonds in positions that are unusual in the body, which may affect the incorporation of the isomers in tissue lipids and their metabolic conversion (140).

The metabolic aspects of isomeric fatty acids studied relate primarily to *trans* positional isomers of mono- and dienoic acids with the *cis* positional isomers having been largely neglected, despite the fact that they can represent 20% or more of the total *cis* fatty acids consumed and have distinct metabolic effects (133).

Hydrogenated fats may also contain unusual very long chain saturated fatty acids (VLCSFA) (111).

1.5.3 ABSORPTION AND METABOLISM OF HYDROGENATED FATTY ACIDS

Cis-trans configuration affects the polarity of a compound, *cis* being the more polar. The higher lipophilic character of TFA can result in increased aggregation with non-absorptive SFA (141), especially with very long chain saturated fatty acids VLCSFA, and lead to reduced absorption compared with *cis* isomers (142).

TFA are incorporated preferentially in the *sn*-1 position in PL, substituting for SFA (135).

A role was proposed for peroxisomal β-oxidation in relation to the metabolism of fatty acids that are poorly β-oxidized by mitochondrias. *Trans* isomers of MUFA were β-oxidized at rates faster than, or similar to, those obtained with corresponding *cis*-isomers in peroxisomal fractions in rat liver induced by diets rich in VLCMUFA (143).

Increased serum TG was found in rats fed a diet with a high amount of VLCSFA, including increased contents of arachidic (20:0) and behenic (22:0) acids, while serum PL only contained increased arachidic acid (142), possibly due to selective incorporation in PL (144).

Unusual VLCSFA and very long chain *trans* fatty acids (VLCTFA) with unknown biological effects may be produced by desaturation and elongation in the human body of TFA from hydrogenated oils. Rats were fed a diet containing 18:1 and 18:2 isomeric substrates which were desaturated and elongated by liver microsomes. Large proportions of unusual isomers of

18:2, 20:2, 20:3, and 20:4 were found, with measurable amounts at each step of the cascade, and incorporated into PL (111).

1.5.4 DECLARATION OF HYDROGENATED FAT

Today, there are no general demands for declaration of nutrients, specification of fat or *trans* fat, or for maximum content of TFA, in Norway (145). Therefore it is difficult to assess whether a product contains TFA.

Although the TFA are chemically unsaturated they are considered so different from the *cis* unsaturated fatty acids that they cannot be legally designated unsaturated for purposes of labelling (125). If *trans* fat is declared in Norway it will be considered a part of saturated fat according to the Norwegian Food Safety Authority. Whether the declaration of *trans* fat will be regulated in Norway, might also be influenced by a presumptive cost/benefit analysis. How this declaration will be regulated, if possible, is at this point of time an open question (145).

In 1997, the Norwegian National Council of Nutrition recommended reduced use of partially hydrogenated oils by the margarine industry. New fat mixtures with lower content of partially hydrogenated oils were desired for baking. New products with lower total content of TFA and SFA of the type C12-C16 should be secured by choice of raw materials (126).

As the first country in the world, Denmark introduced strict national limits for the TFA content in food with an upper limit of industrially produced TFA of 2% after January 1, 2004 (127). In 2003, directives for declaration of nutrients were adopted by Codex Alimentarius, and TFA were included in the format for food labelling. At the moment, a group led by Malaysia and Denmark is working on a definition of *trans* fat and laying the basis for discussions concerning rules (145-147). In the European Union (EU), rules are put in place on the labelling of foodstuffs (148). Through the EOES agreement, Norway is bound to adapt EU's rules concerning production and sale of food (149). The TFA content in the Norwegian diet is today insignificant. If the Danish legislated upper limit of 2% TFA in food fats is adopted by the EU, the result might be an increase in TFA in food produced in Norway, while the quantity of imported *trans* fat would be decreased (150).

1.5.3 ALTERNATIVES TO HYDROGENATION

It is possible for the oil/fat industry to produce edible fats with the wanted functionality through various modification processes. One method is full hydrogenation of liquid vegetable oil (contains mostly C18:0) with addition of liquid oils and interesterification of the fatty acids in TG. Additional methods are to use naturally solid vegetable fats such as palm oil (contains mostly C16:0 and 18:1, MP 37°C) or coco oil (contains mostly C12:0, 14:0 and 10:1, MP 25°C), or to use the solid fraction of palm oil by crystallization of specific fatty acids, (palm stearin contains more 16:0 and less 18:1 than palm oil, MP 25 °C) or of coco oil, with or without interesterification.

Other alternatives are to produce new solid fats by plant improvement (102;151), or to use oils with different kinds of emulgators and other additives (for the baking industry) (152).

1.6 EFFECTS OF FATTY ACIDS

1.6.1 EFFECTS OF FATTY ACIDS ON COLORECTAL CANCER

The most consistent effect of specific fatty acids on colonic tumor growth is the inhibitory influence of fish oils enriched in EPA and DHA, probably mediated via the eicosanoid pathway (153). The 3-series prostanoids and the 5-series leukotrienes produced with EPA as substrate have positive health effects compared with the 2- and 4-series. Human colon tumor growth initiated by subcutaneous inoculation of HT29 cells in athymic mice was promoted by high fat diets of other oils, but not of fish oil (154).

Results emphasize the importance of the dietary ω-6/ω-3 PUFA ratio in determining the effects of supplementation with fish oil on parameters of colon cancer (155). The multiplicity of colon adenocarcinoma was significantly inhibited in rat groups fed 5.9% corn oil + 17.6% menhaden fish oil (ω-6/ω-3 = 0.34) compared to those fed 23.5% corn oil (156). This indicated that the relative proportions of ω-6 and ω-3 fatty acids in the diet might be determinants of the high fat effect (155).

There does not appear to be any specific fatty acid that promotes the development of colon cancer when studied in experimental models (153). No literature was found on effects of specific unusual fatty acids on CRC.

1.6.2 EFFECTS OF *TRANS* FATTY ACIDS

Based on experimental data as well as on clinical studies several reports during the early 1980s concluded that TFA probably were comparable with other MUFA and that they were useful alternatives for dietary SFA without apparent side effects (157;158).

Effects early in life

Trans positional isomeric 18:1 and 18:2 fatty acids from partially hydrogenated soybean oil (PHSO) have, however, been shown to inhibit the regulation of reproduction, foetal growth and development in rats (111;119). Studies on humans have shown that TFA are transferred from the mother to the foetus, and decrease birth weight (159-162). TFA also lower the volume of crème in lactating females of all species, including humans (125).

Effects on the risk of cardiovascular disease

TFA have been associated with risk factors for cardiovascular disease (CVD) such as high levels of plasma LDL cholesterol, TG, cholesteryl ester transfer protein (CETP), lipoprotein (a), decreased HDL cholesterol, and effects on platelet function and PGI_2 production, in humans (163-166). TFA seem to be slightly less hypercholesterolemic than palmitic acid (167). The content of TFA in human adipose tissue, possibly with margarine as the main source, was found associated with increased risk of myocardial infarction (168). TFA from partially hydrogenated vegetable oils have been shown to have adverse effects on obesity, insulin resistance, diabetes, and immunity, also considered risk factors for CVD (125;169).

Effects on cancer

When health effects of TFA, including one study of partially hydrogenated vegetable and fish oils, were extensively reviewed in the 1980s, no adverse effects were found on the occurrence of cancer(158;170;171). Reviewed in 1993, most animals studies indicated that TFA are less carcinogenic compared with *cis* isomers, probably by their inhibition of AA metabolism and the following reduction of PGE_2 related proliferation in tumors, and metastases (172). Two 1995 European reviews concluded that TFA do not seem to have any carcinogenic effects (173;174), while one study suggested a possible association between TFA and cancer (175). A review in 1996, representing the literature available on *trans* fat (at the expense of cis fat) and cancer in animal models, the most recent paper published in 1989, confirmed that there had been no evidence to indicate that, under properly controlled conditions, the intake of TFA is a risk factor for cancer (176).

The Danish Nutrition Council concluded in their 2003 report that there is no evidence for a carcinogenic effect of the dietary TFA, but that the studies undertaken after 1994 constitute a basis of continued alertness on the possibility of such effects (127).

Effects on colorectal cancer

In 1997, a strong positive significant correlation was found between TFA status of fat aspirates and the incidence of breast and CRC in the EURAMIC study (177). An association between breast and CRC was found both in the same individual and also in close relatives (178). However, the EURAMIC study was criticised for not taking into account several important confounding factors (179).

In a case-control study in 1999, a tendency to increased colorectal adenoma prevalence associated with consumption of partially hydrogenated vegetable oils was found in the two food groups "oils and condiments" (margarine, bottled oil containing TFA, oil and vinegar dressing, and mayonnaise or other creamy salad dressing) and "sweetened baked goods", but not with total dietary TFA (180;181). Nevertheless, the use of hospital based controls and a sigmoidoscopy screened population confers disadvantages; there was a potential for incomplete case ascertainment (167), and the possible inclusion of cases in the control group could introduce biases toward the null in estimating the main effect of TFA intake (179).

In a case control study in 2001, statistically significant positive associations were found between TFA intake and colon cancer risk in women and in men and women ≥ 67 years, but not in men. Those who did not use NSAID were at a 50% greater risk of developing colon cancer when they consumed high levels of TFA. Women who were estrogen negative had a twofold increase in risk of colon cancer from high levels of TFA, while estrogen positive women did not experience an increased risk regardless of level of TFA consumed (182).

Many of the risk factors for CVD, on which TFA have adverse effects, such as high levels of cholesterol, triglycerids, obesity, insulin resistance, diabetes, and immunity, have also been related to CRC or colorectal adenomas (183-189).

After a *trans* MUFA diet for 8 weeks, the TFA present in the diet were actively incorporated by tumors in rats (2201) (190).

Potential mechanisms for interference by *trans* fatty acids

Potential mechanisms for effects of TFA have been proposed, including disruption of the cell membrane by incorporation of TFA into membrane PL (180). Possible biological effects might include alteration of basic membrane properties including modification of acyl chain order and "fluidity", phase behaviour, elastic compressibility, permeability, fusion, flip flop and protein activity. Interaction with other membrane lipids, particularly with cholesterol, may play a prominent role in the modulation of the local structure and function of cell membranes (191).

The relationship between CRC susceptibility and the distribution of polymorphic variants of different forms of metabolic enzymes such as CYP450 1A1 and 1A2, glutathione S-transferases (GST), N-acetyltransferase (NAT)2, aldehyde dehydrogenase (ALDH)2 and methylene-tetrahydrofolate reductase (MTHFR), has been in focus because of their important role in the activation of many procarcinogens or chemicals (76).

TFA induce adverse alterations in the activities of the important mixed function oxidase (MFO) CYP-448/450 enzyme system that metabolizes carcinogens and drugs, and produce alterations in adipose cell size and number, lipid class and fatty acid composition (125).

It was shown by many researchers that TFA in membranes inhibited the function of Δ-6 desaturase resulting in decreased conversion of e.g. LA to GLA or AA, and of ω-3 fatty acids to their elongated tissue ω-3 fatty acids, with a resulting escalation of adverse effects of EFA deficiency, in rats fed hydrogenated oil (1991) (111). This may include disruption of eicosanoid synthesis with resulting inhibition of immune and inflammatory responses and cell differentiation Pedersen, 1998 724 /id;Zolfaghari, 2003 472 /id}.

Problems in obtaining suitable control fat

The relatively scarce knowledge about the relationship between TFA and cancer risk, albeit voluminous information is available concerning effects of the amount and type of fat on experimental carcinogenesis, is partially due to difficulties in obtaining a suitable control fat so that the impact of TFA can be properly evaluated. This has led to confounding interpretation of results, particularly in the early studies. Thus, deficiencies in the design of dietary fats used for animal feeding in carcinogenesis experiments have likely been a cause of discrepancy in the database (176).

1.7 INTAKE OF FAT

A hypothetical scheme of intake of fats and antioxidant vitamins through the evolution (Figure 14) indicates that the percent of energy in the human diet from total and saturated fat have been increasing, and the intake of vitamin C and E decreasing, since early 1800s. The energy percent from ω-6 fatty acids has been increasing since mid 1900s. The energy percent from ω-3 fatty acids has been decreasing, while that from *trans* fatty acids has been increasing with the industrial hydrogenation of oils, since early 1900s.

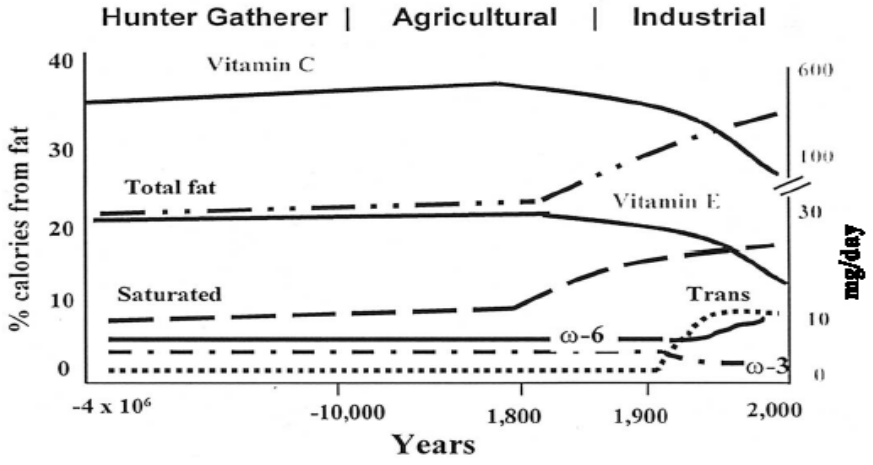

Figure 14 Hypothetical scheme of (% calories) intake of fats and vitamins; total and saturated fat, ω-6, ω-3, and *trans* fatty acids, vitamin E and C (mg/d), data extrapolated from cross-sectional analyses of contemporary hunter-gatherer populations and from longitudinal observations and their putative changes during the preceding 100 years. Simopoulos A. P. (161).

Such changes in fat intake over a short time period are upsetting the characteristic balance during evolution when our genes were programmed to respond to diet and other aspects of the environment (161).

As societies industrialize, consumption of fat may increase from less than 20% of total energy intake to 40% (11). The still dramatically varying lipid content of diets may perhaps explain geographic differences in CRC incidence (66).

1.7.1 RECOMMENDATIONS FOR INTAKE OF FAT

The Norwegian recommendations state that the total fat intake should not exceed 30% and the intake of hard fat (SFA + TFA) should not exceed 10% of total energy intake. The desirable intake of MUFA is 10-15% of energy, of PUFA 5-10% (not >10%), including 1% ω-3 fatty acids (minimum 0.5%) and at least 3% EFA (192).

Suggested intake of polyunsaturated fatty acids

Suggested minimum and optimum intakes of ω-3 and ω-6 PUFA are shown in Table 3. The numbers are based on data from patients with EFA deficiency and on estimation of required and optimal intake in healthy, normal individuals with an energy intake of 2200 kcal/d (102).

The Food and Drug Administration (FDA), U.S.A has ruled that intakes of up to 3 g/d of marine ω-3 acids are Generally Recognized As Safe (GRAS) for inclusion in the diet (193).

Table 3 Suggested intake of polyunsaturated fatty acids.

	Omega-3	Omega-6	Omega-3	Omega-6
	(% of energy)		(mg/d)	
Minimum	0.2-0.3	1-3	400-600	2400-7200
Optimum	1-2	3-7.5*	2400-4800	7200-18000

*Pregnant and breast feeding women. Rustan A. C. (102).

1.7.2 DIETARY FAT CONTENT

After having increased from the start of this century at the expense of starch, except during of World War II 1940-1945, the fat content the Norwegian diet decreased from 40 to 34% of total energy intake from 1975 to 2003 (194). The content of hard fat (SFA + TFA) in the diet is estimated to have decreased from about 21 to 15% of total energy intake from the 1970s to the early 2000s (195). The P/S ratio has increased from 0.34 in 1975 to 0.50 in 2000 (196).

1.7.3 INTAKE OF FATTY ACIDS OF CURRENT INTEREST

In 1998, the mean intake of marine ω-3 fatty acids in a random sample of the Norwegian population between 16 and 79 years was 0.89 g/d (0.4 % of total energy) (197). The estimated intake of TFA in 2004 decreased from 15 g/d (5.5% of energy) in 1958 to 1.5 g/d (0.5% of energy), and TFA from industrial products decreased from two thirds to one third the last 15 years (150;195), see also Figure 15.

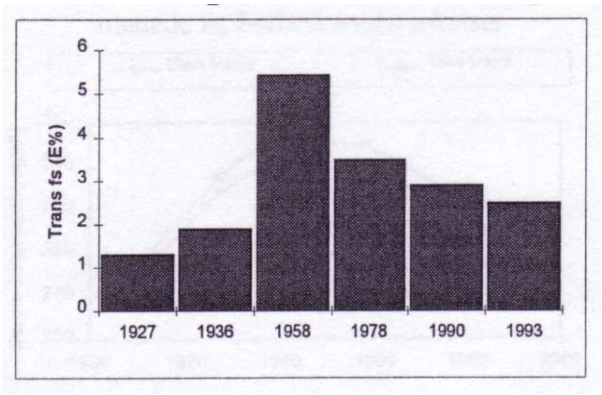

Figure 15 *Trans* fatty acids (% of energy) in the diet of the Norwegian household. Johansson L (195).

1.8 OILS OF CURRENT INTEREST

1.8.1 FISH OIL

Fish oil is extracted from the total fish body and contains mainly TG with variable amounts of PL, hydrocarbons, fatty alcohols, glycerol ethers and wax esters. Fish oil is characterized by a wide range of C14-22 fatty acids and a high degree of unsaturation ranging up to six double bonds per molecule is characteristic of fish oil. The fatty acid patterns vary widely with fish species and with the composition of the plankton and the time of year (198). PUFA, including EPA and DHA may constitute 70-80% of total fat in fat fish (95).

Fish oil contains relatively high cholesterol concentrations dependent of the fish type and smaller amounts of other sterols (199).

Fish oil may also contain prooxidant trace metals such as iron and copper, and sulphur, phosphorus, chlorine, bromine, iodine, and heavy metals (198).

Norway has had access to large amounts of fish at a low price (142), and had in 1968 23%, in 1970 19%, in 1975 17%, in 1980 17%, and in 1983 19% of the world production of fish oils. In the early 1990s, the Norwegian production was the fifth highest in the world (200).

In 1990, 76% of the fish oil was being used in margarines and bakery fats, mainly in Europe. At that time 16% was used for aqua feed and 8% for various industrial purposes. In 2000, the proportion for aqua feed had increased to nearly 60%, the industrial and pharmaceutical usage had not changed much, but the use of fish oil in hardened edible fats had decreased to 31%. In year 2010, practically all of the fish oil production is expected to be used in aqua feed (201). Recent trends in the use of fish oil in Europe and Scandinavia show a significant drop (202).

1.8.2 COD LIVER OIL

Ordinary fish oil does not usually contain vitamin A and D, but cod liver oil does (203). The use of cod liver oil against rickets and rheumatism among several diseases, started in the 1700s, and has long traditions in Norway. In 1854, Peter Möller developed a new extraction process for cod liver oil in Christiania. After World War II, vitamin supplements took over

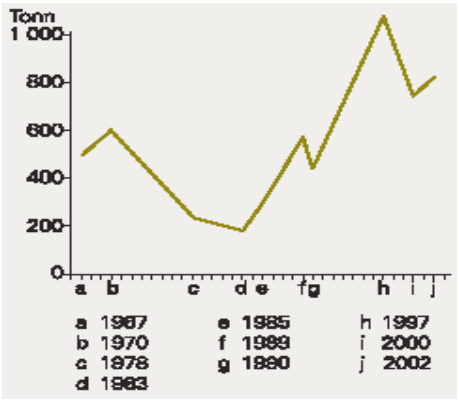

Figure 16 Total consumption of cod liver oil in Norway (tons). Concentrated fish oil based on sales value 2002 and estimated to 113 tons, is not included. Peter Möller AS. (204).

the market, but Bang and Dyerbergs studies among Eskimos lead to increased use of cod liver oil from the 1960s (Figure 16) (204). In Norway, a supplement of 5 ml cod liver oil per day is recommended from 4 weeks of age due to its vitamin D content (203). Cod liver oil contains approximately 28% PUFA, 46% MUFA and 16% SFA (205), and the recommended daily dose contains 1.2 g ω-3 fatty acids, thereof 0.4 g EPA and 0.6 g DHA, 250 µg vitamin A, 10 µg vitamin D, and 10 mg vitamin E (Möllers Cod Liver Oil). Cod liver oil and concentrated fish oil contributed 150-175 mg/d to the intake of very long chain ω-3 fatty acids in Norway in 2002 (196).

1.8.3 BUTTER OIL

Butter oil is produced from cream in a concentration process that almost completely removes water and fat free dry milk substances, and contains 99.9% milk fat including 94.4 % fatty acids and 5.5 % glycerol (206), the rest being MG, DG, FFA, PL, and sterols including 10-20 mg/dl cholesterol (139). The typical distribution of fatty acids in butter is 4% PUFA and 32% MUFA, including up to 8% TFA, and 64% SFA, the ω-6/ω-3 ratio being 3:0% (126).

There are important differences between bovine and human milk fat; the bovine milk containing more SFA including substantial quantities of 4:0-10:0 and less 18:2, and it also contains approximately 2% *t*-18:1. The TG structure id unique with much of the 4:0-10:0 fatty acids at the *sn*-3 position, where they are available to selective milk or added lipases(139).

Butter have lost a sizable portion of its market share due to controversies associated with its cholesterol content and high percentage of LCSFA (207). The butter consumption has decreased from 9 to 3 g/d the last 15 years, while butter alone contributed 1% of total energy and 4% of SFA in the Norwegian diet (194).

1.8.4 SOYBEAN OIL

Soy grows faster and soybeans are cheaper than other raw materials for oils. Soybean oil is the largest edible oil in the world production (208), and was produced in Norway from 1955

(209). This oil contains mostly LA, but also ω-3 fatty acids, MUFA, SFA and lecithin. The ω-6/ω-3 ratio of soybean oil is 54:8% (126).

The greatest perspective in feed production for the Norwegian fish farming seems to lie in the utilization of vegetable fat sources including soybean fat, which is already used in considerable quantities (210).

Preliminary drying of the seeds, steam washing of oils, refining and blending with refined oils, lead to increased TFA in such oils, and the TFA content, although small, show 18:1, 18:2, 18:3, and 20:1 fatty acids (211;212).

Besides being the most important raw materials in margarines, soybean oil and hydrogenated soybean oils are also central raw materials for producers of mayonnaises, salads and salad oils (213). The doubled use of such products in Norway over the last twenty years has made them a considerable source of UFA, especially the ω-6 fatty acid LA (194).

1.8.5 HYDROGENATED OILS

The basic Food and Drug Administration (FDA, USA) definition of a hydrogenated fat is one that is solid at room temperature. Such fats typically contain 15-25% TFA. Partially hydrogenated oils are defined by the FDA as liquid at room temperature and are lower in TFA. Some vegetable fats and oils have been moderately or slightly hydrogenated (133). Light hydrogenation produces almost no TFA (214). However, in the reality, different hydrogenation degrees are not referred to according to such definitions. Available information showed the following use of terms:

- fats with MP 30-45 °C (hydrogenated oils), MP 30-33 °C with 55-60% TFA (hydrogenated fish oil) and 30-42% TFA (hydrogenated soybean oil), were referred to as partially hydrogenated or lightly hydrogenated
- fat with MP 29 ± 2.0 °C and 30.2 ± 4.3% TFA (hydrogenated fish oil) was referred to as partially hydrogenated
- fats with MP 38-42 °C and 38- a little less than 50-55% TFA (hydrogenated fish oil) or 46-51% TFA (hydrogenated soybean oil), fat with MP 49 ± 3.0 °C and 3.6 ± 0.5% TFA (hydrogenated fish oil), and fat with 3.6% TFA, were referred to as highly hydrogenated
- fats with MP 50-60 °C with approximately 0-21% monoene and diene TFA (hydrogenated fish oil) or < 1% TFA (hydrogenated soybean oil) were referred to as totally hydrogenated.

Hydrogenated soybean oil

Light hydrogenation of soybean oil reduces most of the ALA to LA. A low-ALA soybean oil contains 4% ALA, has twice the stability of regular soy oil (214). The changes in the fatty acid content of soybean oil during hydrogenation is shown in Figure 17.

Five species with tri-*cis* and tri-*trans* configurations and three species with *cis*-*trans* mixed configurations were found in lightly hydrogenated soybean oil (LHSO) with an iodine value (IV, a measure of unsaturation) of 111, but at an IV of 96, tri-*cis* was no longer present (215).

In partially hydrogenated soybean oil (PHSO), elaidic acid 9*t*-18:1 is the predominating TFA, and the 9*c*,13*t*,-18:2 isomer is the major *trans* PUFA (95;212;216).

Totally hydrogenated soybean oil (THSO) has essentially been hydrogenated to completion, i.e., > 99% of the fatty acids are saturated (217). THSO may contain 11% palmitic, 88% stearic and 1% longer fatty acids (214).

Hydrogenated soybean oils are used in the baking- and food industry as ingredients in a countless number of foods, such as frying oils, cookies, biscuits, chocolate, liver paste, wiener sausage, sauces, bouillon, rice mixtures, non dairy cream, and nut butters (125;136;218-220).

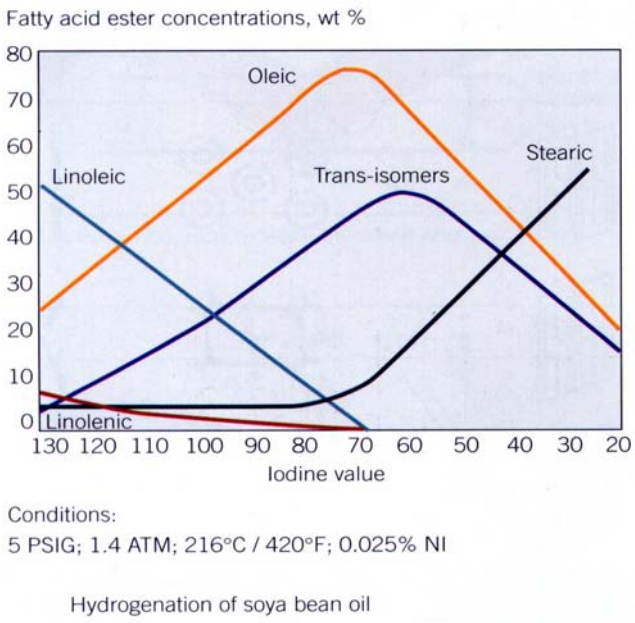

Figure 17 Hydrogenation transfers linoleic and α-linolenic acids into stearic acid via oleic acid and various *trans* fatty acids. Iodine value is a measure of unsaturation. Denofa (126).

Hydrogenated fish oil

Hydrogenation of fish oil forms positional and geometrical isomers of VLCPUFA as well as VLCSFA which do not exist in hydrogenated vegetable oils (221). Hydrogenated fish oil also contains sterols and stanols, i.e. hydrogenated sterols (209).

Usually, the unsaturation of fish oils from capelin, cod liver, and mackerel must be reduced considerably to achieve adequate stability. This reduction is primarily of the 20:5 and 22:6 ω-3 fatty acids. These three fish oils contain substantial proportions of 20:1 (gadoleic and gondoic) and 22:1 (erucic and cetoleic) fatty acids, which are, in most instances, simply converted to *trans* forms during hydrogenation. In consequence, the TFA concentration in hydrogenated oils from these fish types tends to be relatively high, 40-50%, i.e., at the same level as highly hydrogenated soybean oil intended for stick margarines (129), and it may be as high as 60% for partially hydrogenated fish oil (PHFO) with MP 30-45 °C (128).

Menhaden and sardine oils initially have relatively little 20:1 and 22:1. Products of such fish oils were granted GRAS status in the U.S.A. in 1998 (129).

In PHFO, a mixture of different *trans* isomers with chain length 16-24 carbon atoms has been found (142), and also numerous long chain *cis* and *trans* isomers that had to be included in the group of unidentified fatty acids (211). PHFO is used in margarines, shortenings, salad oils, frying fats, low calorie spreads, bread, pastries, cakes, cookies, biscuits and synthetic creams (222).

Figure 18 shows the reduced consumption of hydrogenated fish oil in Norway lately.

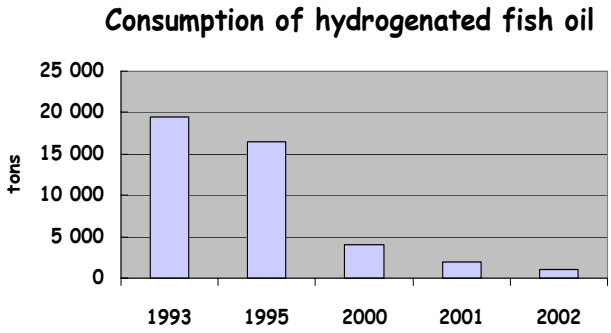

Figure 18 Reduced consumption of hydrogenated fish oil in Norway. Denofa (223).

Totally hydrogenated fish oil (THFO) is so far not used in foods for humans, but the possibility of using this oil in interesterified form has been shown interest (212). Some THFO is used in animal feed (224). THFO contains a minimal amount of TFA, but high amounts of LCSFA C14-19 and VLCSFA C20-24 (142), which lead to considerably higher melting points for THFO compared with PHFO or HHFO (212).

The functionality of hydrogenated fish oil is largely based on the high TFA content and the large range of MP covered by the fatty acids (128), giving margarines an excellent plastic consistency (198). In comparison with a fat consisting of only one kind of fatty acid, which would melt at a certain temperature, a fat such as PHFO, consisting of a mixture of different fatty acids, is melting little by little (Figure 19) (102). Optimal functionality is attained at MP 30-40 °C. In this area is also the maximum TFA content (60-50%) found (128).

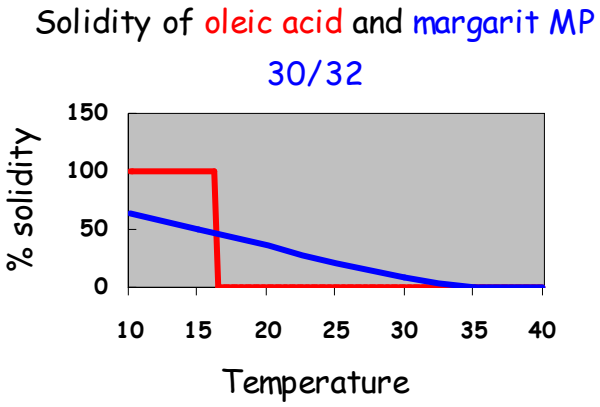

Figure 19 Per cent solidity of margarit and oleic acid at different temperatures. Margarit is the raw material for margarine after hydrogenation of fish oil. Denofa (102).

Partially hydrogenated oils in margarine

Partially hydrogenated whale and fish oils contributed 70% of the raw material for the margarine production in Norway between 1912 and 1962. Between 1950 and 1970, whale oil was gradually interchanged with herring and soybean oils as raw materials (196;209).

The hard margarines Melange and Per were the main source of hydrogenated marine fatty acids and TFA in Norway (194). Figure 20 shows the distribution of margarine containing partially hydrogenated fat: total, marine, or vegetable through the 20th century. During the 1950s, the composition of the Norwegian margarine was least healthy, seen from a nutritional view. In addition to containing a high amount of partially hydrogenated marine fat, the margarine had a high cholesterol content and a low proportion of PUFA (195). In 1992, approximately 35% of the fat used for margarine production was of marine origin (225). After the negative effects of PHFO on risk factors for CRC were discovered in the 1990s, the production of PHFO and PHSO was

Partially hydrogenated fat, weight % of total margarine production in Norway

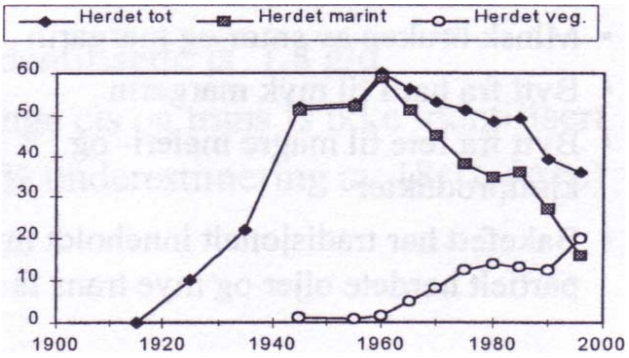

Figure 20 Total, marine, and vegetable partially hydrogenated fat (% of total margarine weight). Johansson L. (195).

gradually reduced, and these oils are no longer used in margarine produced in Norway for direct sale to households (196;225). Instead, a mixture of THSO, liquid vegetable oil and/or palm or coco fat or fractions thereof was used, with the fatty acids interesterified (209).

The consumption of margarine was traditionally higher in Norway than in South Europe(226). The DAFNE II data of approximately 1990 based on Household Budget Surveys firmly document the remarkable disparity of food habits among European countries (Table 4). The disparity presents both qualitative and quantitative aspects. Vegetable fat generally means industrially processed vegetable oils (margarine) (227).

Table 4 Distribution of availability of various added lipids in six European countries, DAFNE II 1990 (227).

Average availability of total added lipids (by type)
(g/ person/ day)

	Greece	Ireland	Luxembourg	Norway	Spain	United Kingdom
Butter	0.8	24	14	3.7	0.9	5.7
Animal fat	0.0	3.4	8.9	3.6	0.2	5.0
Vegetable oil	64	4.2	16	1.4	57	5.9
Vegetable fat	5.6	12	18	27	1.9	16
Total	**70**	**44**	**57**	**36**	**60**	**33**

The distribution pattern shows that partially hydrogenated vegetable oil constituted 75% of total added lipids in Norway, a very high percentage compared to the other countries (3-48%). Partially hydrogenated vegetable oil seemed to be the highest single fat source, representing approximately 28% of total fat intake in Norway, which around 1990 was 98 g/d (194).

The margarine intake in Norway increased strongly during the years after World War II, but has during the last 50 years been decreasing from 24 to 11 kg/person/year in 2000 (Figure 21), which is partly the reason for the decreased intake of total fat, marine fat and TFA (194).

From the early 1990s, the competition on the Norwegian market by international oil and fat producers has increased steadily (223). The proportion of margarine consumed in Norway in 2002 covered by Norwegian producers, was only approximately 8% on energy basis (194).

By considering the cholesterolemic effects of all common individual fatty acids with serum cholesterol predictive equations as a tool, the reformulation into "*trans* free" margarines with < 1% TFA, has resulted in healthier products. A cholesterol reducing margarine (Vita), tested

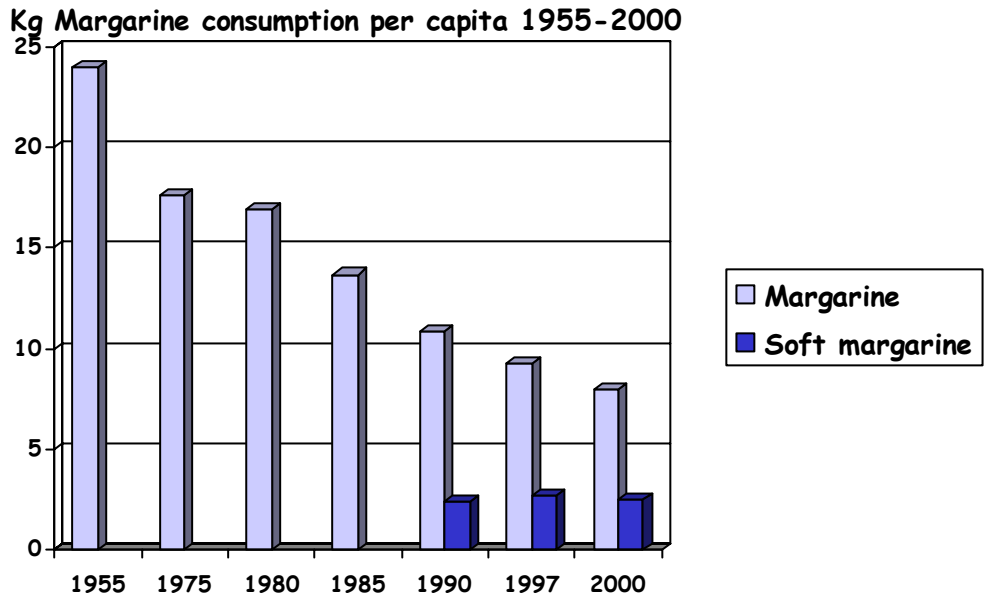

Figure 21 Reduced intake of margarine in Norway. Denofa (223).

tested against butter and a low fat SFA diet, was produced in Norway in 2003 (167).

Fatty acid isomers found in margarines

High values of *t*18:1 in margarines of the 1970s were always accompanied by positional isomers of *c*18:1, and of 9*t*,12*t*-, 9*c*,12*t*-, and 9*t*,12*c*-18:2, and two mixed geometric LA derived isomers could be identified (228). Mono-*t*-di-*c* isomers were found in 1991 (215).

Hydrogenated fish oil in bread

Shortening and bakery margarines have properties different from those of table margarines. The value of hydrogenated fish oil lies in its creaming power, particularly in cake making (198). Hydrogenated fish oil contributed 6% to the fat used for production of margarine in Norway in 2000, and 3% in 2001 (194). In 2002, the main market for hydrogenated fish oil in Norway was still the margarine industry. Of the 165000 tons hydrogenated fish oil sold by Denofa in Norway 1989-2002, 50% was of MP 30-32 °C, 25% of MP 38-40 °C, 25% blends of those two, and very little of MP 50-52 °C (223). Table 5 shows the contents of *trans* fat.

