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Fats and fatty acids in human nutrition

Report of an expert consultation

Food and Agriculture
Organization of
the United Nations

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In memoriam

Professor John C. Waterlow died peacefully on 19 October 2010 at the age of 94 at the Chelsea and Westminster Hospital in London. Over the last years his body had weakened but his mind was as sharp as ever up to his last days. With his passing away, the international nutrition community has lost an exceptional nutritionist. FAO will miss this remarkable, knowledgeable, reliable and loyal friend who put all his expertise and wisdom to the service of the hungry and malnourished in different parts of the world.

Professor Waterlow spent approximately twenty years in the Caribbean region, working in Guyana, Trinidad and Tobago and Jamaica, where he established the Tropical Metabolism Research Unit at the University of the West Indies in Kingston, Jamaica and carried out his cutting-edge work on the pathophysiology and treatment of malnutrition. A trademark of his work was to transform complex scientific and technical issues into simple, practical messages such as his "10 easy-to-remember steps" treatment guidelines for hospital staff in treating malnutrition and its related diseases.

When Professor Waterlow returned to the UK and began his long tenure as Professor of Human Nutrition at the London School of Hygiene and Tropical Medicine (LSHTM), a long-standing and strong relationship continued with FAO. Because of his eclectic interests and knowledge, John's contributions ranged from childhood growth and diseases to nutrition requirements, with particular attention to protein, his specialty. He generously gave his time, expertise and prestige to support FAO and WHO in their nutrition programmes from the early 1970's until 2004, chairing a number of expert committees and consultations and participating in numerous seminars and meetings. Even with his retirement from the LSHTM in 1981 he continued to serve selflessly.

Not only did he serve, but the plethora of students he taught, in the United Kingdom and in Jamaica, served with him and then in his place after he did truly retire. He was seen by many, even those who had never studied formally under him, as "the professor". Once in retirement he was reluctant to fill the place of an active scientist in scientific deliberations, noting that he was no longer current with the scientific literature. However, once the deliberations began no one could quite identify those scientific areas in which he was failing. Perhaps his last scientific tour de force was the 2006 revision of the 1978 classic *Protein turnover in mammalian tissues and in the whole body*, which he did the old fashioned way relying on index cards and little on computer searches.

John Waterlow was never interested in pushing his own research or areas of interest except when it was for the welfare of the children in the developing world or, in fact, children everywhere. When the discussion became too esoteric and argumentative, he would remind all, in an even voice and with carefully chosen words, what was the main reason they were discussing these issues and "those who were the object of the discussion" should not be forgotten.

He will be remembered by all of us who had the benefit to work with him, for his extensive knowledge of nutrition, for his dedication for the cause of combating hunger and malnutrition in all its forms, and for his integrity and wisdom during the nutrition deliberations in international fora.

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Within the Secretariat, the special efforts of Dr Gina Kennedy, who compiled and reviewed draft papers and Dr Robert Weisell who prepared the background papers for publication in the *Annals of Nutrition and Metabolism*, as well as the draft report are gratefully acknowledged.

Each of these outstanding scientists is listed in the annex of this report.

Acronyms and symbols

%E	percent of energy
%E fat	percent of energy from fat
%FA	percentage fatty acid composition ("wt:wt")
AA	arachidonic acid *(trivial name)* 20:4n-6 *(IUPAC notation)** 5z,8z,11z,14z-eicosatetraenoic acid *(systematic name)*
AD	Alzheimer's disease
AI	adequate intake (expressed as a range)
ALA	alpha linolenic acid *(trivial name)* 18:3n-3 *(IUPAC notation)** 9z,12z,15z-octadecatrienoic acid *(systematic name)*
AMDR	acceptable macronutrient distribution range
ANR	average nutrient requirement
ARM	age-related maculopathy
BC	breast cancer
BP	blood pressure
CE	cholesterol ester
CHD	coronary heart disease
CHO	carbohydrate
ChREBP	cholesterol regulatory element binding protein
CLA	conjugated linoleic acid
CLN	conjugated linolenic acid
CNS	central nervous system
COX	cyclooxygenase
CRC	colorectal cancer
CVD	cardiovascular disease
DG	diacylglycerol
DHA	docosahexaenoic acid [cervonic acid] *(trivial name)* 22:6n-3 *(IUPAC notation)** 4z,7z,10z,13z,16z,19z-docosahexaenoic acid *(systematic name)*
DHGLA	dihomo-gamma linolenic acid
DPA	n-6 docosapentaenoic acid
DRI	dietary reference intake
E	energy
EAR	estimated average requirement
EFA	essential fatty acid
EJCN	European Journal of Clinical Nutrition
EPA	eicosapentaenoic acid [timnodonic acid] *(trivial name)* 20:5n-3 *(IUPAC notation)** 5z,8z,11z,14z,17z-eicosapentaenoic acid *(systematic name)*
FA	fatty acid
FAME	fatty acid methyl ester
FAO	Food and Agriculture Organization of the United Nations
FBS	food balance sheet

FDA	US Food and Drug Administration
FER	fat energy ratio
FFA	free fatty acid
FID	flame ionization detector
GC	gas-liquid chromatography
GDP	gross domestic product
GLA	gamma linolenic acid
HDL	high density lipoprotein
HDL-C	high density lipid cholesterol
HETE	hydroxyeicosatetraenoic acid
HM	human milk
HPETE	hydroperoxytetraenoic acid
IBD	inflammatory bowel disease
IDL	intermediate-density lipoproteins
IDS	individual dietary survey
IMF	intramuscular fat
IUPAC	International Union of Pure and Applied Chemistry
JAMA	Journal of the American Medical Association
L-AMDR°	lower value of acceptable macronutrient distribution range
LA	linoleic acid *(trivial name)* 18:2n-6 *(IUPAC notation)** 9z,12z-octadecadienoic acid *(systematic name)*
LCPUFA	long-chain polyunsaturated fatty acid (>2 double bonds; >18 C atoms)
LDL	low density lipoprotein
LDL-C	low density lipoprotein cholesterol
LOX	lipooxygenase
LT	leukotriene
MCT	medium chain triglyceride
MG	monoacylglycerol
MT	metric tonne
MUFA	monounsaturated fatty acid
NIV	nutrient intake value
NOAEL	no observable adverse effect level
NRCD	nutrition-related chronic disease
OA	oleic acid
PC	prostate cancer
PG	prostaglandin
PGI	prostacyclin
PHVO	partially hydrogenated vegetable oils
PL	phospholipid
PPAR	peroxisome proliferator-activated receptor
P/S ratio	polyunsaturated fatty acid/saturate fatty acid ratio
PUFA	polyunsaturated fatty acid (2 or more double bonds)
RA	rheumatoid arthritis
RCT	randomized controlled trial
RDA	recommended dietary allowance
SDA	stearidonic acid
SFA	saturated fatty acid
SHGB	sex-hormone-binding-globulin

SL	structured lipid
SNP	single nucleotide polymorphism
SPE	sucrose polyesters
ST	structured triacylglycerols
TC	total cholesterol
TEI	total energy intake
TFA	*trans* fatty acid
TG	triacylglycerol
TLC	thin-layer chromatography
TX	thromboxane
U-AMDR°	upper value of acceptable macronutrient distribution range
UL°°	tolerable upper intake level
UN	United Nations
UP	upper level
VCAM	vascular cell adhesion molecule
VLDL	very-low-density lipoprotein
WHO	World Health Organization

* Note: C:Dn-#, where C=number of C atoms: D=number of double bonds and # = number of C atoms the first double bond is separated from the Methyl group; n-6 (IUPAC notation) = ω6 (Holman notation)

° This term refers either to the upper or lower value of the AMDR range. It is very similar to the use of UCI or LCI for the upper or lower bounds of confidence intervals. Values in excess or lower than the range do not represent risk of excess or deficit respectively.

°° This term was developed for instances where biochemical indicators are needed to confirm risk of adverse effects for intakes that exceed this intake level. In the case of FA, this only applies to TFA.

Chapter 1: Introduction

The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO), in their roles as technical agencies of the United Nations (UN), are charged with providing science-based guidance on food and nutrition to national governments and the international community. The process used to do this involves periodic and systematic reviews of scientific evidence, which often culminates with the convening of joint expert consultations to review the state of scientific knowledge, deliberate on the issues and translate this knowledge into a definition of requirements and corresponding nutrient-based recommendations. The overall goal of these recommendations is to support health and nutritional well-being of individuals and populations. The topics covered during the recent past include energy, protein and amino acids, fats and oils, most of the vitamins and minerals and carbohydrates, with the objective of providing guidance on nutritional requirements and recommended dietary intakes.

The Joint FAO/WHO Expert Consultation on Fats and Fatty Acids in Human Nutrition (hereafter Expert Consultation) was the most recent expert meeting convened, and was held in Geneva from 10 to 14 November 2008. The Expert Consultation was the third to be held on the subject of fats in human nutrition, the first expert consultation on this topic being held in 1977 (FAO, 1978) and the second in 1993 (FAO, 1994).

The timeliness of this Expert Consultation is also tied to the clear recognition of the increasing global burden of nutrition-related chronic disease. Recent work of FAO and WHO in connection with this includes the 2002 Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases (WHO, 2003), the 2001 Expert Consultation on Human Energy Requirements (FAO, 2004) and its companion 2002 Expert Consultation on Protein and Amino Acid Requirements in Human Nutrition (WHO, 2007), one 2002 Technical Workshop on Food Energy – Methods of Analysis and Conversion Factors (FAO, 2003), and several Scientific Updates; one by FAO/WHO in 2006 on Carbohydrates in Human Nutrition (Nishida *et al*., 2007) and another by WHO on *Trans* Fatty Acids (Nishida and Uauy, 2009). These integrated efforts provide, to varying degrees, the scientific basis that guides strategies, programmes and projects of FAO and WHO and their Member Countries.

During the past fifteen years, the changes in diets and lifestyles resulting from industrialization, urbanization, economic development and market globalization have increased rapidly and particularly in the developing countries where major socio-economic changes are occurring. Whereas general improvement in the standard of living has been observed, this has often been accompanied by unhealthy dietary patterns and insufficient physical activity to maintain an optimal energy balance and a healthy weight. The net result has been increased prevalence of diet-related chronic diseases in all socio-economic groups and which now represent the main cause of deaths and disability worldwide.

SCIENTIFIC DEVELOPMENTS

There have been a number of major developments in the field of fats and fatty acids in human nutrition during the past fifteen years, with the resulting need for an update since the 1994 publication and recommendations. These developments are elaborated

more fully in the chapters that follow. A large number of population-based cohort studies and randomized controlled trials (RCT) have been conducted to address the impact of fats, and specifically of different fatty acids, on human health. Regarding total fat, for example, several recent reports of prospective observational studies found either no or small associations between total dietary fat intake and obesity, weight gain, coronary heart disease (CHD), and cancer risk (Field *et al*., 2007; He *et al*., 2003; Hu *et al*., 1997; Koh-Banerjee *et al*., 2003; Xu *et al*., 2006, Beresford *et al*., 2006; Howard *et al*., 2006; Kushi and Giovannucci, 2002; Prentice *et al*., 2006; WCRF/AICR, 2007). Several RCT of physiological measures have not found evidence for beneficial effects of low-fat diets. For example, a low-fat (27–30% of energy from fat or %E fat), high-carbohydrate diet did not favourably affect serum lipids, fasting serum glucose, fasting serum insulin, or blood pressure, compared with higher fat diets (Appel *et al*., 2005; Gardner *et al*., 2007; Schaefer *et al*., 2005). In a meta-analysis of clinical trials comparing low-fat (<30% of energy from fat or %E fat) energy-restricted diets to low-carbohydrate (<60 g/d), non-energy-restricted diets, it was demonstrated that the low-fat diets induced larger reductions in LDL-cholesterol (LDL-C), but did not improve weight loss after 12 months and they also increased triglyceride levels and lowered HDL-cholesterol (HDL-C) levels (Nordmann *et al.*, 2006). Consistent associations have been found between higher intakes of specific dietary fats, including particular polyunsaturated fatty acids, and between substituting (easily digested) carbohydrates with polyunsaturated fat, and lower risk of heart disease (Mozaffarian and Willett, 2007; Hu *et al*., 2001). At the same time various ecological data from observational studies in developing and transitional countries suggested that shifting from a lower to a higher percentage of energy from fat has been associated with both lower and higher energy intake and to unhealthy weight gain, thus, potentially contributing to the increasing problem of overweight and obesity (Ghafoorunissa, 1996; Li *et al.*, 2007; Longde, 2005; Popkin *et al.*, 1995).

Regarding polyunsaturated fatty acids (PUFA), controlled feeding and cohort studies of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intakes have demonstrated physiological benefits on blood pressure, heart rate, triglycerides, and likely inflammation, endothelial function, and cardiac diastolic function, and consistent evidence for a reduced risk of fatal CHD and sudden cardiac death at consumption of ~250 mg/day of EPA plus DHA (Burr *et al*., 1989; Gissi-Hf, 2008; Mozaffarian and Rimm, 2006; Yokoyama *et al*., 2007). DHA also plays a major role in development of the brain and retina during foetal development and the first two years of life (Cetin and Koletzko, 2008; Decsi and Koletzko, 2005; Helland *et al*., 2008), which is a "window of opportunity" also for preventing avoidable growth failure and undernutrition and reducing death and disease including the development of obesity and noncommunicable diseases later in life. As far as n-6 to n-3 ratio is concerned, the 2002 Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases and its background scientific review had indicated a balanced intake of n-6 and n-3 PUFAs is essential for health (WHO, 2003; Reddy and Katan, 2004). But there is a debate that increasing LA intake does not result in increased arachidonic acid (AA) in plasma or platelet lipids, and does not increase formation of proinflammatory mediators (Adam *et al*., 2003). Furthermore, both n-6 and n-3 fatty acids have been shown to have anti-inflammatory properties that are protective of atherogenic changes in vascular endothelial cells (De Caterina *et al*., 2000).

Another area of interest since the last report relates to *trans* fatty acids. The 1993 expert consultation did not provide any specific recommendations; however this was reviewed by the 2002 expert consultation (WHO, 2003) and more recently during a WHO Scientific Update on *Trans* Fatty Acid (Nishida and Uauy, 2009). Scientific evidence that emerged over the past two decades shows that *trans* fatty acid consumption has unique adverse effects on serum lipids, including increasing LDL-C,

lowering HDL-C, increasing lipoprotein(a), increasing ApoB levels, and decreasing ApoA1 levels (Katan *et al*., 1994; Mensink and Katan, 1992; Mozaffarian and Clarke, 2009; Mozaffarian *et al*., 2006).