Table 5 *Trans* fat content of partially hydrogenated fish oils used in Norway 1989-2002. Denofa (223).

Melting point, °C	% *Trans* (GLC) monoenes and dienes	% Total *trans* fatty acids (IR)
30-32	54-55	55-60
38-40	50-52	50-55
50-52	20-21	?

GLC, Gas-Liquid Chromatography; IR, Infrared spectrophotometry.

Margarines containing hydrogenated fish oils are imported or produced by Russia and East Europe (209). Norway is now importing dough containing this oil from East Europe (229). Due to high flour prices, one third of the bread consumed in Norway in 2003 was of imported origin, mainly half finished bread and rolls (230). The import of finished and half finished food products is steadily increasing. The contents of hydrogenated fish oil is unknown (209).

1.8.6 NORWAY COMPARED WITH NEIGHBOURING COUNTRIES

The consumption of TFA has been especially high in northern Europe (169). The consumption of PHFO in some European countries in 1984 and 1985, including Norway, Denmark, and Sweden, is shown in Table 6 (231). Estimation from national Food Balance Sheet, Food Frequency Questionnaire, 3 day Dietary Record, and Duplicate Diets showed a dietary content of TFA during 1986-1993 of 1.9 g/d per person in Finland, 3.3 g in Sweden, 5 g in Denmark, 6 g in Iceland, and 8-10 g in Norway (221). Thus, Norway appears to have had the highest TFA intake among the Nordic countries (212;225;231;232).

Table 6 Consumption of partially hydrogenated fish oil 1984 and 1985 (kg/y/person), FAO. Norum K. R. (231).

Country	Consumption (kg/year/person)
The Netherlands	8·6
Norway	8·1
Peru	5·0
Belgium	4·6
UK	4·0
Denmark	3·9
West Germany	3·6
Chile	3·2
Sweden	1·6
South Africa	1·5

The main source of TFA in Scandinavia was partially hydrogenated vegetable oils and fats (232), except in Norway, where it was partially hydrogenated marine oils for most of the last century (221). As late as the mid 1990s, when TFA mainly was derived from dairy and meat in Scandinavia, margarines and shortenings produced from hydrogenated marine and vegetable oils were still significant sources of TFA in Norway (80%). Of the partially hydrogenated oils used in Norway at that time, approximately two thirds were PHFO, and the intake of PHFO in Norway was 11 g/person/day. The daily per capita intake of PHFO and partially vegetable oils was approximately 10% and 5% of total fat intake, respectively (233).

The use of fish oil in Norway and colorectal cancer

The increasing CRC incidence rates in both sexes in Norway ever since the registration started in the early 1950s is most remarkable, and the incidence has more than doubled over the years (2;5). The reason for the observed difference in the CRC incidence trend in Norway compared with neighbouring countries is obscure (234). This difference might be due to interaction between genetic predisposition and changes in lifestyle. Known dietary or other

life style associated factors cannot fully explain it (3). Norway has, however, used cod liver oil and margarine based on hydrogenated fish oil to a greater extent than most other countries. The Norwegian diet is to a large degree based on bread with margarine. Thus, in addition to the contribution by margarine, also bakery products have contributed to the TFA intake. Despite of Norway having had one of the world's highest productions and intakes of fish oil and hydrogenated fish oil, consequences on health have not been sufficiently studied. The few controlled human studies undertaken have been associated with circulation diseases.

1.8.7 EFFECTS OF OILS OF CURRENT INTEREST

Unfavourable effects on lipid risk indicators for coronary heart disease by a PHFO diet (8.0% TFA) were found in young men, at least to the same extent as a butterfat diet (0.9% TFA). In addition, unfavourable anti fibrinolytic effects by a PHSO diet (8.5% TFA) relative to the PHFO or butter diets were found, and butter acted as a procoagulant relative to PHFO (233;235). Replacement of PHFO with vegetable oil in margarine led to significant improvements on plasma LP in young women (236).

When rats were fed diets containing FO, PHFO, or HHFO, and the fatty acid composition of plasma, erytrocytes, subcutaneous adipose tissue, hepatic microsomal membranes, and plasma LP profile was compared between groups, the tissue content of MUFA increased and that of PUFA decreased after an increase of the degree of hydrogenation of the dietary fat. Tissues from rats fed PHFO showed significant amounts of TFA only and significant increases in total and LDL cholesterol. Although no direct evidence, the effects on plasma cholesterol may be attributable to the high content of *trans* isomers. The concentration of plasma TG was decreased only by highly hydrogenated fat feeding (237).

It was also shown that FO (0.5% TFA), PHFO (30% TFA), and HHFO (3.6% TFA) had different effects on different important enzymes in hepatic rat microsomes (238), and that young animals were more sensitive than aged animals to the modification of the fatty acid composition and ω-6/ω-3 ratio of hepatic microsomal membranes (239).

Hydrogenation degree of fish oil and induction and growth of colorectal cancer in mice

A study performed with Min mice here at the Norwegian Institute of Public Health showed no statistically significant differences in the effects of fish oil, similarly hydrogenated as in the present study, on incidence, number, or size of colorectal tumors.

1.9 APPROACHES

1.9.1 PROBLEM APPROACH

With the problem field "Extensive use of hydrogenated fish oil over time in Norway, a country with a high colorectal cancer incidence trend compared with its neighbours", as background, the problem approach was to examine whether there is a relation between intake of hydrogenated fish oil and CRC. It would be unethical to perform experiments on humans exposing them to amounts of edible oils considered to be potentially harmful. The relation was therefore studied in mice.

Aims

The first aim of this study was to examine whether hydrogenated fish oil in the diet can increase induction and/or growth of CRC in azoxymethane (AOM) treated A/J mice.

Because of the complex composition of hydrogenated oils, the intension was to correlate the biological effects to the degree of hydrogenation rather than to specific fatty acids in the oils. As controls, native fish oil, butter oil without water and salt, refined soybean oil, and soybean oil similarly hydrogenated as the fish oil, were used. Thereby, oils which were consumed in Norway in the past and/or which may be used in the future are exemplified in this study. This leads to *the second aim*, which was to reveal patterns of effects on induction and/or growth of CRC in the material, even if the results were not statistically significant.

The native fish oil was first analysed for protecting properties regarding induction and/or growth of CRC in the animals in comparison with soybean oil or butter oil (figures are not shown here).

Endpoints and outcome variables

Effects of the exposure to the experimental oils on induction and/or growth of CRC were measured by using colorectal tumors as endpoints. Outcome variables were the following tumor parameters; *incidence* and *number* to express induction, *size* to express growth, and *load* which takes into consideration both induction and growth of CRC.

Hypotheses

The following three hypotheses were tested in 4 series of analyses:

A. The first hypothesis

Hydrogenated fish oil in the diet will increase induction and/or growth of colorectal cancer in AOM treated A/J mice in comparison with unhydrogenated fish oil, thus losing the protecting properties of native fish oil.

1. Fish oils
2. Hydrogenated fish oils and soybean or butter oil

B. The second hypothesis

Induction and/or growth of colorectal cancer in AOM treated A/J mice will increase with increasing hydrogenation degree of fish or soybean oil in the diet.

3. Hydrogenated fish and soybean oils

C. The third hypothesis

Hydrogenated fish oils in the diet will increase induction and/or growth of colorectal cancer in AOM treated A/J mice in comparison with corresponding hydrogenation degrees of soybean oil.

4. Hydrogenated fish and soybean oils

Working hypotheses

The following common null hypotheses and alternative hypotheses were set up:

For associations between dependant and independent variables

1. H_0: "There is no relation between the tumor parameter and type of oil in the diet"
2. H_A: "There is a relation between the tumor parameter and type of oil in the diet"

For differences between treatment groups

1. H_0: "The tumor parameter is equal in the two treatment groups"
2. H_A: "The tumor parameter is different in the two treatment groups"

1.9.2 EXPERIMENTAL APPROACH

The following experimental approach was made:

1. Establishing test system

Before the experiment could be started, a test system for A/J mice had to be established.

Pilot study

A pilot experiment was undertaken with A/J mice already held at house to find the best possible way of increasing the number of colorectal lesions with AOM without damaging the animals unnecessary, and the shortest possible experiment time.

Breeding

A/J mice were imported from the U.S.A. to breed pups for the main experiment.

Mixing experimental oils with the standard feed

To achieve reproducible doses of the oils in the feeds, a food processor was tried out.

Feeding automats

Hanging automats especially fit for feeding non-pelleted diets were tested.

2. Testing of the experimental oils

Nine groups of A/J mice were injected twice with AOM and each fed a diet containing 19 w/w % of one of nine experimental oils (Table 7) and 1 w/w % corn oil mixed with AIN-76M. The animals were killed at week 15 and colons were dissected and examined for tumors.

Table 7 Types and abbreviations of the experimental oils.

Experimental oils	Types	Abbreviation
Butter oil	Natural, without water and salt	BO
Fish oils	Native, raw	FO
	Partially hydrogenated	PHFO
	Highly hydrogenated	HHFO
	Totally hydrogenated	THFO
Soybean oils	Refined	SO
	Partially hydrogenated	PHSO
	Highly hydrogenated	HHSO
	Totally hydrogenated	THSO

2 MATERIALS AND METHODS

2.1 PILOT STUDY

A pilot study was undertaken on A/J mice fed Rat & Mouse No. 3 Breeding Diet (RM3) early in life and Rat & Mouse No. 1 Maintenance Diet (RM1 (E)) later. The animals were injected twice with azoxymethane (AOM) subcutaneously in the neck region on days 6 and 13; 7.5 mg/kg (8 animals), 10.0 mg/kg (2 animals), and 12.5 mg/kg (3 animals) and killed after 7 weeks. In addition, 1 animal injected 12.5 mg/kg AOM was killed after 12 weeks. After cervical translocation, the colons were dissected, prepared, and mucosal lesions examined.

2.2 EXPERIMENT

2.2.1 BREEDING

Fifty female and ten male A/J mice were purchased from Jackson Laboratory (Bar Harbour, ME, US) to breed pups for the main experiment. The animals had been born during week 3 2003, arrived in week 10 approximately 7 weeks of age, and were acclimatized for 4 weeks.

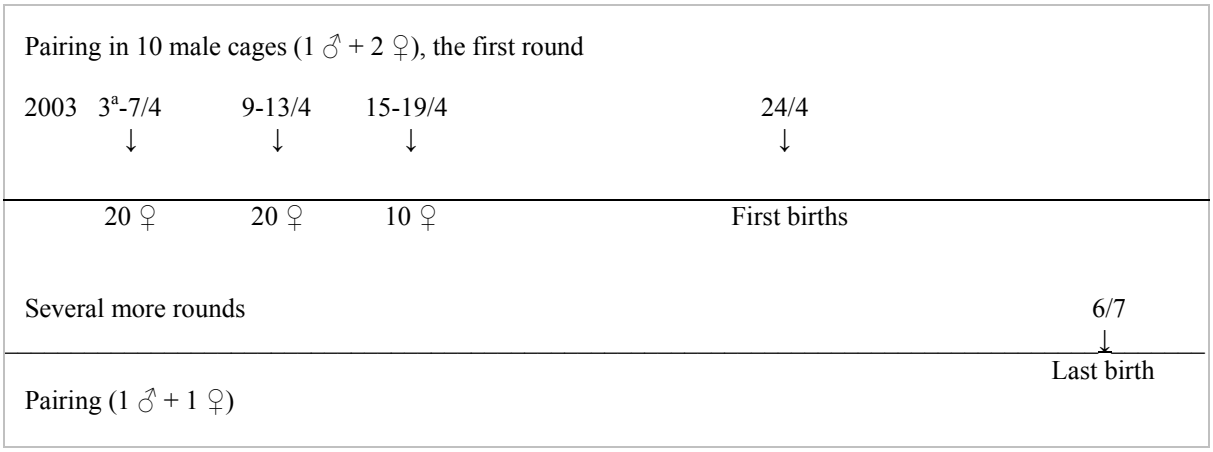

Figure 22 Breeding display. [a]Start of the breeding.

The ten male mice were caged individually (Figure 22) and the fifty female mice two and two together in suspended polysterol cages with filter top and wood chips bedding. The animals received the special breeding diet RM3 *ad libitum* and had free access to tap water. Room conditions were as described in the next paragraph. After pairing, the females were returned to their original cages, but after detection of pregnancy, they were placed in individual cages. Several rounds of pairing were necessary to get the most of the females fertilized. In this

connection, a 4 days pairing routine (a period as long as the oestrus cycle) and two days rest for males was changed to 8 days pairing and 5 days rest to increase the chance of fertilization. The latter cycle was repeated until the planned numbers of living offspring for the experiment had been achieved after approximately 3 months. Only first litters were used.

2.2.2 EXPERIMENTAL ANIMALS

Randomized, placebo controlled studies aiming at preventing the occurrence of adenomatous polyps in voluntary humans are considered the best method within chemopreventive research. A weakness with this method is, however, that the endpoint is not cancer, but relapse of adenomas (240).

In this experiment, the A phenotype of JAX mice named A/J mice were used. This mouse strain was developed by L.C. Strong in 1921 and has been widely used in cancer research (241). Inbred mice differ dramatically in their sensitivity to the colon carcinogen AOM (242). The A/J mice are wild type animals highly susceptible to inducement of ACF and multiple intestinal tumors (243), primarily in the distal colon (244), representative of human adenomas or carcinomas *in situ* (245). For these reasons they can be used as a murine model of human sporadic CRC as a sensitive test system for dietary factors.

Care

The experimental animals were housed in polysterol cages with a layer of wooden chips as bedding in a ventilated holding room maintained under controlled conditions (21 ± 2 ˚C and 50 ± 10% relative humidity) in a 12 h light/dark cycle. A low level of noises was held.

The cages were of two different sizes, suspended cages with filter top designed for up to eight mice, and smaller cages for up to four mice placed in ventilated glass cabinets. The small cages were especially used around the time of birth for one dam with pups. Cages with pups were changed every week and cages with grown animals every two weeks.

The animals had free access to tap water, which was changed twice a week, while the bottles were changed ones a week. The feeding mode was *ad libitum*, and feeding was attended to on a daily basis. Not to disturb dam and litter, feeding the first week after birth was attended to daily only from day 4, when the experimental feeds were introduced.

2.2.3 EXPERIMENTAL FEEDS

Basal feed

American Institute of Nutrition-76M Semi-Purified Diet (AIN-76M) (Table 8), a pulverized feed modified (the 5% corn oil removed) from the standard feed AIN-76A, was bought from Special Diets Services (P.O. Box 705, Witham, Essex CM8 3AD, UK).

Table 8 Ingredients and energy providing nutrients of the basic feed AIN-76M.

Ingredients	g/kg
Casein, High Protein	210.50
DL-Methionine	3.20
Sucrose	526.30
Corn Starch	157.89
Fiber (cellulose)	52.60
Mineral Mix	36.90
Vitamin Mix	10.50
Choline Bitartrat	2.10
Ethoxyquin (antioxidant)	0.01
TOTAL	1,000.00

Energy sources	%	kcal/g
Protein	23.8	
Carbohydrate	76.2	
SUM	100.0	
Metabolizable Energy		3.59

Earlier animal experiments have shown that feeding animals with partially hydrogenated vegetable oils leads to symptoms of EFA deficiency (246). To ensure supply of the essential linoleic and α-linolenic acids for the animals, corn oil from Mills DA (Box 4644 Sofienberg, 0506 OSLO, Norway) bought from the grocery shop was added to the feeds amounting to 1.9% of total energy (Table 11). The corn oil contained 13 mg vitamin E per 100 g oil.

Antioxidants limit degeneration of fat, but might also represent a source of error in the experiment. Therefore, no extra antioxidants or micronutrients were added.

Experimental oils

Fish and soybean oils produced 12^{th}-13^{th} of Dec 2002 and transported frozen at -20 °C to Rikshospitalet University Hospital on the 17^{th} of Des 2002 were donated by Denofa (Øraveien 2, 1631 Gamle Fredrikstad, Norway). Butter oil produced and fetched in week 2, 2003, was donated by Tine Fellesmeieriet BA (Kalbakken, 0902 Oslo, Norway). The oils were ordinary raw materials for the food industry taken directly from the production line, except THFO, which was produced in the laboratory by Denofa.

The experimental oils were not analyzed specifically for TFA contents. In general, the *trans* content in hydrogenated oil will vary within certain limits depending upon the composition of the crude oil (unsaturation degree) and furthermore on the hydrogenation conditions (233). Exact conditions for the hydrogenation of the experimental oils and for the refining of the SO are not known. Hydrogenation conditions for hydrogenated soybean oils in a study by K. Almendingen were; temperature: 155°C (MP 40/42°C), 155-180°C (MP 30/32 and 38/40°C), catalyst: nickel, pressure: 0.5-1.0 bar above atmospheric pressure (233). FO, PHFO, HHFO, and THFO were supposed to contain equal amounts of cholesterols (209), and the same goes for SO, PHSO, HHSO, and THSO. Approximated values for the *trans* and cholesterol contents and melting points are shown in Table 9, and the fatty acid profiles of the experimental oils, as analyzed by Denofa, Table 10.

Table 9 Approximated melting points and *trans* and cholesterol contents of the experimental oils, adapted from Denofa, Tine, and diverse literature.

Experimental oil	MP, ca. °C	*Trans* monoenes and dienes (GLC), ca. %	Total *trans* (IR), ca. %	Cholesterol, mg/100 g (%)
BO	33[A], 40[B]	2.2[A]	3.7[A]-4.84[T]	268, 1933 (~0.3,19)[B], 219[D]
FO	25[S]		0.3[M]	500 (~0.5)[P]
PHFO	31-33[E], 30[S]	54-55 [GA], a little > 54-55[S+GA]	54[S], a little > 55-60[S+GA]	250-800 (0.25-0.80)[GA]
HHFO	40-42[E], 40[S]	50-21[GA], a little < 50-52[S+GA]	38[S], a little < 50-55[S+GA]	480-925 (0.48-0.93)[GA]
THFO	**55-60**[S]	21-0[GA]	< 1[S]	?
SO	0	0.24-0.48[T]	0.40-0.86[T]	0[D]
PHSO	**31-33**[E], 30[S]	?	42[S]	?
HHSO	**40-42**[E], 40[S]	49.5[A]	46[S], 51[A]	?
THSO	**55**[S]	?	< 1[S]	?

BO, butter oil; FO, fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil; MP, melting point; GLC, Gas-Liquid Chromatography; IR, Infrared spectrophotometry. [A]From K. Almendingen (233).[B]By A. S. Biong (206). [D]From The Large Food Table (205), [E]By A. Evennett (247), [GA]By G. Andersen, Denofa (248), [M]From N. Morgado (239), [P]From J. Pettersen (199), [S]By H. Standal, Denofa (128;209), [T]From A. Aro (TRANSFAIR) (211). Real figures of these characteristics for the experimental oils may differ from the values of this table. Supposed melting points for some of the oils are highlighted.

The MP of the experimental PHSO was supposed to be 31-33 °C and of HHSO 40-42 °C, and the MP of PHFO and HHFO probably were in similar areas. There are no common criteria for referring to hydrogenated oils with MP within these areas as "partially" and "highly" hydrogenated, respectively (see point 1.8.5 Hydrogenated oils). The phrases "partially" and "highly" hydrogenated were used here to keep the two hydrogenation degrees apart.

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The fish oils

FO, PHFO, HHFO and THFO were all obtained from the same batch of a mixture of several crude fish oils, mainly from South America. The fish species are not known. A certificate of analysis for a sample of fish oil received earlier from Denofa is presented in Appendix II.

The FO was native and contained approximately 33% PUFA, including 12.1% EPA and 8.4% DHA, 30% MUFA, 2% unidentified fatty acids, and 36% SFA. Thus, the FO might be characterized as containing not far from equal parts of SFA, PUFA, and MUFA.

The PHFO contained approximately 45% PUFA, 12% MUFA, 6% unidentified fatty acids, and 37% SFA, and may be characterized as containing mostly PUFA, a great part of SFA, and some MUFA and unidentified fatty acids, including fatty acids changed by the hydrogenation.

The HHFO contained approximately 38% PUFA, 7% MUFA, 5% unidentified fatty acids, and 51% SFA, and may be characterized as containing mostly SFA, a great part of PUFA, and some MUFA and unidentified fatty acids, including fatty acids changed by the hydrogenation.

The THFO contained approximately 2% PUFA, < 1% MUFA and unidentified fatty acids, and 97% SFA, and may be characterized as containing almost only SFA, including fatty acids changed by the hydrogenation.

The soybean oils

The SO, PHSO, HHSO and THSO were obtained from the same boat load of soybeans.

The SO was refined and contained 61% PUFA, 23% MUFA, < 1% unidentified fatty acids, and 15% SFA, and may be characterized as containing mostly PUFA, a great part of MUFA, including up to 1% TFA (128), and some SFA.

The PHSO contained 1% PUFA, 77% MUFA, < 1% unidentified fatty acids, and 22% SFA and may be characterized as containing mostly MUFA, a great part of SFA, and ≤ 1% PUFA or unidentified fatty acids, including fatty acids changed by the hydrogenation.

The HHSO contained < 1% PUFA, 69% MUFA, < 1% unidentified fatty acids, and 30% SFA and may be characterized as containing mostly MUFA, a great part of SFA, and < 1% PUFA

Table 10 Fatty acid profiles of the experimental oils.[a]

	Butter oil	Fish oil	Soybean oil						
			Degree of hydrogenation				Degree of hydrogenation		
Fatty acid	BO[b]	Raw FO	Partial PHFO	High HHFO	Total THFO	Refined SO	Partial PHSO	High HHSO	Total THSO
4:0	4.9								
6:0	2.4								
8:0	1.3								
10:0	2.7								
11:0	0.3								
12:0	2.9						0.2	0.2	
14:0	10.3	8.3	8.7	8.8	8.0	0.1	0.2	0.1	0.1
15:0	0.8	0.6	0.6	0.6	0.5				
16:0	27.8	21.7	21.4	25.6	35.5	11.2	12.0	11.0	15.0
17:0	0.5	0.5	0.7	0.5	0.2		0.1	0.1	0.2
18:0	12.3	4.2	4.8	9.5	22.4	3.3	8.5	17.1	83.0
19:0		0.3		0.1	0.2			0.5	
20:0	0.2	0.2	0.5	3.4	18.1	0.3	0.4	0.4	0.7
22:0			0.3	1.8	12.5	0.4	0.4	0.4	0.6
24:0				0.2				0.1	0.1
14:1 *cis*	0.9								
14:1 n5		0.2	0.2	0.2	0.2				
16:1 n7		10.5	11.8	6.8	0.2		0.1		
16:1 cis	1.4								
16:1 *trans*	0.8								
17:1		1.5	0.2						
18:1 n7		3.2				1.5			
18:1 n9		10.4				21.5			
18:1 *cis*	25.9								
18:1 *trans*	2.8								
18:1 total							76.2	68.8	
20:1 n7		0.2							
20:1 n9		2.0				0.2	0.2	0.1	
22:1 n9 eruka		0.2							
22:1 n11		1.1							
24:1		0.3							
16:2 n6		1.3		0.1	0.2				
18:2 n6		1.6	0.2	0.1		55.4	1.1	0.9	
18:2 *cis,cis*	1.5								
18:2 conjug.	0.7								
20:2		0.2							
16:3 n3					0.6				
18:3 n3		0.8				5.8			
18:3 *all cis*	0.4								
16:4 n3		0.7		0.1					
18:4 n1		0.2							
18:4 n3		1.7				0.1			
20:4 n3		1.0							
20:4 n6		1.1			0.7				
22:4 n6		0.3			0.6				
20:5 n3 EPA		12.1							
21:5 n3		0.6							
22:5 n3		2.6							
22:6 n3 DHA		8.4							
18:1n9+C 18n7			17.3	11.1					
20 poly			17.2	15.1					
22 poly			9.9	11.5					
Unidentified		2.0	6.2	4.5	0.2	0.2	0.6	0.3	0.3
TOTAL	100.0	100.0	100.0	100.0	100.1	100.0	100.0	100.0	100.0

[a]The hydrogenated fish oils came from the same batch as the native fish oil, and the hydrogenated soybean oils from the same boat load of soybeans as the refined soybean oil. These oils were analyzed by Denofa (% areas). [b]The experimental butter oil was not analyzed, thus the fatty acid profile for this oil (%) is that of a "mean" Norwegian butter oil, analyzed by Tine 1991.

and unidentified fatty acids, including fatty acids changed by the hydrogenation.

The THSO contained 0% PUFA, 0% MUFA, < 1% unidentified fatty acids, and nearly 100% SFA, and may be characterized as containing almost only SFA, including fatty acids changed by the hydrogenation.

The butter oil

Product specification of the experimental BO was not available. However, the biological variation of butter oil is low. The fatty acid profile of the BO in Table 10 is based on 341 samples of butter analyzed in 1991 (TINE FoU). The samples were taken in ten dairies in different parts of Norway, one sample per dairy per month during one year (249). A general product specification of butter oil is presented in Appendix III.

BO contains approximately 3% PUFA, 32% MUFA, 0% unidentified fatty acids, and 66% SFA, and may be characterized as containing mostly SFA, a great part of MUFA, and little PUFA, including 3-8% TFA (126;150).

Mixing of experimental feeds

The experimental oils were mixed by food processor with AIN-76M and corn oil without heating, the hardened oils for 20 minutes, and the viscous oils for a shorter time, with stirring in between, to achieve adequate homogeneity in the standard feed.

Amounts of prepared experimental feeds were recorded, and average feed expenditure (FE) was estimated for each treatment group of animals at the end of the experiment.

Composition of feeds

The distribution of ingredients per 100 gram feed and per production unit is shown in Table 11, and the nutrients of the experimental diets are specified in Table 12, along with their contribution to total energy. The diets were high fat diets, thus the contents of the other nutrients and fibre were proportionally reduced compared with a low fat diet. Experimental oil provided 36.6% and total fat 38.5% of total isocaloric dietary energy. The energy percent from fat lies in an area not unusual for human intakes.

Table 11 Distribution of ingredients in the feeds and production unit by weight, and their contribution to total energy.

Ingredient	g/100 g feed	Kcal/100 g feed	Energy, %	Production unit, g
AIN-76M	80	287	61.5	400
Experimental oil	19	171	**36.6**	95
Corn oil	1	9	**1.9**	5
TOTAL	100	467	100.0	500

AIN-76M, American Institute of Nutrition-76M Semi-Purified Diet.

Table 12 Specification of dietary nutrients and their contribution to total energy.

Nutrient	% w/w	Kcal/100 g feed	% Energy
Protein	17	68	14.6
Fat	20	180	**38.5**
Carbohydrates	55	219	46.9
Fiber	4		
Micro nutrients	4		
TOTAL	100	467	100.0

Storage

The experimental oils were thoroughly stored on nitrogen in containers packed in several layers of aluminium folio and placed in paper boxes at Rikshospitalet University Hospital at -30 C° until the start of the experiment. After transferring the oils to the Norwegian Institute of Public Health, oils and mixed feeds were lettered and stored on nitrogen in airtight buckets in the dark at 4 C°, as done by Duthie *et al*, to avoid degeneration of the fats (171).

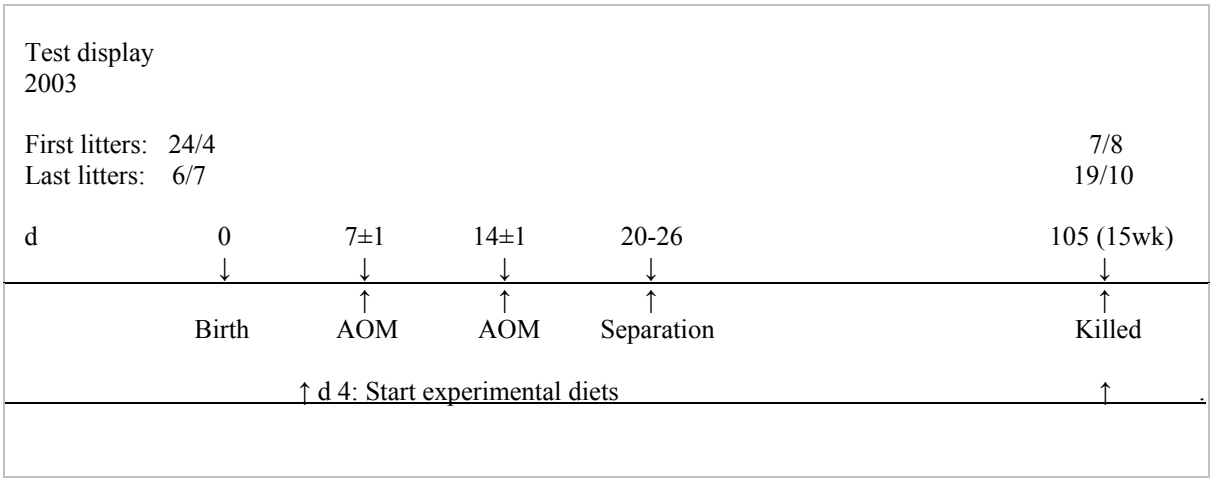
2.2.4 FEEDING AUTOMATS

Due to the different consistencies of the various oils, some feeds were light and fluffy, others light and sticky, and others again dense and sticky. Therefore, several automats of the same type but differing with respect to the size of the feeding openings were used. The automats were checked daily and feeds replenished when needed. In addition, the feeds were stirred down in the automats in order to ensure that feeding could take place *ad libitum*.

2.2.5 EXPERIMENTAL TREATMENT OF THE ANIMALS

Prior to the start of feeding the experimental diets, there was an adjustment period of 3 days for dam with pups. The experiment started on the 28th of April 2003. The experimental design is shown in Figure 23.

The order of introduction of diets to the animals was drawn randomly before the start of births, and the diets were scheduled in the randomized order. The first born litter was presented diet number one, the second born litter diet number two, and so on, until the nine diets were represented. Since births took place over 10.5 weeks, the diets were delegated from the start of the schedule in several turns.



AOM, azoxymethane; d, day; wk, weeks.

Figure 23 Experimental design.

When the diet had been introduced to the last born litter, each of the nine treatments BO, FO, PHFO, HHFO, THFO, SO, PHSO, HHSO, and THSO had been presented to a group constituting ≥ 20 animals which lived through the experimental time period.

Since the diets were introduced to dams with offspring on day 4 after birth, the pups were exposed to the treatment through the mother's milk already before they started being exposed through eating the diets themselves. The exposure lasted until the animals were killed.

In order to increase the number of tumors in the colons, the animals were treated twice, ones a week, with 10 mg/kg body weight AOM, purchased from Sigma Chemical Co (St. Louis, MO, US), dissolved in saline, by subcutaneous injection in the neck region. AOM undergoes

biotransformation in the liver to produce the carcinogenic metabolite methylazoxymethanol (MAM). The metabolizing enzyme system is the alcohol inducible P4502E1, also found in colon tissue (250).

The animals were weighed once a week, for the first time on day 7 ± 1 and the last time on day 105 ± 1, when they were killed by cervical dislocation. Colons were removed and the number and diameter of tumors scored by the use of light microscope (LM) from Nikon (Melville, NY, U.S.A.) in sections (fields of view) of ca 1 cm, from anus towards coecum. Samples of liver and coecum were collected in plastic test tubes, snap-frozen in liquid nitrogen, and stored at - 70 °C.

2.3 PINWORM INFECTION

The summer 2003, a pinworm infection was detected in mice used in another experiment, but housed in the same room as the animals of this experiment. It was decided that ongoing experiments were to continue to the end without medication of the animals, while strict precautions were taken to hinder spreading of the pinworm.

2.4 SCORING OF LESIONS

The mice were killed by cervical dislocation and final weight (FW) registered. The colons were dissected, rinsed in cold (ca. 4°C) PBS (1.14 mM $NaH_2PO_4 \cdot H_2O$, 5053 mM $Na_2HPO_4 \cdot 12\ H_2O$, 0.14 M NaCl, pH 7.4), slit open along their longitudinal median axes, and fixed flat between identification numbered PBS wet filter papers (27). The fixated colons were placed in 10% neutral buffered formalin solution (3.7% formalin in 28.9 mM $NaH_2PO_4 \cdot H_2O$, 44.4 mM $Na_2HPO_4 \cdot H_2O$) for at least 24 h prior to staining. Staining of the colons was performed for 4 s with 0.2% filtered methylene blue from George T. Gurr Ltd. (London, UK) dissolved in the same formalin solution. They were then placed in sterile plastic test tubes from Cellstar (Greiner, Germany) filled with the same neutral buffered formalin solution.

At least 24 h after staining, the surface of the whole mount colons was transilluminated in an inverse LM, and the size of the tumors was scored under x20 magnification. The examination of the specimens was carried out blind. The colons were assessed in the direction anus to coecum. During scoring, the colons were placed between two Petrie discs, with a few drops of neutral buffered formalin solution, to protect the colons from drying up. One of the Petrie

discs was divided into sections by marking stripes with a diameter of 1 cm, corresponding to one field of vision at a magnification of x20. The size of each tumor was scored as the diameter in the length direction of the intestine by an eyepiece graticule.

The diameter and localization of the tumors were recorded on a form shaped as a network, and successively identified by the blind number of the animal. The first colonna indicated the blind number, while the rest of the colonnas indicated the numbers of the fields of vision. In the rows, the diameters were recorded below the field of vision numbers they were found in.

When the recording was finished, the tumors were added up for each animal, and the number of tumors noted on the form under the blind number of the animal. Scored diameter values were later corrected for magnification (scored d/20), and mm^2 areas were calculated by use of the formula $A = \pi \cdot [\text{scored d}/(20 \cdot 2)]^2 = \pi \cdot \text{scored d}^2/1600$.

2.5 STATISTICS

The data material was analysed by the use of the statistical program SPSS, from SPSS Inc. (Chicago, Illinois, US). Data showing normal distributions and homogenous variances were analysed by parametrical tests, but for most of the material non parametrical tests were used. Tests used to look for associations between lesion incidence and treatments or differences in incidence between treatment groups were Fisher's Exact or Pearson Chi-Square tests, and the effect measure for differences in incidence between treatment groups was relative risk (RR) with 95% confidence interval (CI). Tests used to look for associations between lesion number, size, or load and treatments were Kruskal-Wallis Test with Exact Sig. or Asymp. Sig. and very seldom Oneway Anova, while Mann Whitney Test with Exact Sig. (2-tailed) or Asymp. Sig. (2-tailed) and very seldom T-Test were used to look for differences in tumor number, size, or load between treatment groups, and median was used as effect measure.

Since the results of this preliminary study were intended as a basis for generating hypotheses for further experiments in a larger project, a large number of comparisons were performed in the analyses. The results were looked at in series according to hypotheses and questions decided in advance, and the p-values were adjusted manually *à la* Bonferroni in accordance with the number of comparisons in each series.

Due to lack of information, it was not possible to figure out in advance the number of animals needed in the experiment to secure a high power in the study. Hence a cut off point of $p \leq 0.2$ was set, to avoid the risk of not detecting real differences. However, where a full table for these reasons was analysed for one of the genders, it was also analysed for the other gender, even if the overall p-value for the association between the tumor parameter and type of oil in the diet was higher than the cut off point.

The significance level (the probability of finding a difference only by chance, when it is not real) was set at $p \leq 0.05$.

Recorded confounding factors were animal gender and weight. The planned use of General Linear Model to take into consideration the effects of these factors on the results had to be departed because the criteria for the use of this test were not present. Thus, it was not possible to analyse interactions between dietary oils, gender, and final weight. The data material was therefore first checked for differences between genders, and then analysed separately for females and males. FW is dealt with in connection with the tumor parameters in text, tables, and illustrative curves for females and males separately.

2.6 LIMITATIONS

In this thesis, colorectal tumors include tumors between the anal opening and the coecum. Two fast growing large tumors originating in the skin of the outer anal region were therefore excluded, one from a female animal in the HHFO group and one from a male animal in the SO group. One of these tumors was collected as a sample.

3 RESULTS

3.1 ESTABLISHING TEST SYSTEM

3.1.1 PILOT STUDY

Because the animals injected with 12.5 mg/kg AOM were losing fur, maximum tolerable dose (MTD) was set at 10 mg/kg for the main experiment. The animals given this dose seemed to tolerate two subcutaneous injections in the neck region with days 6 and 13 as safe points of time. Lesions were observed in the colon of the animals. To ensure adequate number of lesions in the main experiment, it was decided to treat the experimental animals for 12 weeks (summer vacations postponed the harvesting of colons until week 15).

3.1.2 BREEDING ACCOUNT

114 pairings in total resulted in 45 living litters, 4.4 living offspring per living litter, and 199 animals living throughout the experiment, i.e., 1.75 living offspring per pairing (Table 13).

Table 13 Breeding account.

	Butter oil	Fish oils				Soybean oils				
Animals	BO	FO	PHFO	HHFO	THFO	SO	PHSO	HHSO	THSO	Total
Born										
Total offspring[a]	40	27	24	29	32	25	27	22	48	274
Litters born	5	5	5	5	6	5	5	4	8	48
Mean per litter	8	5.4	4.8	5.8	5.3	5.4	5.4	5.5	6	5.7
Cannibalized[b]										
Before AOM	5	0	1	1	3	1	1	0	2	14
After AOM	5	7	0	5	6	3	5	0	19	50
Died										
After separation	0	0	0	1♀ 1♂	0	1♀	0	0	2+1♂	6
Ill, killed[c]	2♂							1♂	1♂	4
Sacrificed[d]	1♂									1
Living										
Living offspring	27	20	23	21	23	20	21	21	23	199
Living litters	5	5	5	5	6	5	5	4	5	45
Mean per litter	5.4	4	4.6	4.2	3.8	4	4.2	5.3	4.6	4.4

[a]After counting. It is not known how many pups disappeared before counting. [b]The most of these animals disappeared, probably by cannibalization. [c]Hair loss, prepuce infection, obstipation. [d]Occurence of pinworm investigated. FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil; AOM, azoxymethane.

In the THSO group more than 50% of the offspring was lost after introduction of the diet. Of he 50 purchased females, 8 gave no living offspring even after several rounds of pairing. The number of animals cannibalized before counting is not known.

3.1.3 MIXING EXPERIMENTAL OILS WITH AIN-76M, FEEDING AUTOMATS

The mixing of unheated experimental oils by food processor into the basis feed seemed to produce adequate homogeneity for all the different oils. Clumps found during feeding were traced back to the AIN-76M. The feeding automats turned out suitable for *ad libitum* feeding.

3.1.4 ACCOUNT OF EXPERIMENTAL FEEDS

Average expenditure of feed per mouse per day was 4.46 g including wasting by the animals, and varied from 3.70 g in the HHFO group to 5.45 g in the FO group (Table 14).

Table 14 Expenditure of experimental ingredients and feeds.

Expenditure[a]	Butter oil	Fish oils				Soybean oils				
Products	BO	FO	PHFO	HHFO	THFO	SO	PHSO	HHSO	THSO	TOTAL
In total, kg										
AIN 76M	12.200	17.600	10.664	11.864	12.784	13.000	8.800	7.600	9.064	103.576
Experimental oil	2.898	4.180	2.533	2.817	3.036	3.088	2.090	1.805	2.153	24.597
Corn oil	0.153	0.220	0.133	0.148	0.160	0.163	0.110	0.095	0.113	1.295
FEED	15.250	22.000	13.330	14.830	15.980	16.250	11.000	9.500	11.330	129.470
Mouse days	3249	4038	2644	4012	3852	3275	2453	2544	2884	28951
Per mouse per d, g										
AIN-76M	3.76	4.36	4.03	2.96	3.32	3.97	3.59	2.99	3.14	3.57
Experimental oil	0.89	1.04	0.96	0.70	0.79	0.94	0.85	0.71	0.75	0.85
Corn oil	0.05	0.05	0.05	0.04	0.04	0.05	0.04	0.04	0.04	0.04
FE	4.70	5.45	5.04	3.70	4.15	4.96	4.48	3.74	3.93	4.46

[a]Expenditure by 303 animals in total, including the 199 experimental animals, 44 animals in an alternative test procedure, 60 animals which died or were exposed of after separation, and 49 dams. Expenditure by 81 pups which disappeared before they were weaned is not included. FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil; AIN-76M, basis feed; FE, feed expenditure.

3.2 WHAT THE COLONS SHOWED

The colons differed in size by length, width, thickness, strength, and appearance. Some looked “clean” with flat regular appearing mucosae, while others looked “messy” with folds and many vaguely coloured spots. Some colons had blue stained corny appearing large lymphoid nodules with many compartments, while others had small nodules. Some nodules seemed erythematous while others showed some kind of network. Some colons had few and others many light blue or turquoise stained fine-corny appearing lymphoid like tissues which

appeared as small oval or round "holes". Some "holes" had green or turquoise coloured crypts around or against the edge, which looked as if they were decomposing. Other green crypts were seen along straight or bending fissures with or without such "holes".

3.2.1 TUMORS AND ABERRANT CRYPT FOCI

Tumors were identified by their circular appearance, high protruding, bright blue staining, and chaotic looking irregular and distorted crypts (Figure 24). Elevated and flat ACF were seen, and some of the flat ACF were recorded as tumors due to their prominent and chaotic appearance, even if they were not highly elevated or circular. Some of the tumors seemed to originate in lymphoid nodules.

Figure 24 Tumor in the A/J mouse colon. Magnification x20. Photo by Jan Erik Paulsen, Norwegian Institute of Public Health.

3.3 TESTING OF THE EXPERIMENTAL OILS

3.3.1 DISTRIBUTIONS OF SAMPLES

The distribution of *animals* on the nine diets is presented in Table 15. Uneven distribution of animals between the genders led to some quite small samples of animals for examination of tumor incidence, number and load. Uneven distribution of tumors between genders led to some quite small samples less than 10 tumors for examination of tumor size. The distribution of *tumors* on the nine treatment groups is presented in Table 16.

Table 15 Distribution of A/J mice on the nine diets, by gender and in total**.**

A/J mice	BO	FO	PHFO	HHFO	THFO	SO	PHSO	HHSO	THSO	N total
♀	14	11	7	9	12	10	13	16	12	104
♂	13	9	16	12	11	10	8	5	11	95
N total	27	20	23	21	23	20	21	21	23	199

BO, butter oil; FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, refined soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

Table 16 Distribution of tumors on the nine dietary treatment groups of A/J mice in the main experiment, also by gender.

Tumors	BO	FO	PHFO	HHFO	THFO	SO	PHSO	HHSO	THSO	N total
♀	14	2	6	7	15	16	28	5	11	104
♂	26	9	13	10	28	20	16	2	21	145
N total	40	11	19	17	43	36	44	7	32	249

BO, butter oil; FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, refined soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

3.3.2 EFFECTS OF THE EXPERIMENTAL OILS ON ANIMAL BODY WEIGHT

The weight development of the animals is dealt with in the manuscript for an article found in Appendix IV. Final weight (FW) was highly statistically significantly related to gender. Seen in total, males had 1.2 times as high FW as females, the median difference being 3.7 g, $p < 0.0005$. Males also had highly statistically significantly higher FW than females in any of the treatment groups, the median differences ranging from 2.5 to 5.9 g (Table 17). Differences in FW are dealt with in the article in connection with the tumor parameters.

Final weight seen in connection with feed expenditure

Figure 25 shows illustrative curves of median FW and average FE for the total of female and male animals in each treatment group. Average FE in the various treatment groups must not be mistaken for means used in statistical tests, since FE was not measured for each mouse.

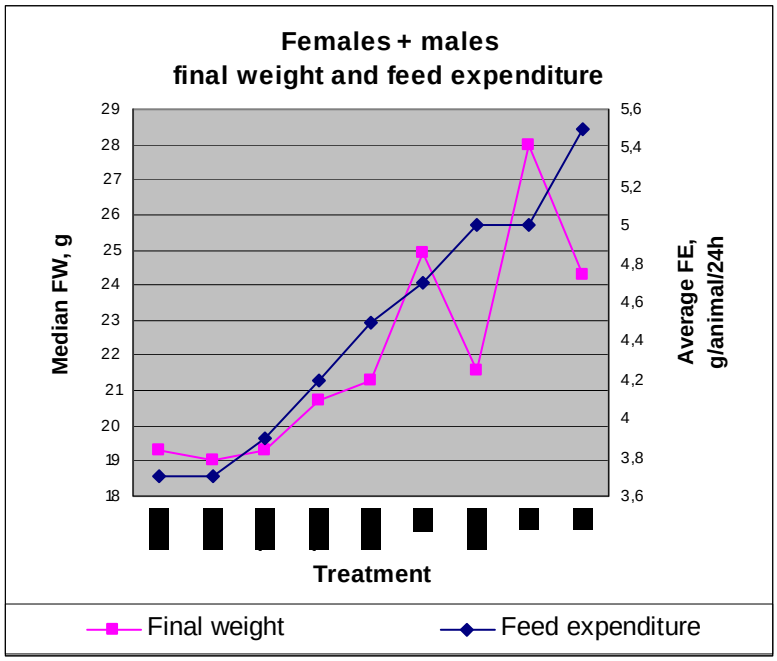


Figure 25 Final animal weight (FW) seen in connection with feed expenditure (FE).

3.3.3 EFFECTS OF THE EXPERIMENTAL OILS ON TUMOR PARAMETERS

The effects of the experimental oils are thoroughly described in the manuscript for the article (Appendix IV). In the present section, the results regarding the three hypotheses are presented based on statistical evidence, with overviews comprising ranked effects and indications of statistical evidence for each series. Median differences between treatment groups with p-values are found in Tables 1-24 (Appendix V) along with data for final weight.

Differences in effects between genders

An overview of the gender differences is shown in Table 17. Males had increased tumor incidence and number, i.e., *induction of CRC* by THFO and increased *tumor load* by FO relative to females, while females had increased tumor size, i.e., *growth of CRC* by SO and THFO relative to males (Table 1-4 Appendix V). In the treatment groups not mentioned, no gender differences were found, or the differences were not statistically significant.

Table 17 Gender differences in colorectal tumor parameters and final weight in AOM treated A/J mice.

Genders Tumor	Ranked estimated median gender differences in tumor parameters and final weight, largest difference first[a]	Statistical evidence[b] for association
Incidence	* ♦ ♦ ♦ **THFO** > FO > THSO > BO > SO > PHSO > HHSO > HHFO > PHFO **0.576** 0.485 0.409 0.346 -0.300 0.260 0.087 0.084 0.054	strong
Number	* ♦ ♦ ♦ **THFO** > THSO > FO > BO > SO = PHSO = HHSO = HHFO = PHFO **2.0** 1.5 1.0 0.5 0.0	strong
Size, mm^2	* * **SO** > FO > **THFO** > THSO > PHFO > HHSO > HHFO > BO > PHSO **-3.21** -2.37 **-2.23** -2.01 1.51 -0.84 -0.23 0.13 0.00	strong
Load, mm^2	♦ ♦ * THFO > PHSO > BO > SO > **FO** > THSO > PHFO > HHSO = HHFO 4.45 -3.83 3.78 -2.39 **0.79** 0.49 0.38 0.00	yes
FW, g	† † † † † † † † † **FO > BO > SO > THFO > HHSO > PHFO > THSO > PHSO > HHFO** **5.9 5.0 5.0 4.1 3.9 3.4 3.0 2.8 2.5**	strong

[a]Figure in black: males have higher tumor parameter value than females. Figure in red: females have higher tumor parameter value than males. [b]Strong or †, $P \leq 0.005$; yes or ∗, $P \leq 0.05$; weak (tendency) or ♦, $0.20 \geq P > 0.05$; no, $P > 0.20$, p-values have not been adjusted *a la* Bonferroni. AOM, azoxymethane; BO, butter oil without water and salt; FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, refined soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil; FW, final weight.

Differences in effects between treatment groups

FO had been analysed for possible protecting properties in comparison with SO or BO in advance. The results will be mentioned in series 2.

Series 1: No statistically significant differences were found in the tumor parameters between HFO and unhydrogenated FO, irrespective of gender (Tables 18-19, Tables 5-8 Appendix V).

Series 2: Because the tumor parameters seemed more increased in females by SO and in males by BO relative to the other gender (Table 17), protective properties of HFO were analysed in comparison with SO for females and BO for males (Tables 18-19, Tables 9-12 Appendix 5).

In the preanalysis, FO clearly reduced tumor incidence, number, and load in females, but there was no statistically significant difference for tumor size, compared with SO. When FO was hydrogenated; THFO reduced tumor incidence (RR SO-THFO = 2.70), PHFO reduced tumor size (md = -3.9 mm^2), and PHFO and HHFO reduced tumor load (md = -6.0 and -6.2 mm^2, respectively) in females relative to SO, and tumor incidence, size, and load were related to type of oil in the diet.

Table 18 Series 1 and 2: Ranked tumor parameters and final weight in female AOM treated A/J mice and statistical evidence for change in effects, or in protection compared with SO, when fish oil was hydrogenated.

Series 1 and 2 Females, tumor	Ranking					Increase by HFO found compared with		Statistical evidence* for differences	association
						FO	SO		
	SO > PHFO > THFO = HHFO > **FO**								
Incidence	0.900	0.571		0.333	0.182	no	no	FO: no, SO: yes	FO: no
								THFO, weak HHFO	SO: yes
	SO > PHFO > THFO = HHFO = **FO**								
Number	1.5	1.0		0.0		no	no	FO: no, SO: no	FO: no
									SO: no
Load, mm^2	6.18	0.16		0.00		no	no	FO: no, SO: yes	FO: no
								PHFO, HHFO	SO: yes
	SO > **FO** > THFO > HHFO > PHFO								
Size, mm^2	4.44	3.70	3.14	1.04	0.50	no	no	FO: no, SO: yes	FO: weak
								PHFO	SO: yes
	SO > **FO** > PHFO > THFO > HHFO								
FW, g	25.0	20.9	19.0	18.9	17.8	no	no	FO: yes all	FO: strong
								SO: strong all	SO: strong

*Statistical evidence: strong, $P \leq 0.005$; yes, $P \leq 0.05$; weak (tendency), $0.20 \geq P > 0.05$; no, $P > 0.20$, p-values have been adjusted *a la* Bonferroni. AOM, azoxymethane; SO, refined soybean oil; FO, native fish oil; HFO, hydrogenated fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; FW, final weight.

In the preanalysis, FO seemed to reduce tumor incidence and load, but to increase tumor size, in males, compared with BO, but these results were not statistically significant. When FO was hydrogenated; THFO seemed to increase tumor incidence, number, and load and PHFO to increase tumor size in males compared with BO, not either statistically significant. HHFO, on the contrary, reduced tumor load (md = -4.0 mm^2), and tumor incidence, number, and load were related to type of oil in the diet.

Table 19 Series 1 and 2: Ranked tumor parameters and final weight in male AOM treated A/J mice and statistical evidence for change in effects, or in protection compared with BO, when fish oil was hydrogenated.

Series 1 and 2 Males, tumor	Ranking	Increase by HFO found compared with FO	BO	Statistical evidence* for differences	association
	THFO > **BO** > **FO** > PHFO > HHFO				
Incidence	0.909 0.846 0.667 0.625 0.417	no		FO: no	FO: weak
			no	BO: weak HHFO	BO: yes
Load, mm^2	4.45 3.97 0.79 0.54 0.00	no		FO: no	FO: yes
			no	BO: yes HHFO weak PHFO	BO: strong
	THFO > **BO** = **FO** = PHFO > HHFO				
Number	2.0 1.0 0.0	tendency THFO		FO: weak THFO	FO: yes
			no	BO: no	BO: yes
	PHFO > **FO** > **BO** > THFO > HHFO				
Size, mm^2	2.01 1.33 1.08 0.91 0.81	no		FO: no	FO: no
			no	BO: no	BO: no
	BO > **FO** > THFO > PHFO > HHFO				
FW, g	27.5 26.7 23.1 22.4 20.3	no		FO: strong all	FO: strong
			no	BO: strong all	BO: strong

*Statistical evidence: strong, $P \leq 0.005$; yes, $P \leq 0.05$; weak (tendency), $0.20 \geq P > 0.05$; no, $P > 0.20$, p-values have been adjusted *a la* Bonferroni. AOM, azoxymethane; BO, butter oil without water and salt; FO, native fish oil; HFO, hydrogenated fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; FW, final weight.

Series 3: In females, THFO increased tumor size relative to PHFO (md = 2.6 mm^2), and PHSO increased tumor number relative to HHSO (md = 2). Tumor size was related to degree of HFO in the diet for females and tumor number to degree of HSO for both females and males. In males, THFO increased tumor number compared with PHFO (md = 1) or HHFO (md = 2) and tumor load compared with HHFO (md = 4.5 mm^2), and tumor number and load were related to degree of HFO in the diet. Tables 20-21 and Tables 13-20 Appendix V.

Table 20 Series 3: Ranked tumor parameters and final weight in female AOM treated A/J mice and statistical evidence for increasing effects with increasing hydrogenation degree of fish oil (a) or soybean oil (b).

Series 3 Females, tumor	Ranking			Increase found with increasing hydrogenation degree of HFO or HSO	Statistical evidence* for differences	association
a) Hydrogenated fish oils						
	PHFO > HHFO = THFO					
Incidence	0.571	0.333		no	no	no
Number	1.0	0.0		no	no	no
Load, mm^2	0.16	0.00		no	no	no
	THFO > HHFO > PHFO					
Size, mm^2	3.14	1.04	0.50	no, but TH>PH	yes TH-PH	yes
	PHFO > THFO > HHFO					
FW, g	19.0	18.9	17.8	no, but weak tendency TH>HH	weak PH-HH weak TH-HH	weak
b) Hydrogenated soybean oils						
	PHSO > THSO > HHSO					
Incidence	0.615	0.500	0.313	no	no	no
Number	2.0	0.5	0.0	no	yes PH-HH	yes
Load, mm^2	8.29	0.32	0.00	no	weak PH-HH	weak
	PHSO > HHSO > THSO					
FW, g	20.5	18.1	18.0	no	yes PH-HH yes PH-TH	strong
	HHSO > PHSO = THSO					
Size, mm^2	3.97	3.14		no	no	no

*Statistical evidence: strong, $P \leq 0.005$; yes, $P \leq 0.05$; weak (tendency), $0.20 \geq P > 0.05$; no, $P > 0.20$, p-values have been adjusted *a la* Bonferroni. AOM, azoxymethane; HFO, hydrogenated fish oils; HSO, hydrogenated soybean oils; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil; PH, the partial hydrogenation degree; HH, the high hydrogenation degree; TH, the total hydrogenation degree; FW, final weight.

Table 21 Series 3: Ranked tumor parameters and final weight in male AOM treated A/J mice and statistical evidence for increasing effects with increasing hydrogenation degree of fish oil (a) or soybean oil (b).

Series 3 Males, tumor	Ranking			Increase found with increasing hydrogenation degree of HFO or HSO	Statistical evidence* for differences	association
a) Hydrogenated fish oils:						
	THFO > PHFO > HHFO					
Incidence	0.909	0.625	0.417	no, but tendency TH>HH	weak TH-HH	weak
Number	2.0	1.0	0.0	no, but TH>PH, TH>HH	yes TH-PH, TH-HH	yes
Load, mm^2	4.45	0.54	0.00	no, but TH>PH, TH>HH	yes TH-PH, TH-HH	strong
FW, g	23.1	22.4	20.3	no, but tendency TH>HH	weak TH-HH, PH-HH	yes
	PHFO > THFO > HHFO					
Size, mm^2	2.01	0.91	0.81	no	no	no
b) Hydrogenated soybean oils:						
	THSO > PHSO > HHSO					
Incidence	0.909	0.875	0.400	no, but weak tendency TH>HH	weak TH-HH	weak
	THSO = PHSO > HHSO					
Number	2.0		0.0	no, but tendency TH>HH	weak TH-HH, weak PH-HH	yes
	PHSO ≈ HHSO > THSO					
Size, mm^2	3.14	3.13	1.13	no	no	no
	PHSO > HHSO > THSO					
FW, g	23.3	22.0	21.0	no, but PH>TH	yes PH-TH	yes
	PHSO > THSO > HHSO					
Load, mm^2	4.46	0.80	0.00	no, but weak tendency TH>HH	weak TH-HH	weak

*Statistical evidence: strong, $P \leq 0.005$; yes, $P \leq 0.05$; weak (tendency), $0.20 \geq P > 0.05$; no, $P > 0.20$, p-values have been adjusted *a la* Bonferroni. AOM, azoxymethane; HFO, hydrogenated fish oils; HSO, hydrogenated soybean oils; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil; PH, the partial hydrogenation degree; HH, the high hydrogenation degree; TH, the total hydrogenation degree; FW, final weight.

Series 4: No statistically significant increases were found HFO increasing any of the tumor parameters relative to corresponding degrees of HSO, irrespective of gender (Tables 22-23 and Tables 21-24 Appendix V). On the contrary; in females, PHSO clearly increased tumor size compared with PHFO (md = 2.6 mm^2), but tumor size was not statistically significantly related to type of hydrogenated oil in the diet. And in males, PHSO increased tumor load (md = 3.9 mm^2) compared with PHFO, and tumor load was clearly related to type of hydrogenated oil in the diet.

Differences in effects with a view to the specific oils

Results are here presented from a different point of view. Some results may in this way be treated several times. Also results that are not statistically significant may be described here.

Table 22 Series 4: Ranked tumor parameters and final weight in female AOM treated A/J mice and statistical evidence for hydrogenated fish oils increasing effects compared with corresponding hydrogenation degrees of soybean oil.

Series 4 Females, tumor	Ranking	HFO found more harmful than HSO, by degree	Statistical evidence* for differences	association
	PHSO>PHFO > THSO>THFO = HHFO>HHSO			
Incidence	0.615 0.571 0.500 0.333 0.333 0.313	no	no	no
	PHSO>PHFO > THSO>THFO = HHFO=HHSO			
Number	2.0 1.0 0.5 0.0 0.0	no	no	no
	PHSO>PHFO < THSO>THFO = HHFO=HHSO			
Load, mm^2	8.29 0.16 0.32 0.00 0.00	no	no	no
	HHSO>HHFO < THFO=THSO = PHSO>PHFO			
Size, mm^2	3.97 1.04 3.14 3.14 0.50	no	strong PH	weak
	PHSO>PHFO > THFO>THSO > HHSO>HHFO			
FW, g	20.5 19.0 18.9 18.0 18.1 17.8	no	no	yes

*Statistical evidence: strong, $P \leq 0.005$; yes, $P \leq 0.05$; weak (tendency), $0.20 \geq P > 0.05$; no, $P > 0.20$, p-values have been adjusted *a la* Bonferroni. AOM, azoxymethane; HFO, hydrogenated fish oils; HSO, hydrogenated soybean oils; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil; PH, the partially hydrogenation degree; HH, the high hydrogenation degree; TH, the total hydrogenation degree; FW, final weight.

Table 23 Series 4: Ranked tumor parameters and final weight in male AOM treated A/J mice and statistical evidence for hydrogenated fish oils increasing effects compared with corresponding hydrogenation degrees of soybean oil.

Series 4 Males, tumor	Ranking	HFO found more harmful than HSO, by degree	Statistical evidence* for differences	association
	THFO=THSO > PHSO>PHFO > HHFO>HHSO			
Incidence	0.909 0.875 0.625 0.417 0.400	no	no	yes
	THFO=THSO = PHSO>PHFO > HHFO=HHSO			
Number	2.0 2.0 1.0 0.0	no	weak PH	strong
	THFO>THSO < PHSO>PHFO > HHFO=HHSO			
Load, mm^2	4.45 0.80 4.46 0.54 0.00	no	yes PH	strong
	PHSO>PHFO < HHSO>HHFO < THSO>THFO			
Size, mm^2	3.14 2.01 3.13 0.81 1.13 0.91	no	no	no
	PHSO>PHFO < THFO>THSO < HHSO>HHFO			
FW, g	23.3 22.4 23.1 21.0 22.0 20.3	only TH	yes TH	yes

*Statistical evidence: strong, $P \leq 0.005$; yes, $P \leq 0.05$; weak (tendency), $0.20 \geq P > 0.05$; no, $P > 0.20$, p-values have been adjusted *a la* Bonferroni. AOM, azoxymethane; HFO, hydrogenated fish oil; HSO, hydrogenated soybean oils; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil; PH, the partial hydrogenation degree; HH, the high hydrogenation degree; TH, the total hydrogenation degree; FW, final weight.

Between genders

PHSO seemed to increase tumor incidence in males {RR = 1.42, with CI = (0.86, 2.35, P = 0.336) and to increase tumor load in females (md = 3.8 mm^2, P = 0.875) relative to each other.

SO increased tumor size (md = 3.2 mm^2, P = 0.05) and also seemed to increased tumor incidence and load (P = 0.303 and 0.251, respectively) in females relative to males.

THFO increased tumor incidence {RR = 2.73, with CI = (1.20, 6.20) and P = 0.009} and number (md = 2.0, P = 0.041), and tended to increase tumor load (md = 4.4 mm^2, P = 0.081), in males relative to females. On the contrary, THFO increased tumor size in females relative to males (md = 2.2 mm^2, P = 0.026).

THSO tended to increase tumor incidence {RR = 1.82, with CI = (1.00, 3.30), P = 0.069}and tumor number (md = 1.5, P = 0.092) and seemed to increase tumor load (md = 0.5 mm^2, P = 0.309) and reduce tumor size in males relative to females (md = -2.8 mm^2, P = 0.218).

PHFO seemed to increase tumor incidence {RR = 1.09 (0.52, 2.31), P = 1.00}, size (md = 0.0 mm^2, P = 0.915) and load (md = 1.5, P = 0.253) in males relative to females, but no statistical evidence was found.

FO increased tumor load (md = 0.8 mm^2, P = 0.035), tended to increase tumor incidence {RR = 3.67, with CI = (0.96, 13.95), P = 0.065) and tumor number (md = 1.0 mm^2, P = 0.053) in males relative to females.

BO tended to increase tumor incidence {RR = 1.69, with CI = (0.95, 3.00), P = 0.103}, number (md = 0.5, P = 0.147) and load (md = 3.8 mm^2, P = 0.086), and seemed to increase tumor size (md = 0.1 mm^2, P = 0.817) in males relative to females.

In the ***HH degree*** of fish and soybean oil, there was probably no difference between the genders in incidence or tumor number (P = 1.00 or near), except that HHFO seemed to increase tumor size in females relative to males (md = 0.2 mm^2, P = 0.239).

Between specific oils

In females, **PHSO** increased tumor number relative to HHSO (md = 2.0 mm^2, P = 0.048, and P = 0.039 for the relation between tumor number and type of HSO in the diet), reduced tumor size (md = -2.6 mm^2, P = 0.030, and P = 0.031 for the relation between tumor number and type of HSO in the diet), and tended to increase tumor load relative to HHSO (md = 8.3 mm^2, P = 0.123, and P = 0.094 for the relation between tumor load and type of HSO in the diet).

In males, PHSO tended to increase tumor number relative to HHSO (md = 2.0 mm^2, P = 0.111, and P = 0.033 for the relation between tumor number and type of HSO in the diet).

In females; ***SO*** increased tumor incidence relative to THFO {RR = 2.70, with CI = (1.18,6.17), P = 0.033, and P = 0.029 for the relation between tumor incidence and type of oil in the diet}, tumor size relative to PHFO (md = 3.9 mm^2, P = 0.048, and P = 0.036 for the relation between tumor size and type of oil in the diet), and tumor load relative to both PHFO and HHFO (md = 6.0 mm^2, P = 0.030, and md = 6.2 mm^2, P = 0.045, respectively, and P = 0.041 for the relation between tumor load and type of oil in the diet).

In the preanalysis of protecting properties of FO, SO increased tumor incidence, number, and load in females relative to FO, while no statistically significant differences were found for males. SO was not tested for males against any other oil.

In females; ***THFO*** reduced tumor incidence relative to SO {RR SO-THFO = 2.7, with CI = (1.18, 6.17), and P = 0.033, and = P = 0.029 for the relation between tumor incidence and type of oil in the diet} and increased tumor size relative to PHFO (md = 2.6 mm^2, P = 0.030, and P = 0.031 for the relation between tumor size and degree of HFO in the diet).

In males; THFO tended to increase tumor incidence relative to HHFO {RR = 2.18 with CI = (1.09, 4.37), P = 0.081, and P = 0.051 for the relation between tumor incidence and degree of HFO in the diet), increased tumor number relative to PHFO and HHFO (md = 1.0, P = 0.030 and md = 2.0, P = 0.045, respectively, and P = 0.011 for the relation between tumor number and degree of HFO in the diet) and also tended to increase it relative to FO (md = 1.0, P = 0.099, and P = 0.023 for the relation between tumor number and type of fish oil in the diet).

THFO increased tumor load in males relative to HHFO (md = 4.4 mm^2, P = 0.006), tended to increase it relative to PHFO (md = 3.9 mm^2, P = 0.051), and P = 0.005 for the relation between tumor load and degree of HFO in the diet, and also seemed to increase it relative to FO (md = 3.7 mm^2, and p = 0.017 for the relation between tumor load and type of fish oil in the diet).

In females, none of the differences regarding ***THSO*** were statistically significant.

In males, THSO tended to increase tumor incidence {RR = 2.27, with CI = (0.76, 6.76), P = 0.189}, tumor number (md = 2.0, P = 0.078), and tumor load (md = 0.8 mm^2, P = 0.156) relative to HHSO, and tumor incidence and load were weakly related to type of HSO in the diet (P = 0.094 and 0.085, respectively) while tumor number was related (P = 0.033).

In females, ***PHFO*** reduced tumor size (md = -3.9 mm^2, P = 0.048) and load (md = -6.0 mm^2, P = 0.030) relative to SO, and P = 0.041 and 0.036, respectively, for the relation between tumor size and type of oil in the diet.

In males, PHFO tended to reduce tumor load relative to BO (md = -3.4 mm^2, P = 0.105, and P = 0.003 for the relation between tumor load and type of oil in the diet).

FO was not found to differ statistically significant from HFO. In the preanalysis of protecting properties, FO reduced tumor incidence, number, and load in females relative to SO, but no statistically significant differences was found for males relative to BO. FO tended to reduce tumor number in males relative to THFO (md = -1.0, P = 0.099), and P = 0.023 for the relation between tumor number and type of fish oil in the diet. FO was not tested against the other oils.

In females, the only test regarding ***BO*** was in the preanalysis of protecting properties of FO. BO seemed to increase tumor incidence {RR FO-BO = 0.36, with CI = (0.09, 1.42), P = 0.624) and number (md = 0.5, P = 0.204), relative to FO. In this analysis, also SO was compared with BO. BO seemed to reduce tumor incidence (RR SO-BO = 1.80, with CI = (1.03, 3.16), P = 0.237}, number (md = -1.0, P = 0.294), size (md = -3.5 mm^2, P = 0.120), and reduced load (md = -6.0 mm^2, P = 0.042), relative to SO, and P = 0.004, 0.002, 0.135, and 0.002, respectively, for the relations between tumor incidence, number, or size and type of oil in the diet.

In males, the differences regarding BO in the preanalysis had higher P-values. BO tended to increase tumor incidence {RR = 0.49, with CI = (0.24, 1.00), P = 0.123}, seemed to increase tumor number (md = 1.0, P = 0.252), and increased tumor load (md = 4 mm^2, P = 0.018), relative to HHFO, and P = 0.045, 0.019, and 0.003, respectively, for the relations between tumor incidence, number, and load and type of oil in the diet.

In females, ***HHFO*** tended to reduce tumor incidence {RR SO-HHFO = 2.70, with CI = (1.05, 6.96), P = 0.060), seemed to reduce tumor number (md = -1.5, P = 0.234), and reduced tumor load (md = -6.2 mm^2, P = 0.045), relative to SO, and P = 0.029, P = 0.236, and 0.041 for the relation between tumor incidence, number, or load and type of oil in the diet)

In males, ***HHFO*** tended to reduce tumor incidence {RR = 0.49 with CI = (0.24, 1.00), P = 0.123}, seemed to reduce tumor number (md = -1.0, P = 0.252), and reduced tumor load (md = -4.0 mm^2, P = 0.018), relative to BO, and P = 0.045, 0.019 and 0.003, respectively, for the relations between tumor incidence, number, or load and type of oil in the diet).

HHFO also tended to reduce tumor incidence relative to THFO {RR THFO-HHFO = 2.18, with CI = (1.09, 4.37), P = 0.081} and number (md = -2.0 mm^2, P = 0.045), and reduced tumor load (md = -4.4 mm^2, P = 0.006), and P = 0.51, 0.011, and 0.005, respectively, for the relations between tumor incidence, number, or load and type of HFO in the diet.

In females, ***HHSO*** seemed to reduce tumor incidence {RR = 0.51, with CI = (0.22, 1.18), P = 0.309}, number (md = -2.0 mm^2, P = 0.048), and load (md = -8.3, P = 0.123), relative to PHSO, and P = 0.254, 0.029, and 0.094, respectively, for the relations between tumor incidence, number, or load and type of HSO in the diet.

In males, ***HHSO*** tended to reduce tumor incidence {RR = 2.27, with CI = (0.76, 6.76), P = 0.189}, tumor number (md = -2.0, P = 0.078), and tumor load (md = -0.8 mm^2, P = 0.156), relative to THSO, and P = 0.094, 0.033, and 0.085, respectively, for the relations between tumor incidence, number, or load and type of HSO in the diet.

HHSO also tended to reduce tumor number relative to PHSO (md = -2.0, P = 0,111) and seemed to reduce tumor load (md = -4.5 mm^2, P = 0.210).

DISCUSSION

4.1 ESTABLISHING TEST SYSTEM

4.1.1 BREEDING

The reproductive performance and behaviour of inbred strains varies depending on the individual strain. Breeding with A/J mice seemed to be difficult due to problems with infertility and cannibalism. Exposure to sudden noises, vibrations, and excess handling may influence breeding (241). Sudden noises from outside the building and noises and vibrations from equipment inside were unavoidable. It is not known whether such disturbances partly were the reason for the behaviour of the animals.

Biological variation

The large variation in effect parameter values within treatment groups might indicate biological variation in the A/J mice. This makes it difficult to obtain statistical significance for real differences, and necessitates larger samples in order to make investigations meaningful (251). Inbred strains of mice are maintained by successive brother x sister matings. The fifty female and ten male animals used as parents in the breeding at the Norwegian Institute of Public Health cannot have come from the same female and male couple. Thus, the breeding performed here was not brother x sister matings. Whether this mating procedure could increase the biological variation of the animals is not known.

Experimental period

When animal models are used in research, the experimental treatment is usually started at the same time for all of the animals to avoid time point dependent variations of influencing factors. Such an approach was not possible in this instance, since several rounds of breeding had to take place. The animals were born during a time period of approximately two and a half months. This method is seldom used. However, delegating the diets from the start of the schedule in several turns should assure representation of all of the treatments throughout the whole experimental period. In this way, a possible error or weakness with the experiment due to time point dependent factors should be more evenly distributed on the treatment groups.

4.1.2 HOMOGENEITY OF THE FEEDS

Homogenous dispersion of the oils in the feeds is important in order to achieve reproducible treatment doses. It has earlier been shown that, due to the high content of tristearin in totally hydrogenated fat and *trans* fatty acids in partially hydrogenated fat, melting of the fat into the feed is unfavourable, since it hardens very quickly. Fat drops are then formed, which may not be eaten by the animals (217).

In this study, unheated fats were mixed into the pulver feed AIN-76M. Even if the experimental oils differed a lot with regard to consistency, the mixing of as much as 20% by weight unheated oils/fats by food processor into the basis feed seemed to produce adequate homogeneity for all the feeds. The employed procedure prevented unnecessary degeneration of the oils by termic oxidation or hydrolysis. This method has also earlier been used with success at the Norwegian Institute of Public Health (252).

4.1.3 FEEDING AUTOMATS

A new feeding automat for pulverized feed was invented at the Norwegian Institute of Public Health in 1994, and further developed in 1995 and 2002 (253-255). One type of these models was used in this study. The differing sized feeding openings turned out to be of major advantage. Through a certain age period, the animals were playing inside the automats, which led to feed wasting. Smeary feeds stuck to their fur, while fluffy feeds just blow away. In such periods, automats with small openings were used. Adult animals were given automats with small openings for fluffy feeds and large openings for sticky feeds. In this way, feed waisting diminished and the access to the feed was enhanced.

Securing continual access to feed for the animals was, however, time consuming. The stickiness of some of the feeds led to extra work, and feeding had to be attended to every day. Secondly, rodents do not normally prefer pulverized feed. In fact, the animals did themselves produce pellet like clumps from some of the feeds, which they dragged around in the cages. Clumps like that were never seen during filling of the automats. Besides, they were too large to pass through the top lattice into the automats. In addition, pulverized feed is easily wasted. Feed in the form of pellets would function much better. There are, however, limitations for pelleting diets. Diets with about 17-21% of weight fats/oils produce fairly soft and usually fragile pellets, and the pelleting capability diminishes with further increases in fat/oil (256). Further attempts to find new pelleting methods for feeds with high fat contents are needed.

4.1.4 CONFOUNDING FACTORS IN THE FEEDS

The feeds included a very high energy percent of fat, 38.5%. Findings indicate that increased ingestion of fats markedly enhances tumor promotion in the colon (257-260). But since fat contributed the same energy percent to all of the diets, the treatment groups would be equally affected. The same goes for the high content of sucrose in AIN-76M (261;262).

4.2 PINWORM INFECTION

The animals were not medicated against the pinworm (*Syphacia obvelata/muris*), since such treatment might influence the effects of the experimental diets at a higher degree than the pinworm infection. An infection like this may be subclinical, but if it develops it can influence the immune system of the animals. An influence on colon cancer models has not been documented, but is not out of the question (263).

The A/J mice were not the source of the infection. One male A/J mouse was sacrificed to check for the worm. After thorough examination, one worm was found. It was visible by the eye. When the colons were dissected after 15 weeks, they were not checked especially for worm, but no worms were seen. If the infection had been severe, worms would have been detected. When the colons were examined in the LM, a bloody bubble was found in 1 of 243 colons. This might have been a result of the infection. It seemed as a high fat diet did not create a desirable environment for the worm.

4.3 TESTING OF THE EXPERIMENTAL OILS

There has been an increasing CRC trend in Norway over the last decades compared with the other Nordic countries. Norway has had a higher consumption of hydrogenated fish oils and cod liver oils relative to its neighbours. The first aim of this study was to examine whether hydrogenated fish oil in the diet can increase induction and/or growth of CRC in azoxymethane (AOM) treated A/J mice, by setting up three hypotheses that were addressed by four series of analyses.