The knowledge of the role of particular fatty acids in determining health and nutritional well-being and how they exert these effects has expanded substantially over the past decade. Whereas fats are energy-dense (37 kilojoules or 9 kilocalories per gram), the health consequences of dietary fats go well beyond their role as energy sources. We now have a better understanding of how fats and fatty acids are metabolized and utilized in the body, how they alter cell membrane function, how they control gene transcription and expression, and how they interact with each other. Fats and fatty acids should now be considered as key nutrients that affect early growth and development and nutrition-related chronic disease later in life. For example, specific n-3 and n-6 fatty acids are essential nutrients and also, as part of the overall fat supply may affect the prevalence and severity of cardiovascular disease, diabetes, cancer and age-related functional decline. Dietary fats provide the medium for the absorption of fat-soluble vitamins; are a primary contributor to the palatability of food; and are crucial to proper development and survival during the early stages of life-embryonic development and early growth after birth on through infancy and childhood. Thus, the role of essential fatty acids during pregnancy and lactation is highlighted, and the role of long-chain n-3 fatty acids as structural components for the development of the brain and central nervous system is now accepted. This makes the process of defining requirements and recommendations more complex and thus the need to focus on the roles of individual fatty acids and how requirements vary with age and physiological status.

With respect to the recommendations arising from the previous expert consultation (FAO, 1994), the 2008 Expert Consultation placed greater emphasis on the role of specific fatty acid categories, an example being the convincing role of long-chain polyunsaturated fatty acids (LCPUFA) in neonatal and infant mental development, as well as their beneficial role in maintenance of long-term health and prevention of specific chronic diseases. The 2008 Expert Consultation also recognized that the entities n-3 PUFA and n-6 PUFA include more than one fatty acid, each with its individual properties, and the umbrella term lacks precision, particularly in the area of food labelling. However, food labelling in most countries must comply with food standards or food codes, which often draw on Codex Alimentarius standards and nomenclature and thus the desired level of precision may not necessarily be up-to-date. Convincing evidence was provided to support the need to reduce *trans* fatty acids and thereby reduce the risk of developing coronary heart disease.

EXPERT CONSULTATION PROCESS

In preparing and conducting the Expert Consultation the Framework for the Provision of Scientific Advice on Food Safety and Nutrition was followed (FAO/WHO, 2007). The process of selecting experts began with a call that was posted on both the FAO and WHO websites and publicized through numerous channels, including the network of the UN Standing Committee on Nutrition. All applications were reviewed by a panel of four persons, consisting of one member each from FAO and WHO and two independent external experts designated by the FAO and WHO Secretariat. Each application was evaluated carefully and ranked based on the combination of an applicant's educational background, field of expertise, including scientific publications and membership or participation in scientific panels related to the subject of the Expert Consultation. After initial evaluation to identify qualified candidates, geographic and gender balance and a mixture of scientific areas of expertise were considered to arrive at a final selection. In addition, all experts, authors and reviewers were required to complete a "declaration

of interest" so as to allow assessment of any conflicts of interest or perceived conflicts of interest regarding positions or opinions on certain issues.

Background papers for the Expert Consultation were commissioned after an extensive review of the topics covered in the two previous expert consultation reports and consultation with experts on additional issues and topics that needed to be addressed given the availability of new scientific evidence. This resulted in thirteen background papers, which were published in a Special Issue of the *Annals of Nutrition and Metabolism* (Burlingame *et al.*, 2009) as a means of providing a useful research and reference source.

In developing their conclusions and recommendations, the authors of the background papers were asked to use the four criteria levels (convincing, probable, possible or insufficient) of the "strength of evidence" developed and applied by the joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Disease (WHO, 2003). The strength of evidence was reviewed and evaluated again during the Expert Consultation to arrive at recommendations and conclusions and to establish requirement levels. As was the case in the past, only evidence that warranted the levels of evidence "convincing" and "probable" were used to formulate recommendations.

All the background papers were peer-reviewed by at least three experts before being forwarded to the Expert Consultation for review and discussion. In addition, consultation participants reviewed all the papers before the consultation was convened. It should be noted and is emphasized, however, that the background papers do not represent the final conclusions of the Expert Consultation. That is the role of this report. The background papers were central in providing the information for this report, but these chapters also include inputs, conclusions and recommendations from the deliberations of the consultation.

REFERENCES

Adam, O., Wolfram, G. & Zollner, N. 2003. Influence of dietary linoleic acid intake with different fat intakes on arachidonic acid concentrations in plasma and platelet lipids and eicosanoid biosynthesis in female volunteers. *Ann. Nutr. Metab.*, 47(1): 31-36.

Appel, L.J., Sacks, F.M., Carey, V.J., Obarzanek, E., Swain, J.F., Miller, E.R., 3rd, Conlin, P.R., Erlinger, T.P., Rosner, B.A., Laranjo, N.M., Charleston, J., McCarron, P. & Bishop, L.M. 2005. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. *JAMA*, 294(19): 2455-2464.

Beresford, S.A., Johnson, K.C., Ritenbaugh, C., Lasser, N.L., Snetselaar, L.G., Black, H.R., Anderson, G.L., Assaf, A.R., Bassford, T., Bowen, D., Brunner, R.L., Brzyski, R.G., Caan, B., Chlebowski, R.T., Gass, M., Harrigan, R.C., Hays, J., Heber, D., Heiss, G., Hendrix, S.L., Howard, B.V., Hsia, J., Hubbell, F.A., Jackson, R.D., Kotchen, J.M., Kuller, L.H., LaCroix, A.Z., Lane, D.S., Langer, R.D., Lewis, C.E., Manson, J.E., Margolis, K.L., Mossavar-Rahmani, Y., Ockene, J.K., Parker, L.M., Perri, M.G., Phillips, L., Prentice, R.L., Robbins, J., Rossouw, J.E., Sarto, G.E., Stefanick, M.L., Van Horn, L., Vitolins, M.Z., Wactawski-Wende, J., Wallace, R.B. & Whitlock, E. 2006. Low-fat dietary pattern and risk of colorectal cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. *JAMA*, 295(6): 643-654.

Burlingame, B., Nishida, C., Uauy, R. & Weisell, R. 2009. Fats and fatty acids in human nutrition; joint FAO/WHO Expert Consultation. *Ann. Nutr. Metab*., 55: 1-3.

Burr, M.L., Fehily, A.M., Gilbert, J.F., Rogers, S., Holliday, R.M., Sweetnam, P.M., Elwood, P.C. & Deadman, N.M. 1989. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet*, 2(8666): 757-761.

Cetin, I. & Koletzko, B. 2008. Long-chain omega-3 fatty acid supply in pregnancy and lactation. *Curr. Opin. Clin. Nutr. Metab. Care*, 11(3): 297-302.

De Caterina, R., Liao, J.K. & Libby, P. 2000. Fatty acid modulation of endothelial activation. *Am. J. Clin. Nutr*., 71(1 Suppl.): 213S-223S.

Decsi, T. & Koletzko, B. 2005. N-3 fatty acids and pregnancy outcomes. *Curr. Opin. Clin. Nutr. Metab. Care*, 8(2): 161-166.

FAO. 1978. *Dietary fats and oils in human nutrition; a joint FAO/WHO report.* FAO Food and Nutrition Paper 3, FAO. Rome.

FAO. 1994. *Fats and oils in human nutrition; Report of a joint FAO/WHO expert consultation*. FAO Food and Nutrition Paper 57, FAO. Rome.

FAO. 2003. *Food energy – methods of analysis and conversion factors. Report of a technical workshop.* FAO Food and Nutrition Paper 77, FAO, Rome.

FAO. 2004. *Human energy requirements; Report of a Joint FAO/WHO/UNU Expert Consultation.* FAO Food and Nutrition Technical Report Series No. 1, FAO, Rome.

FAO/WHO 2007. *FAO/WHO Framework for the Provision of Scientific Advice on Food Safety and Nutrition.* FAO/WHO, Rome & Geneva.

Field, A.E., Willett, W.C., Lissner, L. & Colditz, G.A. 2007. Dietary fat and weight gain among women in the Nurses' Health Study. *Obesity*, 15(4): 967-976.

Gardner, C.D., Kiazand, A., Alhassan, S., Kim, S., Stafford, R.S., Balise, R.R., Kraemer, H.C. & King, A.C. 2007. Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. *JAMA*, 297(9): 969-977.

Ghafoorunissa. 1996. Fats in Indian diets and their nutritional and health implications. *Lipids*, Mar; 31 (Suppl):S287-91.

Gissi-Hf, I. 2008. Effect of n-3 polyunsaturated fatty acids in patients with chronic heart failure (the GISSI-HF trial): a randomised, double-blind, placebo-controlled trial. *Lancet*, 372: 1223-1230.

He, K., Merchant, A., Rimm, E.B., Rosner, B.A., Stampfer, M.J., Willett, W.C. & Ascherio, A. 2003. Dietary fat intake and risk of stroke in male US healthcare professionals: 14 year prospective cohort study. *Br. Med. J*., 327(7418): 777-782.

Helland, I.B., Smith, L., Blomen, B., Saarem, K., Saugstad, O.D. & Drevon, C.A. 2008. Effect of supplementing pregnant and lactating mothers with n-3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age. *Pediatrics*, 122(2): e472-479.

Howard, B.V., Manson, J.E., Stefanick, M.L., Beresford, S.A., Frank, G., Jones, B., Rodabough, R.J., Snetselaar, L., Thomson, C., Tinker, L., Vitolins, M. & Prentice, R. 2006. Low-fat dietary pattern and weight change over 7 years: the Women's Health Initiative Dietary Modification Trial. *JAMA*, 295(1): 39-49.

Hu, F.B., Stampfer, M.J., Manson, J.E., Rimm, E., Colditz, G.A., Rosner, B.A., Hennekens, C.H. & Willett, W.C. 1997. Dietary fat intake and the risk of coronary heart disease in women. *N. Engl. J. Med.*, 337(21): 1491-1499.

Hu, F.B., Manson, J.E. & Willett, W.C. 2001. Types of dietary fat and risk of coronary heart disease: a critical review. *J. Am. Coll. Nutr.*, 20(1): 5-19.

Katan, M.B., Zock, P.L. & Mensink, R.P. 1994. Effects of fats and fatty acids on blood lipids in humans: an overview. *Am. J. Clin. Nutr.*, 60(6 Suppl.): 1017S-1022S.

Koh-Banerjee, P., Chu, N.F., Spiegelman, D., Rosner, B., Colditz, G., Willett, W. & Rimm, E. 2003. Prospective study of the association of changes in dietary intake, physical activity, alcohol consumption, and smoking with 9-y gain in waist circumference among 16 587 US men. *Am. J. Clin. Nutr.*, 78(4): 719-727.

Kushi, L. & Giovannucci, E. 2002. Dietary fat and cancer. *Am. J. Med.*, 113 Suppl. 9B: 63S-70S.

Li, Y., Zhai, F., Yang, X., Schouten, E.G., Hu X., He, Y., Luan, D. & Ma, G. 2007. Determinants of childhood overweight and obesity in China. *British Journal of Nutrition.*, 97 (1):210–215.

Longde, Wang. 2005. *Synthesis Report - 2002 China National Nutrition and Health Status Survey Report I.* ISBN: 9787117063739, 7117063734, People's Health Press, Beijing, China.

Mann, J.I. 2002. Diet and risk of coronary heart disease and type 2 diabetes. *Lancet*, 360(9335): 783-789.

Mensink, R.P. & Katan, M.B. 1992. Effect of dietary fatty acids on serum lipids and lipoproteins. A meta-analysis of 27 trials. *Arterioscler. Thromb.*, 12(8): 911-919.

Mozaffarian, D. & Rimm, E.B. 2006. Fish intake, contaminants, and human health: evaluating the risks and the benefits. *JAMA*, 296(15): 1885-1899.

Mozaffarian, D. & Willett, W.C. 2007. Trans fatty acids and cardiovascular risk: a unique cardiometabolic imprint? *Curr. Atheroscler. Rep.*, 9(6): 486-493.

Mozaffarian, D. & Clarke, R. 2009. Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. *EJCN*, 63, Suppl. 2: S22–S33.

Nishida, C., Martinez Nocito, F. & Mann, J. 2007. Joint FAO/WHO Scientific Update on Carbohydrates in Human Nutrition. *EJCN.*, 61 (Suppl. 1).

Nishida, C. & Uauy, R. 2009. WHO Scientific Update on Trans Fatty Acids (TFA). *EJCN*, 63 (Suppl. 2).

Nordmann, A.J., Nordmann, A., Briel, M., Keller, U., Yancy, W.S., Jr., Brehm, B.J. & Bucher, H.C. 2006. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Arch. Intern. Med*., 166(3): 285-293.

Popkin, B.M., Paeratakul, S., Zhai, F. & Ge ,K. 1995. Dietary and environmental correlates of obesity in a population study in China. *Obes Res.,* Sep; 3 (Suppl 2):135s-143s.

Prentice, R.L., Caan, B., Chlebowski, R.T., Patterson, R., Kuller, L.H., Ockene, J.K., Margolis, K.L., Limacher, M.C., Manson, J.E., Parker, L.M., Paskett, E., Phillips, L., Robbins, J., Rossouw, J.E., Sarto, G.E., Shikany, J.M., Stefanick, M.L., Thomson, C.A., Van Horn, L., Vitolins, M.Z., Wactawski-Wende, J., Wallace, R.B., Wassertheil-Smoller, S., Whitlock, E., Yano, K., Adams-Campbell, L., Anderson, G.L., Assaf, A.R., Beresford, S.A., Black, H.R., Brunner, R.L., Brzyski, R.G., Ford, L., Gass, M., Hays, J., Heber, D., Heiss, G., Hendrix, S.L., Hsia, J., Hubbell, F.A., Jackson, R.D., Johnson, K.C., Kotchen, J.M., LaCroix, A.Z., Lane, D.S., Langer, R.D., Lasser, N.L. & Henderson, M.M. 2006. Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. *JAMA*, 295(6): 629-642.