The effects of the experimental oils are dealt with thoroughly in the manuscript for an article (Appendix IV), where also patterns of interest in the material are discussed based on the same series of analyses, which was the second aim. In the present paragraph, only the most important results are dealt with.

4.3.1 DIFFERENCES IN EFFECTS BETWEEN TREATMENT GROUPS

Consistent with results in Min mice (224), no statistically significant evidence was found in support of the first two hypotheses concerning increased effects of HFO in the diet, neither relative to unhydrogenated FO, nor with increasing hydrogenation degree, irrespective of gender. For increasing hydrogenation degree, this result also applied to the HSO. There was however some support, regarding males; for the first hypothesis by a tendency to increased tumor number and loss of protecting properties by THFO relative to FO, and for the latter hypothesis by a tendency to 2.2 times increased tumor risk by THFO relative to HHFO, and statistically significantly increased tumor number and load by THFO relative to HHFO and tumor number and size with a tendency to increased tumor load by THFO relative to PHFO. And, regarding females, statistically significantly increased tumor size was found by THFO relative to PHFO. Similar support was found for HSO; a tendency to increased tumor number and weak tendencies to increased tumor risk and load by THSO relative to HHSO.

Patterns in this material seemed to indicate that the HFO had effects different from FO, and that these effects rather than being caused by a change in fish oil by the hydrogenation process *per se*, might be related to specific hydrogenation degrees of the oils. This notion also seemed to apply to the effects of HFO in the study with Min mice, which neither showed statistically significant differences. This was not supported by statistically significant findings for HFO, but there was some support for HSO; increased tumor number and a weak tendency to increased tumor load in females and a tendency to increased tumor number also in males were found by PHSO relative to HHSO. If the effects had been caused by the hydrogenation process *per se*, all of the hydrogenated oils would have been expected to increase the effects both relative to their respective unhydrogenated oils and with increasing hydrogenation degree. Thus, one cannot due to the supporting results in the first paragraph of this section rule out the possibility that also the effects of HFO were related to specific hydrogenation degrees.

Unhydrogenated FO had been analysed in advance for protecting properties in comparison with SO and BO. When HFO were compared with FO, no statistically significant differences in effects were found, thus showing no evidence of change in protective properties when FO was hydrogenated. When HFO, however, in addition were analysed for protecting properties in comparison with the same control oils that had been used for FO, protecting properties were still found for some of the HFO; in females, for THFO regarding tumor incidence, for

PHFO regarding growth, and for PHFO and HHFO regarding tumor load, relative to SO, and in males, for HHFO regarding tumor load relative to BO. Also weak tendencies to protective properties were found for HHFO regarding tumor incidence in both genders and for PHFO regarding tumor load in males, compared with the two respective control oils.

Patterns in the material seemed to indicate that protecting properties might not only be lost, but also gained, when FO was hydrogenated. This seemed to pertain to for example the above mentioned PHFO regarding growth in females and HHFO regarding tumor load in males. There was, however not conclusive evidence.

It has been stated that the relatively scarce knowledge about the relationship between TFA and cancer risk partly is due to difficulties in obtaining a suitable control fat for proper evaluation of the impact of TFA (176). Patterns of effects in the material seemed to indicate that when protecting properties of HFO were studied with SO and BO as controls, the control oils might contain substances affecting the figures describing these properties. This could have led to confounding interpretation of the results, if it were not for the fact that both FO and the HFO were studied in comparison with the same control oils. Since the control oils were SO for females and BO for males, the figures for protection cannot be compared directly for females and males. But this was done because in the preanalysis of protecting properties of FO, it seemed as FO did not protect males like it protected females, and SO seemed to increase the tumor parameters less and BO more in males than in females. This way the chance of revealing effects was larger, and the number of comparisons somewhat reduced.

No statistically significant evidence was either found in support of the third hypothesis, i.e., of increased effects of HFO in the diet relative to corresponding hydrogenation degrees of soybean oil, irrespective of gender, even though HHFO seemed to increase tumor incidence slightly in both genders relative to HHSO, and THFO to increase tumor load substantially relative to THSO (there was some doubt about this result). On the contrary, patterns in the material seemed to indicate that all the hydrogenation degrees of soybean oil might increase some effects relative to the corresponding hydrogenation degrees of fish oil, seemingly in 14 of the 24 comparisons. Indeed, relative to PHFO; PHSO clearly increased growth in females (although the relation between tumor size and type of hydrogenated oil in the diet was weak) and tumor load in males considerably, and also a tendency to increased tumor number was found for males. But there was no evidence for the rest of the comparisons.

4.3.2 DIFFERENCES IN EFFECTS BETWEEN GENDERS

Even if the analyses were undertaken based on specific hypotheses, many comparisons were made in total in this study. This increases the risk of finding false positive results (251). The p-values for the gender differences were not adjusted *a la* Bonferroni; if they had been, many of them would not have been statistically significant. It seems, however, important to mention the effects of THFO, SO, and FO, see below. Relations were found between tumor incidence, number, size or load and gender (P = 0.001, 0.003, 0.001 and 0.033, respectively). Totally seen, males had 1.5 times as high tumor risk as females in this experiment {RR = 1.53, with CI = (1.20, 1.95)}. The effects seemed to be depending on gender for at least some of the experimental oils.

4.3.3 EFFECTS OF THE EXPERIMENTAL OILS ON BODY WEIGHT

Irrespective of gender, the oils mostly seemed to influence body weight the same way through the whole experimental period. All of the experimental oils each seemed to have a similar effect on body weight in both genders, perhaps except PHFO and THFO, and was seemingly ranked as follows: SO > BO > FO > PHSO > PHFO (♂THFO) > THFO (♂PHFO) > HHSO > THSO > HHFO. The weights were increased more in males than in females for all of the oils. The differences in FW between males and females seemed to be ranked as follows: FO > BO > SO > THFO > HHSO > PHFO > THSO > PHSO > HHFO, and the differences in feed expenditure: FO > SO > PHFO > BO > PHSO > THFO > THSO > HHSO > HHFO.

Statistically significantly increased FW were found for FO relative to HFO in both genders gender (highly statistically significant for males), for SO relative to HFO in females and for BO relative to HFO in males (highly statistically significant), for PHSO relative to HHSO or THSO in females and for PHSO relative to THSO in males, and for THFO relative to THSO in males. Tendencies to increased FW were found for PHFO or THFO relative to HHFO in both genders.

4.3.4 INFLUENCE OF GENDER AND BODY WEIGHT

The data material did not fit the criteria for the use of tests which take into consideration putative interference with the effects of the experimental oils by gender and body weight simultaneously. Therefore, females and males were analysed separately. No conclusions can be made on interference by body weight on the tumor parameters. Patterns in the material did, however, give some clues.

It looked like body weight might affect induction and/or growth of CRC, possibly dependent on experimental oil and gender. High body weight is known to affect sex hormones through leptin and to increase CRC, and, in humans, it was earlier found that the relationship between leptin and the sex hormone testosterone is influenced by progress of puberty and is different in obese girls and boys (75;264). It has been proposed that a high fat intake, which the animals in this experiment were exposed to, raises the concentrations of circulating sex hormones such as estrogen (265). Conversion of estradiol generates free radicals and chemically reactive intermediates (101). In recent years, several lines of epidemiologic, clinical and experimental evidences have been reported showing that estrogen hormones may be involved in malignant colorectal tumors (266). Most estrogen action appears to be exerted via estrogen receptors (ERs) on target cells (265). ERs have been reported in CRC tumors. An ER subtype found in rat, mouse, and human homologs has been demonstrated in normal and neoplastic human colorectal tissues and in vitro in colonic epithelial cells (266).

The different effects of sex hormones on body weight and its fat proportion and vice versa, and the putative effects of sex hormones and body weight on colorectal carcinogenesis, should lead to the inferences that gender differences in effects on CRC exist, and that the effects would increase with increasing body weight. Since the latter mostly was not the fact in this study, additional risk factors for CRC not only to gender but also to body weight had to be present. At the same time, this shows that gender and body weight, rather than being confounding risk factors of their own, were intermediate risk factors for substances in the experimental oil in the carcinogenesis. If it had been possible to use a test taking into account the interference by gender and body weight, such a model would have explained the percentage of the effect variation for gender and body weight, and, in the end for the more direct effects of substances in the experimental oils.

4.3.5 WEAKNESS OF THE STUDY

This study was characterized by large variances, weak tests, and occasionally small samples which when less than ten may have weakened the usefulness of specific tests (251). The uneven distribution between genders led to samples of animals less than ten in the PHFO and HHFO groups for females and in the FO, PHSO, and HHSO groups for males, and of tumors less than ten in the FO, PHFO, HHFO, and HHSO groups for females and the FO and HHSO groups for males. These weaknesses of the study may have led to many false negative results, i.e., many of the results that were not statistically significant, but led to possible indications

when the patterns of effects in the material were studied, might possibly have been true. The probability of not detecting a real difference is depending on the power of the study, which, judged by the many large variances, was low. Thus the magnitude of false negative results might be high.

With the chosen significance level, there was a 5% risk of getting false positive results, i.e., of presenting a difference as real when it was not.

In this study, the effect measure in comparisons of groups is estimated median difference (md). The real figure for the difference may vary grossly from the estimated one. Since the statistical tests that were used do not calculate variance for median differences, the uncertainty of the estimated effects is not known. When effect sizes are assessed by figures, they may differ a lot from the real ones. This also applies to the assessments by words in the next paragraph.

4.3.6 EFFECTS OF SPECIFIC OILS

Some of the experimental oils seemed to be more harmful to the animals than others regarding tumor load, such as PHSO to both genders, SO to females, and THFO to males. THSO seemed to be a special case, giving a high induction rate and fairly large growth in males, and a fairly high induction rate and quite large growth in females, but unexpectedly not very large tumor load in either gender.

Several results seem to indicate that the effects might be dependent on phase of colorectal carcinogenesis for most of the oils.

PHSO and SO has been increasingly used in Norway since production of these oils was started in here in the 1960s. PHSO is now used to a low degree. THFO is not used in human foods. THSO has been used in interesterified form during the last decade, since it was substituted for PHSO. In addition, PHFO has been used since 1912, and FO (as cod liver oil) and BO from even earlier times. Thus, it seems important to elucidate the effects of these oils.

The **PHSO** contained a large amount of TFA (42%) of C18:1, a putative risk factor for CRC, at least in humans (177). Relative to the other oils tested, PHSO seemed to give both genders

about the highest induction and growth of CRC and tumor load, but tumor incidence seemed to be increased and tumor load reduced in males relative to females. PHSO clearly increased growth in females and tumor load in males considerably relative to PHFO. Although no direct evidence, these results seem to support the idea that TFA as risk factors for CRC.

The **SO** contained a large amount of ω-6 fatty acids (55%) and a high ω-6/ω-3 ratio (9.4:1), both putative risk factors for CRC possibly in both humans and rodents (267). It has been proposed that exocyclic adducts might be formed in the colonic epithelium via lipid peroxidation of ω-6 PUFA, and that this is related to *induction* of tumors (268). Results from animal models indicate that ω-6 PUFA have a tumor enhancing effect, predominantly during the *post initiation phase* (154;269).

In addition, a high intake of the ω-6 PUFA linoleic acid inhibits the detoxification of estrogens (267), and synergism between high dietary intake and estrogen catabolism has been suggested for females (101). It has been suggested that estrogens and their receptors play an important role in the *progression* stage of colorectal tumors (266). In this study, SO seemed to give females about the highest induction and growth of CRC and the second highest tumor load, relative to the other oils tested. This seems to fit well with the above mentioned propositions and findings.

If lipid peroxidation of ω-6 PUFA increases colorectal carcinogenesis in female animals, it seems logic that also males would be affected. The tumor parameter figures also seemed to be high in males. The high fat diets may have increased the body weight of the animals, and in addition, SO seemed to increase body weight more than the other oils. Increased body weight is a risk factor for CRC. Thus, the high body weight may also explain a part of the large effect in both genders, both alone and as an intermediate risk factor for substances in SO. Increased body weight also affect sex hormones, which might be one of the mechanisms that makes it a risk factor for CRC (see *Influences of gender and body weight*).

The effects of SO still seemed to be considerably lower in males than in females. Estrogen is typically a female hormone, but is also produced in males. If a high intake of ω-6 PUFA inhibits the detoxification of estrogens in females, it would probably do so in males too, but the effects would be less since males have a much lower level of estrogen than females. Also, the proportion of leptin and estrogen producing body fat is usually less in males than in

females. This might partly explain the lower effects in males (264;270). Male hormones may also influence these questions.

The **THFO** contained a high amount (97%) of VLCSFA (31%) and LCSFA (64%), which even though they are poorly absorbed in male rodents (142), have been suspected to be risk factor for CRC. Relative to the other oils tested, **THFO** seemed to give males about the highest induction of CRC and tumor load but fairly low growth, and to give females low induction and tumor load, but large growth. Induction and tumor load by THFO seemed to be increased and growth reduced in males relative to females. This seems to support the idea of substances in THFO being risk factors for CRC, whether it could be the content of VLCSFA or LCSFA, or the high total amount of SFA that increases the effects.

The THFO is used in animal feed (224), but so far not in foods for humans. The possibility of using THFO in interesterified form in foods for humans has been shown interest. If the interesterification is non specific, a part of the SFA will change place in the TG from their natural *sn*-1 or 3 positions to the *sn*-2 position, where they might be absorbed to a larger degree. This might increase the putative pathological consequences of THFO (271).

The **THSO** contained nearly 100% SFA, of which 83% were C18 and 15% C16. Earlier evidence seems consistent with no relationship between palmitic or stearic acids and CRC in humans (179). The results of the present study did not seem to be consistent with this. Relative to the other oils tested, THSO seemed to give males about the highest induction of CRC but not so large growth and tumor load, and to also give females fairly high induction and large growth but low tumor load. Induction by THSO seemed to be increased and growth reduced in males relative to females. THSO seemed to give males equal induction of CRC relative to THFO, somewhat higher growth, and strangely, much lower tumor load. The results seem to indicate the possibility that high amounts of C18 and 16 SFA might be risk factors for CRC, contrary to earlier findings.

The **PHFO** contained a large amount of TFA (55-60%) mostly of VLCFA C20-22 PUFA, C18:1 and 16:1. Relative to the other oils tested, PHFO seemed to give both genders a fairly high induction of CRC and a fairly low growth and tumor load. Growth seemed to be increased, and tumor load a little increased, in males relative to females. This seems to indicate that PHFO even if it hydrogenated still contains protective substances.

FO consists to a large degree of ω-3 fatty acids, especially the EPA and DHA (212). The FO in this study contained EPA/DHA (20.5%), other PUFA (12.5%), MUFA (30%), unidentified fatty acids (2%), and a high content of LCSFA (36%). An impressive body of epidemiological data suggests an inverse relationship between CRC risk and consumption of diets rich in ω-3 fatty acids, and earlier evidence show that ω-3 PUFA act at different stages of cancer development (9;272). The present results seemed to be in accordance with this, with the exception of gender differences. Relative to the other oils tested, FO seemed to give females about the lowest induction of CRC and tumor load, but large growth, and seemed to give males a high induction, but not very large growth and tumor load. Tumor incidence, number, and load seemed to be increased in males relative to females.

Besides the dependence of phase of the carcinogenesis, both the effects on both genders and the gender differences in the effects of FO might be related to body weight as well. There is a possibility that the high contents of fat and/or sucrose in the diets may have affected the animals differently due to differences in the amounts eaten. According to the feed expenditure measured for females and males in total, the intake of the FO diet might have been the highest, and not far from 50% higher than the possibly lowest, which was that of the HHFO diet. The intake would probably also have been higher for males than for females. The higher intake of fat and sucrose may have affected the males relative to the females.

A 20% by weight FO diet has earlier been shown to increase both secondary tumor formation in colon cancer involving liver metastases in rats, and to develop 1000 fold more metastases than in rats fed a low fat diet or a diet enriched with safflower oil (273).

The **BO** contained 65% SFA mostly of C16, 18 and 14. The MCFA content was 21%. Relative to the other oils tested, BO seemed to give males a fairly high induction of CRC and large tumor load, and tended to increase them considerably relative to females. But also females had fairly high induction. Incidence and tumor load tended to be increased in males relative to females. The growth was fairly low in both genders. BO seemed to increase tumor load at least 7.4 times relative to PHFO and HHFO in males.

Rises in the intake of the MCFA lauric and myristic acids among subjects at high risk for CRC and CRC patients have been reported (179). These fatty acids are able to induce COX-2 overexpression in human tissues (274), and COX-2 is upregulated from 2 to 50 fold in 85-

90% of CRC patients (275). If this applies to these animals, the high intake of MCFA might explain some of the increased effects. In addition, the results regarding THFO and THSO also points to the high amount of C16 and 18.

There is some doubt about the amounts of TFA in the **HH degree** of the fish and soybean oil in this study. But, there was a predominance of changed C18:1 fatty acids in the HHSO (30% SFA, mostly C16), and a predominance of changed C20-22 PUFA and SFA (51% SFA, mostly C18) in the HHFO. Relative to the other oils tested; irrespective of gender, the HH degree of fish and soybean oil seemed to give the animals the lowest induction of CRC and tumor load, only second to FO regarding induction in females. HHSO seemed to give the largest growth, only next to SO in females, while HHFO did not give very large growth. There were possibly no gender differences in effects, except that tumor size seemed to be increased in females relative to males.

Both the HH degree oils seemed to be the most protecting oils in both induction and growth of CRC, only second to FO in induction, and almost without gender differences. There probably are several reasons for the seemingly protective capacity of the HHs. HHFO has earlier also shown protective potential against heart disease by reducing the plasma TG concentration in male rats compared with FO or PHFO (237).

On the background of foregoing results; since HHFO contains large proportions of TFA including VLCTFA, SFA including VLCSFA, and unusual VLCPUFA of the *cis* configuration, and less protecting fatty acids than FO or PHFO, most of the seemingly large protecting capacity of HHFO must be based on other factors than EPA and DHA.

Low body weight may partly have been the reason for the protection. Both the HHs seemed to give about the lowest FW. This might partly have been due to low fat absorption, low feed intake (seemingly the lowest feed expenditure in this experiment was measured), and increased energy expenditure by hyperactivity at a young age.

No literature was found on absorption of HH oils. But, the absorption of fatty acids in HHFO might possibly be lower than that of PHFO (95%) and higher than that of THFO (61%) (142). The HH oils had in common with the TH oils almost white faeces, an extremely harsh odour coming from their cages, like the smell of trimethylamines, and hyperactivity at a young age.

If the light coloured faeces was due to soapification of SFA with calcium and magnesium the absorption coefficient for fatty acids in HHFO would have been closest to that of THFO. Then the intake of the suspicious fatty acids would have been reduced. This may also have contributed to the reduced effects on the carcinogenesis.

The protecting properties of the HH oils do not indicate that they are harmless in colorectal carcinogenesis. Seemingly, HHSO led to the second largest growth of CRC, while it was reduced by HHFO, although not relative to BO or PHFO in females and to THFO and HHFO in males.

4.3.7 THE APPLICATION OF MOUSE DATA TO HUMANS

Even if mice are small animals with increased metabolism and short life span compared with humans, they develop tumors in the same tissues, in most cases with histology remarkably similar to that of humans (276). It must, however, be emphasized that animal models cannot substitute for humane clinical studies. Compared to small rodents, humans must have evolved more complex genetic controls over cell growth in at least some of their tissues because of their greater size and longevity (60). Animal model are, however, used for preexaminations to make the human studies more specific with a higher degree of validity. And, the mouse is the model organism which is closest to humans (62).

This experiment was performed over 15 weeks. It is possible that longer time effects might be different from those found here.

4.3.8 OVERALL CONCLUSION

Certain effect patterns in this material seemed to indicate that the effects of the experimental oils on colorectal carcinogenesis in the AOM treated A/J mice, in addition to being influenced by gender and body weight, might be different in the induction and growth phase of CRC, and dependent on type of oil in the diet and hydrogenation degree, the latter might differ between HFO and HSO. These indications were not based on statistically significant results only, so they must be taken for what they are worth. Some evidence was however found.

No statistically significant evidence was found of increasing CRC by HFO relative to unhydrogenated FO, with loss of protecting properties, nor of increasing effects with

increasing hydrogenation degree; which would have indicated that the effects of HFO were due to a change in fish oil by the hydrogenation process *per se*.

That the HH degree of HSO reduced induction of CRC relative to the TH degree, indicated that the effects of HSO were dependent on specific hydrogenation degrees, i.e., were related to specific substances in these oils. Even if the null hypotheses for HFO could not be rejected, the possibility that also the effects of HFO might be dependent on specific hydrogenation degrees could not be ruled out.

No statistically significant evidence was either found of increased effects of HFO relative to corresponding hydrogenation degrees of HSO. On the contrary, when the effects were examined with a view to the specific oils, however, it seemed as HSO were more harmful than HFO; all the tumor parameters seem to be increase by PHSO relative to PHFO, and statistical evidence was found for tumor number and load in males.

In the view to the specific oils, it actually seemed as any of the experimental oils, including those that were exemplifying oils consumed in Norway over different time periods, might contain substances that are risk factors in colorectal carcinogenesis; in any way in these animals. Even if it had been possible to draw this conclusion for the A/J mice, findings in experimental animals will not necessarily apply to man.

If the oils in this experiment really may exemplify oils used in Norway, and if the results of this study might apply to humans; the results would have indicated that of the examined oils, HHFO might not be the most likely fat to play a role in the increasing CRC trend in Norway compared with its neighbouring countries. More likely candidates would be PHFO or FO (as cod liver oil), which besides HHFO were the only of the experimental oils with increased consumption relative to the other Nordic countries, and which were used to a great degree long enough and early enough to have affected the rising trend in Norway, which started at least as late as in the 1950s, when the registering of the CRC incidence started.

4.3.9 FURTHER RESEARCH

It seems important to look into the effects of those of the experimental oils that are still in use in human foods, such as SO, THSO, PHSO, PHFO, BO and cod liver oil, or that may be put

to use in the future, such as THFO. This might also give some answers with respect to the effects of the two suspected candidates in the Nordic region.

There is some doubt about in what proportions PHFO and HHFO has been used in Norway in the earlier hard margarines. If possible, such information should be found.

It might also be interesting to look closer at what fish species have been dominating in the hydrogenation of fish oil in Norway, since some species may have larger amounts of VLCPUFA, which may make a difference for the substances in the end products after hydrogenation.

HHFO could be examined for increasing effects on CRC relative to a more neutral normal mouse diet.

Studying effects of whale oil, which was also used to a great degree in Norway from 1912 and for approximately 50 years might also give some answers regarding the CRC trend.

REFERENCES

(1) Ilyas M, Straub J, Tomlinson IP, Bodmer WF. Genetic pathways in colorectal and other cancers. Eur J Cancer 1999; 35(14):1986-2002.

(2) Møller B, Fekjær H, Hakulinen T, Tryggvadóttir L, Storm HH, Talbäck M et al. Prediction of cancer incidence in the Nordic countries up to the year 2002. Eur J Cancer Prev 2002; 11(Supplement 1).

(3) Malila N, Hakulinen T. Epidemiological trends of colorectal cancer in the Nordic countries. Scand J Surg 2003; 92(1):5-9.

(4) Cancer Registry of Norway.www.kreftregisteret.no. Cancer in Norway 2000. 2002. Report

(5) Cancer Registry of Norway. Cancer in Norway 2001. 2004. Report

(6) Hanahan D, Weinberg RA. The hallmarks of cancer. Cell 2000; 100(1):57-70.

(7) Bach SP, Renehan AG, Potten CS. Stem cells: the intestinal stem cell as a paradigm. Carcinogenesis 2000; 21(3):469-476.

(8) Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000; 408(6810):307-310.

(9) Roynette CE, Calder PC, Dupertuis YM, Pichard C. n-3 polyunsaturated fatty acids and colon cancer prevention. Clin Nutr 2004; 23(2):139-151.

(10) Leslie A, Carey FA, Pratt NR, Steele RJ. The colorectal adenoma-carcinoma sequence. Br J Surg 2002; 89(7):845-860.

(11) World Cancer Research Fund. Food, Nutrition and the Prevention of Cancer: A Global Perspective, 1st ed. Washington, DC. 1997. Am Inst Cancer Res. Report

(12) Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998; 338(21):1481-1487.

(13) Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. Cell 1990; 61(5):759-767.

(14) Schulmann K, Reiser M, Schmiegel W. Colonic cancer and polyps. Best Pract Res Clin Gastroenterol 2002; 16(1):91-114.

(15) Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996; 87(2):159-170.

(16) Baylin SB, Herman JG. DNA hypermethylation in tumorigenesis: epigenetics joins genetics. Trends Genet 2000; 16(4):168-174.

(17) Tejpar S, Van Cutsem E. Molecular and genetic defects in colorectal tumorigenesis. Best Pract Res Clin Gastroenterol 2002; 16(2):171-185.

(18) Bjerknes M. A test of the stochastic theory of stem cell differentiation. Biophys J 1986; 49(6):1223-1227.

(19) Cheng H, Bjerknes M. Whole population cell kinetics and postnatal development of the mouse intestinal epithelium. Anat Rec 1985; 211(4):420-426.

(20) Maskens AP. Histogenesis of colon glands during postnatal growth. Acta Anat (Basel) 1978; 100(1):17-26.

(21) Maskens AP, Dujardin-Loits RM. Kinetics of tissue proliferation in colorectal mucosa during post-natal growth. Cell Tissue Kinet 1981; 14(5):467-477.

(22) St Clair WH, Osborne JW. Crypt fission and crypt number in the small and large bowel of postnatal rats. Cell Tissue Kinet 1985; 18(3):255-262.

(23) Totafurno J, Bjerknes M, Cheng H. The crypt cycle. Crypt and villus production in the adult intestinal epithelium. Biophys J 1987; 52(2):279-294.

(24) Wasan HS, Park HS, Liu KC, Mandir NK, Winnett A, Sasieni P et al. APC in the regulation of intestinal crypt fission. J Pathol 1998; 185(3):246-255.

(25) Wong WM, Mandir N, Goodlad RA, Wong BC, Garcia SB, Lam SK et al. Histogenesis of human colorectal adenomas and hyperplastic polyps: the role of cell proliferation and crypt fission. Gut 2002; 50(2):212-217.

(26) Bird RP. Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen: preliminary findings. Cancer Lett 1987; 37(2):147-151.

(27) Paulsen JE, Namork E, Steffensen IL, Eide TJ, Alexander J. Identification and quantification of aberrant crypt foci in the colon of Min mice--a murine model of familial adenomatous polyposis. Scand J Gastroenterol 2000; 35(5):534-539.

(28) Reddy BS, Rao CV, Seibert K. Evaluation of cyclooxygenase-2 inhibitor for potential chemopreventive properties in colon carcinogenesis. Cancer Res 1996; 56(20):4566-4569.

(29) Kune GA. Hereditary. In: Kune GA, editor. Causes and control of colorectal cancer: a model for cancer prevention. Boston: Kluwer Academic Publishers, 1996: 48-68.

(30) Cannon-Albright LA, Skolnick MH, Bishop DT, Lee RG, Burt RW. Common inheritance of susceptibility to colonic adenomatous polyps and associated colorectal cancers. N Engl J Med 1988; 319(9):533-537.

(31) Houlston RS, Collins A, Slack J, Morton NE. Dominant genes for colorectal cancer are not rare. Ann Hum Genet 1992; 56 (Pt 2):99-103.

(32) Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, Albertsen H et al. Identification and characterization of the familial adenomatous polyposis coli gene. Cell 1991; 66(3):589-600.

(33) Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, Horii A et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science 1991; 253(5020):665-669.

(34) Ichii S, Horii A, Nakatsuru S, Furuyama J, Utsunomiya J, Nakamura Y. Inactivation of both APC alleles in an early stage of colon adenomas in a patient with familial adenomatous polyposis (FAP). Hum Mol Genet 1992; 1(6):387-390.

(35) Levy DB, Smith KJ, Beazer-Barclay Y, Hamilton SR, Vogelstein B, Kinzler KW. Inactivation of both APC alleles in human and mouse tumors. Cancer Res 1994; 54(22):5953-5958.

(36) Hawk ET, Umar A, Viner JL. Colorectal cancer chemoprevention--an overview of the science. Gastroenterology 2004; 126(5):1423-1447.

(37) Balmain A, Nagase H. Cancer resistance genes in mice: models for the study of tumour modifiers. Trends Genet 1998; 14(4):139-144.

(38) Demant P. Genetic resolution of susceptibility to cancer--new perspectives. Semin Cancer Biol 1992; 3(3):159-166.

(39) Dragani TA, Manenti G, Pierotti MA. Genetics of murine lung tumors. Adv Cancer Res 1995; 67:83-112.

(40) Lang NP, Butler MA, Massengill J, Lawson M, Stotts RC, Hauer-Jensen M et al. Rapid metabolic phenotypes for acetyltransferase and cytochrome P4501A2 and putative exposure to food-borne heterocyclic amines increase the risk for colorectal cancer or polyps. Cancer Epidemiol Biomarkers Prev 1994; 3(8):675-682.

(41) Perera FP, Weinstein IB. Molecular epidemiology: recent advances and future directions. Carcinogenesis 2000; 21(3):517-524.

(42) Rajagopalan H, Nowak MA, Vogelstein B, Lengauer C. The significance of unstable chromosomes in colorectal cancer. Nat Rev Cancer 2003; 3(9):695-701.

(43) Steffensen IL, Paulsen JE, Alexander J. [Genetic and environmental factors in colorectal cancer. Mutations in the familial adenomatous polyposis gene]. Tidsskr Nor Laegeforen 1997; 117(14):2046-2051.

(44) Luebeck EG, Moolgavkar SH. Multistage carcinogenesis and the incidence of colorectal cancer. Proc Natl Acad Sci U S A 2002; 99(23):15095-15100.

(45) Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B et al. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 1997; 275:1787-1790.

(46) Sparks AB, Morin PJ, Vogelstein B, Kinzler KW. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. Cancer Res 1998; 58(6):1130-1134.

(47) Powell SM, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN et al. APC mutations occur early during colorectal tumorigenesis. Nature 1992; 359(6392):235-237.

(48) Polakis P. The adenomatous polyposis coli (APC) tumor suppressor. Biochim Biophys Acta 1997; 1332(3):F127-F147.

(49) Fodde R, Smits R, Clevers H. APC, signal transduction and genetic instability in colorectal cancer. Nat Rev Cancer 2001; 1(1):55-67.

(50) Fodde R, Kuipers J, Rosenberg C, Smits R, Kielman M, Gaspar C et al. Mutations in the APC tumour suppressor gene cause chromosomal instability. Nat Cell Biol 2001; 3(4):433-438.

(51) Kaplan KB, Burds AA, Swedlow JR, Bekir SS, Sorger PK, Nathke IS. A role for the Adenomatous Polyposis Coli protein in chromosome segregation. Nat Cell Biol 2001; 3(4):429-432.

(52) Munemitsu S, Albert I, Souza B, Rubinfeld B, Polakis P. Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. Proc Natl Acad Sci U S A 1995; 92(7):3046-3050.

(53) Yamada Y, Oyama T, Hirose Y, Hara A, Sugie S, Yoshida K et al. beta-Catenin mutation is selected during malignant transformation in colon carcinogenesis. Carcinogenesis 2003; 24(1):91-97.

(54) Blobe GC, Schiemann WP, Lodish HF. Role of transforming growth factor beta in human disease. N Engl J Med 2000; 342(18):1350-1358.

(55) Miyaki M, Kuroki T. Role of Smad4 (DPC4) inactivation in human cancer. Biochem Biophys Res Commun 2003; 306(4):799-804.

(56) Takeda A, Stoeltzing O, Ahmad SA, Reinmuth N, Liu W, Parikh A et al. Role of angiogenesis in the development and growth of liver metastasis. Ann Surg Oncol 2002; 9(7):610-616.

(57) Laboratory Animal Unit. Compendium in Laboratory Animal Science. Oslo, Norway: 1999.

(58) Reddy BS. Colon carcinogenesis models for chemoprevention studies. Hematol Oncol Clin North Am 1998; 12(5):963-973.

(59) Pories SE, Ramchurren N, Summerhayes I, Steele G. Animal models for colon carcinogenesis. Arch Surg 1993; 128(6):647-653.

(60) Nunney L. Lineage selection and the evolution of multistage carcinogenesis. Proc R Soc Lond B Biol Sci 1999; 266(1418):493-498.

(61) Goodrow TL. One decade of comparative molecular carcinogenesis. Prog Clin Biol Res 1996; 395:57-80.

(62) Hauge JG. Biochemistry. Oslo: The University Press, 2001.

(63) UK Co-ordinating Committee on Cancer Research. UKCCCR Guidelines for the welfare of animals in experimental neoplasia. Second. 1997. London.
Serial (Book,Monograph)

(64) The Norwegian reference Centre. Norwegian legislation. http://oslovet.veths.no/ . 2004.
Electronic Citation

(65) Laboratory Animal Unit. Compendium in Laboratory Animal Science. Annelise Hem, Dag Marcus Eide, Espen Engh and Adrian Smith ed. Oslo, Norway: 2001.

(66) Giovannucci E, Willett WC. Dietary factors and risk of colon cancer. Ann Med 1994; 26(6):443-452.

(67) Ames BN. Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. Science 1983; 221(4617):1256-1264.

(68) Gaston B, Drazen JM, Loscalzo J, Stamler JS. The biology of nitrogen oxides in the airways. Am J Respir Crit Care Med 1994; 149(2 Pt 1):538-551.

(69) Otamiri T, Sjodahl R. Increased lipid peroxidation in malignant tissues of patients with colorectal cancer. Cancer 1989; 64(2):422-425.

(70) Reddy BS, Wynder EL. Large-bowel carcinogenesis: fecal constituents of populations with diverse incidence rates of colon cancer. J Natl Cancer Inst 1973; 50(6):1437-1442.

(71) Romagnolo DF, Chirnomas RB, Ku J, Jeffy BD, Payne CM, Holubec H et al. Deoxycholate, an endogenous tumor promoter and DNA damaging agent, modulates BRCA-1 expression in apoptosis-sensitive epithelial cells: loss of BRCA-1 expression in colonic adenocarcinomas. Nutr Cancer 2003; 46(1):82-92.

(72) Gibney MJ. Optimal macronutrient balance. Proc Nutr Soc 1999; 58(2):421-425.

(73) Almendingen K, Hofstad B, Trygg K, Hoff G, Hussain A, Vatn MH. Current diet and colorectal adenomas: a case-control study including different sets of traditionally chosen control groups. Eur J Cancer Prev 2001;(10):395-406.

(74) Le Marchand L, Wilkens LR, Hankin JH, Kolonel LN, Lyu LC. A case-control study of diet and colorectal cancer in a multiethnic population in Hawaii (United States): lipids and foods of animal origin. Cancer Causes Control 1997; 8(4):637-648.

(75) World Health Organization. Diet, nutrition and the prevention of chronic diseases: report of a Joint WHO/FAO Expert Consultation. Genova. 2003.
Report

(76) Kiyohara C. Genetic polymorphism of enzymes involved in xenobiotic metabolism and the risk of colorectal cancer. J Epidemiol 2000; 10(5):349-360.

(77) Kushi L, Giovannucci E. Dietary fat and cancer. Am J Med 2002; 113 Suppl 9B:63S-70S.

(78) Terry PD, Rohan TE, Wolk A. Intakes of fish and marine fatty acids and the risks of cancers of the breast and prostate and of other hormone-related cancers: a review of the epidemiologic evidence. Am J Clin Nutr 2003; 77(3):532-543.

(79) Hakama M. Chemoprevention of cancer. Acta Oncol 1998; 37(3):227-230.

(80) Corpet DE, Tache S. Most effective colon cancer chemopreventive agents in rats: a systematic review of aberrant crypt foci and tumor data, ranked by potency. Nutr Cancer 2002; 43(1):1-21.

(81) Umar A, Viner JL, Richmond E, Anderson WF, Hawk ET. Chemoprevention of colorectal carcinogenesis. Int J Clin Oncol 2002; 7(1):2-26.

(82) Chan TA. Nonsteroidal anti-inflammatory drugs, apoptosis, and colon-cancer chemoprevention. Lancet Oncol 2002; 3(3):166-174.

(83) Martin C, Connelly A, Keku TO, Mountcastle SB, Galanko J, Woosley JT et al. Nonsteroidal anti-inflammatory drugs, apoptosis, and colorectal adenomas. Gastroenterology 2002; 123(6):1770-1777.

(84) Kakizoe T. Chemoprevention of cancer--focusing on clinical trials. Jpn J Clin Oncol 2003; 33(9):421-442.

(85) Anderson D. Antioxidant defences against reactive oxygen species causing genetic and other damage. Mutat Res 1996; 350(1):103-108.

(86) Fernandez E, Chatenoud L, La Vecchia C, Negri E, Franceschi S. Fish consumption and cancer risk. Am J Clin Nutr 1999; 70(1):85-90.

(87) Franceschi S, Favero A, La Vecchia C, Negri E, Conti E, Montella M et al. Food groups and risk of colorectal cancer in Italy. Int J Cancer 1997; 72(1):56-61.