Reddy, W.S. & Katan, M.B. 2004. Diet, nutrition and the prevention of hypertension and cardiovascular diseases. *Special Issue: Diet, Nutrition and the Prevention of Chronic Diseases: Scientific Background Papers of the Joint WHO/FAO Expert Consultation (Geneva, 28 Jan–1 Feb 2002) Public Health Nutr. 7*: 167–186.

Schaefer, E.J., Gleason, J.A. & Dansinger, M.L. 2005. The effects of low-fat, high-carbohydrate diets on plasma lipoproteins, weight loss, and heart disease risk reduction. *Curr. Atheroscler. Rep*., 7(6): 421-427.

WHO. 2003. *Diet, Nutrition and the Prevention of Chronic Diseases. Report of a Joint WHO/FAO Expert Consultation,* WHO Technical Report Series 916, WHO, Geneva.

WHO. 2007. *Protein and amino acid requirements in human nutrition; Report of a Joint WHO/FAO/UNU Expert Consultation,* WHO Technical Report Series 935, WHO, Geneva.

WCRF/AICR (World Cancer Research Fund/American Institute for Cancer Research). 2007. *Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective.* Washington DC.

Xu, J., Eilat-Adar, S., Loria, C., Goldbourt, U., Howard, B.V., Fabsitz, R.R., Zephier, E.M., Mattil, C. & Lee, E.T. 2006. Dietary fat intake and risk of coronary heart disease: the Strong Heart Study. *Am. J. Clin. Nutr.*, 84(4): 894-902.

Yokoyama, M., Origasa, H., Matsuzaki, M., Matsuzawa, Y., Saito, Y., Ishikawa, Y., Oikawa, S., Sasaki, J., Hishida, H., Itakura, H., Kita, T., Kitabatake, A., Nakaya, N., Sakata, T., Shimada, K. & Shirato, K. 2007. Effects of eicosapentaenoic acid on major coronary events in hypercholesterolaemic patients (JELIS): a randomised open-label, blinded endpoint analysis. *Lancet*, 369(9567): 1090-1098.

Chapter 2: Summary of conclusions and dietary recommendations on total fat and fatty acids

DEFINITIONS

There are inherent limitations with the convention of grouping fatty acids based only on the number of double bonds, i.e. saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) insofar as describing the effects of fatty acids on human health and in developing dietary recommendations. The large body of epidemiological evidence about total fats, fatty acids, and human health apply these groupings and show that the major groups of fatty acids are associated with different health effects. However, the Expert Consultation recognized that individual fatty acids within each broad classification of fatty acids may have unique biological properties and health effects. This has relevance in making global recommendations because intakes of the individual fatty acids that make up the broad groupings will differ across regions of the world depending on the predominant food sources of total fats and oils. The Expert Consultation also recognized that in spite of these limitations, the scientific community in general and an increasing proportion of the general population continues to use the groupings based on chemical structure and thus, there would be disadvantages in abandoning them. Moreover, few countries have food composition databases that enable dietary assessment of individual fatty acid intake.

For the sake of clarity and in recognition that often we use generalized terms to refer to specific fatty acids, the Expert Consultation thought it appropriate to provide details as to the use in this document. In particular:

- The Expert Consultation recognises that grouping of fatty acids into these three broad groups (SFA, MUFA and PUFA) is based on chemical classifications, but it is clear that individual fatty acids within these groups have distinct biological properties. However, most of the epidemiological evidence reviewed by the experts uses broad groupings, which makes it difficult to distinguish and disentangle the effects of individual fatty acids.
- SFA refers to the major SFA in our diet, namely C14, C16, C18, except in the case of milk and coconut oil where SFA range from C4 to C18.
- MUFA refers to the major monounsaturated fatty acid in Western diets, which is oleic acid (C18:1n-9). It should be recognized that in some populations, a major monounsaturated fatty acid is erucic acid (C22:1n-9), as for example, found in culinary oils derived from some Brassica spp. such as rapeseed and mustard seed.
- PUFA refers to the major PUFA in our diet, which includes mainly linoleic acid (C18:2n-6), a lower proportion of alpha-linolenic acid (C18:3n-3), and depending on seafood intake a variable but relatively low proportion of long chain PUFA such as AA, EPA, DPA and DHA. For the purposes of food labelling, the terms EFA,

PUFA, long chain PUFA, n-6 and n-3 lack precision and should not be used without fully specifying the actual fatty acids and their amounts. Many different fatty acids with quite different properties fall under these umbrella terms.

- TFA refers to the major *trans* fatty acids in our diet which are typically isomers of 18:1 *trans* derived from partially hydrogenated vegetable oils.
- Some fatty acids (e.g. *trans* monoenes, conjugated linoleic acid [CLA], etc.) are members of more than one chemical classification but by convention are interpreted as in only one category (*trans* monoenes in MUFA, CLA in PUFA, etc.).
- There are many fatty acids that are usually minor components of most foods but are major components of some specialty foods and/or of supplements. FAO/WHO recommendations must be carefully interpreted with respect to unusual fatty acids ["usual" = straight chain, all-cis, methylene-interrupted (homoallylic); "unusual" = *trans*, branched chain, non-methylene interrupted double bond structure].

LEVELS AND STRENGTH OF EVIDENCE

During the preparatory process for the Expert Consultation the participants agreed on the criteria that would be used to judge the levels and strength of evidence required to conclude that total fat and fatty acids affect major health and disease outcomes. It was decided to follow the same criteria employed in the report *Diet, Nutrition, and the Prevention of Chronic Diseases; Report of a Joint WHO/FAO Expert Consultation* (WHO, 2003), which had based its criteria on a modified version of that used by the World Cancer Research Fund, (WICF/AICF, 2007). In doing so the experts acknowledged other equally valid criteria that exist.

Four levels of judgment were identified:

- Convincing
- Probable
- Possible
- Insufficient

Given the limited number of randomized controlled trials of dietary fat and chronic disease or death it was agreed that only evidence of sufficient strength to be "**convincing**" or "**probable**" would allow a dietary recommendation to be formulated.

SUMMARY OF TOTAL FAT AND FATTY ACID REQUIREMENTS FOR ADULTS, INFANTS (0-24 MONTHS) AND CHILDREN (2-18 YEARS)

There was **convincing** evidence that energy balance is critical to maintaining healthy body weight and ensuring optimal nutrient intakes, regardless of macronutrient distribution expressed in energy percentage (%E). The requirements on total fat and different fatty acid groups are summarized in the following tables: Table 2.1 for adults and Table 2.2 for infants and children. It was emphasized that requirements should be tailored to individuals and that the general requirements for certain groups, e.g. children and elderly subjects, have not yet been adequately established.

TABLE 2.1
Recommended dietary intakes for total fat and fatty acid intake: Adults

			Level of Evidence			
Fat/FA	Measure	Numeric amount	Convincing	Probable	Possible	Insufficient
Total fat	AMDR U-AMDR L-AMDR	20–35%E 35%E 15%E		No relation with CHD events, fatal CHD, total cancer, or cancer subtypes		Risk of diabetes, metabolic syndrome components, body weight/adiposity
SFA	U-AMDR	10%E	C12:0–16:0 ↑ LDL and total/HDL ratio in comparison to cis MUFA or PUFA; ↑ LDL but no effect on total/HDL in comparison to carbohydrate		↑ risk of diabetes	Risk of hypertension, body weight/adiposity
MUFA	AMDR	By difference [a], [b]	↓ LDL and total/HDL ratio when substituting SFA (C12:0–16:0)		↓ risk of metabolic syndrome components	Risk of diabetes, body weight/adiposity, CHD events, total cancer or cancer subtypes
Total PUFA	AMDR (LA + ALA + EPA + DHA) U-AMDR L-AMDR AI	6–11%E 11%E 6%E 2.5–3.5%E	See above, for exchange of SFA for PUFA Essential (LA, ALA) ↓ risk of CHD events when PUFA replace SFA		↓ risk of metabolic syndrome components, diabetes ↑ lipid peroxidation with high consumption, especially when tocopherol intake is low Specific minimum to prevent deficiency unclear	Risk of body weight/adiposity, total cancer or cancer subtypes
n-6 PUFA	AMDR (LA) EAR AI	2.5–9%E 2%E (SD of 0.5%) 2–3%E	See above, for exchange of SFA for PUFA Essential (LA)	↓ risk of metabolic syndrome components, diabetes	Specific minimum to prevent deficiency unclear	Risk of body weight/adiposity, total cancer or cancer subtypes
n-3 PUFA	AMDR (n-3[c]) L-AMDR (ALA) AMDR (EPA + DHA)	0.5–2%E > 0.5%E 0. 250–2* g/day	↓ risk of fatal CHD events (EPA+DHA) Essential (ALA)		↓ risk of total CHD events, stroke Specific minimum to prevent deficiency unclear	
TFA[d]	UL	<1%E	↓ HDL and ↑ total/HDL ratio in comparison to SFA (C12:0–C16:0), cis MUFA or PUFA ↑ risk of CHD events	↑ risk of fatal CHD and sudden cardiac death ↑ risk of metabolic syndrome components, diabetes		Risk of body weight/adiposity, diabetes, total cancer or cancer subtypes

(Explanations of the abbreviations are found in the list of acronyms and symbols)

[a] Total fat [%E] – SFA [%E] – PUFA [%E] – TFA [%E] [b] can be up to 15 – 20 %E, according to total fat intake [c] ALA + n-3 long-chain PUFA [d] total TFA from ruminant and industrially-produced sources

* for secondary prevention of CHD

TABLE 2.2

Recommended dietary intakes for total fat and fatty acid intake: Infants (0-24 months) and children (2-18 years)

Fat/FA	Age Group	Measure	Numeric Amount	Level of Evidence
Total fat	0-6 mo	AMDR	40-60%E	Convincing
		AI	based on composition % of total fat in HM,	Convincing
	6-24 mo	AMDR	gradual reduction, depending on physical activity, to 35%E[a]	Convincing
	2-18 yr	AMDR	25-35%E*	Probable
SFA	2-18 yr	U-AMDR	8%E* Children from families with evidence of familiar dyslipidemia (high LDL cholesterol) should receive lower SFA but not reduced total fat intake	Probable
MUFA	2-18 yr	AMDR	total fat [%E] - SFA [%E] - PUFA [%E] - TFA [%E]	Probable
Total PUFA	6-24 mo	U-AMDR	<15%E	Probable
	2-18 yr	U-AMDR	11%E	Probable
LA & ALA	0-24 mo	Comment	essential and indispensable	Convincing
n-6 PUFA				
AA	0-6 mo	AI	0.2-0.3%E[b]	Convincing
		U-AMDR	Based on HM composition as %E of total fat	Convincing
LA	0-6 mo	AI	HM composition as %E of total fat	Convincing
	6-12 mo	AI	3.0-4.5%E	Convincing
	6-12 mo	U-AMDR	<10%E	Probable
	12-24 mo	AI	3.0-4.5%E	Convincing
	12-24 mo	U-AMDR	<10%E	Probable
n-3 PUFA				
ALA	0-6 mo	AI	0.2-0.3%E[b]	Convincing
	6-24 mo	AI	0.4-0.6%E	Probable
	6-24 mo	U-AMDR	<3%E	Probable
DHA	0-6 mo	AI	0.1-0.18%E[b]	Convincing
	0-6 mo	U-AMDR	no upper value within the HM range up to 0.75%E	Convincing
	0-6 mo	Comment	conditionally essential due to limited synthesis from ALA	Probable
	6-24 mo	AI	10-12 mg/kg	Probable
	0-24 mo	Comment	critical role in retinal and brain development	Convincing
EPA+DHA	2-4 yr	AI	100-150 mg (age adjusted for chronic disease prevention)[c]	Probable
	4-6 yr	AI	150-200 mg (bridged from an infant value of 10 mg/kg)	Probable
	6-10 yr	AI	200-250 mg (to the adult value assigned at age 10 years)	Probable
TFA[d]	2-18 yr	UL	<1%E	Convincing

(Explanations of the abbreviations are found in the list of acronyms and symbols)

* Simell *et al*., 2009

[a] For infants 6-12 mo, the proposed fat intake as a %E is lower than those recommended in the 1994 report. The primary reasons are the concern over increased obesity rates and the redefined growth standards based on human milk-fed infants, associated with leaner growth in later infancy (WHO 2006).

[b] The amounts are expressed as %E in order to be consistent with the other entries in the table. However based on human milk composition as is often the case when referring to infants of breast feeding age, the amounts for AA and ALA would be expressed as 0.4-0.6%FA and for DHA as 0.20-0.36%FA. This conversion assumes that half of the energy in human milk comes from fat. For children 6-24 months of age the estimation is based on provision of breast milk to meet half of the daily energy needs, the rest of the energy would come from non breast milk diet.

[c] Although there is no specific data from long term studies on the relationship between fatty acid intake and chronic disease prevention from children the assumption is that children also benefit from lower saturated fat and higher PUFA intakes.

[d] Total TFA from ruminant and industrially-produced sources.

CONCLUSIONS AND RECOMMENDATIONS FOR TOTAL FAT

The Expert Consultation examined the background papers, scientific reports and various studies assessing the relationship between total dietary fats as well as selected fatty acids and various physiological conditions and illnesses. The experts agreed with the evidence summarized in two recent reports (WHO, 2003; WCRF/AICR, 2007) that there is no probable or convincing evidence for significant effects of total dietary fats on coronary heart disease or cancers. Therefore, of primary concern and importance was the potential relationship between total dietary fats and body weight (overweight and obesity).

There was **convincing** evidence that energy balance is critical to maintaining healthy body weight and ensuring optimal nutrient intakes, regardless of macronutrient distribution of energy as % total fat and % total carbohydrates.

Although the specific evidence was not reviewed in-depth at the consultation it was felt sensible that maintaining appropriate dietary patterns and energy levels, and adequate physical activity levels were critical in preventing unhealthy weight gain (i.e. overweight and obesity) and to ensure optimal health for those predisposed to insulin resistance.

Some older intervention studies from industrialized countries suggest that diets with lower % of energy from fat (i.e. %E fat) tend to be hypocaloric and are therefore associated with short term weight loss. Conversely, more recent randomized controlled trials in predominantly overweight populations from industrialized countries, which compared isocaloric diets with different levels of total fat, have shown that a higher %E fat can lead to greater weight loss than observed with low fat diets. However, the differences in the intake of other macronutrients such as amount and type of carbohydrates and the relatively high drop-out rate in some studies limit the strength of the evidence and the generalization of these results.