(88) Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Intake of fat, meat, and fiber in relation to risk of colon cancer in men. Cancer Res 1994; 54(9):2390-2397.

(89) Kato I, Akhmedkhanov A, Koenig K, Toniolo PG, Shore RE, Riboli E. Prospective study of diet and female colorectal cancer: the New York University Women's Health Study. Nutr Cancer 1997; 28(3):276-281.

(90) Willett WC, Stampfer MJ, Colditz GA, Rosner BA, Speizer FE. Relation of meat, fat, and fiber intake to the risk of colon cancer in a prospective study among women. N Engl J Med 1990; 323(24):1664-1672.

(91) Frølich W. The fish fat - an important and healt bringing part of our food. Næringsmiddelindustrien 1997; 2.

(92) Willett WC. Diet, nutrition, and avoidable cancer. Environ Health Perspect 1995; 103((Suppl. 8)):S165-S170.

(93) Champe PC, Harvey RA. Biochemistry. Second ed. Philadelphia, Pennsylvania, USA: Lippincott - Raven, 1994.

(94) Marks DB, Marks AD, Smith CM. Basic medical biochemistry. Baltimore, Maryland, USA: Williams & Wilkins, 1996.

(95) Nes M, Müller H, Pedersen JI. Nutrition Theory. [Book in Norwegian]. 4 ed. Oslo, Norway: 1998.

(96) Fredholm L, Gustafsson I-B, Jonsson L. What happens to the food fats when cooked of fried? Näringsforskning 2004;(35):132-140.

(97) Helland E. Shortenings. [In Norwegian]. Oslo: Yrkeslitteratur, 1999.

(98) Drevon CA. The long chain fatty acids from fish and our health. Næringsmiddelindustrien 1997; 2.

(99) Allan Walker W, Blackburn G. Symposium introduction: nutrition and gene regulation. J Nutr 2004; 134:2434S-2436S.

(100) Mahan LK, Escott-Stump S. Krause's Food, nutrition, & diet therapy. Tenth ed. USA: W.B.Saunders Company, 2000.

(101) Bartsch H, Nair J, Owen RW. Dietary polyunsaturated fatty acids and cancers of the breast and colorectum: emerging evidence for their role as risk modifiers. Carcinogenesis 1999; 20(12):2209-2218.

(102) Omega-3 fatty acids: structure and characteristics. keff/NFE Conference Proceeding.: 2001.

(103) Jones PJH, Kubow S. Modern nutrition in health and disease. 9th edition ed. Baltimore, MD: Williams & Wilkins, 1998.

(104) Papamandjaris AA, MacDougall DE, Jones PJ. Medium chain fatty acid metabolism and energy expenditure: obesity treatment implications. Life Sci 1998; 62(14):1203-1215.

(105) Pohl J, Ring A, Ehehalt R, Herrmann T, Stremmel W. New concepts of cellular fatty acid uptake: role of fatty acid transport proteins and of caveolae. Proc Nutr Soc 2004; 63(2):259-262.

(106) Ramirez M, Amate L, Gil A. Absorption and distribution of dietary fatty acids from different sources. Early Hum Dev 2001; 65 Suppl:S95-S101.

(107) Salway JG. Metabolism at a Glance. 4th ed. Blackwell Science, 1998.

(108) Nelson GJ, Ackman RG. Absorption and transport of fat in mammals with emphasis on n-3 polyunsaturated fatty acids. Lipids 1988; 23(11):1005-1014.

(109) Carroll KK. DIETARY FAT AND THE FATTY ACID COMPOSITION OF TISSUE LIPIDS. J Am Oil Chem Soc 1965; 42:516-528.

(110) Guthrie N, Carroll KK. Specific versus non-specific effects of dietary fat on carcinogenesis. Prog Lipid Res 1999; 38(3):261-271.

(111) Holman RT, Pusch F, Svingen B, Dutton HJ. Unusual isomeric polyunsaturated fatty acids in liver phospholipids of rats fed hydrogenated oil. Proc Natl Acad Sci U S A 1991; 88(11):4830-4834.

(112) Calder PC, Grimble RF. Polyunsaturated fatty acids, inflammation and immunity. Eur J Clin Nutr 2002; 56 Suppl 3:S14-S19.

(113) Sprecher H. An update on the pathways of polyunsaturated fatty acid metabolism. Curr Opin Clin Nutr metab Care 1999; 2(2):135-138.

(114) Brenner RR. Hormonal modulation of delta6 and delta5 desaturases: case of diabetes. Prostaglandins Leukot Essent Fatty Acids 2003; 68(2):151-162.

(115) Garg ML, Thomson AB, Clandinin MT. Interactions of saturated, n-6 and n-3 polyunsaturated fatty acids to modulate arachidonic acid metabolism. J Lipid Res 1990; 31(2):271-277.

(116) Burdge G. Alpha-linolenic acid metabolism in men and women: nutritional and biological implications. Curr Opin Clin Nutr Metab Care 2004; 7(2):137-144.

(117) Holman RT, Johnson SB, Ogburn PL. Deficiency of essential fatty acids and membrane fluidity during pregnancy and lactation. Proc Natl Acad Sci U S A 1991; 88(11):4835-4839.

(118) James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. Am J Clin Nutr 2000; 71(1 Suppl):343S-348S.

(119) Zolfaghari R, Ross AC. Recent advances in molecular cloning of fatty acid desaturase genes and the regulation of their expression by dietary vitamin A and retinoic acid. Prostaglandins Leukot Essent Fatty Acids 2003; 68(2):171-179.

(120) Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A. Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr 2004; 79(6):935-945.

(121) Cowing BE, Saker KE. Polyunsaturated fatty acids and epidermal growth factor receptor/mitogen-activated protein kinase signaling in mammary cancer. J Nutr 2001; 131(4):1125-1128.

(122) Bishop-Bailey D, Calatayud S, Warner TD, Hla T, Mitchell JA. Prostaglandins and the regulation of tumor growth. J Environ Pathol Toxicol Oncol 2002; 21(2):93-101.

(123) Almendingen K. Risk of coronary heart disease with special emphasis on intake of trans fatty acids from hardened fish oils. Institute for Nutrition Research, University of Oslo, 1999.

(124) Bugge F. The margarine industry in Norway 1876-1985. [In Norwegian]. Oslo: 1985.

(125) Passwater RA. What your doctor should be reading: explaining hydrogenation. Interview with dr. Mary Enig. HealthWorld Online . 2004.
Electronic Citation

(126) Vegetable oils, trans free fat and margarine. NFE and keff: Are oils only good for your healt? 01 Nov 1; Fredrikstad, Norway.: 2001.

(127) Stender S, Dyerberg J. The influence of trans fatty acids on health. 2003. Søborg, Denmark, The Danish Nutrition Council.
Report

(128) Production of fat for the food industry - Teknisk Matforum.: 2003.

(129) Ackman RG, Mag TK. Trans fatty acids and the potential for less in technical products. In: Sebedio JL, Christie WW, editors. Trans fatty acids in human nutrition. Dundee: The Oily Press, 1998: 35-58.

(130) Pfalzgraf A, Timm M, Steinhart H. [Content of trans-fatty acids in food]. Z Ernahrungswiss 1994; 33(1):24-43.

(131) Howard FA, Henderson C. Hydrogenation of polyunsaturated fatty acids by human colonic bacteria. Lett Appl Microbiol 1999; 29(3):193-196.

(132) Lamberto M, Ackman RG. Confirmation by gas chromatography/mass spectrometry of two unusual trans-3-monoethylenic fatty acids from the Nova Scotian seaweeds Palmaria palmata and Chondrus crispus. Lipids 1994; 29(6):441-444.

(133) Valenzuela A, Morgado N. Trans fatty acid isomers in human health and in the food industry. Biol Res 1999; 32(4):273-287.

(134) Simopoulos AP. The role of fatty acids in gene expression: health implications. Ann Nutr Metab 1996; 40(6):303-311.

(135) Vessby B, Becker W, Aro A. Trans fatty acids - a Scandinavian perspective. International Society for the Study of Fatty Acids and Lipids ISSFAL NEWSLETTER 1996; 3:6-10.

(136) Andersson JE. Wants harmful fat surveyed [In Norwegian]. The Consumer Council of Norway . 2003.
Electronic Citation

(137) Beare-Rogers JL. Trans- and postional isomers of common fatty acids. Adv Nutr Res 1983; 5:171-200.

(138) Enig MG. Trans fatty acids - an update. Nutr Q 1993; 17:79-95.

(139) Jensen RG, Ferris AM, Lammi-Keefe CJ. The composition of milk fat. J Dairy Sci 1991; 74(9):3228-3243.

(140) Position of double bond of trans isomers of linoleic acid affects fatty acid desaturation and elongation. Nutr Rev 1992; 50(2):54-56.

(141) Peters JC, Lawson KD, Middleton SJ, Triebwasser KC. Assessment of the nutritional effects of olestra, a nonabsorbed fat replacement: introduction and overview. J Nutr 1997; 127(8 Suppl):1539S-1546S.

(142) Granlund L, Larsen LN, Christiansen EN, Pedersen JI. Absorption of very-long-chain saturated fatty acids in totally hydrogenated fish oil. Br J Nutr 2000; 84(5):681-688.

(143) Neat CE, Thomassen MS, Osmundsen H. Effects of high-fat diets on hepatic fatty acid oxidation in the rat. Isolation of rat liver peroxisomes by vertical-rotor centrifugation by using a self-generated, iso-osmotic, Percoll gradient. Biochem J 1981; 196(1):149-159.

(144) Larsen LN, Bremer J, Flock S, Skattebol L. Alpha- and beta- alkyl-substituted eicosapentaenoic acids: incorporation into phospholipids and effects on prostaglandin H synthase and 5-lipoxygenase. Biochem Pharmacol 1998; 55(4):405-411.

(145) Trans fat and legislation and the new Norwegian Food Safety Authority. Norway.: 2003.

(146) Trans fat now listed. U.S.Food and Drug Administration. 2004.
Electronic Citation

(147) Sibbald B. New food labels to reveal nutritional content. Canadian Medical Association. CMAJ . 2000.
Electronic Citation

(148) Byrne D. Food labelling - Introduction. European Union. 2004.
Electronic Citation

(149) [No author listed]. Internasjonal cooperation. Norwegian Food Safety Authority . 2004.
Electronic Citation

(150) Tobiassen L. New focus on trans fatty acids. Comments by Professor Jan Ivar Pedersen. [Article in Norwegian]. Norsk tidsskrift for ernæring [2], 12-13. 2004.
Magazine Article

(151) Edible oil processing. Sheffield: Sheffield Academic Press, 2000.

(152) Matforsk. What do the researchers working in the food industry know about trans fat? 03 Dec 2; 2003.

(153) Klurfeld DM, Bull AW. Fatty acids and colon cancer in experimental models. Am J Clin Nutr 1997; 66(6 Suppl):1530S-1538S.

(154) Calder PC, Davis J, Yaqoob P, Pala H, Thies F, Newsholme EA. Dietary fish oil suppresses human colon tumour growth in athymic mice. Clin Sci (Lond) 1998; 94(3):303-311.

(155) Simopoulos AP, Cleland LG. Omega-6/Omega-3 Essential Fatty acid Ratio: The Scientific Evidence. Washington, D.C., USA: Karger, 2003.

(156) Reddy BS, Sugie S. Effect of different levels of omega-3 and omega-6 fatty acids on azoxymethane-induced colon carcinogenesis in F344 rats. Cancer Res 1988; 48(23):6642-6647.

(157) British Nutrition Foundation, Trans Fatty Acids. London: 1987.

(158) Senti FRed. Health Aspects of Dietary Trans Fatty Acids. Bethesda, MD: Life Sciences Research Office, Federation of American Societies for Experimental Biology, 1985.

(159) Berghaus TM, Demmelmair H, Koletzko B. Fatty acid composition of lipid classes in maternal and cord plasma at birth. Eur J Pediatr 1998; 157(9):763-768.

(160) Elias SL, Innis SM. Infant plasma trans, n-6, and n-3 fatty acids and conjugated linoleic acids are related to maternal plasma fatty acids, length of gestation, and birth weight and length. Am J Clin Nutr 2001; 73(4):807-814.

(161) Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother 2002; 56(8):365-379.

(162) Stender S, Dyerberg J. Influence of trans fatty acids on health. Ann Nutr Metab 2004; 48(2):61-66.

(163) Gatto LM, Sullivan DR, Samman S. Postprandial effects of dietary trans fatty acids on apolipoprotein(a) and cholesteryl ester transfer. Am J Clin Nutr 2003; 77(5):1119-1124.

(164) Tholstrup T, Samman S. Postprandial lipoprotein(a) is affected differently by specific individual dietary fatty acids in healthy young men. J Nutr 2004; 134(10):2550-2555.

(165) Troisi R, Willett WC, Weiss ST. Trans-fatty acid intake in relation to serum lipid concentrations in adult men. Am J Clin Nutr 1992; 56(6):1019-1024.

(166) Turpeinen AM, Wubert J, Aro A, Lorenz R, Mutanen M. Similar effects of diets rich in stearic acid or trans-fatty acids on platelet function and endothelial prostacyclin production in humans. Arterioscler Thromb Vasc Biol 1998; 18(2):316-322.

(167) Pedersen JI, Kirkhus B, Muller H. Serum cholesterol predictive equations in product development. Eur J Med Res 2003; 8(8):325-331.

(168) Pedersen JI, Ringstad J, Almendingen K, Haugen TS, Stensvold I, Thelle DS. Adipose tissue fatty acids and risk of myocardial infarction - a case-control study. Eur J Clin Nutr 2000;(54):618-625.

(169) Mann GV. Metabolic consequences of dietary trans fatty acids. Lancet 1994; 343(8908):1268-1271.

(170) Trans fatty acids: Report of the British Nutrition Foundation's Task Force on Trans Fatty Acids. 1987. London: British Nutrition Foundation.
Report

(171) Duthie IF, Barlow SM, Ashby R, Tesh JM, Whitney JC, Saunders A et al. Feeding of partially hydrogenated fish oils to rats in comparison with partially hydrogenated soybean oil and refined rapeseed oil: a combined chronic oral toxicity and carcinogenicity study with in utero phase. Acta Med Scand Suppl 1988; 726:1-89.

(172) Skjelle K, Thorstein B. Trans fatty acids in in the Norwegian diet - occurence and effects on health. 1993.
Unpublished Work

(173) Trans fatty acids. British Nutrition Foundation. 1995. London.
Report

(174) Stender S, Dyerberg J, Holmer G, Ovesen L, Sandstrom B. The influence of trans fatty acids on health: a report from the Danish Nutrition Council. Clin Sci (Lond) 1995; 88(4):375-392.

(175) Kohlmeier L, Simonsen N, Margolin B, Thamm M. Stores of trans fatty acids and breast cancer. Am J Clin Nutr 1995; 61:896.

(176) Ip C, Marshall JR. Trans fatty acids and cancer. Nutr Rev 1996; 54(5):138-145.

(177) Bakker N, Van't Veer P, Zock PL. Adipose fatty acids and cancers of the breast, prostate and colon: an ecological study. EURAMIC Study Group. Int J Cancer 1997; 72(4):587-591.

(178) Stoll BA. Association between breast and colorectal cancers. Br J Surg 1998; 85(11):1468-1472.

(179) Nkondjock A, Shatenstein B, Maisonneuve P, Ghadirian P. Specific fatty acids and human colorectal cancer: an overview. Cancer Detect Prev 2003; 27(1):55-66.

(180) McKelvey W, Greenland S, Chen MJ, Longnecker MP, Frankl HD, Lee ER et al. A case-control study of colorectal adenomatous polyps and consumption of foods containing partially hydrogenated oils. Cancer Epidemiol Biomarkers Prev 1999; 8(6):519-524.

(181) McKelvey W, Greenland S, Sandler RS. A second look at the relation between colorectal adenomas and consumption of foods containing partially hydrogenated oils. Epidemiology 2000; 11(4):469-473.

(182) Slattery ML, Benson J, Ma KN, Schaffer D, Potter JD. Trans-fatty acids and colon cancer. Nutr Cancer 2001; 39(2):170-175.

(183) Almendingen K, Hofstad B, Vatn MH. Does high body fatness increase the risk of presence and growth of colorectal adenomas followed up in situ for 3 years? Am J Gastroenterol 2001; 96(7):2238-2246.

(184) Giovannucci E. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. J Nutr 2001; 131(11 Suppl):3109S-3120S.

(185) Hardwick JC, Van Den Brink GR, Offerhaus GJ, Van Deventer SJ, Peppelenbosch MP. Leptin is a growth factor for colonic epithelial cells. Gastroenterology 2001; 121(1):79-90.

(186) Komninou D, Ayonote A, Richie JP, Jr., Rigas B. Insulin resistance and its contribution to colon carcinogenesis. Exp Biol Med (Maywood) 2003; 228(4):396-405.

(187) Macarthur M, Hold GL, El Omar EM. Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. Am J Physiol Gastrointest Liver Physiol 2004; 286(4):G515-G520.

(188) McKeown-Eyssen GE, Bright-See E, Bruce WR, Jazmaji V, Cohen LB, Pappas SC et al. A randomized trial of a low fat high fibre diet in the recurrence of colorectal polyps. Toronto Polyp Prevention Group. J Clin Epidemiol 1994; 47(5):525-536.

(189) Trevisan M, Liu J, Muti P, Misciagna G, Menotti A, Fucci F. Markers of insulin resistance and colorectal cancer mortality. Cancer Epidemiol Biomarkers Prev 2001; 10(9):937-941.

(190) Moreira NX, Curi R, Padovese R, Mancini-Filho J. Incorporation of dietary trans monounsaturated fatty acids into tissues of Walker 256 tumor-bearing rats. Braz J Med Biol Res 2001; 34(4):501-508.

(191) Stillwell W, Wassall SR. Docosahexaenoic acid: membrane properties of a unique fatty acid. Chem Phys Lipids 2003; 126(1):1-27.

(192) Norwegian Nutrition Council. Norwegian nutrient recommendations 1997. 1998.
Pamphlet

(193) Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Arterioscler Thromb Vasc Biol 2003; 23(2):e20-e30.

(194) Bjørneboe G-EAa, Haga Rimestad A. The development in the Norwegian diet 2003. Directorate for Health and Social affairs, editor. 2003. Oslo, Norway, Printhouse AS.
Report

(195) Johansson L. Trans fatty acids in the Norwegian diet. [Lecture in Norwegian]. University of Oslo, 1999.

(196) Pedersen JI, Tverdal A, Kirkhus B. Diet changes and the rise and fall of cardiovascular disease mortality in Norway [Article in Norwegian]. Tidsskr Nor Lægeforen 2004; 124(11):1532-1536.

(197) Johansson LR, Solvoll K, Bjorneboe GE, Drevon CA. Intake of very-long-chain n-3 fatty acids related to social status and lifestyle. Eur J Clin Nutr 1998; 52(10):716-721.

(198) FAO FID. FAO Fisheries Technical Papers - T142. FAO Fisheries Department . 1986.
Electronic Citation

(199) Pettersen J, Oterhals Å, Opstvedt J. Unhydrogenated fish oil in food products. Lipidforum 1999; 57:11-15.

(200) Beare-Rogers J, Ghafoorunissa, Korver O, Rocquelin G, Sundram K, Uauy R. Dietary fat in developing countries. IUNS Committee 1/4.United Nations University . 2001.
Electronic Citation

(201) Barlow S. Fish meal and oil - supplies and markets. International Fishmeal and Fish Oil Organization, UK . 2001.
Electronic Citation

(202) Fish oils and their future in international fats and oils scene. 97 Mar; LIPIDEX'97, Belgium: 1997.

(203) Granli G. Cod liver oil and n-3 fatty acids. Peter Møller. Eutrophia 2000; 3.

(204) Lien AM, Saarem K. Cod liver oil - not only in months with "R". Næringsmiddelindustrien 1997; 2.

(205) Rimestad AH, Borgejordet Å, Vesterhus KN, Sygnestveit K, Løken EB, Trygg K et al. The Large Food Table.[Book in Norwegian]. Oslo: Gyldendal: 2001.

(206) Biong AS. Butter oil. Almendingen K, editor. TINE RaD Centre, Kalbakken, Oslo. 2004.
Internet Communication

(207) Fouad FM, Mamer OA, Sauriol F, Shahidi F. Chemical and epidemiological aspects of modified butter oil fractions. J Toxicol Environ Health B Crit Rev 1998; 1(2):149-179.

(208) Sargent J. Omega-3, omega-6 fatty acids and vegetables, good for salmon and humans? Havbruk. 2001.
Electronic Citation

(209) Standal H. Margarine history in Norway. 2002.
Personal Communication

(210) Skrede A. Nutrition and feeds. Akvaforsk-Alliance . 2004.
Electronic Citation

(211) Aro A, Van Amelsvoort J, Becker W, van Erp-Baart M-A, Kafatos A, Leth T et al. Trans fatty acids in dietary fats and oils from 14 European countries: The TRANSFAIR study. J Food Compost Anal 1998; 11:137-149.

(212) Granlund L. Absorption and metabolism of totally hydrogenated fish oil. Thesis in Norwegian. University of Oslo, 1999.

(213) Oil and fat. Information from the Government and Departments. ODIN. 2004.
Electronic Citation

(214) [No author]. In the matter of trans fats. Baking Business. 4-11-2004.
Electronic Citation

(215) Mossoba MM, McDonald RE, Armstrong DJ, Page SW. Identification of minor C18 triene and conjugated diene isomers in hydrogenated soybean oil and margarine by GC-MI-FT-IR spectroscopy. J Chromatogr Sci 1991; 29(8):324-330.

(216) Ratnayake WM, Chen ZY, Pelletier G, Weber D. Occurrence of 5c,8c,11c,15t-eicosatetraenoic acid and other unusual polyunsaturated fatty acids in rats fed partially hydrogenated canola oil. Lipids 1994; 29(10):707-714.

(217) Kaplan RJ, Greenwood CE. Poor digestibility of fully hydrogenated soybean oil in rats: a potential benefit of hydrogenated fats and oils. J Nutr 1998; 128(5):875-880.

(218) [No authors listed]. Tracing the trans fatty acids. [Article in Norwegian]. IFORM. 2004.
Magazine Article

(219) [No authors listed]. What is gene food [In Norwegian]. Norwegian Society for the Concervation of Nature. 6-10-2004.
Electronic Citation

(220) [No authors listed]. Western meat consumption deforesting Amazonas [Press statement in Norwegian]. Rainforest Fund. 21-4-2004.
Electronic Citation

(221) Johansson L, Pedersen JI, Alexander J. Trans fatty acids and health. Nordic conference. Scand J Nutr 1996; 40:19-21.

(222) Ifoma. Fish Oil Bulletin No. 20. 1986.
Report

(223) Standal H. Fat. Presentation. 2002.
Slide

(224) Molin M. The effect of hydrogenated fish oil on the development of intestinal cancer in Min mice. Høgskolen i Akershus, 2004.

(225) Johansson L, Rimestad AH, Frost Andersen L. Trans fatty acids in the Norwegian diet. Article in Norwegian. Scand J Nutr 1994; 38(62):66.

(226) Johnson LK, Hjermann I, Tonstad S. Diet and secondary prevention of coronary heart disease. Tidsskr Nor Lægeforen 2001;(121):1092-1098.

(227) Trichopoulou A, Breslin L. DAFNE II (Data Food Networking). Network for the Pan-European Food Data Bank based on Household Budget Surveys, editor. 1994. Report

(228) Meyer WH. Trans-isomeric fatty acids in West German margarines, shortenings, frying, and cooking fats. Am J Clin Nutr 1980; 33(4):732-733.

(229) Hydrogenated marine fat in bread. (Personal observation). 2004.

(230) Thommessen M. The increasing bread import. [In Norwegian]. Nationen. 2003. Electronic Citation

(231) Norum KR, Christiansen EN, Christoperson BO, Bremer J. Metabolic and nutritional aspects of long-chain fatty acids of marine origin. Rotterdam, The Netherlands: Academic Press Limited, 1989: 118-140.

(232) Becker W. Intake of trans fatty acids in the Nordic countries. Scand J Nutr 1996; 40:16-18.

(233) Almendingen K, Jordal O, Kierulf P, Sandstad B, Pedersen JI. Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on serum lipoproteins and Lp[a] in men. J Lipid Res 1995; 36(6):1370-1384.

(234) Svensson E, Grotmol T, Hoff G, Langmark F, Norstein J, Tretli S. Trends in colorectal cancer incidence in Norway by gender and anatomic site: an age-period-cohort analysis. Eur J Cancer Prev 2002; 11:489-495.

(235) Almendingen K, Seljeflot I, Sandstad B, Pedersen JI. Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on hemostatic variables in men. Arterioscler Thromb Vasc Biol 1996;(16):375-380.

(236) Muller H, Jordal O, Seljeflot I, Kierulf P, Kirkhus B, Ledsaak O et al. Effect on plasma lipids and lipoproteins of replacing partially hydrogenated fish oil with vegetable fat in margarine. Br J Nutr 1998;(80):243-251.

(237) Morgado N, Sanhueza J, Galleguillos A, Garrido A, Nieto S, Valenzuela A. Effect of dietary hydrogenated fish oil on the plasma lipoprotein profile and on the fatty acid composition of different tissues on the rat. Ann Nutr Metab 1999; 43(5):310-318.

(238) Morgado N, Galleguillos A, Sanhueza J, Garrido A, Nieto S, Valenzuela A. Effect of the degree of hydrogenation of dietary fish oil on the trans fatty acid content and enzymatic activity of rat hepatic microsomes. Lipids 1998; 33(7):669-673.

(239) Morgado N, Sanhueza J, Nieto S, Valenzuela A. Effect of the degree of hydrogenation of fish oil on the enzymatic activity and on the fatty acid composition of hepatic microsomes from young and aged rats. Ann Nutr Metab 2003; 47(3-4):124-131.

(240) Corpet DE, Pierre F. Point: From animal models to prevention of colon cancer. Systematic review of chemoprevention in min mice and choice of the model system. Cancer Epidemiol Biomarkers Prev 2003; 12(5):391-400.

(241) Mouse models for cancer research. The Jaxon Laboratory. 2002.
Electronic Citation

(242) Dong M, Guda K, Nambiar PR, Nakanishi M, Lichtler AC, Nishikawa M et al. Azoxymethane-induced preadipocytes factor 1 (Pref-1) functions as a differentiation inhibitor in colonic epithelial cells. Carcinogenesis 2004.

(243) Guda K, Giardina C, Nambiar P, Cui H, Rosenberg DW. Aberrant transforming growth factor-beta signaling in azoxymethane-induced mouse colon tumors. Mol Carcinog 2001; 31(4):204-213.

(244) Papanikolaou A, Wang QS, Delker DA, Rosenberg DW. Azoxymethane-induced colon tumors and aberrant crypt foci in mice of different genetic susceptibility. Cancer Lett 1998; 130(1-2):29-34.

(245) Guda K, Upender MB, Belinsky G, Flynn C, Nakanishi M, Marino JN et al. Carcinogen-induced colon tumors in mice are chromosomally stable and are characterized by low-level microsatellite instability. Oncogene 2004; 23(21):3813-3821.

(246) Svensson L. The effect of dietary partially hydrogenated marine oils on desaturation of fatty acids in rat liver microsomes. Lipids 1983; 18(3):171-178.

(247) Evennett A. Almendingen K, editor. Denofa. 2002.
Internet Communication

(248) Andersen G. Average from analyzes of hydrogenated fish oils. Denofa. 2002.
Internet Communication

(249) Biong A. Product specification. 1999.
Pamphlet

(250) Sohn OS, Ishizaki H, Yang CS, Fiala ES. Metabolism of azoxymethane, methylazoxymethanol and N-nitrosodimethylamine by cytochrome P450IIE1. Carcinogenesis 1991; 12(1):127-131.

(251) Altman DG. Practical statistics for medical research. 8th ed. Boca Raton, Florida: CRC Press LLC, 1999.

(252) Paulsen JE, Elvsaas IK, Steffensen IL, Alexander J. A fish oil derived concentrate enriched in eicosapentaenoic and docosahexaenoic acid as ethyl ester suppresses the formation and growth of intestinal polyps in the Min mouse. Carcinogenesis 1997; 18(10):1905-1910.

(253) Kolstad MC. Effects of the omega-3/omega-6 ratio in PhIP-induced carcinogenesis in Min mice. [Thesis in Norwegian]. Akershus University College, 2003.

(254) Ørjasæter Elvsaas I-K. Polyunsaturated omega-3 fatty acids and inheritable colorectal cancer in mice. [Thesis in Norwegian]. Akershus University College, 1996.

(255) Østensvig Stamm TM. Omega-3 polyunsaturated fatty acids and colorectal cancer. [Thesis in Norwegian]. Akershus University College, 1995.

(256) What do I need to know about fats and oils? Harlan. 2005.
Electronic Citation

(257) Birt DF, Kris ES, Choe M, Pelling JC. Dietary energy and fat effects on tumor promotion. Cancer Res 1992; 52(7 Suppl):2035s-2039s.

(258) Klurfeld DM, Weber MM, Kritchevsky D. Inhibition of chemically induced mammary and colon tumor promotion by caloric restriction in rats fed increased dietary fat. Cancer Res 1987; 47(11):2759-2762.

(259) Pariza MW, Boutwell RK. Historical perspective: calories and energy expenditure in carcinogenesis. Am J Clin Nutr 1987; 45(1 Suppl):151-156.

(260) Poirier LA. Brief history of the role of nutrition in carcinogenesis. Adv Exp Med Biol 1986; 206:5-10.

(261) Dragsted LO, Daneshvar B, Vogel U, Autrup HN, Wallin H, Risom L et al. A sucrose-rich diet induces mutations in the rat colon. Cancer Res 2002; 62(15):4339-4345.

(262) Slattery ML, Benson J, Berry TD, Duncan D, Edwards SL, Caan BJ et al. Dietary sugar and colon cancer. Cancer Epidemiol Biomarkers Prev 6[9], 677-685. 1997.
Abstract

(263) Eide DM. Pinworm infeksjon ved ADFD. Dyreavdelingen, FHI. 2003.
Internet Communication

(264) Pilcova R, Sulcova J, Hill M, Blaha P, Lisa L. Leptin levels in obese children: effects of gender, weight reduction and androgens. Physiol Res 2003;(52):53-60.

(265) Campbell TC. Nutrition and drug-metabolizing enzymes. Clin Pharmacol Ther 1977; 22(5 Pt 2):699-706.

(266) Di Leo A, Messa C, Cavallini A, Linsalata M. Estrogens and colorectal cancer. Curr Drug Targets Immune Endocr Metabol Disord 2001; 1(1):1-12.

(267) Davis DL, Bradlow HL, Wolff M, Woodruff T, Hoel DG, Anton-Culver H. Medical hypothesis: xenoestrogens as preventable causes of breast cancer. Environ Health Perspect 1993; 101(5):372-377.

(268) Bartsch H, Barbin A, Marion MJ, Nair J, Guichard Y. Formation, detection, and role in carcinogenesis of ethenobases in DNA. Drug Metab Rev 1994; 26(1-2):349-371.

(269) Dommels YEM, Alink GM, van Bladeren PJ, van Ommen B. Dietary n-6 and n-3 polyunsaturated fatty acids and colorectal carcinogenesis: results from cultured

colon cells, animal models and human studies. Envir Toxicol Pharmacol 2002; 12:233-244.

(270) Martin LJ, Mahaney MC, Almasy L, MacCluer JW, Blangero J, Jaquish CE et al. Leptin's sexual dimorphism results from genotype by sex interactions mediated by testosterone. Obes Res 2002; 10(1):14-21.

(271) Granlund L. Absorption and metabolism of totally hydrogenated fish oil. Thesis University of Oslo. 1999.

(272) Reddy BS. Studies with the azoxymethane-rat preclinical model for assessing colon tumor development and chemoprevention. Environ Mol Mutagen 2004; 44(1):26-35.

(273) Griffini P, Fehres O, Klieverik L, Vogels IM, Tigchelaar W, Smorenburg SM et al. Dietary omega-3 polyunsaturated fatty acids promote colon carcinoma metastasis in rat liver. Cancer Res 1998; 58(15):3312-3319.

(274) Lee JY, Sohn KH, Rhee SH, Hwang D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through toll-like receptor 4. J Biol Chem 2001;(276):16683-16689.

(275) DuBois RN, Giardiello FM, Smalley WE. Nonsteroidal anti-inflammatory drugs, eicosanoids, and colorectal cancer prevention. Gastroenterol Clin North Am 1996; 25(4):773-791.

(276) Balmain A, Harris CC. Carcinogenesis in mouse and human cells: parallels and paradoxes. Carcinogenesis 2000; 21(3):371-377.

APPENDICES

APPENDIX I Terminology for classification and judgement of documentation (evidence) put forward by the American Institute for Cancer Research (4)

Causal relationship	Criteria for judgement of evidence	Implications for dietary advice
Convincing	Epidemiologic studies show consistent associations, with little or no evidence to the contrary. There should be a substantial number of acceptable studies, (i.e., for dietary variables more than 20 studies) preferably including prospective designs, conducted in different population groups, controlled for possible confounding factors. Dietary intake data should refer to time preceding occurrence of cancer. Any dose-response relationships should be supportive of a causal relationship. Associations should be biologically plausible. Laboratory evidence is usually supportive or strongly supportive.	The conclusion is that a causal relationship exists. Such a "convincing" relationship is an adequate basis for giving preventive advice to health personnel, politicians and populations.
Probable	Epidemiological studies showing associations are either not so consistent, with a number and/or proportion of studies not supporting the association, or else the number or type of studies is not extensive enough to make a more definite judgement. Mechanistic and laboratory evidence are usually supportive or strongly supportive.	The conclusion is that a causal relationship is probable. A probable increased or reduced risk is also a basis for preventive recommendations.
Possible	Epidemiological studies are generally supportive, but are limited in quantity, quality or consistency. There may or may not be supportive mechanistic or laboratory evidence. Alternatively, there are few or no epidemiological data, but strongly supportive evidence from other disciplines.	There is no basis for preventive recommendations.
Insufficient	There are only a few studies, which are generally consistent, but really do no more than hint at a possible relationship. Often, more well-designed research is needed.	No advice can be given.
No judgement	If a significant portion of the data is inconsistent, amounting to possible or insufficient evidence of a causal relationship, the inconsistent evidence is noted as such. In some cases, the data are extremely limited and/or inconsistent: for such relationships, no judgement can be made.	No advice can be given.

APPENDIX II Certificate of analysis for a sample of fish oil

DENOFA

CERTIFICATE OF ANALYSIS

Product: Fish Oil	Customer:	Customers orderno.:
		Item No.:
Product name: 30 % Fiskeolje	Quantity: 500 ml	Date: 2002-05-07
Batch.nr.: Sample	Our Ref.No.:	Ref: G. Andersen

Oxidative quality		Limits	Analysis	Method
Acid value		: max 1	0,3	DL.01
Peroxide value	meq/kg	: max 5	0,8	DL.02
[illegible]	0°C	: min 3 hours	pass	DL.05
Iodine value		: min 190	203,5	DL.07
Colour gardener		: max 6	pass	DL.10

Fatty acid profile

Eicosapentaenoic (EPA)w/w% :	min 18	18,1	DL.15
Docosahexaenoic (DHA)w/w%:	min 12	12,7	DL.15

" w/w% denotes percentage of the glycerides containing the given fatty acid chain."

Antioxydant

DL-αtocopherol	ppm :	min 1000	added

Kvalitetskontroll
Denofa Leknes
Quality control

Terje Bilsen
Laboratorieleder

Gunnar Andersen
QC Manager

APPENDIX III Product specification for usual butter oil

PS 4101 - Smørolje på tank, utgave 3 (2 sider)

KVALITETSSIKRING	Dokumentasjon: PRODUKTSPESIFIKASJON	
	Artikkeltype:	Andre fettprodukter
	Artikkelgruppe:	Smørolje
	Artikkel:	Smørolje på tank

Forfatter: Solveig Lise	Godkjent av: Willy E Eriksen	Godkj. dato: 03.11.1999	Gyldig: 25.02.1999	Utgave: 3	Sider: 2	Dok.nr: PS-4101

KONFIDENSIELL
Produktspesifikasjonen er TINE Norske Meieriers eiendom og skal ikke overlates til andre, eller endres, uten samtykke fra produktansvarlig person i FoU-senteret.

En fullstendig produktspesifikasjon består av en generell del, denne delen og en ernæringsdel.

ENHETSSTØRRELSE OG ARTIKKELNUMMER:

1331	1340					

PRODUKTBESKRIVELSE:
Sammensetning pr. 100 g vare:

		Kilde:			Kilde:
energi	3696 kJ 899 kcal	Beregnet Beregnet			
fett	99,8 g	Analyser			
vitamin A (retinol)	1400 µg	Beregnet			

Utfyllende produktbeskrivelse:
Vannfri smørolje er produsert av fløte i en konsentreringsprosess som medfører nesten fullstendig fjerning av vann og tørrstoff (melkekomponenter).

Vannfri smørolje skal ha en ren smak og ha en klar gul farge ved 40°C.

HOLDBARHET / OPPBEVARING:
Maks. 1 uke ved 45°C - 55°C (smørolje på tank).