Various ecological data from observational studies in developing and transitional countries suggest that shifting from a lower to a higher %E fat has been associated with both lower and higher total energy intake and to unhealthy weight gain; thus, potentially contributing to the increasing problem of overweight and obesity. The opposite is observed in industrialized countries where %E fat has decreased while obesity has increased.

The insufficient evidence and conflicting interpretation of results on the nature of the relationship between the %E fat and adult body weight convinced the Expert Consultation that at this time it was not possible to determine at a probable or convincing level the causal relationship of excess % energy intake from fat and unhealthy weight gain.

Full agreement among the experts regarding the upper value of acceptable macronutrient distribution range (AMDR) for %E fat was not achieved; thus maintaining the current recommendation for a maximum intake value of 30-35%E fat was considered prudent. Further studies and a systematic review of all available evidence are needed to provide better evidence on which to base a recommendation on AMDR for %E fat that are applicable globally.

There was agreement among the experts that in populations with inadequate total energy intake, such as seen in many developing regions, dietary fats are an important macronutrient that contribute to increase energy intake to more appropriate levels.

Based on the considerations provided in the preceding section, the Expert Consultation proposed the following AMDR which are consistent with the existing 2002 expert consultation recommendations (WHO, 2003):

***Minimum total fat* intakes for adults** [a]

- 15%E to ensure adequate consumption of total energy, essential fatty acids, and fat soluble vitamins for most individuals.
- 20%E for women of reproductive age and adults with BMI <18.5, especially in developing countries in which dietary fat may be important to achieve adequate energy intake in malnourished populations.

***Maximum total fat* intakes for adults** [a]

- 30–35%E for most individuals.

[a] To optimize health, special attention should be given to both the overall dietary pattern, in terms of types of food consumed, and total energy intakes, in relation also to anthropometric (age group, BMI) and lifestyles characteristics.

CONCLUSIONS AND RECOMMENDATIONS FOR SATURATED FATTY ACIDS (SFA)

Individual saturated fatty acids (SFA) have different effects on the concentration of plasma lipoprotein cholesterol fractions. For example, lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids increase LDL cholesterol whereas stearic (C18:0) has no effect.

There is convincing evidence that:

- Replacing SFA (C12:0–C16:0) with polyunsaturated fatty acids (PUFA) decreases LDL cholesterol concentration and the total/HDL cholesterol ratio. A similar but lesser effect is achieved by replacing these SFA with monounsaturated fatty acids (MUFA).
- Replacing dietary sources of SFA (C12:0–C16:0) with carbohydrates decreases both LDL and HDL cholesterol concentration but does not change the total/HDL cholesterol ratio.
- Replacing SFA (C12:0–C16:0) with *trans*-fatty acids (TFA) decreases HDL cholesterol and increases the total /HDL cholesterol ratio.

Based on coronary heart disease (CHD) morbidity and mortality data from epidemiological studies and controlled clinical trials (using CHD events and death), it was also agreed that:

- There is **convincing** evidence that replacing SFA with PUFA decreases the risk of CHD.
- There is **probable** evidence that replacing SFA with largely refined carbohydrates has no benefit on CHD, and may even increase the risk of CHD and favour metabolic syndrome development (Jakobsen *et al*., 2009).
- There is a possible positive relationship between SFA intake and increased risk of diabetes.
- There is insufficient evidence relating to the effect on the risk of CHD in replacing SFA with either MUFA or largely whole grain carbohydrates; however, based on indirect lines of evidence this could result in a reduced risk of CHD.
- There is insufficient evidence that SFA affects the risk for alterations in indices related to the components of the metabolic syndrome.

Based on cancer morbidity and mortality data, it was also agreed that:

- There is insufficient evidence for establishing any relationship of SFA consumption with cancer.

Therefore, it is recommended that SFA should be replaced with PUFA (n-3 and n-6) in the diet and the total intake of SFA not exceed 10%E.

CONCLUSIONS AND RECOMMENDATIONS FOR MONOUNSATURATED FATTY ACIDS (MUFA)

- There is **convincing** evidence that replacing carbohydrates with MUFA increases HDL cholesterol concentrations.
- There is **convincing** evidence that replacing SFA (C12:0–C16:0) with MUFA reduces LDL cholesterol concentration and total/HDL cholesterol ratio.
- There is possible evidence that replacing carbohydrates with MUFA improves insulin sensitivity.
- There is insufficient evidence for relationships of MUFA consumption with chronic disease end points such as CHD or cancer.
- There is insufficient evidence for relationships of MUFA consumption and body weight and percent adiposity.
- There is insufficient evidence of a relationship between MUFA intake and risk of diabetes.

The determination of intake of MUFA is unique in that it is calculated by difference, i.e. MUFA = Total fat intake (%E)– SFA (E%) – PUFA (E%) – TFA (%E). Therefore, the MUFA intake resulting may cover a wide range depending on the total fat intake and dietary fatty acid pattern.

CONCLUSIONS AND RECOMMENDATIONS FOR POLYUNSATURATED FATTY ACIDS (PUFA)

- There is **convincing** evidence that linoleic acid (LA) and alpha-linolenic acid (ALA) are indispensable since they cannot be synthesized by humans.
- There is **convincing** evidence that replacing SFA with PUFA decreases the risk of CHD.
- There is **convincing** and sufficient evidence from experimental studies to set an acceptable intake to meet essential FA needs for linoleic acid (LA) and alpha-linolenic acid (ALA) consumption.
- There is possible evidence that PUFA affect the risk of alterations in indices related to the metabolic syndrome.
- There is possible evidence of a relationship between PUFA intake and reduced risk of diabetes.
- There is insufficient evidence for establishing any relationship of PUFA consumption with cancer.
- There is insufficient evidence for relationships of PUFA consumption and body weight and percent adiposity.

The minimum intake values for essential fatty acids to prevent deficiency symptoms are estimated at a **convincing** level to be 2.5%E LA plus 0.5%E ALA. Based on epidemiologic studies and randomized controlled trials of CHD events, the minimum recommended value of total PUFA consumption for lowering LDL and total cholesterol concentrations, increasing HDL cholesterol concentrations and decreasing the risk of CHD events is 6%E. Based on experimental studies, risk of lipid peroxidation may increase with high (>11%E) PUFA consumption, particularly when tocopherol intake is low. Therefore, the resulting acceptable range for total PUFA (n-6 and n-3 fatty

acids) can range between 6 and 11%E. The adequate intake to prevent deficiency is 2.5–3.5%E.

Thus, the recommended range (ADMR) for PUFA is 6–11%E.

CONCLUSIONS AND RECOMMENDATIONS FOR N-3 POLYUNSATURATED FATTY ACID INTAKE

The available evidence indicates that 0.5-0.6%E alpha-linolenic acid (ALA) per day corresponds to the prevention of deficiency symptoms. The total n-3 fatty acid intake can range between 0.5–2%E whereas the minimum dietary requirement of ALA (>0.5%E) for adults prevents deficiency symptoms. The higher value 2%E (ALA) plus n-3 long-chain polyunsaturated fatty acids (LCPUFA) eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (AMDR 0.250 g–2.0 g) can be part of a healthy diet. Whilst ALA may have individual properties in its own right, there is evidence that the n-3 LCPUFA may contribute to the prevention of CHD and possibly other degenerative diseases of aging. For adult males and non-pregnant/non-lactating adult females 0.250 g/day of EPA plus DHA is recommended, with insufficient evidence to set a specific minimum intake of either EPA or DHA alone; both should be consumed. For adult pregnant and lactating females, the minimum intake for optimal adult health and fetal and infant development is 0.3 g/d EPA+DHA, of which at least 0.2 g/d should be DHA.

The U-AMDR for EPA + DHA consumption is set at 2 g/d due to experimental evidence indicating that high supplement intakes of n-3 LCPUFA may increase lipid peroxidation and reduce cytokine production. However, this Expert Consultation also acknowledged that higher consumption values, as high as 3 g/d reduce other cardiovascular risk factors and have not had adverse effects in short- and intermediate-term randomized trials, and that some individuals in populations with high seafood consumption consume higher values with no apparent evidence of harm. In this regard, the experts noted that the Australian and New Zealand reference value for the upper value of intake of EPA + DPA + DHA has been set at 3 g/d (NHMRC, 2006) and the US Food and Drug Administration having set a 'Generally Regarded as Safe' value of 3000 mg/day for n-3 LCPUFA (IOM, 2005). Following careful consideration and extensive debate and considering the issue of sustainability of the supply of fish, the experts agreed on the value of 2 g/d as the U-AMDR for EPA plus DHA with the acknowledgement that future randomised controlled trials (RCT) and other research may justify raising this figure in the future. It was decided not to include DPA in the recommendations due to the fact that DPA is currently a research issue with limited evidence from RCT studies.

CONCLUSIONS AND RECOMMENDATIONS FOR N-6 POLYUNSATURATED FATTY ACIDS

It is recognized that only a sparse amount of human data is available for establishing a precise quantitative estimate of the linoleic acid (LA) requirement to prevent deficiency; thus a range rather than an average LA requirement is recommended. Animal and human studies demonstrate that the prevention of deficiency signs (e.g. in rats reduced growth, scaliness of skin, necrotic tail) occurs when 1–2% of total energy is provided by LA. Therefore, an estimated average requirement (EAR) for LA of 2%E and an adequate intake (AI) for LA of 2–3% E are proposed. In accepting that the U-AMDR values of total PUFA and total n-3 fatty acids are 11%E and 2%E respectively, the resulting acceptable range (AMDR) for n-6 fatty acids (LA) intake

is 2.5–9%E. The lower value or AI (2.5–3.5%E) corresponds to the prevention of deficiency symptoms, whereas the higher value as part of a healthy diet contributing to long term health by lowering LDL and total cholesterol levels and therefore the risk for CHD. For infants 6–12 months of age as well as children 12–24 months of age, an AI range of 3.0–4.5%E is recommended with a U-AMDR of <10%E. There is insufficient evidence for establishing any relationship of n-6 PUFA consumption with cancer.

Arachidonic acid (AA) is not essential for a healthy adult whose habitual diet provides LA > 2.5%E. For infants 0-6 months AA should be supplied in the diet within the range of 0.2-0.3%E[1] based on human milk composition as a criterion.

CONCLUSIONS AND RECOMMENDATIONS FOR N-6 TO N-3 RATIO

Based on the evidence and conceptual limitation, there is no rational for a specific recommendation for n-6 to n-3 ratio, or LA to ALA ratio, if intakes of n-6 and n-3 fatty acids lie within the recommendation established in this report.

CONCLUSIONS AND RECOMMENDATIONS FOR *TRANS*-FATTY ACID INTAKE (TFA)

The Expert Consultation devoted substantial time and discussion to the issue of *trans*-fatty acid (TFA) but in doing so drew heavily from the conclusions of the recently completed and published reports of the WHO Scientific Update on *trans* fatty acids (Nishida and Uauy, 2009). There is convincing evidence that TFA from commercial partially hydrogenated vegetable oils (PHVO) increase CHD risk factors and CHD events – more so than had been thought in the past. There also is probable evidence of an increased risk of fatal CHD and sudden cardiac death in addition to an increased risk of metabolic syndrome components and diabetes. In promoting the removal of TFA, which are predominantly a by-product of industrial processing (partial hydrogenation) usually in the form of PHVO, particular attention must be given to what would be their replacement; this is a challenge for the food industry. It was noted that among adults, the estimated average daily ruminant TFA intake in most societies is low. The experts acknowledged the current recommendation of a mean population intake of TFA of less than 1%E may need to be revised in light of the fact that it does not fully take into account the distribution of intakes and thus the need to protect substantial subgroups from having dangerously high intakes. This could well lead to the need to remove partially hydrogenated fats and oils from the human food supply.

CONSIDERATIONS FOR FOOD-BASED DIETARY GUIDELINES

The experts agreed that in addition to dietary requirements for total fat and fatty acids, food-based dietary guidelines are essential for promoting health and preventing disease. However, the consultation did not conduct a review of this subject. A general recommendation is to follow a dietary pattern predominantly

[1] If based on human milk composition as is often the case when referring to infants of breast feeding age, the amount would be expressed as 0.4–0.6%FA. This conversion assumes that half of the energy in human milk comes from fat.

based on whole foods (i.e., fruits and vegetables, whole grains, nuts, seeds, legumes, other dietary fibre sources, LCPUFA-rich seafood) with a relatively lower intake of energy dense processed and fried foods, and sugar-sweetened beverages; and to avoid consumption of large portion sizes. Moderate consumption of dairy products and lean meats and poultry can also be an important part of recommended food-based dietary guidelines. Maintaining recommended dietary patterns, appropriate energy intake and adequate physical activity levels are critical to prevent unhealthy weight levels (i.e. overweight and obesity) and to ensure optimal health for those predisposed to insulin resistance.

RECOMMENDATIONS FOR FURTHER RESEARCH

Further research and investigation are needed on:

- The effects of total fat consumption as a percentage of energy on weight gain, weight, maintenance, and weight loss in developing countries;
- The effects of different saturated fatty acids of varying chain lengths on CHD, diabetes, and metabolic syndrome risk and endpoints;
- The influence of different saturated fatty acids of varying chain lengths on *de novo* synthesis of fatty acids, and the implications for health outcomes;
- The effects of monounsaturated fatty acids on CHD, diabetes, and metabolic syndrome risk and endpoints;
- The effects of n-3 and n-6 polyunsaturated fatty acids on diabetes and metabolic syndrome risk and endpoints;
- Human studies to determine the dose-dependent effects of LA and ALA on formation of long-chain PUFA as well as the assessment of conversion rates of LA to AA in relation to the intakes;
- The effects of ALA on cardiovascular outcomes;
- Establishing the adult brain daily requirement of AA and DHA and translating these into daily dietary intakes of AA and DHA;
- The effects of long chain n-3 PUFA on depression and other mood disorders; and on aggression, hostility and antisocial behaviour; These studies should include:
    - both prospective observational studies and randomized clinical trials;
    - in trials, purified preparations of long chain n-3 PUFA (alone and in combination);
    - dose response studies;
    - studies on the duration of dietary consumption required for greatest benefit;
    - larger numbers of subjects in each treatment group;
    - delineating the importance of n-3 PUFA as monotherapy or adjunct therapy and identifying the mechanism(s) of action of these PUFA in mood disorders;
    - sufficiently sensitive tests designed to measure effects in mood and cognition.
- The effects of long-chain n-3 PUFA on the prevention and treatment of cognitive decline and Alzheimer's disease, including larger and longer duration randomized clinical trials;
- The relationship of *trans* fatty acid and saturated fatty acids with prostate cancers;
- The relationship of n-3 PUFA and fish with colorectal, prostate and breast cancers, including both incidence and progression;
- Simplified, low-cost rapid methods for analyzing fatty acid profiles of biological and food samples.