MERKING:

KVALITETSNORMER:

	Norm	Nedre tillske-grense	Øvre tillske-grense	Avvik	Met nr.
KJEMISK					
Vann	<0,2 %		0,2 %	>0,3 %	MA 800
Syretall	< 0,6 mg KOH pr. g		0,6 mg KOH pr. g		MA 810
Peroksydtall	< 0,2 mekv. O: pr. kg		0,2 mekv. O: pr. kg		AOAC 965.33
SENSORISK					
Hovedpoeng	5,0 - 4,0 p			< 2,7 p	MA 849 MA 843
MIKROBIOLOGISK					
Koliforme bakterier	< 10 pr. g		10 pr. g	> 100 pr. g	MA 850
B. cereus	<10 pr. g		10 pr. g	> 100 pr. g	NMKL 67 2 MA 481 MA 482
L. monocytogenes	Ikke påvist i 25 g			Påvist	IDF 143
Salmonella spp	Ikke påvist i 25 g			Påvist	NMKL 71

SENSORISK KVALITET
(Bakterier 5 poengs vare)

Utseende: Klar, gul ved +40°C

Lukt og smak: Ren, fyldig, en anelse kokt smørsmak

Effects of hydrogenated fish oil in experimental colorectal carcinogenesis in A/J mice

by

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Key words: Tumor, azoxymethane, colorectal carcinogenesis, A/J mice, hydrogenated oils.

The abbreviations used are: CRC, colorectal cancer; AOM, azoxymethane; HFO, hydrogenated fish oils; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; HSO, hydrogenated soybean oils; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil; PH, partially hydrogenated; HH, highly hydrogenated; TH, totally hydrogenated; FW, final weight; md, estimated median difference; SFA, saturated fatty acids; LCSFA, long chain saturated fatty acids; VLCSFA, very long chain saturated fatty acids; MCFA, medium chain fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; VLCPUFA, very long chain polyunsaturated fatty acids; TFA, *trans* fatty acids.

Abstract

The colorectal cancer (CRC) incidence in Norway has increased considerably during the last fifty years. The main aim of this study was to examine whether hydrogenated fish oil can induce or increase CRC in AOM treated A/J mice. The effects of three diets containing hydrogenated fish oil (HFO) (19 w/w %); partially hydrogenated (PHFO), highly hydrogenated (HHFO), or totally hydrogenated (THFO), mixed with corn oil (1 w/w %) in AIN-76M, was studied. Fish oil (FO), butter oil (BO), soybean oil (SO), and soybean oil similarly hydrogenated as the fish oil; PHSO, HHSO and THSO were controls. Tumors identified by surface examination of unsectioned methylene blue stained colon preparations transilluminated in the inverse light microscope, revealed no statistically significant evidence of HFO increasing induction and/or growth of CRC compared with FO, or with increasing degrees of HFO or HSO, or in comparison with corresponding hydrogenation degrees of HSO. On the contrary, PHSO clearly increased growth in females and tumor load in males, compared with PHFO. In females; THFO increased growth compared with PHFO, and PHSO tumor number compared with HHSO. In males; THFO increased number compared with PHFO and HHFO, and load compared with HHFO. Compared with SO; THFO protected females regarding incidence, PHFO regarding growth which FO did not, and PHFO and HHFO regarding load. Compared with BO; HHFO protected males regarding load which FO did not. Patterns in the material showed that all the oils, dependent of gender, showed signs of being related to induction and/or growth of CRC in these animals.

Introduction

Findings indicate that increased ingestion of fats enhances colonic tumor promotion [1-4]. Consumption of fat rose in the industrial world from early in the twentieth century, especially due to the new fat source margarine[5]. Partially hydrogenated vegetable and fish oils have been increasingly used, especially in margarine [6-14]. Norway has experienced an increasing colorectal cancer (CRC) incidence trend over time compared with its neighbouring countries. The reason for this increasing trend is obscure [15]. Norway is, however, diverging in at least the following factors; we have been consuming hydrogenated fish oils (HFO) in margarine and cod liver oil as a vitamin D source to a greater extent than most other countries during the last century [16-18]. Negative effects on risk factors for circulation diseases from intake of HFO have been shown in humans and rodents [19,20]. It seems important to study the consequences of HFO in the diet on CRC. This experiment was performed at the Norwegian Institute of Public Health preliminary to a larger project.

Since it would be unethical to expose humans to amounts of edible oils considered to be potentially harmful, the influence of HFO was tested on A/J mice treated with the colon carcinogen azoxymethane (AOM) [21]. These mice are wild type animals highly susceptible to inducement of multiple intestinal tumors [22], primarily in the distal colon [23], representative of human adenomas or carcinomas *in situ* [24]. For these reasons they can be used as a murine model of human sporadic CRC as a sensitive test system for dietary factors.

The first aim was to examine whether partially hydrogenated fish oil (PHFO), highly hydrogenated fish oil (HHFO), or totally hydrogenated fish oil (THFO) in the diet can increase induction and/or growth of CRC in AOM treated A/J mice. Native fish oil (FO), butter oil exclusive water and salt (BO), refined soybean oil (SO), partially hydrogenated

soybean oil (PHSO), highly hydrogenated soybean oil (HHSO), or totally hydrogenated soybean oil (THSO) were used as controls. The objective was to relate the biological effects of HFO to the degree of hydrogenation. *The second aim* was to reveal patterns of effects on CRC on induction and/or growth of CRC in the material, also taking into account results which were not statistically significant. The data material did not fill the criteria for the use of tests that take into consideration confounding effects of both gender and body weight [25]. The effects of the experimental oils were analysed separately for female and male animals in four series which had been decided in advance, and final weight (FW) is treated in the text, tables, and figures of all series in connection with the tumor parameters.

Materials and methods

Animals, chemicals, and experimental oils. Female and male A/J mice bred in house from mice originally purchased from Jackson Laboratory, Bar Harbour, ME, USA were housed in polysterol cages in a room with 12 hr light/dark cycle and controlled humidity and temperature. AOM from Sigma Chemicals Company, St. Louis, MO, USA was dissolved in 0.9% NaCl. The animals were given water and diet *ad libitum*. Fish oils and soybean oils were delivered by Denofa, Gamle Fredrikstad, Norway, and butter oil was delivered by Tine Fellesmeieriet BA, 0902 Oslo, Norway. The animals were randomly assigned to nine isocaloric diets, in which fat contributing 38.5 % of total energy, i.e. 19 w/w % experimental oil and 1 w/w % corn oil, were mixed in the standard feed AIN-76M composed by the American Institute of Nutrition, bought from Special Diets Services, P.O. Box 705, Witham, Essex CM8 3AD, UK. Diets containing three different hydrogenation degrees of fish oil; PHFO, HHFO or THFO, were compared with six different diets containing native FO, BO without salt and water, refined SO, or soybean oil similarly hydrogenated as the fish oil; PHSO, HHSO and THSO. The melting point of the partially hydrogenated oils was 31-33, of the highly hydrogenated oils 40-42, and of the totally hydrogenated oils 55-60. The experimental oils were taken directly from the ordinary production line and correspond to the raw materials used in commercial margarines and butter, except for THFO which was produced in the laboratory especially for this experiment.

Experimental design. 104 female and 95 male A/J mice were injected subcutaneously with AOM (10 mg/kg body weight) once weekly for 2 weeks. The animals received the diets from d 4, were weighed once a week, and killed after 15 weeks. Colons were dissected, and their unsectioned whole mount preparations examined in order to identify, characterize and score

tumors, and to study differences between genders and between diets for each gender separately in tumor incidence (number of animals with tumor/number of animals in the group) and number (total number of tumors per animal in the group) as indices for induction of CRC, size (area of aberrant tissue per tumor per animal with tumor in the group) as index for growth of CRC, and load (total tumor area per animal in the group) which takes into consideration both induction and growth of CRC. For the differences between diets, four series of analyses were set up to search for answers to questions that were decided in advance.

Scoring of tumors. The colons were dissected, rinsed in cold PBS, slit open longitudinally and fixed flat between wet (PBS) filter papers for 24 h in 10% neutral buffered formalin prior to 4 s staining with 0.2% methylene blue from George T. Gurr Ltd., UK dissolved in the same formalin solution. The examination of tumors was carried out blindly by the same investigator by transillumination of colons in an inverse light microscope at a magnification of x20, and 2 types of tumors identified. Type 1 tumors were characterized by their bright blue staining, high protruding into the colonic lumen, circular appearance, and irregular and distorted crypts. Type 2 tumors were characterized by their bright blue or turquoise staining, irregular and distorted crypts, and slightly elevated and prominent appearance, but flat at the boundary line to the surrounding mucosa, which some times seemed normal and flat, and some times normal but discoloured and giving the impression of being elevated. Since the type 2 tumors were not observed as highly elevated structures, their bright blue or turquoise appearance and irregular and distorted crypts seen with transillumination were used as criteria for their identification. Tumor number and size were recorded. Tumor size was scored by an eyepiece graticule as longitudinal diameter, and mm^2 areas calculated.

Statistical analysis. Fisher's Exact or Pearson Chi-Square tests were used to calculate relations between tumor incidence and type of oil in the diet, and Relative Risk with 95% Confidence Interval as a measure of effect with variance when comparing groups. Kruskal-Wallis Test and Oneway Anova (seldom) were used to calculate relations between tumor number, size, or load and type of oil in the diet, Mann Whitney Test and T-Test (seldom) to compare groups, and median difference was used as effect measure. For each series of analyses, P-values were manually adjusted *à la* Bonferroni for differences between groups, but not for differences between genders. The significance level was set at $p \leq 0.05$.

Results

Weight development is illustrated by curves for female and male animals separately for each treatment group (Figures 3 and 4). Besides some minor temporary disturbances, animal body weights seemed to develop normally, and the animals were not visibly ill. The weight level seemed more or less differently influenced by the various experimental oils from early in life.

Final body weights (FW) could be ranked almost equally for females and males by the diets as follows (deviation for males in parentheses): SO > BO > FO > PHSO > PHFO (THFO) > THFO (PHFO) > HHSO > THSO > HHFO (range estimated median = ♀ 24.9-17.8 g and ♂ 29.9-20.3 g). Differences in FW between treatment groups are dealt with in connection with the tumor parameters. FW was increased in males compared with females without regard to type of oil in the diet, and the gender differences were ranked by the diets as follows; FO > BO = SO > THFO > HHSO > PHFO > THSO > PHSO > HHFO (range estimated median difference (md) = 5.9-2.5 g, and P-values differed between <0.0005 and 0.004).

Effects of experimental oils on final body weight and on induction and growth of CRC. Differences in induction (tumor incidence and number) and growth (tumor size) of CRC, tumor load, and FW, between genders and between treatment groups within each gender were examined. An overview of the results is given in Figures 1 and 2.

Results corresponding to p-values within the interval $0.2 \geq p > 0.05$ are referred to as tendencies and described by the words "tend" or "weak", while results corresponding to p-values > 0.2 are described by the word "seem", consistently in the Results Chapter.

Gender differences. Effects of the experimental oils are illustrated along with FW by separate curves for each gender in Figures 5-8. In this paragraph, statistically significant results are marked (s) and tendencies (t). *Induction of CRC* was, tended to be, or seemed to be substantially increased in males compared with females by THFO (s), THSO (t), FO (t), BO (t), and PHSO and seemingly less by HHSO, HHFO and PHFO, while SO was the only oil seemingly increasing tumor incidence in females compared with males, also substantially. *Growth of CRC* was increased by SO (s) and THFO (s) and seemed to be increase by FO and THSO, substantially, and HHSO and HHFO seemingly less, in females compared with males, while it seemed to be substantially increased by PHFO and less by BO in males compared with females, and the result for PHSO seemed unclear. *Tumor load* tended to be substantially increased in males compared with females by THFO (t) and BO (t), less by FO (s), and seemingly even lesser by THSO and PHFO, while it seemed to be increased substantially in females compared with males by PHSO and SO, and to be equal in both genders by HHSO and HHFO.

Differences between treatment groups

Statistically significant results are indicated by underlined effect values in parenthesis.

1. Will HFO in the diet increase induction and/or growth of CRC in AOM treated A/J mice in comparison with unhydrogenated FO, thus losing the protecting properties of native FO? No statistically significant differences were found between HFO and FO regarding *induction and/or growth of CRC* irrespective of gender (Table 1, Figures 9 and 10).

In females, all the HFO seemed to increase *induction of CRC* by tumor incidence, and PHFO by tumor number, and in males, THFO seemed to increase induction by tumor incidence and

tended to increase it by tumor number, compared with FO. HHFO, on the contrary, seemed to reduce tumor incidence and number in males compared with unhydrogenated FO. Tumor incidence tended to be related (P = 0.104), and tumor number was related (P = 0.023), to type of fish oil in the diet for males, but no relations were found for females.

In males, PHFO seemed to increase *growth of CRC*, while THFO and HHFO, on the contrary, seemed to reduce tumor size compared with FO, but tumor size was not related to type of fish oil in the diet. In females, all the HFO seemed to reduce tumor size compared with FO, and tumor size was weakly related to type of fish oil in the diet (P = 0.082).

In females, PHFO seemed to increase *tumor load* compared with FO, but tumor load was not related to type of fish oil in the diet. In males, THFO seemed to increase tumor load, while PHFO and HHFO, on the contrary, seemed to reduce it, compared with FO. Tumor load was related to type of fish oil in the diet for males (P = 0.017).

All the HFO reduced *FW* compared with FO irrespective of gender, and FW was clearly related to type of fish oil in the diet for both females and males (P = 0.003 and < 0.0005, respectively).

2. Will FO, when it is hydrogenated, lose its protective properties for AOM treated A/J mice in comparison with SO or BO? Possible protecting properties of FO in the diet had been looked for in comparison with SO or BO in advance (figures not shown). Because the tumor parameters seemed more increased in females by SO and in males by BO, the protective properties of fish oil after hydrogenation were analysed in comparison with SO for females and BO for males (Table 2, Figures 11 and 12).

Females. FO tended to protect females more than males regarding *induction of CRC*, and compared with SO, FO protected females, while protection was not clear for males (not shown). When FO was hydrogenated, compared with SO; THFO still had (RR SO-THFO = 2.70), HHFO tended to have, and PHFO seemed to have protecting properties for females regarding tumor incidence, although seemingly less than FO had, and all the HFO still seemed to have protecting properties regarding tumor number, HHFO and THFO equal to FO, but PHFO seemingly less than FO had. Tumor incidence was related to type of oil in the diet for females ($P = 0.029$), but tumor number was not.

FO seemed to protect males more than females against *growth of CRC*, and seemed to protect females, but not males, compared with SO (not shown). When FO was hydrogenated, compared with SO; PHFO protected females regarding growth (md = -3.9 mm^2), seemingly gaining protecting properties relative to FO, and HHFO and THFO seemed to do the same. Tumor size was related to type of oil in the diet for females ($P = 0.036$).

FO protected females more than males regarding *tumor load*, and protected females, and seemed to also protect males, compared with SO (not shown). When FO was hydrogenated, compared with SO; PHFO (md = -6.0 mm^2) and HHFO (md = -6.2 mm^2) still had, and THFO seemed to have, protecting properties for females regarding tumor load, although PHFO seemingly less than FO had. Tumor load was related to type of oil in the diet for females ($P = 0.041$).

FO tended to reduce *FW* in females compared with SO (not shown). When FO was hydrogenated, all the HFO reduced FW in females compared with SO and seemingly more than FO did. FW was clearly related to type of oil in the diet ($P < 0.0005$).

Males. FO seemed to protect both genders regarding *induction of CRC*, males only by tumor incidence, compared with BO (not shown). When FO was hydrogenated, compared with BO; THFO seemed to increase tumor number in males, tending to increase it relative to FO, and also seemed to increase tumor incidence, thus to have lost the seemingly protecting properties, while on the contrary, HHFO tended to protect regarding tumor incidence and seemed to protect regarding tumor number, thus to have gained protective properties relative to FO. Tumor incidence and number were both related to type of oil in the diet for males ($P = 0.045$, and $P = 0.019$, respectively).

FO did not seem to protect either gender regarding *growth of CRC* compared with BO, but rather to increase it (not shown). When FO was hydrogenated, compared with BO; PHFO seemed to increase growth even more than FO seemed to do, while on the contrary, HHFO and THFO seemed to protect males against growth, seemingly gaining protecting properties which FO lacked. Tumor size was not related to type of oil in the diet for males.

FO seemed to protect both genders regarding *tumor load* compared with BO (not shown). When FO was hydrogenated, compared with BO; THFO seemed to increase tumor load in males and to have lost the seemingly protecting properties, while on the contrary, PHFO tended to protect, and HHFO (md = -4.0 mm^2) protected males, and seemed to have gained protecting properties relative to FO, regarding tumor load. Tumor load was strongly related to type of oil in the diet for males ($P = 0.003$).

FO seemed to reduce *FW* in males compared with BO (not shown). When FO was hydrogenated, all the HFO reduced FW in males compared with BO, and more than FO did, and FW was strongly related to type of oil in the diet ($P < 0.0005$).

3. Will induction and/or growth of CRC in AOM treated A/J mice increase with increasing hydrogenation degree of fish or soybean oil in the diet? (Table 3, Figures 13 and 14). ***HFO.*** In females, PHFO seemed to increase *induction of CRC* by tumor incidence and number compared with HHFO or THFO, but neither tumor incidence nor number was related to degree of HFO in the diet. In males, THFO seemed to increase induction by tumor incidence and increased it by tumor number (md = 1) compared with PHFO, tended to increase tumor incidence and increased tumor number (md = 2) compared with HHFO, and both tumor incidence and number were related to degree of HFO in the diet (P = 0.051 and 0.011, respectively).

In females, THFO seemed to increase *growth of* CRC by tumor size compared with HHFO and increased it compared with PHFO (md = 2.6 mm^2), the difference between HHFO and PHFO not being statistically significant, and tumor size was related to degree of HFO in the diet (P = 0.031). In males, PHFO seemed to increase tumor size compared with THFO or HHFO, but tumor size was not related to degree of HFO in the diet. In females, PHFO seemed to increase *tumor load* compared with HHFO or THFO, but tumor load was not related to degree of HFO in the diet. In males, THFO tended to increase tumor load compared with PHFO and increased it compared with HHFO (md = 4.5 mm^2), the difference between PHFO and HHFO not being statistically significant, and tumor load was clearly related to degree of HFO in the diet (P = 0.005).

Weak evidence was found that *FW* did neither increase nor decrease with increasing degree of HFO irrespective of gender, since HHFO tended to reduce FW compared with both PHFO and THFO, the differences between PHFO and THFO being far from statistically significant.

FW was weakly related to degree of HFO in the diet for females (P = 0.061) and related for males (P = 0.044).

HSO. In females, PHSO seemed to increase *induction of* CRC by tumor incidence compared with THSO or HHSO, to increase tumor number compared with THSO, and increased tumor number compared with HHSO (md = 2), and tumor number was related to degree of HSO in the diet (P = 0.039), but tumor incidence was not. In males, THSO tended to increase tumor incidence compared with HHSO, PHSO seemed to do the same, and both THSO and PHSO tended to increase tumor number compared with HHSO. Tumor incidence was weakly related, and tumor number related, to degree of HSO in the diet for males (P = 0.094 and 0.033, respectively).

In females, HHSO seemed to increase *growth of* CRC compared with PHSO or THSO, and in males, PHSO and HHSO seemed to increase it compared with THSO. But tumor size was not related to degree of HSO in the diet for either gender.

In females, PHSO seemed to increase *tumor load* compared with THSO and tended to increase it compared with HHSO. In males, PHSO seemed to increase tumor load compared with both THSO and HHSO, and THSO tended to increase it compared with HHSO. Tumor load was weakly related to degree of HSO for both females (P = 0.094) and males (P = 0.085).

HHSO and THSO (ns for males) reduced *FW* compared with PHSO, and THSO seemed to give lowest FW, in both genders. Thus, FW seemed to decrease with increasing degree of HSO irrespective of gender, and therefore to be related to the hydrogenation process as such.

But there was no evidence for this, since the differences in FW between THSO and HHSO were far from statistically significant. FW was related to degree of HSO in the diet for both females (P = 0.004) and males (P = 0.021).

4. Will HFO in the diet increase induction and/or growth of CRC in AOM treated A/J mice in comparison with HSO when compared by corresponding hydrogenation degrees? (Table 4, Figures 15 and 16). HHFO seemed to increase *induction of CRC* by tumor incidence slightly compared with HHSO in both genders. On the contrary; PHSO seemed to increase induction by both tumor incidence and number in females, and seemed to increase tumor incidence and tended to increase tumor number in males, compared with PHFO, and THSO seemed to increase induction by both tumor incidence and number in females, but not in males, compared with THFO.

The HFO seemed not to increase *growth of CRC* compared with corresponding hydrogenation degrees of HSO. On the contrary, in females, PHSO clearly increased tumor size compared with PHFO (md = 2.6 mm^2), and also HHSO seemed to increase tumor size compared with HHFO, while THFO and THSO seemed to increase growth equally. Tumor size was weakly related to hydrogenated oils in the diet for females. In males, PHSO seemed to increase tumor size compared with PHFO, HHSO compared with HHFO, and THSO compared with THFO.

THFO seemed to increase *tumor load* in males compared with THSO. On the contrary, PHSO seemed to increase tumor load in females and increased it in males (md = 3.9 mm^2) compared with PHFO, and THSO seemed to increase it in females compared with THFO. HHFO and HHSO seemed to give equal tumor load in both genders.

THFO increased *FW* in males and seemed to increase it also in females compared with THSO. On the contrary, PHSO seemed to increase FW compared with PHFO and HHSO compared with HHFO in both genders. FW was related to type of hydrogenated oil in the diet irrespective of gender (P = 0.007 for both).

Discussion

The CRC incidence rate in Norway has doubled the last fifty years and has the last decade been the highest in the Nordic countries [26]. On this background, the main aim of this study was to examine the effects of PHFO, HHFO, and THFO in the diet on induction (tumor incidence and number) and growth (tumor size) of CRC, and on tumor load (induction and/or growth of CRC) by transillumination of unsectioned colon preparations from AOM treated A/J mice, with FO, BO, SO, PHSO, HHSO, and THSO as controls. Several degrees of hydrogenated fish oil have not been tested before in the A/J mouse model. An experiment with Min mice, a model for Familial Adenomatous Polyposis (FAP), dealing with similar hydrogenation degrees of fish oil, was performed simultaneously here at the Norwegian Institute of Public Health [27]. No additional literature was found with regard to effects of the experimental oils on the tumor parameters studied. The second aim was to reveal patterns of effects in the material, even if the results were not statistically significant. The discussion therefore includes also the results that were not statistically significant.

Weight development and final weight. Besides some temporary disturbances in the weekly animal weights, the weight development seemed normal and even. There were no signs of major illness. *This seemed to indicate that the experimental oils might contribute to the more or less different body weight levels found in the treatment groups during the experimental period more than the colorectal cancer illness they induced did*. The oils could be ranked almost equally for females and males with respect to the FW they gave. *This seemed to indicate that all of the oils each might have a similar effect on body weight in both genders, perhaps except PHFO and THFO*. Males had increased FW compared with females regardless of type of oil in the diet. Body weight is known to be controlled by sex hormones, and it is normal for males to have higher body weight than females. Body weight is also

known to be a confounding factor in carcinogenesis. *When some oil in comparison with some other oil in this experiment increases induction and/or growth of CRC while it decreases FW, additional risk factors to body weight should be present.*

Gender differences. Induction of CRC seemed to be increased in males compared with females, most substantially by THFO, THSO, FO, BO and PHSO, while SO was the only oil increasing tumor incidence in females compared with males. Growth of CRC seemed to be increased in females compared with males by most of the oils, except by PHFO, PHSO and BO, while tumor load seemed to be increased in females compared with males only by PHSO and SO. *The results seemed to indicate that the effects of the experimental oils might be differently influenced by sex hormones depending on oil and hydrogenation degree, and might be different in induction and growth of CRC.*

1. Change in effects when FO was hydrogenated. ***Induction of CRC and tumor load.*** Compared with unhydrogenated FO; even though all the HFO reduced FW in the animals, mostly supported in a similar experiment with Min mice [27]; in females, all the HFO seemed to increase induction of CRC considerably, PHFO most (seemingly also in Min mice), and PHFO seemed to increase tumor load, while in males, THFO seemed to increase induction (seemingly also in Min mice by tumor incidence) and tumor load considerably, while PHFO seemed to reduce tumor load. PHFO seemed to increase tumor incidence and load slightly in males compared with females (seemingly also in Min mice), while THFO increased induction (seemingly also in Min mice), and tended to increase tumor load considerably in males compared with females. *This seemed to indicate that additional risk factors to body weight are present, and that protecting substances in FO against induction of CRC may have been destroyed and perhaps substituted by harmful fatty acids during the hydrogenation.*

The PHFO contained a high amount of *trans* fatty acids (TFA) (55-60%), a putative risk factor for CRC, at least in humans [28]. The estrogen level has been shown to play a role in colorectal carcinogenesis in women [29]. If this also applies to these experimental animals, the seemingly lower tumor incidence by PHFO in females compared with males might partly be accounted for by the higher estrogen level in females. But the contribution of TFA to total energy intake would probably have been much higher for these female animals (approximately 20%) than for the mentioned women, and therefore might partly explain the harmfulness of PHFO for these animals.

Earlier evidence seems consistent with no relationship between palmitic or stearic acids and CRC in humans [30]; if these results should apply to these experimental animals, it seems as the high content of very long chain fatty acids (VLCSFA) in THFO, even though they are poorly absorbed in male rodents [31], might be a significant risk factor for CRC for these male animals. No literature was found on effects of VLCSFA on induction and/or growth of CRC. *These results seemed to indicate that in induction of CRC, PHFO might contain substances more harmful to the female and THFO substances more harmful to the male animals than substances in the other HFO*.

HHFO seemed to be the most protecting fish oil against induction of CRC for males (seemingly also in Min mice by tumor number), and second to unhydrogenated FO also for females (seemingly the most protecting in Min mice). Still HHFO seemed to increase induction considerably in males compared with females (seemingly also in Min mice).

Growth of CRC. Compared with unhydrogenated FO; in females, all the HFO seemed to reduce growth of CRC considerably (not supported in Min mice), PHFO most, while in males, PHFO seemed to increase growth considerably and THFO to reduce it considerably (not supported in Min mice). Since all the HFO reduced FW in the animals compared with FO, lower body weight might be involved in the reduction of growth, but if so, PHFO transcended this protection in the males. While THFO reduced growth considerably in males compared with females (seemingly also in Min mice), PHFO seemed to increase growth considerably in males compared with females (not supported in Min mice). *These results seemed to indicate that in growth of CRC, PHFO might contain substances more harmful to the male and THFO substances more harmful to the female animals than substances in the other HFO, effects quite opposite to those observed in induction of CRC.*

What THFO did not have in common with FO (36% C14-20 including 0.2% C20, 4% C18, 22% C16, and 8% C14) was the much higher content of VLCSFA and long chain saturated fatty acids (LCSFA) in THFO (97% C14-22 including 13% C22, 18% C20, 22% C18, 36% C16, and 8% C14). Lower absorption of THFO may be the reason for the lower body weight, and lead to lower bioavailability of VLCSFA, both of these might partly explain the reduced growth of CRC in the animals by THFO compared with FO. The absorption of VLCSFA has been shown to be as low as 61% in rats and 19-41% in hamsters [32,33], and of LCSFA 31-39 % in humans [34], because of melting points substantially above body temperatures and a strong tendency to form insoluble soap in the small intestine [35,36]. The difference between THFO and FO might also partly have been due to hydrogenation of risk factors in FO such as cholesterols, tocopherols, carotenes, or deltanoids [37-40].

Even if THFO reduced growth compared with FO, both of these oils increased growth substantially compared with the other fish oils, especially in females. What THFO and FO had in common was a large content of saturated fatty acids (SFA). A 20% by weight FO diet has earlier been shown to increase both secondary tumor formation in colon cancer involving liver metastases in rats, and to develop 1000 fold more metastases than rats fed a low fat diet or a diet enriched with safflower oil [41]. *These results seemed to indicate that unhydrogenated FO might have a harmful effect on growth of CRC.*

HHFO seemed to be the most protecting fish oil also against growth of CRC for males (not supported in Min mice), and second to PHFO also for females (seemingly second to FO in Min mice). Still HHFO seemed to increase growth considerably in females compared with males (not supported in Min mice).

The results seemed to indicate that HHFO might be the most protecting fish oil for males and the second most protecting for females in both induction and growth of CRC.

There probably are several reasons for the seemingly protective capacity of HHFO in colorectal carcinogenesis in both genders compared with the other fish oils. HHFO tended to give lower FW than the other fish oils in both genders. This might possibly partly have been due to lower feed intake; the HHFO group seemed to have the lowest feed expenditure of all the treatment groups, and possibly due to low absorption of the fatty acids. In addition, HHFO contained > 10% less *trans* and unnatural fatty acids than PHFO, of which less possibly would be absorbed, and 46% less SFA than THFO of which possibly some more would be absorbed. HHFO has earlier shown protective potential against heart disease by reducing the plasma TG concentration in male rats compared with FO or PHFO [20].

2. Change in protective properties when FO was hydrogenated. FO tended to protect females more than males against induction of CRC, and compared with SO, FO clearly protected females considerably, while protection for males was not clear. For females; when FO was hydrogenated, all the HFO still seemed to have protecting properties against ***induction of CRC*** compared with SO, although seemingly less than before. All the HFO reduced FW in females also compared with SO. That PHFO seemed to have lost most protecting properties, may be a consequence of better bioavailability of the fatty acids from PHFO than from the other HFO. The absorption degree for PHFO has been shown to be as high as 95% in rats, which is closer to that of SO (99%) than to that of THFO (61%) [42].

FO seemed to protect females considerably less than males against ***growth of CRC***, and to protect females moderately, but not males, compared with SO. When FO was hydrogenated; compared with SO, all the HFO seemed to have gained considerably larger protecting properties against growth in females at the same time as FW was reduced by all the HFO, compared with SO. *This seemed to indicate that reduced body weight may play a larger protecting role in growth than in induction of CRC for the females.* But the reduced body weight did not seem to explain all of the gain in protecting properties of HFO against growth for females, since PHFO seemed to have gained more, and THFO much less, protecting properties than the other HFO.

The SO contained 61% PUFA, 23% MUFA, < 1% TFA, and 15% SFA, including a high amount of ω-6 fatty acids (55%) and a high ω-6/ω-3 ratio (9.4:1), both putative risk factors for CRC possibly in both humans and rodents. Studies in animal models and recent observations in humans have provided evidence that a high intake of ω-6 PUFA stimulates several stages in the development of colon cancer, also by influences on free estrogen levels

and hormonal catabolism [43-47]. Indeed, in females; SO seemed to increase the risk of CRC 1.6-2.7 times, growth of CRC 1.4-8.9 times, and tumor load at least 38.6 times, relative to the HFO. *The results seemed to indicate that SO might be extremely harmful in induction and growth of CRC in the female animals, and that the effects of substances in the SO may affect the figures describing protective properties of the HFO.*

For males: FO seemed to possibly protect both genders regarding induction of CRC compared with BO. When FO was hydrogenated; HHFO seemingly showed a considerable and PHFO a minimal gain in protecting properties against ***induction of CRC*** in males, while THFO on the contrary seemed to increase induction in males and lose the possible protecting properties. HHFO protected substantially regarding tumor load compared with BO.

FO possibly seemed not to protect either gender against growth of CRC compared with BO, but rather to increase it. When FO was hydrogenated, compared with BO, HHFO and THFO seemed to protect males against ***growth of CRC***, seemingly gaining protecting properties which FO seemed to lack, while PHFO on the contrary seemed to increase growth, even more than FO did.

The seemingly protecting properties for males of HHFO and PHFO against induction, and of HHFO and THFO against growth of CRC, compared with BO may partly be explained by reduced body weights. All the HFO reduced FW in males compared with BO. For discussion of substances in, and absorption of, the HFO, see above.

The BO contained 3% PUFA and 32% MUFA including a few percents of TFA, and 65% SFA of C16, 18 and 14 mostly. The content of medium chain fatty acids (MCFA) was

approximately 21%. Rises in the intake of the MCFA lauric and myristic acids among subjects at high risk for CRC and CRC patients have been reported [30]. These fatty acids are able to induce COX-2 overexpression in human tissues [48], and COX-2 is upregulated from 2 to 50 fold in 85-90% of CRC patients [49]. If this should also apply to the animals in this study, effects of such fatty acids might partly explain the increased induction and growth of CRC by BO in comparison with some of the HFO in the male animals. Indeed, BO, which seemingly increased tumor load in males compared with females, seemed to increase it at least 7.4 times relative to PHFO and HHFO in males. BO seemed, however, not to give very large tumors. *These results seemed to indicate that BO might be harmful in induction and/or growth of CRC in these animals, especially to the males, and that possible effects of substances in the BO might affect the figures describing protective properties of the HFO.*

The results also seemed to indicate that protecting properties might not only be lost, but also gained, by hydrogenation of FO

3. Change in effects with increasing hydrogenation degree of HFO or HSO. All the HFO and HSO reduced FW in the animals compared with their respective unhydrogenated oils, and FW seemed to decrease with increasing hydrogenation degree of HSO, but not of HFO. The effects of HFO or HSO on induction and/or growth of CRC did not seem to increase with increasing hydrogenation degree in a consistent manner. *The results seemed to indicate that the effects of HFO or HSO on induction and/or growth of CRC might not be caused by the hydrogenation process per se, but might rather be related to specific hydrogenation degrees of the oils.*

4. HFO compared with HSO by corresponding hydrogenation degrees. HHFO seemed to increase tumor incidence slightly in both genders compared with HHSO, and THFO seemed to increase tumor load considerably in males compared with THSO. On the contrary, HSO seemed to be considerably more harmful than HFO in fourteen of the comparisons.

Irrespective of gender, the most substantial increases seemed to be by THSO compared with THFO in increased female incidence, by HHSO compared with HHFO in increased growth, and by PHSO compared with PHFO in increases in all the tumor parameters. *The results seemed to indicate that all the hydrogenation degrees of soybean oil might contain substances more harmful regarding induction and/or growth of CRC in these animals than the substances in the corresponding hydrogenation degrees of fish oil.*

HFO and HSO judged by hydrogenation degree. Irrespective of gender, the PH degree of fish or soybean oil seemed to be the most harmful degree in increasing ***induction of CRC*** in these animals (except the TH degree in males). Partial hydrogenation of fish oil creates positional and geometrical isomers of very long chain polyunsaturated fatty acids (VLCPUFA) as well as VLCSFA not existing in partially hydrogenated vegetable oils [50]. Still, of the two partially hydrogenated oils only PHSO led to seemingly extremely large tumor loads. *These results seemed to indicate the presence of harmful substances in the PH oils, that PHFO still might contain protecting substances, and/or that PHSO might contain extremely harmful substances, for these animals in induction of CRC, irrespective of gender.* PHSO seemed to increase induction by tumor incidence, but not growth, substantially and to almost double tumor load in females compared with males. *This seemed to indicate that in induction of CRC, substances in PHSO might be more harmful to the female than to the male animals.*

For males, the TH degree of fish or soybean oil seemed to be at least equally harmful to PHSO in induction of CRC, but only THFO and PHSO led to equally substantially large tumor load in males. *This seemed to indicate the presence of harmful substances for males in THFO compared with THSO*. One reason might be that total hydrogenation of fish oil creates VLCSFA which do not exist in hydrogenated vegetable oils [47], such as C20-22 fatty acids. THFO also led to considerably higher FW than THSO. THFO seemed substantially more harmful for males than for females regarding induction of CRC and tumor load, but substantially more harmful for females than for males regarding growth of CRC. *The results seemed to indicate that in induction of CRC, substances in THFO might be more harmful to the male than to the female animals, and in growth of CRC more harmful to the female than to the male animals.* These indications also seemed to pertain to THSO, albeit to a lesser degree, and THFO seemingly increased tumor load substantially compared with THSO, but only in males. The difference in tumor load did not seem real, however, and might possibly be a result of the large variance in tumor load in the THSO group related to tumor size.

Compared with the other hydrogenation degrees; irrespective of gender, the HH degree of both fish and soybean oil seemed to give body weights among the lowest, and seemed to protect regarding induction and growth (second to PHFO in females) of CRC and tumor load (together with THFO in females), except HHSO which seemed to give the most extreme increases in growth of CRC (together with PHSO in males). Including fatty acids changed by the hydrogenation, the HHSO contained 70% MUFA and 30% SFA, and the HHFO contained 50% UFA (mostly PUFA) and 50% SFA. There was a predominance of changed C18:1 fatty acids in the HHSO, and a predominance of changed C20-22 PUFA and SFA in the HHFO. *These results might indicate that the HH degree of the hydrogenated fish and soybean oils*

might be less harmful to these animals in induction and/or growth of CRC than the PH or TH degrees, except HHSO in growth.

In spite of the protection of both genders, The HH degree of both oils seemed to increase tumor incidence in males compared with females and to increase growth in females compared with males, but the gender differences still seemed to be among the lowest in this experiment. *The results seemed to indicate that in induction of CRC, substances in the HH degree of fish and soybean oils might be more harmful to the male than to the female animals, and in growth of CRC more harmful to the female than to the male animals.*

Patterns of effects in the material. It looked like the effects of the HFO rather than being caused by the hydrogenation process *per se* might be related to gender, hydrogenation degree, and phase of colorectal carcinogenesis. This seemed to apply also to the control oils. Since these results were not built on statistically significant evidence, no such conclusions can be made. The indications put forward in italics during the discussion are only intended to be a background for generating new hypotheses.