RECOMMENDATIONS ON DIETARY INFORMATION AND PROGRAMME NEEDS

- To provide sufficient and adequate information on dietary fatty acid intakes, it is strongly recommended that countries monitor food consumption patterns of their population groups; data on country-specific fatty acid composition of foods, on bioavailability of fatty acids from food sources and supplements, and on biomarker levels in specific populations are also required for designing and monitoring the impacts of national dietary guidelines and programmes that are aiming to make changes in dietary patterns over time to improve nutrition, including the promotion of appropriate intakes of different dietary fats and oils.

- Fatty acid analysis of whole blood is a representative biological specimen for the assessment of the fatty acid status in tissues in relation to physiopathological conditions. Analysis of whole blood or other samples (e.g., adipose, erythrocytes, phospholipids) should be conducted to monitor the fatty acid status in populations. This information is useful in relating to dietary fat intakes to health outcomes; whole blood analyses can be performed on drops of blood collected from fingertips.

RECOMMENDATIONS FOR NOMENCLATURE

The following definitions for the sub-classes of saturated fatty acids are recommended:

- *Short-chain fatty acids*: These are fatty acids with carbon atoms from three to seven.
- *Medium chain fatty acids*: These are fatty acids with carbon atoms from eight to thirteen.
- *Long-chain fatty acids*: These are fatty acids with carbon atoms from fourteen to twenty.
- *Very-long chain fatty acids*: These are fatty acids with twenty one or more carbon atoms.

The following designations for the sub-classes of polyunsaturated fatty acids are recommended:

- *Long-chain polyunsaturated fatty acids*: These are polyunsaturated fatty acids with twenty to twenty-four carbon atoms.
- *Very-long chain polyunsaturated fatty acids*: These are polyunsaturated fatty acids with twenty-five or more carbon atoms.

REFERENCES

IOM (Institute of Medicine). 2005. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients).* National Academies of Science, Washington DC.

NHMRC (National Health and Medical Research Council) (Dept. of Health and Ageing). 2006. *Nutrient Reference Values for Australia and New Zealand.* NHMRC, Canberra.

Nishida, C. & Uauy, R. 2009. WHO Scientific Update on *trans* fatty acids, *EJCN*, 63: Suppl 2.

Jakobsen, M.U., O'Reilly, E.J., Heitmann, B.L., Pereira, M.A., Bälter, K., Fraser, G.E., Goldbourt, U., Hallmans, G., Knekt, P., Liu, S., Pietinen, P., Spiegelman, D., Stevens, J., Virtamo, J., Willett, W.C. & Ascherio, A. 2009. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *Am. J. Clin. Nutr*., 89: 1425-1432.

Simell, O., Niinikoski, H., Rönnemaa, T., Raitakari, O.T., Lagström, H., Laurinen, M., Aromaa, M., Hakala, P., Jula, A., Jokinen, E., Välimäki, I., Viikari, J. & STRIP Study Group. 2009. Cohort Profile: the STRIP Study (Special Turku Coronary Risk Factor Intervention Project), an Infancy-onset Dietary and Life-style Intervention Trial. *Int J Epidemiol*. Jun;38(3): 650-5.

WHO. 2003. *Diet, Nutrition and the Prevention of Chronic Diseases.* Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series 916, WHO, Geneva.

WHO. 2006. *WHO Multicentre Growth Reference Study Group. WHO Child Growth Standards: Wength/height-for-age, Weight-forage, Weight-for-length, Weight-for-height and Body mass index-for-age: Methods and Development*. WHO, Geneva.

WCRF/AICR (World Cancer Research Fund/American Institute for Cancer Research). 2007. *Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective*, Washington DC.

Chapter 3: Fat and fatty acid terminology, methods of analysis and fat digestion and metabolism

DEFINITION AND CLASSIFICATION OF LIPIDS

Fats, oils and lipids consist of a large number of organic compounds, including fatty acids (FA), monoacylglycerols (MG), diacylglycerols (DG), triacylglycerols (TG), phospholipids (PL), eicosanoids, resolvins, docosanoids, sterols, sterol esters, carotenoids, vitamins A and E, fatty alcohols, hydrocarbons and wax esters. Classically, lipids were defined as substances that are soluble in organic solvents. However, over time this definition was thought to be no longer adequate or accurate and a novel definition and comprehensive system of classification of lipids were proposed in 2005 (Fahy *et al.*, 2005). The novel definition is chemically based and defines lipids as small hydrophobic or amphipathic (or amphiphilic) molecules that may originate entirely or in part through condensations of thioesters and/or isoprene units. The proposed lipid classification system enables cataloguing of lipids and their properties in a way that is compatible with other macromolecular databases. Using this approach, lipids from biological tissues are divided into eight categories, as shown in Table 3.1. Each category contains distinct classes and subclasses of molecules (Fahy *et al.*, 2005).

TABLE 3.1
Lipid categories and typical examples

Category	Example
fatty acyls	oleic acid
glycerolipids	triacylglycerol
glycerophospholipids	phosphatidylcholine
sphingolipids	sphingosine
sterol lipids	cholesterol
prenol lipids	farnesol
saccharolipids	UDP-3-0-(3hydroxy-tetradecanoyl)-N-acetylglucosamine
polyketides	aflatoxin

Modified from Fahy *et al.*, 2005

FATTY ACID NOMENCLATURE

There are a number of systems of nomenclature for fatty acids, but some do not provide sufficient information on their structure. A chemical name must describe the chemical structure unambiguously. The systematic nomenclature recommended by the International Union of Pure and Applied Chemistry (IUPAC-IUB Commission on Nomenclature, 1978) is used for fatty acids. The IUPAC system names fatty acids solely on the basis of the number of carbon atoms, and the number and position of unsaturated fatty acids relative to the carboxyl group. The configuration of double bonds, location of branched chains and hetero atoms and other structural features are also identified. The carbon atom of the carboxyl group is considered to be first and the

carbons in the fatty acid chain are numbered consequently from the carboxylic carbon. By convention, a specific bond in a chain is identified by the lower number of the two carbons that it joins. The double bonds are labelled with Z or E where appropriate, but are very often replaced by the terms *cis* and *trans*, respectively. For example, the systematic name of linoleic acid (LA) is "Z-9, Z-12-octadecadienoic acid" or "*cis*-9, *cis*-12-octadecadienoic acid".

Although the IUPAC nomenclature is pre*cis*e and technically clear, fatty acid names are long and therefore, for convenience, "trivial" or historical names and shorthand notations are frequently used in scientific articles. This is not surprising since those working in the scientific area of dietary fats are familiar with the chemical structures. The conflict between assuring pre*cis*ion and accuracy while allowing brevity and con*cis*eness has always existed.

There are several shorthand notations for dietary fatty acids, but all of them adopt the form C:D, where C is the number of carbon atoms and D is the number of double bonds in the carbon chain. Biochemists and nutritionists very often use the "n minus" system of notation for naturally occurring *cis* unsaturated fatty acids. The term "n minus" refers to the position of the double bond of the fatty acid closest to the methyl end of the molecule. This system defines easily the different metabolic series, such as n-9, n-6 and n-3, etc. The "n minus" system is applicable only to *cis* unsaturated fatty acids and to those *cis* polyunsaturated fatty acids whose double bonds are arranged in a methylene interrupted manner. LA, which has its second double bond located at 6 carbons from the methyl end, is abbreviated to 18:2n-6. The "n minus" system is also referred to as the omega system, but omega-3 is not recommended (IUPAC-IUB Commission on Nomenclature, 1978).

Another system widely used is the delta (Δ) system, in which the classification is based on the number of carbon atoms interposed between the carboxyl carbon and the nearest double bond to the carboxylic group. This system specifies the position of all the double bonds as well as their *cis*/*trans* configuration. It is applicable to a large number of fatty acids, except those with branched chains, hetero atoms, triple bonds and other fatty acids with unusual structural features. According to the delta system, the shorthand notation for LA is "*cis*-Δ9, *cis*-Δ12-18:2". For convenience, it could be expressed as "*cis*,*cis*-Δ9,Δ12-18:2". In some scientific papers, authors drop the "Δ" notation and write it simply as "*cis*-9,*cis*-12-18:2" or "9c,12c-18:2". This report, wherever appropriate, employs the IUPAC, trivial names, delta and n minus shorthand notations.

DIETARY FATS AND FATTY ACIDS

Dietary fat includes all the lipids in plant and animal tissues that are eaten as food. The most common fats (solid) or oils (liquid) are glycerolipids, which are essentially composed of TG. The TG are accompanied by minor amounts of PL, MG, DG and sterols/sterol esters. Fatty acids constitute the main components of these lipid entities and are required in human nutrition as a source of energy, and for metabolic and structural activities.

The most common dietary fatty acids have been subdivided into three broad classes according to the degree of unsaturation; saturated fatty acids (SFA) have no double bonds, monounsaturated fatty acids (MUFA) have one double bond and polyunsaturated fatty acids (PUFA) have two or more double bonds. In general, these fatty acids have an even number of carbon atoms and have unbranched structures. The double bonds of naturally occurring unsaturated fatty acids are very often of the *cis* orientation. A *cis* configuration means that the hydrogen atoms attached to the double bonds are on the same side. If the hydrogen atoms are on opposite sides, the configuration is termed *trans*.

SATURATED FATTY ACIDS

The SFA have the general formula R-COOH. They are further classified into four sub-classes according to their chain length: short, medium, long and very long. There are a various definitions used in the literature for the SFA sub-classes. The Expert Consultation recognized that there is a need for universal definitions and recommends the following definitions for the SFA sub-classes.

- Short-chain fatty acids: Fatty acids with from three to seven carbon atoms.
- Medium-chain fatty acids: Fatty acids with from eight to thirteen carbon atoms.
- Long-chain fatty acids: Fatty acids with from fourteen to twenty carbon atoms.
- Very-long-chain fatty acids: Fatty acids with twenty one or more carbon atoms.

Table 3.2 lists some of the most common dietary SFA, which are mainly provided by animal and especially ruminant dairy fats. Appreciable levels of SFA are also present in some tropical oils, especially in palm oil and coconut oil.

TABLE 3.2
Common saturated fatty acids in food fats and oils

Trivial name	Systematic name	Abbreviation	Typical sources
butyric	butanoic	C4:0	dairy fat
caproic	hexanoic	C6:0	dairy fat
caprylic	octanoic	C8:0	dairy fat, coconut and palm kernel oils
capric	decanoic	C10:0	dairy fat, coconut and palm kernel oils
lauric	dodecanoic	C12:0	coconut oil, palm kernel oil
myristic	tetradecanoic	C14:0	dairy fat, coconut oil, palm kernel oil
palmitic	hexadecanoic	C16:0	most fats and oils
stearic	octadecanoic	C18:0	most fats and oils
arachidic	eicosanoic	C20:0	peanut oil
behenic	docosanoic	C22:0	peanut oil
lignoceric	tetracosanoic	C24:0	peanut oil

UNSATURATED FATTY ACIDS

The unsaturated fatty acids are also further classified into three sub-groups according their chain lengths. Various definitions have also been used in the literature for the sub-classes of unsaturated fatty acids, but no universally accepted definitions exist. Therefore, the Expert Consultation recommends the following definitions.

- Short-chain unsaturated fatty acids: Fatty acids with nineteen (19) or fewer carbon atoms.
- Long-chain unsaturated fatty acids: Fatty acids with twenty (20) to twenty four (24) carbon atoms.
- Very-long-chain unsaturated fatty acids: Fatty acids with twenty five (25) or more carbon atoms.

Monounsaturated fatty acids

More than one hundred *cis*-MUFA occur in nature, but most are very rare compounds. Oleic acid (OA) is the most common MUFA and it is present in considerable quantities in both animal and plant sources. Table 3.3 lists the most common dietary MUFA.

TABLE 3.3
Some common *cis*-monounsaturated fatty acids in fats and oils

Common name	Systematic name	Delta abbreviation	Typical sources
palmitoleic	*cis*-9-hexadecenoic	16:1Δ9c (9c-16:1)	marine oils, macadamia oil, most animal and vegetable oils.
oleic	*cis*-9-octadecenoic	18:1Δ9c (9c-18:1) (OA)	all fats and oils, especially olive oil, canola oil and high-oleic sunflower and safflower oil
cis-vaccenic	*cis*-11-octadecenoic	18:1Δ11c (11c-18:1)	most vegetable oils
gadoleic	*cis*-9-eicosenoic	20:1Δ9c (9c-20:1)	marine oils
	cis-11-eicosenoic	20:1Δ11c (11c-20:1)	marine oils
erucic acid	*cis*-13-docosenoic	22:1Δ13c (13c-22:1)	mustard seed oil, high erucic rapeseed oil
nervonic	*cis*-15-tetracosenoic	24:1Δ15c (15c-24:1)	marine oils

Polyunsaturated fatty acids

Natural PUFA with methylene-interrupted double bonds and all of *cis* configuration can be divided into 12 families, ranging from double bonds located at the n-1 position to the n-12 position (Gunstone, 1999). The most important families, in terms of extent of occurrence and human health and nutrition, are the n-6 and n-3 families. The members of these two families are listed in Tables 3.4 and 3.5. Linoleic acid (LA) is the parent fatty acid of the n-6 family. It has 18 carbon atoms and two double bonds and the first double bond is 6 carbon atoms from the methyl end of the fatty acid chain, and hence the n-6 name. LA can be desaturated and elongated in humans to form a series of n-6 PUFA (Table 3.4). α-linolenic acid (ALA) is the parent fatty acid of the n-3 family. It also has 18 carbon atoms, but three double bonds. In contrast to LA, the first double bond in ALA is 3 carbon atoms from the methyl end of the fatty acid chain, and hence the n-3 name. Similarly to LA, ALA can also be desaturated and elongated to form a series of n-3 PUFA (Table 3.5).

LA and ALA occur in almost all dietary fats and attain major proportions in most vegetable oils (White, 2008). ALA is primarily present in plants, occurring in high concentrations in some seeds and nuts and also in some vegetable oils, although its presence in conventional diets is much lower than that of LA. Arachidonic acid (AA) is the most important n-6 PUFA of all the n-6 fatty acids because it is the primary precursor for the n-6 derived eicosanoids. AA is present at low levels in meat, eggs, fish, algae and other aquatic plants (Wood *et al*., 2008; Ackman, 2008a). Eiocosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most important n-3 fatty acids in human nutrition. EPA and DHA are components of marine lipids. Marine fish such as mackerel, salmon, sardine, herring and smelt are excellent sources of EPA and DHA (Ackman, 2008a). Fish oils containing 60% EPA and DHA are sold as sources of these important n-3 fatty acids. Algal oils and single-cell oil sources of the LCPUFA are now becoming available (to provide EPA+DHA+AA). Furthermore, genetically modified oils, produced by genetic manipulation of soy and other plants, are currently being developed and will be widely available in the near future.

In addition to the mentioned fatty acids, the human diet contains *trans* fatty acids, originating from ruminant deposits and milk fats (Huth, 2007) and also from foods prepared from partially hydrogenated oils (Craig-Schmidt and Teodorescu, 2008), the latter source dominating. In recent years, researchers have focused their attention on unusual, minor dietary fatty acids such as conjugated linoleic acid isomers (CLA) (Tricon *et al*., 2005), conjugated linolenic acid isomers (CLN) (Tsuzuki *et al*., 2004) and furan fatty acids (Spiteller, 2005) because of their potentially beneficial health effects.

TABLE 3.4
Nutritionally important n-6 PUFA

Common name	Systematic name	N minus abbreviation	Typical sources
linoleic acid	*cis*-9,*cis*-12-octadecadienoic	18:2n-6 (LA)	most vegetable oils
γ-linolenic acid	*cis*-6, *cis*-9,*cis*-12-octadecatrienoic acid	18:3n-6 (GLA)	evening primrose, borage and blackcurrant seed oils
dihomo-γ-linolenic acid	*cis*-8,*cis*-11,*cis*-14-eicosatrienoic acid	20:3n-6 (DHGLA)	very minor component in animal tissues
arachidonic acid	*cis*-5,*cis*-8,*cis*-11,*cis*-14-eicosatetraenoic acid	20:4n-6 (AA)	animal fats, liver, egg lipids, fish
docosatetraenoic acid	*cis*-7,*cis*-10,*cis*-13,*cis*-16-docosatetrtaenoic acid	22:4n-6	very minor component in animal tissues
docosapentaenoic acid	*cis*-4,*cis*-7,*cis*-10,*cis*-13,*cis*-16-docosapentaenoic acid	22:5n-6	very minor component in animal tissues

TABLE 3.5
Nutritionally important n-3 PUFA

Common name	Systematic name	N minus abbreviation	Typical sources
α-linolenic	*cis*-9,*cis*-12-*cis*-15-octadecatrienoic acid	18:3n-3 (ALA)	flaxseed oil, perilla oil, canola oil, soybean oil
stearidonic acid	*cis*-6,*cis*-9,*cis*-12,*cis*-15-octadecatetraenoic acid	18:4n-3 (SDA)	fish oils, genetically enhanced soybean oil, blackcurrant seed oil, hemp oil
	cis-8,*cis*-11,*cis*-14,*cis*-17-eicosatetraenoic acid	20:4n-3	very minor component in animal tissues
eicosapentaenoic acid	*cis*-5, *cis*-8,*cis*-11,*cis*-14,*cis*-17-eicosapentaenoic acid	20:5n-3 (EPA)	fish, especially oily fish (salmon, herring, anchovy, smelt and mackerel)
docosapentaenoic acid	*cis*-7,*cis*-10,*cis*-13,*cis*-16, *cis*-19-docosapentaenoic acid	22:5n-3 (n-3 DPA)	fish, especially oily fish (salmon, herring, anchovy, smelt and mackerel)
docosahexaenoic acid	*cis*-4,*cis*-7,*cis*-10,*cis*-13,*cis*-16,*cis*-19-docosahexaenoic acid	22:6n-3 (DHA)	fish, especially oily fish (salmon, herring, anchovy, smelt and mackerel)

ANALYTICAL METHODS

Analysis of fatty acids from biological or food samples generally involves three steps: extraction of lipids, conversion of the extracted lipids to fatty acid methyl esters (FAME) and analysis of the FAME using gas-liquid chromatography (GC) for the fatty acid profile.

Several excellent methods are available for extraction of fat (Christie, 2003 and 2008). Simple extraction procedures using non-polar organic solvents can be used for triacylglycerol-rich samples. Quantitative recovery of the complex lipid mixture from animal tissues is most conveniently achieved using procedures that employ a mixture of polar solvents such as the chloroform-methanol of Folch *et al*. (1957) or that of Bligh and Dyer (1959). If the procedures are followed exactly as described in the original papers, both methods provide reliable results.

The lipids of plant material and photosynthetic tissues are liable to undergo extensive enzyme-catalyzed degradation when extracted with chloroform-methanol. The problem is best overcome by means of conducting a preliminary extraction with

propan-2-ol, followed by re-extraction of the residue with chloroform-methanol (Nicholos, 1963).

For analysis of foods for fatty acid information, the AOAC Official method 996.06 is recommended (AOAC, 2005). The procedure involves hydrolysis of the food samples using either an acid or a base, followed by ether extraction of the released fat, transesterification of the extracted fat to FAME and determination of the fatty acid profile by capillary GC.

An elegant procedure has recently been established for analysis fatty acid composition of whole blood lipids by GC without lipid extraction. Drops of blood (50 µL) collected from fingertips are placed on a piece of chromatography paper that is inserted into a test tube and directly subjected to transmethylation for GC analysis (Marangoni *et al*., 2004a). This is a rapid and inexpensive method for the analysis of circulating fatty acids. The method has the potential for application to other biological specimens. In addition, the collection of blood samples on chromatographic paper is a very convenient technique for securing long-term storage and transportation of blood samples.

FAME are usually prepared by transesterification using hydrochloric acid, sulphuric acid, or borontrifluoride in methanol (Christie, 2008). Acidic methylation reagents, however, should not be used for samples containing CLA isomers. In such cases methylation using sodium methoxide is recommended.

Analyses of fatty acid profiles are best performed with GC using a flame ionization detector. For analyses of FAME mixtures containing no *trans* fatty acids, bonded polar 30 m x 0.32 (or 0.25 mm) capillary columns prepared from Carbowax-20m are recommended. A typical GC run time for FAME from fish oils and other lipids, which contain long-chain highly unsaturated fatty acids such as DHA, is about 65 minutes when the column temperature is operated isothermally at 190°C with helium carrier gas at 12 psig. With column temperature programming (hold at 190°C for 8 min, programme at 30°C/min to 240°C) the same analysis can be executed in a run time of about 25 min (Ackman, 2008b).

For analysis of samples containing *cis* and *trans* isomers, 100 m FFSC columns coated with highly polar cyanopolysiloxane stationary phases are recommended. The best separation of all the fatty acids of partially hydrogenated vegetable oils, with minimum overlaps of *cis*-*trans* isomers of 18:1, as well as other fatty acids, is achieved when the column temperature is operated isothermally at 180°C using hydrogen as the carrier gas at a flow rate of 1.0 ml/min (Ratnayake, 2004; Ratnayake *et al*., 2006; AOCS, 2005).

The application of innovative technical developments to fatty acid analysis, such as fast GC, has allowed the procedure to be greatly simplified, especially by reducing the duration of the analysis, which is now more easily applicable to analyse large numbers of samples in clinical studies. This approach is based on the use of increased carrier gas velocity and pressure, chromatographic columns of smaller diameter, and faster temperature ramping. As a consequence the analytical time for plasma fatty acids can be reduced to 12 minutes or less (Masood *et al*., 2005)

There is very often a need to examine the fatty acid composition of lipid classes in lipid analysis of biological specimens, including for PL, TG and cholesterol esters (CE) in plasma, liver and other tissues. Thin-layer chromatography (TLC) is the most convenient technique for isolation of small amounts of lipid components (Christie, 2008). It permits excellent separations with comparatively short elution times.

Lipidomics

Studies in genomics, proteomics and metabolomics have led to the new science of lipidomics, the full characterization of the molecular species of lipids in biological samples. Lipidomics aims to relate the lipid compositions of biological systems to their

biological roles with respect to the expression of genes involved in lipid metabolism and function, including gene regulation (Spener *et al*., 2003). The molecular species of lipids of biological samples are extremely diverse and are arranged in various combinations and permutations. Identification of these complex molecules is a considerable challenge. Another challenge is to relate the analytical data to their biological functions. Nevertheless, the underlying strategy in lipidomics involves firstly isolation of the biological sample and the sub-fractions, and secondly, extraction of the complex lipids free from proteins and other non-lipid components (Wolf and Quinn, 2008). The extracted lipids are then fractionated, usually using a multi-step chromatography process. In the final step individual molecular species are identified and quantified. Identification of the lipid molecular species is performed using sophisticated mass spectrometry technology (Wolf and Quinn, 2008). Modern mass spectrometry methods, involving ionization by electrospray, fast atom bombardment, atmospheric pressure chemical ionization, atmospheric pressure photo-ionization, and matrix-assisted laser desorption techniques, are highly sensitive and can produce excellent quantitative data.

An important outcome of lipidomics has been the development of the comprehensive classification system for lipids that was discussed previously (Table 3.1). This new classification system will facilitate international communication about lipids and help to deal with the massive amounts of data that will be generated by lipidomilogists.

FAT DIGESTION, ABSORPTION AND TRANSPORT

The digestive process is very complex and requires coordinated lingual, gastric, intestinal, biliary and pancreatic functions. Initially, the dietary fatty acid is masticated and mixed with lingual lipase, followed by hydrolysis by gastric lipase in the stomach and then by pancreatic lipase in the small intestine. Hydrolysis of TG yields 2-monoacyl-sn-glycerols and free fatty acids as final products. The formation of 2-mono-sn-glycerols facilitates the absorption of PUFA at the sn-2 position and the retention of these fatty acids in the glycerol lipids that are subsequently generated and transferred to tissues. Hydrolysis of PL yields sn-1-lysophospholipids and free fatty acids. Dietary esters are hydrolyzed to cholesterol and free fatty acids.

The short and medium-chain fatty acids released are absorbed across the gut and travel through the portal vein to the liver, where they are rapidly oxidized (Gurr and Harwood, 1991). The other products of hydrolysis (e.g., long-chain fatty acids, 2-monoacylglycerol, lysophospholipids and cholesterol) are mixed with bile salts and lecithin to form micelles, which are absorbed through the wall of the intestine. The fatty acids are then converted to TG. Cholesterol and lysophospholipids are also converted to their fatty acid esters. The newly synthesized TG, PL and cholesterol esters are combined with *de novo*-synthesized apolipoproteins to form chylomicrons and are transported out of the enterocyte and into bloodstream via the lymph vessels. While in the bloodstream, the TG of the chylomicrons are hydrolyzed to free fatty acids and glycerol by lipoprotein lipase. The fatty acids and glycerol then pass through the capillary walls to be used by cells as energy or stored as fats in adipose tissue. Some of the free fatty acids released bind to albumin and are cleared by the liver.

The remnants of chylomicron material are cleared from circulation by the liver low-density lipoprotein (LDL) receptor and LDL receptor related proteins. Though both contribute to chylomicron remnant clearance, the LDL receptor normally predominates. The liver catabolizes cylomicron remnants, resynthesizes TG from fatty acids and forms very-low-density lipoprotein (VLDL), which consists primarily of TG and small amounts of cholesterol and phospholipids, and releases them into circulation. VLDL represent the main carrier of TG and also substrates for endothelial lipoprotein lipase and supply

free fatty acids to adipose and muscle tissues. Through lipase hydrolysis they lose some of the TG and are transformed into intermediate-density lipoproteins (IDL) and finally into low-density LDL. LDL is taken by the LDL receptor of peripheral tissue and liver. LDL primarily transfers cholesterol esters in plasma to the peripheral tissues where they are hydrolyzed to free cholesterol and are then re-esterified. High-density lipoproteins (HDL) also play an important role in lipid transport. In humans, HDL carry 15–40% of plasma total cholesterol and are involved in the transport of cholesterol from peripheral tissues to the liver. The absorption (intake-excreted)/intake of most common dietary fatty acids is >95% in humans. However, the absorption of stearic from high stearic acid sources is low (65%), but in mixed diets the absorption is as high as 94% (Baer *et al*., 2003).

Food structure can influence the apparent bioavailability of lipids from foods. EPA and DHA from fish are more effectively incorporated into plasma lipids than when administered as capsules (Visioli *et al*., 2003). Pre-emulsification of an oil mixture prior to ingestion could also increase the absorption of EPA and DHA (Garaiova *et al*., 2007). The physical nature in which TG are found in dairy foods can affect the rate of their digestion. A diet containing 40 g dairy fat, eaten daily for 4 weeks as cheese, did not raise total and LDL cholesterol when compared with butter (Nestel *et al*., 2005). Another study found that the physical structure of fat-rich foods (milk, mozzarella cheese, butter) had no major effect on postprandial plasma TG concentrations (Clemente *et al*., 2003).

The composition of sn-2 of TG and PL is of great importance because, as discussed above, sn-2 facilitates the absorption of these fatty acids as 2-monoacyl-sn-glycerols that are utilized in the re-synthesis of TG and glycerol phospholipids that takes place after fat absorption (Lehner and Kuksis, 1996). In seed oils, PUFA are greatly enriched in the sn-2 position while SFA are concentrated in the sn-1 and sn-3 positions, and MUFA are relatively evenly distributed. In most dietary animal fats, the SFA are predominantly in the sn-1 position, although an appreciable amount of oleic acid is usually present also. The sn-2 position tends to contain mainly PUFA, especially LA. In cow milk, however, all the butyric acid (C4:0) and most of the hexaenoic acid (C6:0) are in the sn-3 position, whereas the long-chain SFA (C14:0, C16:0 and C18:0) are equally distributed at the sn-1 and sn-2 positions. In human milk, palmitic acid (C16:0) is predominantly in the sn-2 position, whereas stearic acid (C18:0) is in the sn-1 position. In marine lipids, SFA and MUFA are preferentially in the sn-1 and sn-3 positions, whereas PUFA are greatly concentrated in the position sn-2 with substantial amounts also being in position sn-3.