For induction of CRC, most results in series 1 were, however, consistent with the results of an experiment in Min mice, which were not either statistically significant, but for growth of CRC they were not consistent. The reason for this might be that in Min mice, most tumors appear in the small intestine [27], and few colorectal tumors were present as samples for the analysis of tumor size.

This study was characterized by large variances, weak tests, and occasionally samples less than ten that may have weakened the usefulness of specific tests [51].

Conclusion

Certain effect patterns in this material seemed to indicate that the effects of the experimental oils on colorectal carcinogenesis in the AOM treated A/J mice, in addition to being influenced by gender and body weight, might be different in the induction and growth phase of CRC, and dependent on type of oil in the diet and hydrogenation degree, the latter might differ between HFO and HSO. These indications were not based on statistically significant results only, so they must be taken for what they are worth. Some evidence was however found.

No statistically significant evidence was found of increasing CRC by HFO relative to unhydrogenated FO, with loss of protecting properties, nor of increasing effects with increasing hydrogenation degree; which would have indicated that the effects of HFO were due to a change in fish oil by the hydrogenation process *per se*.

That the HH degree of HSO reduced induction of CRC relative to the TH degree, indicated that the effects of HSO were dependent on specific hydrogenation degrees, i.e., were related to specific substances in these oils. Even if the null hypotheses for HFO could not be rejected, the possibility that also the effects of HFO might be dependent on specific hydrogenation degrees could not be ruled out.

No statistically significant evidence was either found of increased effects of HFO relative to corresponding hydrogenation degrees of HSO. On the contrary, when the effects were examined with a view to the specific oils, however, it seemed as HSO were more harmful than HFO; all the tumor parameters seem to be increase by PHSO relative to PHFO, and statistical evidence was found for tumor number and load in males.

In the view to the specific oils, it actually seemed as any of the experimental oils, including those that were exemplifying oils consumed in Norway over different time periods, might contain substances that are risk factors in colorectal carcinogenesis; in any way in these animals. Even if it had been possible to draw this conclusion for the A/J mice, no conclusions can be drawn from mouse to man. Findings in experimental animals will not necessarily apply to man. The mouse is, however, the model organism which is closest to humans [52].

If the oils in this experiment really may exemplify oils used in Norway, and if the results of this study might apply to humans; the results would have indicated that of the examined oils, HHFO might not be the most likely fat to play a role in the increasing CRC trend in Norway compared with its neighbouring countries. More likely candidates would be PHFO or FO (as cod liver oil), which besides HHFO were the only of the experimental oils with increased consumption relative to the other Nordic countries, and which were used to a great degree long enough and early enough to have affected the rising trend in Norway, which started at least as late as in the 1950s, when the registering of the CRC incidence started.

Further research. It seems important to look into the effects of those of the experimental oils that are still in use in human foods, such as SO, THSO, PHSO, PHFO, BO and cod liver oil, or that may be put to use in the future, such as THFO. This might also give some answers with respect to the effects of the two suspected candidates in the Nordic region.

It might also be interesting to look closer at what fish species have been dominating in the hydrogenation of fish oil in Norway, since some species may have larger amounts of VLCPUFA, which may make a difference for the substances in the end products after hydrogenation.

Studying effects of whale oil, which was also used to a great degree in Norway from 1912 and for approximately 50 years might also give some answers regarding the CRC trend.

HHFO could be examined for increasing effects on CRC relative to a more neutral normal mouse diet.

This article was a part of a thesis in clinical nutrition for the degree of Candidata Scientiarum.

Acknowledgements

The fish and soybean oils were donated by Denofa and the butter oil by Tine Fellesmeieriet BA. The authors wish to thank Development Manager Håkon Standal and staff at Denofa and Nutrition Supervisor Anne Sofie Biong at Tine for valuable help.

References

1. Birt, D. F., Kris, E. S., Choe, M., and Pelling, J. C. Dietary energy and fat effects on tumor promotion. Cancer Res., *52*: 2035s-2039s, 1992.

2. Klurfeld, D. M., Weber, M. M., and Kritchevsky, D. Inhibition of chemically induced mammary and colon tumor promotion by caloric restriction in rats fed increased dietary fat. Cancer Res., *47*: 2759-2762, 1987.

3. Pariza, M. W. and Boutwell, R. K. Historical perspective: calories and energy expenditure in carcinogenesis. Am J Clin.Nutr, *45*: 151-156, 1987.

4. Poirier, L. A. Brief history of the role of nutrition in carcinogenesis. Adv.Exp.Med.Biol., *206*: 5-10, 1986.

5. World Cancer Research Fund. Food, Nutrition and the Prevention of Cancer: A Global Perspective, 1st ed. Washington, DC. 1997. Am Inst Cancer Res.
Report

6. Oil and fat. Information from the Government and Departments. ODIN. 2004.
Electronic Citation

7. [No authors listed]. What is gene food [In Norwegian]. Norwegian Society for the Conservation of Nature. 6-10-2004.
Electronic Citation

8. [No authors listed]. Western meat consumption deforesting Amazons [Press statement in Norwegian]. Rainforest Fund. 21-4-2004.
Electronic Citation

9. [No authors listed]. Tracing the trans fatty acids. [Article in Norwegian]. IFORM . 2004.
Magazine Article

10. Andersson, J. E. Wants harmful fat surveyed [In Norwegian]. The Consumer Council of Norway. 2003.
Electronic Citation

11. Bjørneboe, G.-E. Aa. and Haga Rimestad, A. The development of the Norwegian diet 2003. Directorate for Health and Social affairs. 2003. Oslo, Norway, Printhouse AS.
Report

12. Johansson, L., Rimestad, A. H., and Frost Andersen, L. Trans fatty acids in the Norwegian diet. Article in Norwegian. Scand J Nutr, *38*: 66, 1994.

13. Passwater, R. A. What your doctor should be reading: explaining hydrogenation. Interview with dr. Mary Enig. HealthWorld Online. 2004.
Electronic Citation

14. Pedersen, J. I., Tverdal, A., and Kirkhus, B. Diet changes and the rise and fall of cardiovascular disease mortality in Norway [Article in Norwegian]. Tidsskr Nor Lægeforen, *124*: 1532-1536, 2004.

15. Svensson, E., Grotmol, T., Hoff, G., Langmark, F., Norstein, J., and Tretli, S. Trends in colorectal cancer incidence in Norway by gender and anatomic site: an age-period-cohort analysis. Eur.J.Cancer Prev., *11*: 489-495, 2002.

16. Becker, W. Intake of trans fatty acids in the Nordic countries. Scand J Nutr, *40*: 16-18, 1996.

17. Lien, A. M. and Saarem, K. Cod liver oil - not only in months with "R". The Food Industry, *2*: 1997.

18. Norum, K. R., Christiansen, E. N., Christoperson, B. O., and Bremer, J. Metabolic and nutritional aspects of long-chain fatty acids of marine origin., Second ed, pp. 118-140. Rotterdam, The Netherlands: Academic Press Limited, 1989.

19. Almendingen, K., Jordal, O., Kierulf, P., Sandstad, B., and Pedersen, J. I. Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on serum lipoproteins and Lp[a] in men. J Lipid Res, *36*: 1370-1384, 1995.

20. Morgado, N., Sanhueza, J., Galleguillos, A., Garrido, A., Nieto, S., and Valenzuela, A. Effect of dietary hydrogenated fish oil on the plasma lipoprotein profile and on the fatty acid composition of different tissues on the rat. Ann.Nutr Metab, *43*: 310-318, 1999.

21. Mouse models for cancer research. The Jaxon Laboratory. 2002.
Electronic Citation

22. Guda, K., Giardina, C., Nambiar, P., Cui, H., and Rosenberg, D. W. Aberrant transforming growth factor-beta signaling in azoxymethane-induced mouse colon tumors. Mol.Carcinog., *31*: 204-213, 2001.

23. Papanikolaou, A., Wang, Q. S., Delker, D. A., and Rosenberg, D. W. Azoxymethane-induced colon tumors and aberrant crypt foci in mice of different genetic susceptibility. Cancer Lett., *130*: 29-34, 1998.

24. Guda, K., Upender, M. B., Belinsky, G., Flynn, C., Nakanishi, M., Marino, J. N., Ried, T., and Rosenberg, D. W. Carcinogen-induced colon tumors in mice are

chromosomally stable and are characterized by low-level microsatellite instability. Oncogene, *23*: 3813-3821, 2004.

25. World Health Organization. Diet, nutrition and the prevention of chronic diseases: report of a Joint WHO/FAO Expert Consultation. Genova. 2003.
Report

26. Møller, B., Fekjær, H., Hakulinen, T., Tryggvadóttir, L., Storm, H. H., Talbäck, M., and Haldorsen, T. Prediction of cancer incidence in the Nordic countries up to the year 2002. Eur J Cancer Prev, *11*: 2002.

27. Molin, M. The effect of hydrogenated fish oil on the development of intestinal cancer in Min mice. 2004. Høgskolen i Akershus.
Thesis/Dissertation

28. Bakker, N., Van't Veer, P., and Zock, P. L. Adipose fatty acids and cancers of the breast, prostate and colon: an ecological study. EURAMIC Study Group. Int.J Cancer, *72*: 587-591, 1997.

29. Slattery, M. L., Benson, J., Ma, K. N., Schaffer, D., and Potter, J. D. Trans-fatty acids and colon cancer. Nutr Cancer, *39*: 170-175, 2001.

30. Nkondjock, A., Shatenstein, B., Maisonneuve, P., and Ghadirian, P. Specific fatty acids and human colorectal cancer: an overview. Cancer Detect.Prev, *27*: 55-66, 2003.

31. Granlund, L., Larsen, L. N., Christiansen, E. N., and Pedersen, J. I. Absorption of very-long-chain saturated fatty acids in totally hydrogenated fish oil. Br.J Nutr, *84*: 681-688, 2000.

32. Granlund, L., Larsen, L. N., Christiansen, E. N., and Pedersen, J. I. Absorption of very-long-chain saturated fatty acids in totally hydrogenated fish oil. Br.J Nutr, *84*: 681-688, 2000.

33. Jandacek, R. J., Hollenbach, E. J., Kuehlthau, C. M., and Steimle, A. R. Effects of dietary behenate and a caprenin-like fat on lipids in the hamster. J Nutr Biochem, *4*: 243-249, 1993.

34. Hashim, S. A. and Babayan, V. K. Studies in man of partially absorbed dietary fats. Am J Clin Nutr, *31*: S273-S276, 1978.

35. Ramirez, M., Amate, L., and Gil, A. Absorption and distribution of dietary fatty acids from different sources. Early Hum.Dev., *65 Suppl*: S95-S101, 2001.

36. Tomarelli, R. M., Meyer, B. J., Weaber, J. R., and Bernhart, F. W. Effect of positional distribution on the absorption of the fatty acids of human milk and infant formulas. J Nutr, *95*: 583-590, 1968.

37. Baron, J. A., Cole, B. F., Mott, L., Haile, R., Grau, M., Church, T. R., Beck, G. J., and Greenberg, E. R. Neoplastic and antineoplastic effects of beta-carotene on colorectal adenoma recurrence: results of a randomized trial. J Natl Cancer Inst., *95*: 717-722, 2003.

38. De Stefani, E., Mendilaharsu, M., Deneo-Pellegrini, H., and Ronco, A. Influence of dietary levels of fat, cholesterol, and calcium on colorectal cancer. Nutr.Cancer, *29*: 83-89, 1997.

39. Martinez-Gonzalez, M. A. and Estruch, R. Mediterranean diet, antioxidants and cancer: the need for randomized trials. Eur J Cancer Prev, *13*: 327-335, 2004.

40. Sergeev, I. N. Calcium signaling in cancer and vitamin D. [Article in Press]. J Steroid Biochem mol Biol, 2005.

41. Griffini, P., Fehres, O., Klieverik, L., Vogels, I. M., Tigchelaar, W., Smorenburg, S. M., and Van Noorden, C. J. Dietary omega-3 polyunsaturated fatty acids promote colon carcinoma metastasis in rat liver. Cancer Res, *58*: 3312-3319, 1998.

42. Granlund, L., Larsen, L. N., Christiansen, E. N., and Pedersen, J. I. Absorption of very-long-chain saturated fatty acids in totally hydrogenated fish oil. Br.J Nutr, *84*: 681-688, 2000.

43. Bartram, H. P., Gostner, A., Scheppach, W., Reddy, B. S., Rao, C. V., Dusel, G., Richter, F., Richter, A., and Kasper, H. Effects of fish oil on rectal cell proliferation, mucosal fatty acids, and prostaglandin E2 release in healthy subjects. Gastroenterology, *105*: 1317-1322, 1993.

44. Bartram, H. P., Gostner, A., Reddy, B. S., Rao, C. V., Scheppach, W., Dusel, G., Richter, A., Richter, F., and Kasper, H. Missing anti-proliferative effect of fish oil on rectal epithelium in healthy volunteers consuming a high-fat diet: potential role of the n-3:n-6 fatty acid ratio. Eur J Cancer Prev, *4*: 231-237, 1995.

45. Calder, P. C., Davis, J., Yaqoob, P., Pala, H., Thies, F., and Newsholme, E. A. Dietary fish oil suppresses human colon tumour growth in athymic mice. Clin Sci (Lond), *94*: 303-311, 1998.

46. Dommels, Y. E. M., Alink, G. M., van Bladeren, P. J., and van Ommen, B. Dietary n-6 and n-3 polyunsaturated fatty acids and colorectal carcinogenesis: results from cultured colon cells, animal models and human studies. Envir Toxicol Pharmacol, *12*: 233-244, 2002.

47. Johansson, L., Pedersen, J. I., and Alexander, J. Trans fatty acids and health. Nordic conference. Scand J Nutr, *40*: 19-21, 1996.

48. Lee, J. Y., Sohn, K. H., Rhee, S. H., and Hwang, D. Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through toll-like receptor 4. J Biol Chem, 16683-16689, 2001.

49. DuBois, R. N., Giardiello, F. M., and Smalley, W. E. Nonsteroidal anti-inflammatory drugs, eicosanoids, and colorectal cancer prevention. Gastroenterol Clin North Am, *25*: 773-791, 1996.

50. Johansson, L., Pedersen, J. I., and Alexander, J. Trans fatty acids and health. [Article in Norwegian]. Nordic seminarium. Scand J Nutr, *40*: 19-21, 1996.

51. Altman, D. G. Practical statistics for medical research, 8th ed.Boca Raton, Florida: CRC Press LLC, 1999.

52. Hauge, J. G. Biochemistry.Oslo: The University Press, 2001.

Tables

Table 1 Effects of HFO versus FO on final weight and colorectal tumor parameters in AOM treated A/J mice.

Diet	Sex	N	N[a]	FW, g	Incidence[b]	Number[c]	Size[d], mm^2	Load[e], mm^2
FO	♀	11	2	20.85 (15.24, 28.08)***	2/11 (0.182)♦	0.0 (0.0, 1.0)♦	3.70 (0.33, 7.07)	0.00 (0.00, 7.07)*
	♂	9	9	26.72 (23.92, 33.00)†††	6/9 (0.667)♦	1.0 (0.0, 4.0)♦♦	1.33 (0.13, 11.04)	0.79 (0.00, 14.77)*
PHFO	♀	7	6	19.01 (18.25, 20.63)*	4/7 (0.571)	1.0 (0.0, 3.0)	0.50 (0.16, 2.83)	0.16 (0.00, 2.83)
	♂	16	13	22.41 (20.06, 26.91)†	10/16 (0.625)	1.0 (0.0, 2.0)	2.01 (0.20, 11.04)	0.54 (0.00, 13.58)
HHFO	♀	9	7	17.82 (14.97, 21.59)*	3/9 (0.333)	0.0 (0.0, 3.0)	1.04 (0.39, 4.91)	0.00 (0.00, 8.87)
	♂	12	10	20.27 (17.43, 23.83)†	5/12 (0.417)	0.0 (0.0, 4.0)	0.81 (0.16, 3.63)	0.00 (0.00, 6.77)
THFO	♀	12	15	18.93 (16.30, 22.98)*	4/12 (0.333)*	0.0 (0.0, 7.0)*	3.14 (0.44, 12.56)*	0.00 (0.00, 27.98)♦
	♂	11	28	23.11 (20.32, 25.76)†	10/11 (0.909)*	2.0 (0.0, 7.0)*♦	0.91 (0.16, 9.62)*	4.45 (0.00, 13.16)♦

Final weight, number, size, and load are given by median (minimum, maximum). N, Number of animals; FW, final weight; FO, native fish oil; HFO, hydrogenated fish oils; AOM, azoxymethane; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil. [a]Number of tumors for analysis of their size. [b]Tumor incidence, number of animals with tumor/number of animals in the group. [c]Tumor number, total number of tumors per animal in the group. [d]Tumor size, area of aberrant tissue per tumor per animal with tumor in the group. [e]Tumor load, total tumor area per animal in the group. ♦$0.20 \geq P > 0.05$, *$P \leq 0.05$, † $P \leq 0.005$.†

Table 2 Effects of HFO versus SO for females, and versus BO for males on final weight and colorectal tumor parameters in AOM treated A/J mice.

Diet	N	N[a]	FW, g	Incidence[b]	Number[c]	Size[d], mm^2	Load[e], mm^2
♀							
SO	10	16	24.95 (20.51, 32.35)†††	9/10 (0.900)*♦	1.5 (0.0, 3.0)	4.44 (0.24, 10.75)*	6.18 (0.00, 3.00)**
PHFO	7	6	19.01 (18.25, 20.63)†	4/7 (0.571)	1.0 (0.0, 3.0)	0.50 (0.16, 2.83)*	0.16 (0.00, 2.83)*
HHFO	9	7	17.82 (14.97, 21.59)†	3/9 (0.333)♦	0.0 (0.0, 3.0)	1.04 (0.39, 4.91)	0.00 (0.00, 8.87)*
THFO	12	15	18.93 (16.30, 22.98)†	4/12 (0.333)*	0.0 (0.0, 7.0)	3.14 (0.44, 12.56)	0.00 (0.00, 27.98)
♂							
BO	13	26	27.53 (21.92, 33.11)†††	11/13 (0.846)♦	1.0 (0.0, 10.0)	1.08 (0.16, 5.94)	3.97 (0.00, 10.84)♦*
PHFO	16	13	22.41 (20.06, 26.91)†	10/16 (0.625)	1.0 (0.0, 2.0)	2.01 (0.20, 11.04)	0.54 (0.00, 13.58)♦
HHFO	12	10	20.27 (17.43, 23.83)†	5/12 (0.417)♦	0.0 (0.0, 4.0)	0.81 (0.16, 3.63)	0.00 (0.00, 6.77)*
THFO	11	28	23.11 (20.32, 25.76)†	10/11 (0.909)	2.0 (0.0, 7.0)	0.91 (0.16, 9.62)	4.45 (0.00, 13.16)

Final weight, number, size and load are given by median (minimum, maximum). N, Number of animals; FW, final weight; SO, refined soybean oil; BO, butter oil without water and salt. HFO, hydrogenated fish oils; AOM, azoxymethane; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; [a]Number of tumors for analysis of their size. [b]Tumor incidence, number of animals with tumor/number of animals in group. [c]Tumor number, total number of tumors per animal in the group. [d]Tumor size, area of aberrant tissue per tumor per animal with tumor in the group. [e]Tumor load, total tumor area per animal in the group. ♦$0.20 \geq P > 0.05$, *$P \leq 0.05$, † $P \leq 0.005$.

Table 3 Effects of HFO or HSO on final weight and colorectal tumor parameters in AOM treated A/J mice compared by increasing hydrogenation degree.

Diet	N	N[a]	FW, g	Incidence[b]	Number[c]	Size[d], mm^2	Load[e], mm^2
♀							
PHFO	7	6	19.01 (18.25, 20.63)♦	4/7 (0.571)	1.0 (0.0, 3.0)	0.50 (0.16, 2.83)*	0.16 (0.00, 2.83)
HHFO	9	7	17.82 (14.97, 21.59)♦♦	3/9 (0.333)	0.0 (0.0, 3.0)	1.04 (0.39, 4.91)	0.00 (0.00, 8.87)
THFO	12	15	18.93 (16.30, 22.98)♦	4/12 (0.333)	0.0 (0.0, 7.0)	3.14 (0.44, 12.56)*	0.00 (0.00, 27.98)
PHSO	13	28	20.51 (17.56, 23.12)**	8/13 (0.615)	2.0 (0.0, 7.0)*	3.14 (0.44, 9.62)	8.29 (0.00, 21.85)♦
HHSO	16	5	18.08 (15.21, 20.86)*	5/16 (0.313)	0.0 (0.0, 1.0)*	3.97 (0.20, 18.47)	0.00 (0.00, 18.47)♦
THSO	12	11	17.96 (16.62, 19.98)*♦	6/12 (0.500)	0.5 (0.0, 3.0)	3.14 (0.20, 12.56)	0.32 (0.00, 15.01)
♂							
PHFO	16	13	22.41 (20.06, 26.91)♦	10/16 (0.625)	1.0 (0.0, 2.0)*	2.01 (0.20, 11.04)	0.54 (0.00, 13.58)♦
HHFO	12	10	20.27 (17.43, 23.83)♦♦	5/12 (0.417)♦	0.0 (0.0, 4.0)*	0.81 (0.16, 3.63)	0.00 (0.00, 6.77)*
THFO	11	28	23.11 (20.32, 25.76)♦*	10/11 (0.909)♦	2.0 (0.0, 7.0)**	0.91 (0.16, 9.62)	4.45 (0.00, 13.16)♦*
PHSO	8	16	23.26 (20.67, 26.72)*	7/8 (0.875)	2.0 (0.0, 4.0)♦	3.14 (0.24, 9.62)	4.46 (0.00, 23.19)
HHSO	5	2	21.97 (19.74, 24.20)*	2/5 (0.400)♦	0.0 (0.0, 1.0)♦♦	3.13 (0.33, 5.94)	0.00 (0.00, 5.94)♦
THSO	11	21	20.96 (18.78, 23.91)*♦	10/11 (0.909)♦	2.0 (0.0, 6.0)♦	1.13 (0.16, 15.90)	0.80 (0.00, 29.30)♦

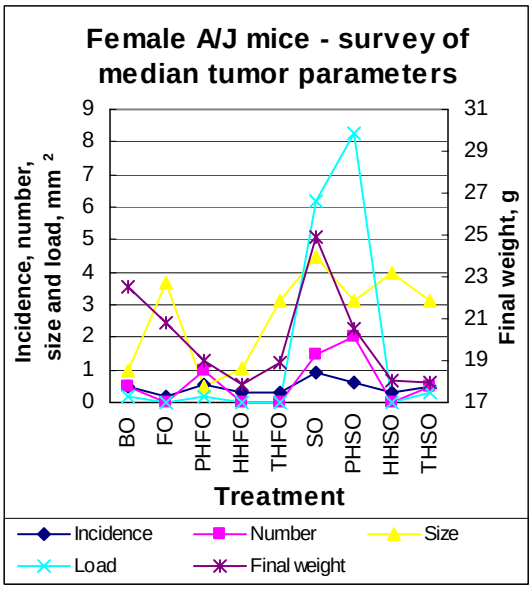
Final weight, number, size and load are given by median (minimum, maximum). HFO, hydrogenated fish oils; HSO, hydrogenated soybean oils; AOM, azoxymethane; N, number of animals; FW, final weight; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil. [a]Number of tumors for analysis of their size. [b]Tumor incidence, number of animals with tumor/number of animals in the group. [c]Tumor number, total number of tumors per animal in the group. [d]Tumor size, area of aberrant tissue per tumor per animal with tumor in the group. [e]Tumor load, total tumor area per animal in the group. ♦$0.20 \geq P > 0.05$, *$P \leq 0.05$, † $P \leq 0.005$.

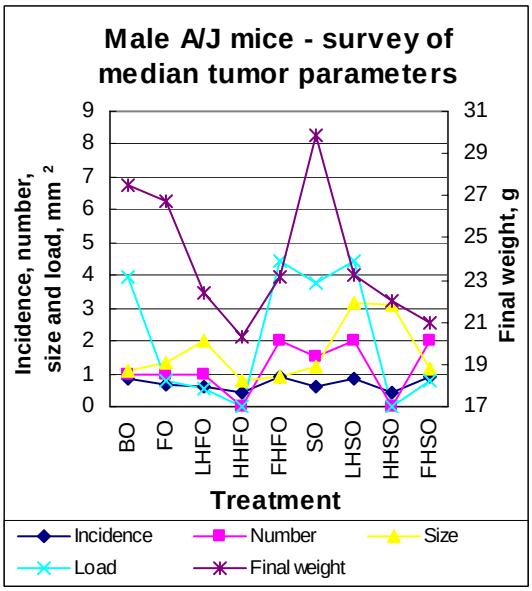
Table 4 Effects of HFO versus HSO on final weight and colorectal tumor parameters in AOM treated A/J mice by corresponding hydrogenation degrees.

Diet	N	N[a]	FW, g	Incidence[b]	Number[c]	Size[d], mm^2	Load[e], mm^2
♀							
PHFO	7	6	19.01 (18.25, 20.63)	4/7 (0.571)	1.0 (0.0, 3.0)	0.50 (0.16, 2.83)†	0.16 (0.00, 2.83)
PHSO	13	28	20.51 (17.56, 23.12)	8/13 (0.615)	2.0 (0.0, 7.0)	3.14 (0.44, 9.62)†	8.29 (0.00, 21.85)
HHFO	9	7	17.82 (14.97, 21.59)	3/9 (0.333)	0.0 (0.0, 3.0)	1.04 (0.39, 4.91)	0.00 (0.00, 8.87)
HHSO	16	5	18.08 (15.21, 20.86)	5/16 (0.313)	0.0 (0.0, 1.0)	3.97 (0.20, 18.47)	0.00 (0.00, 18.47)
THFO	12	15	18.93 (16.30, 22.98)	4/12 (0.333)	0.0 (0.0, 7.0)	3.14 (0.44, 12.56)	0.00 (0.00, 27.98)
THSO	12	11	17.96 (16.62, 19.98)	6/12 (0.500)	0.5 (0.0, 3.0)	3.14 (0.20, 12.56)	0.32 (0.00, 15.01)
♂							
PHFO	16	13	22.41 (20.06, 26.91)	10/16 (0.625)	1.0 (0.0, 2.0)♦	2.01 (0.20, 11.04)	0.54 (0.00, 13.58)*
PHSO	8	16	23.26 (20.67, 26.72)	7/8 (0.875)	2.0 (0.0, 4.0)♦	3.14 (0.24, 9.62)	4.46 (0.00, 23.19)*
HHFO	12	10	20.27 (17.43, 23.83)	5/12 (0.417)	0.0 (0.0, 4.0)	0.81 (0.16, 3.63)	0.00 (0.00, 6.77)
HHSO	5	2	21.97 (19.74, 24.20)	2/5 (0.400)	0.0 (0.0, 1.0)	3.13 (0.33, 5.94)	0.00 (0.00, 5.94)
THFO	11	28	23.11 (20.32, 25.76)*	10/11 (0.909)	2.0 (0.0, 7.0)	0.91 (0.16, 9.62)	4.45 (0.00, 13.16)
THSO	11	21	20.96 (18.78, 23.91)*	10/11 (0.909)	2.0 (0.0, 6.0)	1.13 (0.16, 15.90)	0.80 (0.00, 29.30)

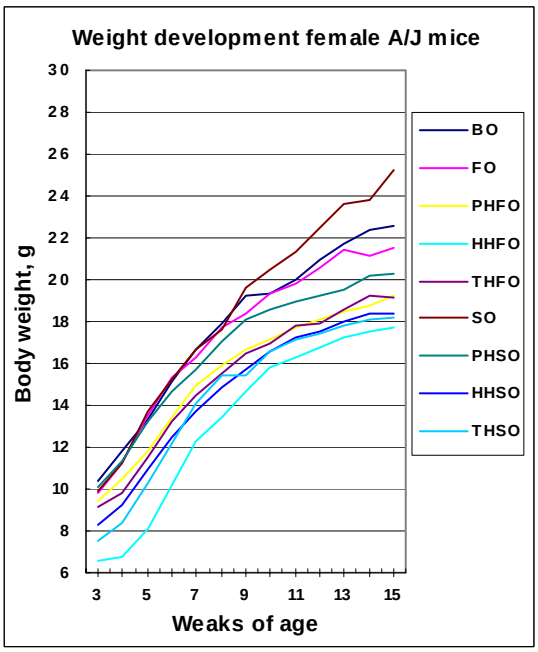
Final weight, number, size and load are given by median (minimum, maximum). HFO, hydrogenated fish oils; HSO, hydrogenated soybean oils; AOM, azoxymethane; N, number of animals; FW, final weight; PHFO, partially hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHFO, highly hydrogenated fish oil; HHSO, highly hydrogenated soybean oil; THFO, totally hydrogenated fish oil; THSO, totally hydrogenated soybean oil. [a]Number of tumors for analysis of their size. [b]Tumor incidence, number of animals with tumor/number of animals in the group. [c]Tumor number, total number of tumors per animal in the group. [d]Tumor size, area of aberrant tissue per tumor per animal with tumor in the group. [e]Tumor load, total tumor area per animal in the group. ♦$0.20 \geq P > 0.05$, *$P \leq 0.05$, † $P \leq 0.005$.

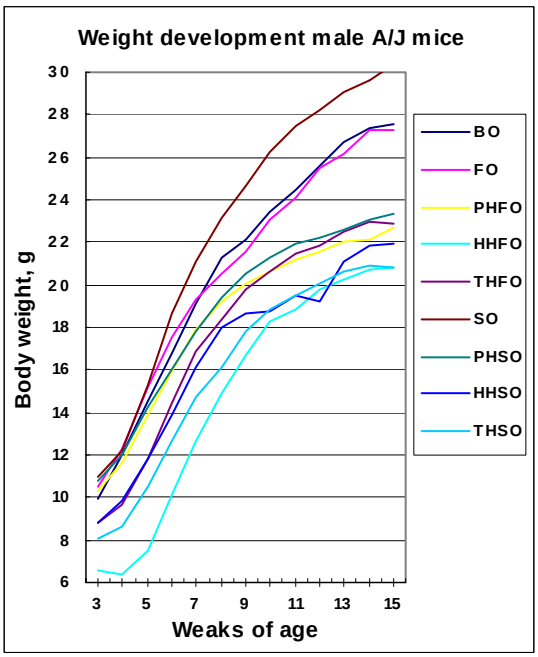
Figures



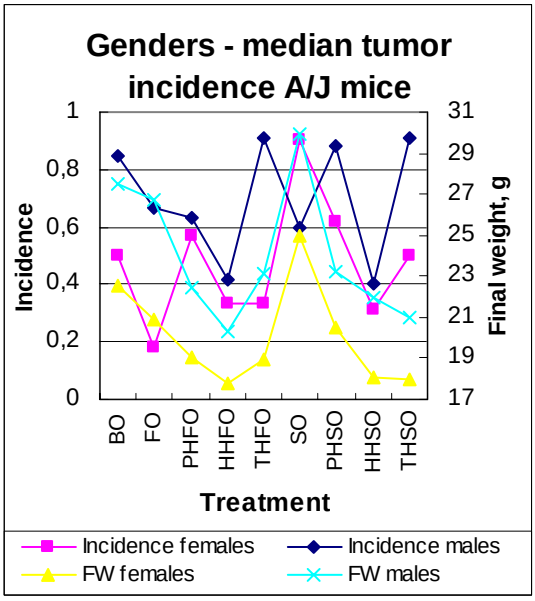


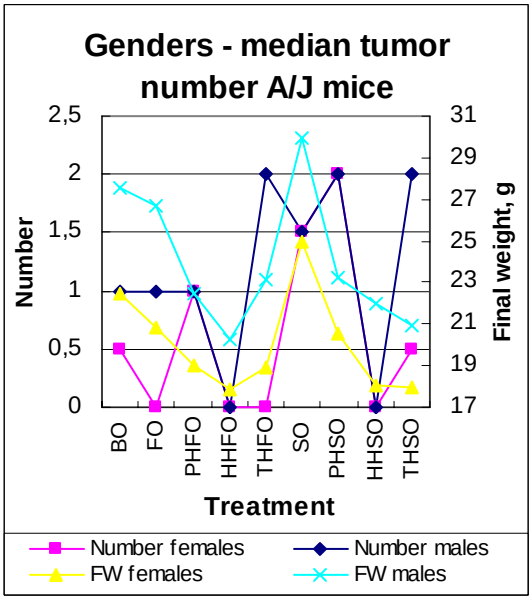
Figures 1 and 2. A survey of tumor incidence, number, size, and load along with final weight by all the experimental oils, separate curves for female and male AOM treated A/J mice. BO, butter oil; FO, fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.



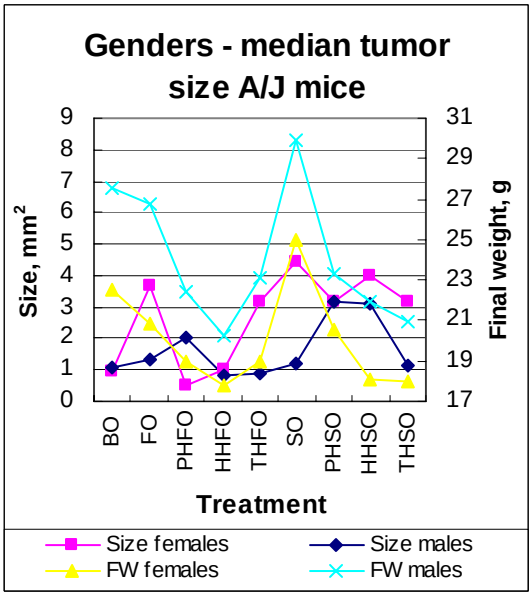


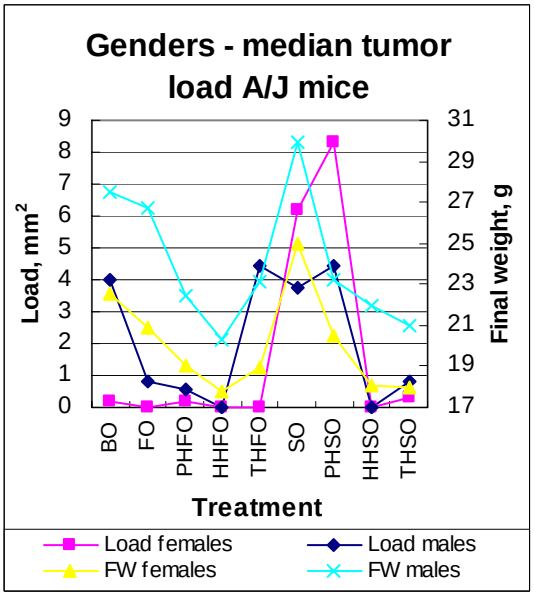
Figures 3 and 4. Weight development wk 3-15 in AOM treated A/J mice fed butter oil (BO), fish oil (FO), partially hydrogenated fish oil (PHFO), highly hydrogenated fish oil (HHFO), totally hydrogenated fish oil (THFO), soybean oil (SO), partially hydrogenated soybean oil (PHSO), highly hydrogenated soybean oil (HHSO), and totally hydrogenated soybean oil (THSO), separate curves for female and male animals.



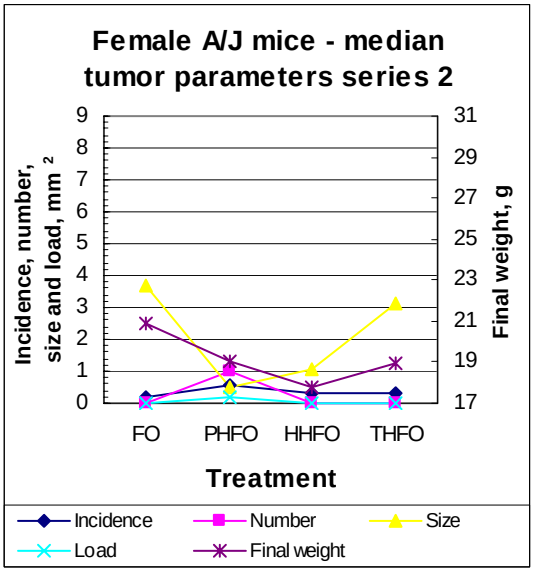


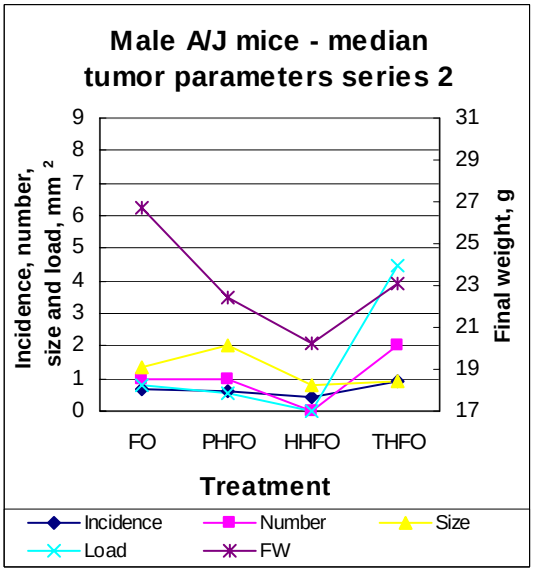
Figures 5 and 6. Separate curves for tumor incidence and number along with final weight (FW) for female and male AOM treated A/J mice. BO, butter oil; FO, fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.