Phospholipids (PL) are constituents of cell membranes, which occur in foods and extracted oils in small quantities. A SFA is usually esterified at the sn-1 position and a PUFA at the sn-2 position. Thus, although a minor component of foods, PL can be important sources of PUFA.

Metabolism of fatty acids

Oxidation

Fat stored as TG is the body's most concentrated source of energy because TG are both reduced and anhydrous. The energy yield from a gram of fat catabolism is approximately 9 Kcal (37.7 kj)/g, compared with 4 Kcal (16.8 kj)/g from protein or carbohydrates.

Fatty acids yield energy by ß-oxidation in the mitochondria. Overall the ß-oxidation process is not very efficient since it requires transport into the mitochondria by carnitine, which involves four steps. As a result, fatty acids are less efficiently used for energy production than carbohydrates, and are preferentially stored in the adipose tissue. In addition, oxidation of long-chain fatty acids initially takes place in peroxisomes and is

also not very efficient. For individuals eating high-fat diets with excess caloric intake, much of the dietary fatty acids are readily stored in the adipose tissue.

The fatty acid structure affects the rate of oxidation. In general, long-chain fatty acids are oxidized more slowly and unsaturated fatty acids oxidized more rapidly than saturated fatty acids. Oxidation of saturated fatty acids decreases with increasing carbon chain length (laurate>myristate>palmitate>stearate) (Leyton *et al*., 1987). For unsaturated fatty acids, 24-h oxidation is in the order, ALA>OA>LA>AA.

De novo synthesis of fatty acids

The synthetic process involves the breakdown of excess dietary carbohydrates to acetate units and condensation of acetate, as acetyl coenzyme A (CoA), with bicarbonate to form malonyl CoA. Acetyl CoA then combines with a series of malonyl CoA molecules to form saturated fatty acids of different carbon lengths, of which the end product is palmitic acid (16:0). The fatty acid synthetic reactions up to this stage take place within the fatty acid synthetic complex. Once palmitic acid is released from the synthetic complex, it can be elongated to stearic acid and even higher saturated fatty acids by further additions of acetyl groups, through the action of fatty acid elongation systems.

In animal tissues the desaturation of *de novo* synthesized saturated fatty acids stops with the formation of the n-9 series MUFA. This conversion is performed by Δ9 desaturase, which is a very active desaturase enzyme in mammalian tissues, and introduces double bonds at the 9-10 position of the fatty acid chain. Oleic acid (18:1Δ9 or 18:1n-9) is the main product. The products of *de novo* synthesis are esterified with glycerol to form TG. In liver these TG are incorporated into VLDL and transported out into circulation. In adipose tissue they are stored as lipid droplets. If a low-fat, high-carbohydrate diet is eaten consistently, the adipose tissue consists mostly of 16:0, 18:0 and 18:1n-9, which are the main products of *de novo* synthesis (Vemuri and Kelly, 2008). Individuals eating large amounts of LA will deposit this fatty acid in adipose tissue (Thomas *et al*., 1987). In the absence of dietary LA and other PUFA, 18:1n-9 is further desaturated and this step is followed immediately by elongation to form the n-9 family of PUFA.

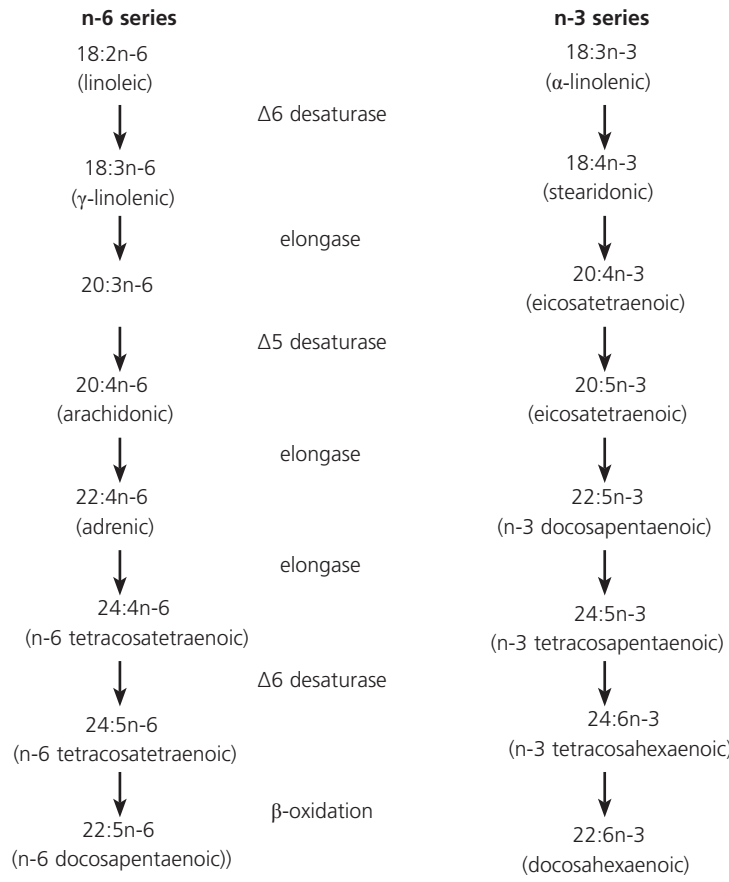
Dietary fatty acids have a significant influence on *de novo* synthesis and it is likely that all dietary fatty acids, except short-chain fatty acids, suppress it (Vemuri and Kelly, 2008). Free-living healthy humans have a significant capacity for *de novo* synthesis, which contributes on average approximately 20% of newly formed adipose TG (Strawford *et al*., 2004). Factors such as background diet, physical activity, genetics, hormones etc. can influence *de novo* synthesis. More research is required to determine how these factors, especially excess dietary fats, influence *de novo* synthesis.

Metabolism of LA and ALA to LCPUFA

Although mammals can readily introduce double bonds at the Δ9 position, they cannot introduce additional double bonds between Δ10 and the methyl terminal end. Thus, LA and ALA cannot be synthesized by mammals, but plants can synthesize both by introducing double bonds at Δ12 and Δ15. Because they are necessary precursors for the synthesis of LCPUFA and eicosanoids, LA and ALA are essential fatty acids and they must be obtained from plants in the diet.

Once LA and ALA are obtained from the diet, they can be converted to n-6 and n-3 families of C20 and C22 LCPUFA by a series of alternating desaturation and elongation reactions (Figure 3.1). The pathways need only Δ6- and Δ5-desaturases, an elongase of the microsomal system and a chain-shortening step involving β-oxidation in the peroxisomes (Moore *et al*., 1995; Sprecher, 2002). The first step involves insertion of a double bond at the Δ6 position of LA and ALA by the action of Δ6 desaturase, which is followed by chain elongation of two carbon units by elongase and insertion of another double bond at the Δ5 position by Δ5-desaturase to form arachidonic acid (20:4n-6 or

FIGURE 3.1
Metabolic pathways for the conversion of dietary linoleic and α-linolenic acids to their long-chain polyunsaturated fatty acids



AA) and eicosapentaenoic acid (20:5n-3 or EPA), respectively. In the next step, AA and EPA are elongated by two carbon units to 22:4n-6 and 22:5n-3 (n-3 DPA), respectively. Further elongation of 22:4n-6 and 22:5n-3 by two carbon units produces 24:4n-6 and 24:5n-3, respectively. These C24 PUFA are then desaturated by Δ6 desaturase to yield 24:5n-6 and 24:6n-3. This is the same desaturase enzyme that desaturates LA and ALA (D'Andrea *et al*., 2002). DHA is formed from 24:6n-3 through chain shortening by two carbon units during one cycle of the β-peroxisomal pathway. By the same chain shortening mechanism 22:5n-6 is produced from 24:5n-6.

The two pathways are independent of each other and there are no crossover reactions. However, since both pathways use the same enzymes, there is competition between the two series for the conversions. Since LA is the predominant PUFA in human diets and intakes of ALA are generally low, plasma and cell levels of n-6 LCPUFA derived from LA tend to be higher than the n-3 LCPUFA levels.

Efficiency of conversion

Although humans and animals have the capacity to convert ALA to EPA and DHA, the efficiency of conversion is low, in particular to DHA. Generally, ALA intake increases ALA, EPA and n-3 DPA, but there is very little increase in DHA in plasma fractions (platelets, white cells and red blood cells) or breast milk (Gerster, 1998; Li *et al*., 1999;

Mantzioris *et al*. 1994; Brenna, 2002; Li *et al*., 2002; Francois *et al*., 2003; Burdge and Calder, 2005). Many studies also report a tendency for DHA to decline when ALA consumption is markedly increased (Burdge and Calder, 2005). Stable isotope tracer studies estimated that the efficiency of conversion of ALA to EPA is 0.2%, to n-3 DPA is 0.13% and to DHA is 0.05% (Pawlosky *et al*., 2001). There are several possible explanations for the poor conversion of ALA to DHA. A large proportion of ingested ALA is oxidized to acetyl CoA, which is recycled into *de novo* synthesis of cholesterol, saturated and monounsaturated fatty acids, or further metabolized to carbon dioxide (DeLany *et al*., 2000). It is also the most rapidly oxidized unsaturated fatty acid (Nettleton, 1991). Unlike LA, the rate of acylation of ALA to tissue lipids is low. The concentration of ALA in plasma and tissue phospholipids is usually less than 0.5% of total fatty acids (Burdge and Calder, 2005). It is most likely that this low content of ALA is not sufficient to compete with LA for the Δ6 desaturase.

There are some reports, however, showing that DHA status can be improved by long-term intakes of vegetable oils containing ALA and less LA (Ezaki *et al*., 1999; Ghafoorunissa *et al*., 2002). This observation is very important for vegetarians and for those who for various reasons do not include fish in their regular diets. Further research needs to be performed to confirm these findings. However, the increase in DHA may not be immediate and also may not be as effective as direct consumption of DHA from fish or fish oil supplements (Burdge and Calder, 2005).

Animal studies have shown that maximum incorporation of DHA into tissues can be achieved using diets with LA-ALA ratios between 4:1 and 2:1 (Woods *et al*., 1996; Bowen *et al*., 1999; Blank *et al.*, 2002). A human feeding study, however, demonstrated that the absolute amount of ALA, more than the LA/ALA ratio, influences the conversion of ALA to its derivatives (Goyens *et al*., 2006). It appears that a reduction in dietary LA together with an increase in ALA intake would be the most appropriate way to enhance EPA and DHA synthesis. Limited data suggest that the conversion of ALA to EPA and DHA is substantially greater in young women than in men of similar age, possibly due to activation of the peroxisomal pathway by estrogens (Burdge and Wootton, 2002; Burdge *et al*., 2002).

In summary, the biosynthetic pathway in humans does not appear to provide sufficient levels of ALA for it to be a substitute for dietary EPA and DHA. High levels of EPA and DHA in blood or other cells are attained only when they are provided as such in the diet and this would occur mostly from the consumption of fish and fish oils, which are rich sources of these n-3 LCPUFA.

Influence of environmental factors on the conversion of LA and ALA to n-6 and n-3 LCPUFA

Some environmental factors affect the activity of Δ5- and Δ6-desaturases, and therefore the conversion of LA and ALA to their LCPUFA. Dietary cholesterol decreases the activity of the desaturases (Huang *et al.,* 1985, 1990; Garg et *al.*, 1986). High-fat diets also decrease the activity of desaturases (Garg *et al.,* 1986). The activity of Δ5 desaturase appears to be low in diabetic humans (Jones *et al.,* 1986; Bassi *et al.,* 1996). Low insulin levels, deficiency of protein and minerals such as iron, zinc, copper and magnesium, which are often associated with malnutrition, decrease Δ6 desaturase activity and therefore the conversions of LA and ALA to LCPUFA. These observations may have significance for populations from developing countries whose diets are deficient in energy and several nutrients. An additional issue concerning PUFA metabolism that would need to be studied in detail, considering that the conversion of LA and ALA to their long chain products takes place mainly in the liver, is the efficiency of the conversion steps in relation to liver function and disease conditions. This topic has not been adequately studied, but it appears from the limited data available that levels of AA and EPA are low in cirrhotic patients.

Alcohol consumption (Horrobin, 1987; Pawlosky and Salem, 2004) and cigarette smoking (Santos *et al*., 1984; Simon *et al*., 1996; Leng *et al*., 1994; Marangoni *et al*., 2004b; Agostoni *et al*., 2008) also decrease tissue LCPUFA concentrations.