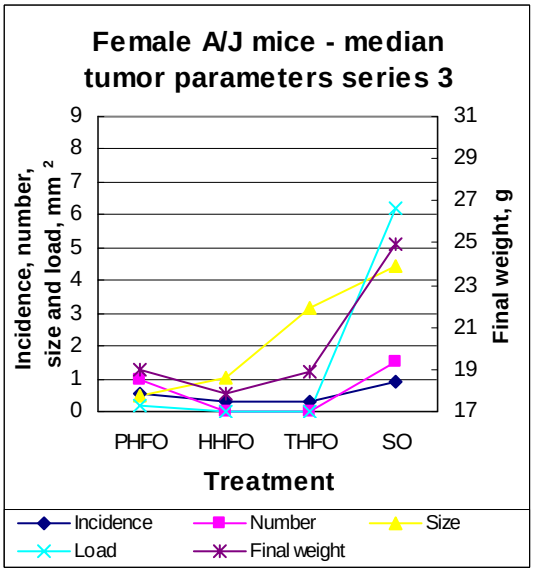


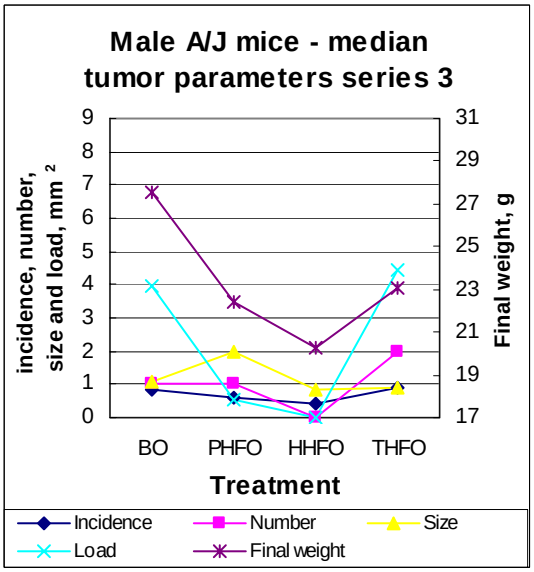
Figures 7 and 8. Separate curves for tumor size and load along with final weight (FW) for female and male AOM treated A/J mice. BO, butter oil; FO, fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.



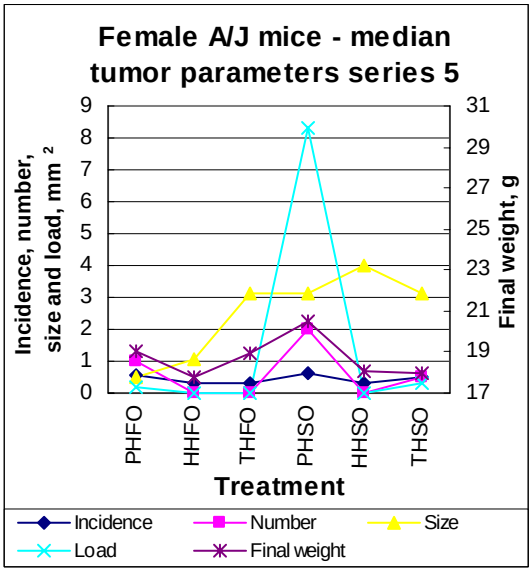


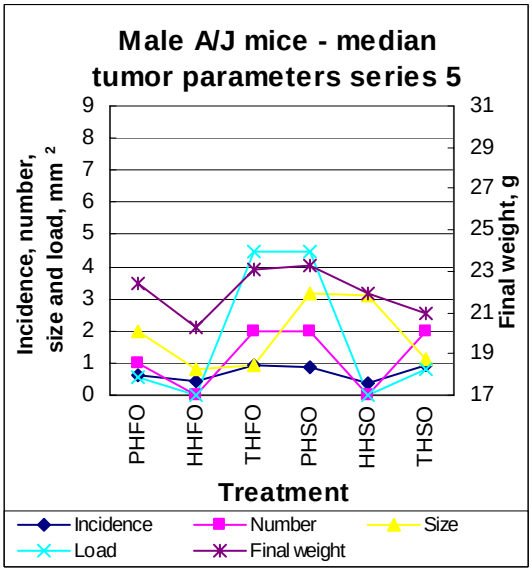
Figures 9 and 10. Series 1 Change in effects when FO was hydrogenated. Tumor incidence, number, size, and load along with final weight, separate curves for female and male AOM treated A/J mice. FO, fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil.



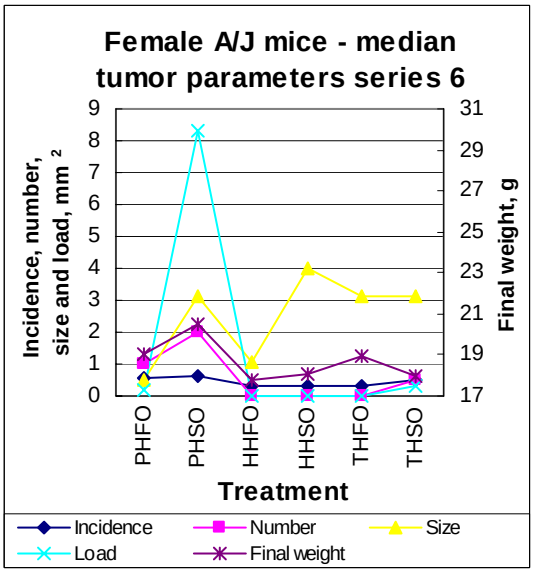


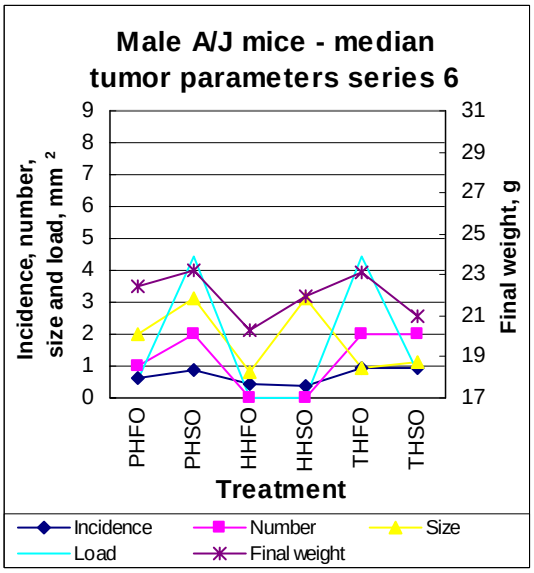
Figures 11 and 12. Series 2 Change in protective properties when FO was hydrogenated. Tumor incidence, number, size, and load along with final weight, separate curves for female and male AOM treated A/J mice. PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, soybean oil; BO, butter oil.





Figures 13 and 14. Series 3 Effects of increasing hydrogenation degree of fish or soybean oil. Tumor incidence, number, size, and load along with final weight, separate curves for female and male AOM treated A/J mice. PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.





Figures 15 and 16. Series 4 Effects of corresponding hydrogenation degrees of fish and soybean oil. Tumor incidence, number, size, and load along with final weight, separate curves for female and male AOM treated A/J mice. PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

APPENDIX V Tables for differences in effects of the experimental oils

DIFFERENCES IN EFFECTS BETWEEN GENDERS

DIFFERENCES IN EFFECTS BETWEEN TREATMENT GROUPS FOR FEMALE AND MALE ANIMALS SEPARATELY

DIFFERENCES IN EFFECTS BETWEEN GENDERS

Induction of CRC, shown by tumor incidence and number

Table 1 Risk estimates with confidence intervals and p-values for gender differences in AOM treated A/J mice fed butter oil, fish oils, and soybean oils.

Gender differences[a]	Animals		TUMOR INCIDENCE		Risk estimate
Animals with and without tumors	N	♂ - ♀	Df	P-value[b]	RR ♂/♀ (95 % CI)
Association	199	95 - 104	1	0.001†	1.528 (1.196, 1.952)*
Differences within groups					
BO	27	13 - 14	(1)	0.103♦	1.692 (0.954, 3.001)
FO	20	**9** - 11	(1)	0.065♦	3.667 (0.964, 13.947)
PHFO	23	16 - **7**	(1)	1.000	1.094 (0.519, 2.305)
HHFO	21	12 - **9**	(1)	1.000	1.250 (0.399, 3.912)
THFO	23	11 - 12	(1)	0.009*	2.727 (1.199, 6.203)*
SO	20	10 - 10	(1)	0.303	0.667 (0.386, 1.152)
PHSO	21	**8** - 13	(1)	0.336	1.422 (0.860, 2.352)
HHSO	21	**5** - 16	(1)	1.000	1.280 (0.350, 4.680)
THSO	23	11 - 12	(1)	0.069♦	1.818 (1.002, 3.299)*

[a]Pearson Chi-Square Test was used and Fisher's Exact Test when Df figure in parenthesis. [b]P-values have not been adjusted *à la* Bonferroni. *P ≤ 0.05 or 95 % CI not including 1. † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; RR, relative risk; CI, confidence interval; BO, butter oil without water and salt; FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, refined soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

Table 2 Estimated median gender differences for tumor number and final weight in AOM treated A/J mice fed butter oil, fish oils, and soybean oils, with p-values.

Gender differences[a]	Animals		TUMOR NUMBER			Final weight		
Animals with and without tumors	N	♂ - ♀	P-value[b]	md	.	md, g	♂/♀	P-value
Association	199	95 - 104	0.003†		.	3.7	1.2	< 0.0005†[c]
Differences within groups ♂-♀					.			
BO	27	13 - 14	0.147♦	0.5	.	5.0	1.2	< 0.0005†
FO	20	**9** - 11	0.053♦	1.0	.	5.9	1.3	0.001†
PHFO	23	16 - **7**	0.915	0.0	.	3.4	1.2	< 0.0005†
HHFO	21	12 - **9**	0.877	0.0	.	2.5	1.1	0.003†
THFO	23	11 - 12	0.041*	2.0	.	4.2	1.2	< 0.0005†
SO	20	10 - 10	0.845	0.0	.	5.0	1.2	0.004†
PHSO	21	**8** - 13	0.814	0.0	.	2.8	1.1	0.001†
HHSO	21	**5** - 16	1.000	0.0	.	3.9	1.2	0.002†
THSO	23	11 - 12	0.092♦	1.5	.	3.0	1.2	< 0.0005†

[a]Mann-Whitney Test with Exact Sig. (2-sided) was used. [b]P-values have not been adjusted *à la* Bonferroni. [c]Asymp. Sig. (2-sided). *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. md, estimated median difference; BO, butter oil without water and salt; FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, refined soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

Growth of CRC, shown by tumor size

Table 3 Estimated median gender differences for tumor size and final weight in AOM treated A/J mice fed butter oil, fish oils, and soybean oils, with p-values.

Gender differences[a]	Tumors		TUMOR SIZE		Final weight		
Animals with tumors	N	♂ - ♀	P-value[b]	md, mm^2	md, g	♂/♀	P-Value
Association	249	145 - 104	0.001†[c]		3.7	1.2	< 0.0005†[c]
Differences within groups ♂ - ♀							
BO	40	26 - 14	0.817	0.1	5.0	1.2	< 0.0005†
FO	11	**9** - **2**	0.909	-2.4	5.9	1.3	0.001†
PHFO	19	13 - **6**	0.253	1.5	3.4	1.2	< 0.0005†
HHFO	17	10 - **7**	0.239	-0.2	2.5	1.1	0.003†
THFO	43	28 - 15	0.026*	-2.2	4.2	1.2	< 0.0005†
SO	36	20 - 16	0.050*	-3.2	5.0	1.2	0.004†
PHSO	44	16 - 28	0.517	0.0	2.8	1.1	0.001†
HHSO	7	**2** - **5**	0.857	-0.8	3.9	1.2	0.002†
THSO	32	21 - 11	0.218	-2.8	3.0	1.2	< 0.0005†

[a]Mann-Whitney Test with Exact Sig. (2-sided) was used. [b]P-values have not been adjusted *à la* Bonferroni. [c]Asymp. Sig. (2-sided). *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. md, estimated median difference; BO, butter oil without water and salt; FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, refined soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

Tumor load, shows induction and/or growth of CRC

Table 4 Estimated median gender differences for tumor load and final weight in AOM treated A/J mice fed butter oil, fish oils, and soybean oils, with p-values.

Gender differences[a]	Animals		TUMOR LOAD		Final weight		
animals with and without tumors	N	♂ - ♀	P-value[b]	md, mm^2	md, g	♂/♀	P-value
Association	199	95 - 104	0.033*		3.7	1.2	< 0.0005†[c]
Differences within groups ♂ - ♀							
BO	27	13 - 14	0.086♦	3.8	5.0	1.2	< 0.0005†
FO	20	**9** - 11	0.035*	0.8	5.9	1.3	0.001†
PHFO	23	16 - **7**	0.531	0.4	3.4	1.2	< 0.0005†
HHFO	21	12 - **9**	1.000	0.0	2.5	1.1	0.003†
THFO	23	11 - 12	0.081♦	4.4	4.2	1.2	< 0.0005†
SO	20	10 - 10	0.251	-2.4	5.0	1.2	0.004†
PHSO	21	**8** - 13	0.874	-3.8	2.8	1.1	0.001†
HHSO	21	**5** - 16	0.893	0.0	3.9	1.2	0.002†
THSO	23	11 - 12	0.309	0.5	3.0	1.2	< 0.0005†

[a]Mann-Whitney Test with Exact Sig. (2-sided) was used. [b]P-values have not been adjusted *à la* Bonferroni. [c]Asymp. Sig. (2-sided). *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. md, estimated median difference; BO, butter oil without water and salt; FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; SO, refined soybean oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

DIFFERENCES IN EFFECTS BETWEEN TREATMENT GROUPS FOR FEMALE AND MALE ANIMALS SEPARATELY

Table 5 Series 1. Risk estimates with confidence intervals and p-values for AOM treated A/J mice. Hydrogenated fish oils compared with unhydrogenated fish oil.

Series 1[a]	Animals		TUMOR INCIDENCE			Risk Estimate
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	RR (95 % CI)
Associations						
The fish oils ♀	39		(3)	0.419		
♂	48		(3)	0.104♦		
Differences between groups						
♀ PHFO - FO	18	**7** - 11	(1)	0.141	0.423	3.143 (0.769, 12.850)
HHFO - FO	20	**9** - 11	(1)	0.617	1.000	1.833 (0.386, 8.701)
THFO - FO	23	12 - 11	(1)	0.640	1.000	1.833 (0.414, 8.112)
♂ PHFO - FO	25	16 - **9**	(1)	1.000	1.000	0.938 (0.516, 1.705)
HHFO - FO	21	12 - **9**	(1)	0.387	1.000	0.625 (0.277, 1.410)
THFO - FO	20	11 - **9**	(1)	0.285	0.855	1.364 (0.828, 2.245)

[a]Fisher's Exact Test was used. [b]P-value adjusted *à la* Bonferroni. *P ≤ 0.05 or 95 % CI not including 1. † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; RR, relative risk; CI, confidence interval; FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil.

Table 6 Series 1. Estimated median differences for tumor number and final weight in AOM treated A/J mice, with p-values. Hydrogenated fish oils compared with unhydrogenated fish oil.

Series 1[a]	Animals		TUMOR NUMBER				Final weight	
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	md	P-value	P x 3
Associations								
The fish oils ♀	39		3	0.455			0.003[c]†	
♂	48		3	0.023*			< 0.0005[c]†	
Differences between groups							md, g	
♀ PHFO - FO	18	**7** - 11		0.107	0.321	1.0	-1.8	0.045*
HHFO - FO	20	**9** - 11		0.408	1.000	0.0	-3.0	0.012*
THFO - FO	23	12 - 11		0.245	0.735	0.0	-1.9	0.039*
♂ PHFO - FO	25	16 - **9**		1.000	1.000	0.0	-4.3	< 0.0015†
HHFO - FO	21	12 - **9**		0.581	1.000	-1.0	-6.5	< 0.0015†
THFO - FO	20	11 - **9**		0.033	0.099♦	1.0	-3.6	< 0.0015†

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil.

Table 7 Series 1. Estimated median differences for tumor size and final weight in AOM treated A/J mice, with p-values. Hydrogenated fish oils compared with unhydrogenated fish oil.

Series 1[a]	Tumors		TUMOR SIZE				Final weight	
Animals with tumors	N	n - n	Df	P-value	P x 3[b]	md, mm^2	P-value	P x 3
Associations								
The fish oils ♀	30		3	0.082♦[c]			0.003[c]	
♂	60		3	0.456[c]			< 0.0005[c]†	
Differences between groups							md, g	
♀ PHFO - FO	8	**6** - **2**		0.643	1.000	-3.2	-1.8	0.045*
HHFO - FO	9	**7** - **2**		1.000	1.000	-2.7	-3.0	0.012*
THFO - FO	17	15 - **2**		0.706	1.000	-0.6	-1.9	0.039*
♂ PHFO - FO	22	13 - **9**		0.935	1.000	0.7	-4.3	< 0.0015†
HHFO - FO	19	10 - **9**		0.305	0.915	-0.5	-6.5	< 0.0015†
THFO - FO	37	28 - **9**		0.595	1.000	-0.4	-3.6	< 0.0015†

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil.

Table 8 Series 1. Estimated median differences for tumor load and final weight in AOM treated A/J mice, with p-values. Hydrogenated fish oils compared with unhydrogenated fish oil.

Series 1[a]	Animals		TUMOR LOAD				Final weight	
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	md, mm^2	P-value	P x 3
Associations								
The fish oils ♀	39		3	0.598			0.003[c]	
♂	48		3	0.017[c]*			< 0.0005[c]†	
Differences between groups							md, g	
♀ PHFO - FO	18	**7** - 11		0.143	0.429	0.2	-1.8	0.045*
HHFO - FO	20	**9** - 11		0.438	1.000	0.0	-3.0	0.012*
THFO - FO	23	12 - 11		0.385	1.000	0.0	-1.9	0.039*
♂ PHFO - FO	25	16 - **9**		0.887	1.000	-0.2	-4.3	< 0.0015†
HHFO - FO	21	12 - **9**		0.200	0.600	-0.8	-6.5	< 0.0015†
THFO - FO	20	11 - **9**		0.181	0.543	3.7	-3.6	< 0.0015†

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; FO, native fish oil; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil.

Table 9 Series 2. Risk estimates with confidence intervals and p-values for AOM treated A/J mice fed hydrogenated fish oils or soybean oil (females) or butter oil (males).

Series 2[a]	Animals		TUMOR INCIDENCE			Risk Estimate
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	RR (95 % CI)
Associations						
HFO and SO ♀	38		(3)	0.029*		
HFO and BO ♂	52		(3)	0.045*		
Differences between groups						
♀ SO - PHFO	17	10 - **7**	(1)	0.250	0.750	1.575 (0.803, 3.090)
SO - HHFO	19	10 - **9**	(1)	0.020	0.060♦	2.700 (1.048, 6.959)*
SO - THFO	22	10 - 12	(1)	0.011	0.033*	2.700 (1.182, 6.170)*
♂ PHFO - BO	29	16 - 13	(1)	0.238	0.714	0.739 (0.473, 1.152)
HHFO - BO	25	12 - 13	(1)	0.041	0.123♦	0.492 (0.242, 1.000)
THFO - BO	24	11 - 13	(1)	1.000	1.000	1.074 (0.798, 1.447)

[a]Fisher's Exact Test was used. [b]P-value adjusted *à la* Bonferroni. *P ≤ 0.05 or 95 % CI not including 1. † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; RR, relative risk; CI, confidence interval; HFO, hydrogenated fish oils; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; BO, butter oil without water and salt; SO, refined soybean oil;

Table 10 Series 2. Estimated median differences for tumor number and final weight in AOM treated A/J mice fed hydrogenated fish oils or soybean oil (females) or butter oil (males), with p-values.

Series 2[a]	Animals		TUMOR NUMBER				Final weight	
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	md	P-value	P x 3
Associations								
HFO and SO ♀	38		3	0.206			< 0.0005[c]†	
HFO and BO ♂	52		3	0.019[c]*			< 0.0005[c]†	
Differences between groups							md, g	
♀ SO - PHFO	17	10 - **7**		0.128	0.384	0.5	6.0	< 0.0015†
SO - HHFO	19	10 - **9**		0.078	0.234	1.5	7.2	< 0.0015†
SO - THFO	22	10 - 12		0.115	0.345	1.5	6.1	< 0.0015†
♂ PHFO - BO	29	16 - 13		0.103	0.309	0.0	-5.1	< 0.0015†
HHFO - BO	25	12 - 13		0.084	0.252	-1.0	-7.2	< 0.0015†
THFO - BO	24	11 - 13		0.252	0.756	1.0	-4.4	0.003†

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; HFO, hydrogenated fish oils; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; BO, butter oil without water and salt; SO, refined soybean oil.

Table 11 Series 2. Estimated median differences for tumor size and final weight in AOM treated A/J mice fed hydrogenated fish oils or soybean oil (females) or butter oil (males), with p-values.

Series 2[a]	Tumors		TUMOR SIZE				Final weight	
Animals with tumors	N	n - n	Df	P-value	P x 3[b]	md, mm^2	P-value	P x 3
Associations								
HFO and SO ♀	44		3	0.036*[c]			< 0.0005[c]†	
HFO and BO ♂	77		3	0.471[c]			< 0.0005[c]†	
Differences between groups							md, g	
♀ SO - PHFO	22	16 - **6**		0.016	0.048*	3.9	6.0	< 0.0015†
SO - HHFO	23	16 - **7**		0.138	0.414	3.4	7.2	< 0.0015†
SO - THFO	31	16 - 15		0.747	1.000	1.3	6.1	< 0.0015†
♂ PHFO - BO	39	13 - 26		0.924	1.000	0.9	-5.1	< 0.0015†
HHFO - BO	36	10 - 26		0.197	0.591	-0.3	-7.2	< 0.0015†
THFO - BO	54	28 - 26		0.754	1.000	-0.2	-4.4	0.003†

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; HFO, hydrogenated fish oils; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; BO, butter oil without water and salt; SO, refined soybean oil.

Table 12 Series 2. Estimated median differences for tumor load and final weight in AOM treated A/J mice fed hydrogenated fish oils or soybean oil (females) or butter oil (males), with p-values.

Series 2[a]	Animals		TUMOR LOAD				Final weight	
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	md, mm^2	P-value	P x 3
Associations								
HFO and SO ♀	38		3	0.041*[c]			< 0.0005[c]†	
HFO and BO ♂	52		3	0.003[c]†			< 0.0005[c]†	
Differences between groups							md, g	
♀ SO - PHFO	17	10 - **7**		0.010	0.030*	6.0	6.0	< 0.0015†
SO - HHFO	19	10 - **9**		0.015	0.045*	6.2	7.2	< 0.0015†
SO - THFO	22	10 - 12		0.073	0.219	6.2	6.1	< 0.0015†
♂ PHFO - BO	29	16 - 13		0.035	0.105♦	-3.4	-5.1	< 0.0015†
HHFO - BO	25	12 - 13		0.006	0.018*	-4.0	-7.2	< 0.0015†
THFO - BO	24	11 - 13		0.399	1.000	0.5	-4.4	0.003†

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; HFO, hydrogenated fish oils; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; BO, butter oil without water and salt; SO, refined soybean oil.

Table 13 Series 3a. Risk estimates with confidence intervals and p-values for AOM treated A/J mice fed increasing hydrogenation degrees of fish oil.

Series 3a[a]	Animals		TUMOR INCIDENCE			Risk Estimate
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	RR (95 % CI)
Associations ♀	28		(2)	0.616		
♂	39		(2)	0.051(*)		
Differences between groups						
♀ THFO - PHFO	19	12 - **7**	(1)	0.377	1.000	0.583 (0.209, 1.627)
HHFO - PHFO	16	**9** - **7**	(1)	0.615	1.000	0.583 (0.189, 1.796)
THFO - HHFO	21	12 - **9**	(1)	1.000	1.000	1.000 (0.295, 3.395)
♂ THFO - PHFO	27	11 - 16	(1)	0.183	0.549	1.455 (0.953, 2.221)
HHFO - PHFO	28	12 - 16	1	0.274	0.822	0.667 (0.309, 1.439)
THFO - HHFO	23	11 - 12	(1)	0.027	0.081♦	2.182 (1.089, 4.372)*

[a]Pearson Chi-Square Test was used and Fisher's Exact Test when Df figure in parenthesis. [b]P-value adjusted *à la* Bonferroni. *P ≤ 0.05 or 95 % CI not including 1. † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; RR, relative risk; CI, confidence interval; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil.

Table 14 Series 3a. Estimated median differences for tumor number and final weight in AOM treated A/J mice fed increasing hydrogenation degrees of fish oil, with p-values.

Series 3a[a]	Animals		TUMOR NUMBER				.	Final weight	
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	md	.	P-value	P x 3
Associations ♀	28		2	0.851			.	0.061[c]♦	
♂	39		2	0.011*			.	0.044[c]*	
Differences between groups							.	md, g	
♀ HHFO - PHFO	16	**9** - **7**		0.629	1.000	-1.0	.	-1.2	0.165♦
THFO - PHFO	19	12 - **7**		0.735	1.000	-1.0	.	-0.1	1.000
THFO - HHFO	21	12 - **9**		0.857	1.000	0.0	.	1.1	0.123♦
♂ HHFO - PHFO	28	12 - 16		0.600	1.000	-1.0	.	-2.1	0.078♦
THFO - PHFO	27	11 - 16		0.010	0.030*	1.0	.	0.7	1.000
THFO - HHFO	23	11 - 12		0.015	0.045*	2.0	.	2.8	0.111♦

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil.

Table 15 Series 3b. Risk estimates with confidence intervals and p-values for AOM treated A/J mice fed increasing hydrogenation degrees of soybean oil.

Series 3b[a]	Animals		TUMOR INCIDENCE			Risk Estimate
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	RR (95 % CI)
Associations ♀	41		2	0.254		
♂	24		(2)	0.094♦		
Differences between groups						
♀ THSO - PHSO	25	12 - 13	1	0.561	1.000	0.813 (0.399, 1.653)
HHSO - PHSO	29	16 - 13	1	0.103	0.309	0.508 (0.218, 1.181)
THSO - HHSO	28	12 - 16	(1)	0.441	1.000	1.600 (0.637, 4.019)
♂ THSO - PHSO	19	11 - **8**	(1)	1.000[c]	1.000	1.039 (0.753, 1.433)
HHSO - PHSO	13	**5** - **8**	(1)	0.217	0.651	0.457 (0.151, 1.380)
THSO - HHSO	16	11 - **5**	(1)	0.063	0.189♦	2.273 (0.764, 6.757)

[a]Pearson Chi-Square Test was used and Fisher's Exact Test when Df figure in parenthesis. [b]P-value adjusted *à la* Bonferroni. [c]The minimum expected count is 0.84. *P ≤ 0.05 or 95 % CI not including 1. † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; RR, relative risk; CI, confidence interval; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

Table 16 Series 3b. Estimated median differences for tumor number and final weight in AOM treated A/J mice fed increasing hydrogenation degrees of soybean oils, with p-values.

Series 3b[a]	Animals		TUMOR NUMBER				.	Final weight	
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	md	.	P-value	P x 3
Associations ♀	41		2	0.039*			.	0.004[c]†	
♂	24		2	0.033*			.	0.021[c]*	
Differences between groups							.	md, g	
♀ HHSO - PHSO	29	16 - 13		0.016	0.048*	-2.0	.	-2.4	0.012*
THSO - PHSO	25	12 - 13		0.223	0.669	-1.5	.	-2.5	0.009*
THSO - HHSO	28	12 - 16		0.152	0.456	-0.5	.	-0.1	1.000
♂ HHSO - PHSO	13	**5** - **8**		0.037	0.111♦	-2.0	.	-1.3	0.666
THSO - PHSO	19	11 - **8**		0.661	1.000	0.0	.	-2.3	0.015*
THSO - HHSO	16	11 - **5**		0.026	0.078♦	2.0	.	-1.0	0.960

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

Table 17 Series 3a. Estimated median differences for tumor size and final weight in AOM treated A/J mice fed increasing hydrogenation degrees of fish oil, with p-values.

Series 3a[a]	Tumors		TUMOR SIZE				.	Final weight	
Animals with tumors	N	n - n	Df	P-value	P x 3[b]	md, mm^2	.	P-value	P x 3
Associations ♀	28		2	0.031[c]*			.	0.061[c]♦	
♂	51		2	0.282[c]			.	0.044[c]*	
Differences between groups							.	md, g	
♀ HHFO - PHFO	13	**7** - **6**		0.168	0.504	0.5	.	-1.2	0.165♦
THFO - PHFO	21	15 - **6**		0.010	0.030*	2.6	.	-0.1	1.000
THFO - HHFO	22	15 - **7**		0.216	0.648	2.1	.	1.1	0.123♦
♂ HHFO - PHFO	23	10 - 13		0.118	0.354	-1.2	.	-2.1	0.078♦
THFO - PHFO	41	28 - 13		0.917	1.000	-1.1	.	0.7	1.000
THFO - HHFO	38	28 - 10		0.173	0.519	0.1	.	2.8	0.111♦

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil.

Table 18 Series 3b. Estimated median differences for tumor size and final weight in AOM treated A/J mice fed increasing hydrogenation degrees of soybean oil, with p-values.

Series 3b[a]	Tumors		TUMOR SIZE				.	Final weight	
Animals with tumors	N	n - n	Df	P-value	P x 3[b]	md, mm^2	.	P-value	P x 3
Associations ♀	44		2	0.812[c]			.	0.004[c]†	
♂	39		2	0.304[c]			.	0.021[c]*	
Differences between groups							.	md, g	
♀ HHSO - PHSO	33	**5** - 28		0.634	1.000	0.8	.	-2.4	0.012*
THSO - PHSO	39	11 - 28		0.740	1.000	0.0	.	-2.5	0.009*
THSO - HHSO	16	11 - **5**		0.597	1.000	-0.8	.	-0.1	1.000
♂ HHSO - PHSO	18	**2** - 16		0.824	1.000	0.0	.	-1.3	0.666
THSO - PHSO	37	21 - 16		0.124	0.372	-2.0	.	-2.3	0.015*
THSO - HHSO	23	21 - **2**		0.775	1.000	-2.0	.	-1.0	0.960

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

Table 19 Series 3a. Estimated median differences for tumor load and final weight in AOM treated A/J mice fed increasing degrees of hydrogenated fish oil, with p-values.

Series 3a[a]	Animals		TUMOR LOAD				Final weight	
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	md, mm^2	P-value	P x 3
Association ♀	28		2	0.911			0.061[c]♦	
♂	39		2	0.005[c]†			0.044[c]*	
Differences between groups							md, g	
♀ HHFO - PHFO	16	**9** - **7**		0.721	1.000	-0.2	-1.2	0.165♦
THFO - PHFO	19	12 - **7**		0.882	1.000	-0.2	-0.1	1.000
THFO - HHFO	21	12 - **9**		0.724	1.000	0.0	1.1	0.123♦
♂ HHFO - PHFO	28	12 - 16		0.198	0.594	-0.5	-2.1	0.078♦
THFO - PHFO	27	11 - 16		0.017	0.051♦	3.9	0.7	1.000
THFO - HHFO	23	11 - 12		0.002	0.006*	4.4	2.8	0.111♦

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil.

Table 20 Series 3b. Estimated median differences for tumor load and final weight in AOM treated A/J mice fed increasing degrees of hydrogenated soybean oil, with p-values.

Series 3b[a]	Animals		TUMOR LOAD				Final weight	
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	md, mm^2	P-value	P x 3
Associations ♀	41		2	0.094♦			0.004[c]†	
♂	24		2	0.085[c]♦			0.021[c]*	
Differences between groups							md, g	
♀ HHSO - PHSO	29	16 - 13		0.041	0.123♦	-8.3	-2.4	0.012*
THSO - PHSO	25	12 - 13		0.189	0.567	-8.0	-2.5	0.009*
THSO - HHSO	28	12 - 16		0.337	1.000	0.3	-0.1	1.000
♂ HHSO - PHSO	13	**5** - **8**		0.070	0.210	-4.5	-1.3	0.666
THSO - PHSO	19	11 - **8**		0.406	1.000	-3.7	-2.3	0.015*
THSO - HHSO	16	11 - **5**		0.052	0.156♦	0.8	-1.0	0.960

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

Table 21 Series 4. Risk estimates with confidence intervals and p-values for AOM treated A/J mice fed corresponding hydrogenation degrees of fish and soybean oil.

Series 4[a]	Animals		TUMOR INCIDENCE			Risk Estimate
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	RR (95 % CI)
Associations ♀	69		(5)	0.526		
♂	63		(5)	0.024*		
Differences between groups						
♀ PHSO - PHFO	20	13 - **7**	(1)	1.000	1.000	1.077 (0.498, 2.331)
HHSO - HHFO	25	16 - **9**	(1)	1.000	1.000	0.938 (0.289, 3.037)
THSO - THFO	24	12 - 12	1	0.408	1.000	1.500 (0.563, 3.997)
♂ PHSO - PHFO	24	**8** - 16	(1)	0.352	1.000	1.400 (0.883, 2.220)
HHSO - HHFO	17	**5** - 12	(1)	1.000	1.000	0.960 (0.271, 3.402)
THSO - THFO	22	11 - 11	(1)	1.000	1.000	1.000 (0.768, 1.302)

[a]Pearson Chi-Square Test was used and Fisher's Exact Test when Df figure in parenthesis. [b]P-value adjusted *à la* Bonferroni. *P ≤ 0.05 or 95 % CI not including 1. † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; RR, relative risk; CI, confidence interval; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

Table 22 Series 4. Estimated median differences for tumor number and final weight in AOM treated A/J mice fed corresponding hydrogenation degrees of fish and soybean oil, with p-values.

Series 4[a]	Animals		TUMOR NUMBER				Final weight	
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	md	P-value	P x 3
Associations ♀	69		5	0.256			0.007[c]*	
♂	63		5	0.005†			0.007[c]*	
Differences between groups							md, g	
♀ PHSO - PHFO	20	13 - **7**		0.330	0.990	1.0	1.5	0.489
HHSO - HHFO	25	16 - **9**		0.688	1.000	0.0	0.3	1.000
THSO - THFO	24	12 - 12		0.771	1.000	0.5	-1.0	0.384
♂ PHSO - PHFO	24	**8** - 16		0.028	0.084♦	1.0	0.9	0.792
HHSO - HHFO	17	**5** - 12		0.748	1.000	0.0	1.7	0.984
THSO - THFO	22	11 - 11		0.498	1.000	0.0	-2.2	0.048*

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

Table 23 Series 4. Estimated median differences for tumor size and final weight in AOM treated A/J mice fed corresponding hydrogenation degrees of fish and soybean oil, with p-values.

Series 4[a]	Tumors		TUMOR SIZE				Final weight	
Animals with tumors	N	n - n	Df	P-value	P x 3[b]	md, mm^2	P-value	P x 3
Associations ♀	72		5	0.065[c]♦			0.007[c]*	
♂	90		5	0.265[c]			0.007[c]*	
Differences between groups							md, g	
♀ PHSO - PHFO	34	28 - **6**		0.001	0.003†	2.6	1.5	0.489
HHSO - HHFO	12	**5** - **7**		0.321	0.963	2.9	0.3	1.000
THSO - THFO	26	11 - 15		0.655	1.000	0.0	-1.0	0.384
♂ PHSO - PHFO	29	16 - 13		0.187	0.561	1.1	0.9	0.792
HHSO – HHFO	12	**2** - 10		0.485	1.000	2.3	1.7	0.984
THSO - THFO	49	21 - 28		0.760	1.000	0.2	-2.2	0.048*

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.

Table 24 Series 4. Estimated median differences for tumor load and final weight in AOM treated A/J mice fed corresponding hydrogenation degrees of fish and soybean oils, with p-values.

Series 4[a]	Animals		TUMOR LOAD				Final weight	
Animals with and without tumors	N	n - n	Df	P-value	P x 3[b]	md, mm^2	P-value	P x 3
Associations ♀	69		5	0.314			0.007[c]*	
♂	63		5	0.004[c]†			0.007[c]*	
Differences between groups							md, g	
♀ PHSO - PHFO	20	13 - **7**		0.148	0.444	8.1	1.5	0.489
HHSO - HHFO	25	16 - **9**		0.897	1.000	0.0	0.3	1.000
THSO - THFO	24	12 - 12		0.690	1.000	0.3	-1.0	0.384
♂ PHSO - PHFO	24	**8** - 16		0.014	0.042*	3.9	0.9	0.792
HHSO - HHFO	17	**5** - 12		1.000	1.000	0.0	1.7	0.984
THSO - THFO	22	11 - 11		0.468	1.000	-3.6	-2.2	0.048*

[a]Kruskal-Wallis Test with Exact Sig. and Mann-Whitney Test with Exact Sig. (2-sided) were used. [b]P-value adjusted *à la* Bonferroni. [c]Asymp. Sig. *P ≤ 0.05, † P ≤ 0.005, ♦0.20 ≥ P > 0.05. Df, degrees of freedom; md, estimated median difference; PHFO, partially hydrogenated fish oil; HHFO, highly hydrogenated fish oil; THFO, totally hydrogenated fish oil; PHSO, partially hydrogenated soybean oil; HHSO, highly hydrogenated soybean oil; THSO, totally hydrogenated soybean oil.