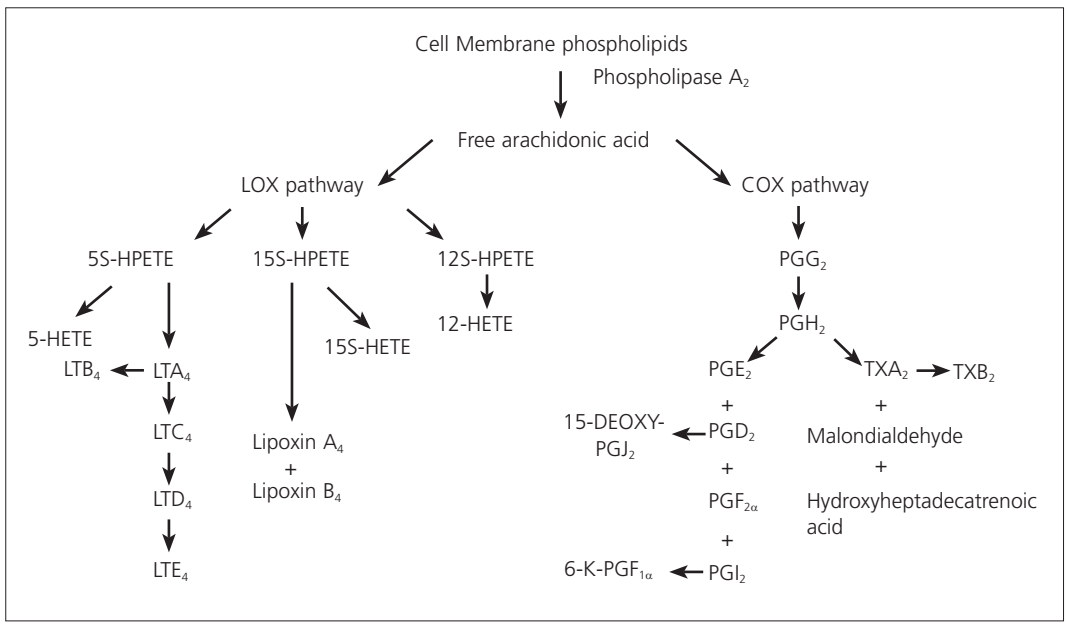
Another current concern is the extremely high level of LA in diets in many Western countries (Lands, 2008). Typical consumption of LA in Europe, Australia and North America ranges between 8.3 and 19.0 g per day in men and 6.8 and 13.2 g per day in women (Burdge and Calder, 2005). This is typically about 10-fold higher than the consumption of ALA. These levels of LA can greatly exceed those needed to prevent essential fatty acid deficiency. Drastic reduction in the intakes of LA is warranted in Western countries. This would result in greater conversion of ALA to EPA and DHA (Lands, 2008). However, caution needs to be exer*cis*ed in this regard since a Nurses Health Study over many years showed that nurses with higher intakes of LA had lower risks of cardiovascular disease and related mortality (Hu *et al*., 1997). It is possible that the deficit in physiological levels of EPA and DHA may be more important than higher intakes of ALA. However, for those who do not consume n-3 LCPUFA as EPA/DHA, the competition for conversion to n-3 LCPUFA from ALA may be compromised. It should be noted that plant-based n-3 PUFA may reduce CHD risk, in particular when seafood-based n-3 PUFA intake is low. This has implications for populations that consume little fatty fish (Mozaffarian *et al*., 2005).

Eicosanoid and docosanoid formations

Formation of eicosanoids is an important biological function of the long-chain n-6 and n-3 C20 PUFA. The eicosanoids include prostaglandins (PG), prostacyclins (PGI), thromboxanes (TX), leukotrienes (LT), hydroperoxytetraenoic acids (HPETE), hydroxyeicosatetraenoic acids (HETE) and lipoxins (Lee and Hwang, 2008).

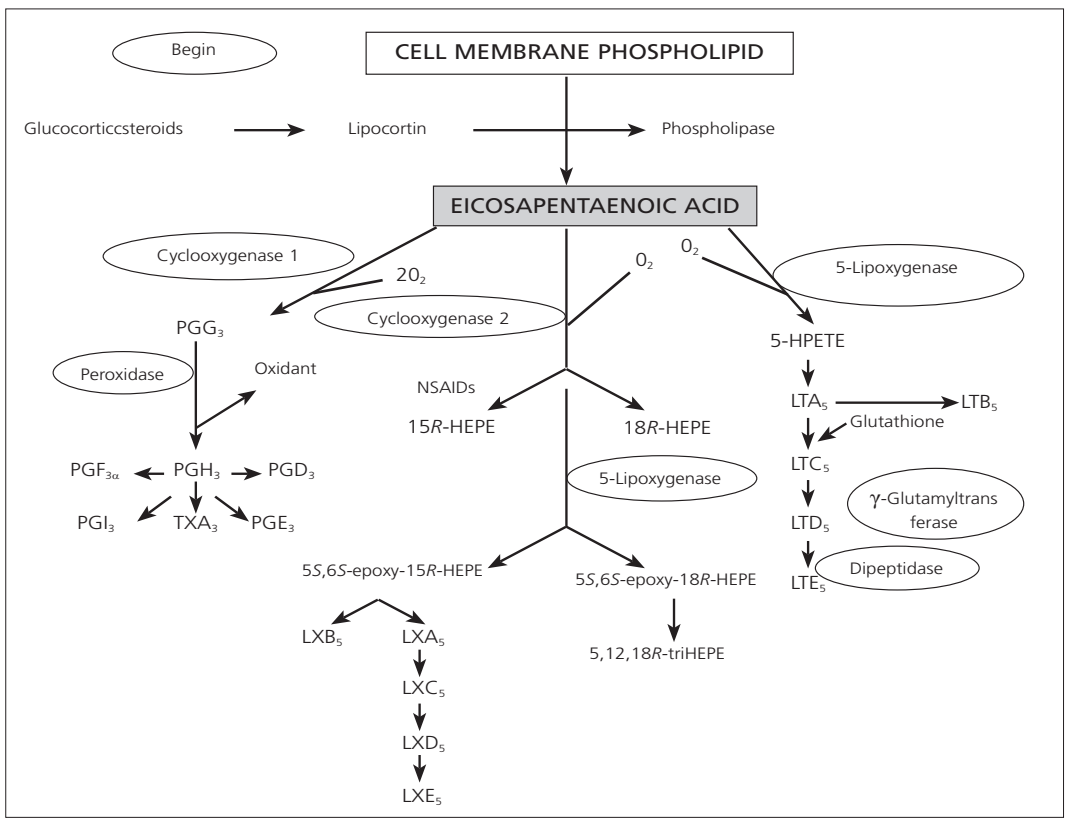
There are two primary pathways involving two microsomal enzymes. Cyclooxygenase (COX) converts the C20 fatty acids to prostanoids (PG, PGI and TX) whereas lipooxygenase (LOX) converts them to HPETE, which are quickly converted to LT, HETE and lipoxins (Smith *et al*., 1991; Samuelson, 1987). There are two types of COX: COX 1, which is constitutive, responsible for the physiological roles of eicosanoids, and COX 2, which is inducible, and is activated by processes such as inflammation. The three important fatty acids involved in eicosanoid production are DHGLA, AA and EPA. As they have different numbers of double bonds, they each give rise to a different series of eicosanoids. Prostanoids of 1-series and LT of 3-series are formed from DHGLA. Prostanoids of 2-series and LT of 4-series are formed from AA, while 3-series prostanoids and 5-series LT are produced from EPA. The eicosanoids from AA and EPA are biologically more active and more important than those derived from DHGLA. Figures 3.2 and 3.3 show the eicosanoid pathways for AA and EPA, respectively. These fatty acids are derived from cell membrane PL by the action phospholipase A2. AA and EPA compete for the same enzymes, and hence the relative levels of products formed depend on the cell membrane concentrations of AA and EPA. Cell membranes typically contain a high proportion of AA and low proportions of EPA and DHA, and therefore AA is the dominant substrate for eicosanoid synthesis. However, a high dietary intake of EPA/DHA can inhibit the production of eicosanoids derived from AA (Corey *et al*., 1983; Culp *et al*., 1979). In addition to the eicosanoids, in recent years a novel group of mediators formed from EPA by aspirin-modified COX-2, termed E-series resolvins has been identified (Serhan *et al*., 2000) (Figure 3.4). DHA is poor substrate for COX and therefore, DHA was not known to produce bioactive mediators until recently. However, DHA-derived bioactive docosanoids, termed D-series resolvins and protectins (neuroprotectins D1) by COX-2 and 5-LOX, have been identified (Serhan *et al*., 2002; Bazan, 2007; Lee and Hwang, 2008) (Figure 3.4).

FIGURE 3.2
Eicosanoid formation from arachidonic acid (AA) via the cyclooxygenase (COX) and lipoxygenase (5-LOX) pathways. HPETE = hydroxyperoxyeicosatetraenoic acid; HETE = hydroxyeicosatetraenoic acid; LT = leukotriene; TX, = thromboxanes; PG = prostaglandins



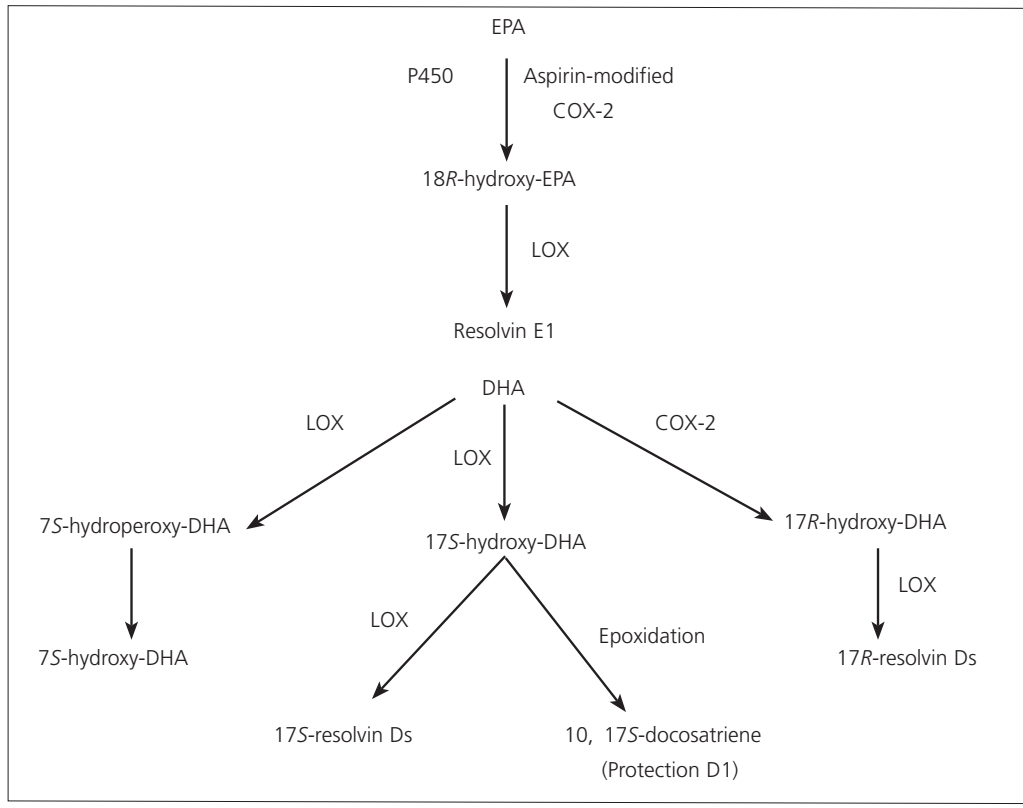
Adapted from Lee and Hwang, 2008

FIGURE 3.3
Eicosanoid formation from eicosapentaenoic acid (EPA) via the cyclooxygenase (COX) and lipoxygenase (5-LOX) pathways. HPETE, hydroxyperoxy-eicosapentaenoic acid; HETE = hydroxyeicosatetraenoic acid; LT = leukotriene; TX = thromboxanes; PG = prostaglandins



Adopted from Li *et al*., 2002

FIGURE 3.4
Metabolic pathways for the conversion of eicosapentaenoic (EPA) and docosahexaenoic (DHA) to resolvins and protectins. LOX = Lipooxygenase. COX = Cyclooxygenase



Adapted from Lee and Hwang, 2008

Physiological functions of n-6 and n-3 PUFA and eicosanoids

The two essential fatty acids, LA and ALA and their long-chain products (AA, EPA and DHA) play prominent physiological roles in different organs. Upon incorporation into structural lipids, these fatty acids can modify membrane fluidity, membrane thickness, and alter specific interactions with membrane proteins (Carrillo-Tripp and Feller, 2005). Although the key anti-inflammatory effects of EPA and DHA are mediated via antagonizing AA metabolism, these n-3 fatty acids have a number of other anti-inflammatory effects, as described in detail in another section. The n-3 fatty acids affect cytokines and other factors. Cytokines are a family of proteins produced and released by cells involved in the inflammatory process and in the regulation of the immune system. Cell culture studies have shown that n-3 fatty acids can decrease the endothelial expression of a variety of cytokine-induced leukocyte adhesion molecules and of secretable protein products implicated in leukocyte recruitment and local amplification of inflammation. DHA, but not EPA, is effective in reducing endothelial expression of E-selectin, ICAM-1 (intercellular cell-adhesion molecule1) and VCAM-1 (vascular cell adhesion molecule 1), and impaired the ability of ligand bearing monocytes to adhere (De Caterina and Libby, 1996). The magnitude of this effect parallels that of incorporation of DHA into cellular phospholipids.

Another important biological role of n-3 and n-6 PUFA is the regulation of enzymes involved in lipid metabolism. PUFA activate the expression of genes of fatty acid transport and oxidation (acyl CoA synthetase, acyl CoA oxidase, liver fatty acid binding protein, carnitine palmityoyltransferase-1 and cytochrome P450A1) and suppress the expression of genes regulating *de novo* synthesis of lipids (stearoyl CoA desaturase, acetyl CoA carboxylase, and fatty acid synthase) (Jump, 2002; Jump, 2008; Sampath

and Ntambi, 2005). PUFA exert these effects on gene expression by regulating three major transcriptional factors controlling multiple pathways involved in lipid metabolism. PUFA activate peroxisome proliferators activated receptor (PPARα) and suppress the nuclear abundance of carbohydrate regulatory element binding protein (ChREBP)/Max-like factor X (MLX) and sterol regulatory element binding protein (SREBP-1). PUFA activation of PPARα enhances fatty acid oxidation, while PUFA suppression of SREBP-1 and ChREBP)/MLX results in inhibition of *de novo* synthesis of fatty acids. As such PUFA promote a shift in metabolism toward fatty acid oxidation and away from fatty acid synthesis and storage. The result of this two-fold action is a negative fat balance, thereby making PUFA a good candidate for the dietary management of hyperlipidemia. The transcription factors display a differential response to PUFA. EPA is potent activator of PPARα and DHA controls nuclear abundance of SREBP-1. Nuclear abundance of carbohydrate regulatory element binding protein (ChREBP)/MLX, appears equally responsive to a wide range of C18-C22 carbon n-3 and n-6 PUFA (Jump, 2008). More studies are required to evaluate the significance of these differences. Assessments of these differences may provide new insights into disorders of lipid metabolism associated with chronic metabolic diseases such as diabetes and obesity.

N-3 and n-6 PUFA inhibit fatty acid synthase in adipose tissue. PUFA also repress transcription of the leptin gene. Leptin is an adipose derived hormone that regulates appetite, body weight and adiposity. Increased plasma leptin levels have been correlated with increased adiposity, while weight loss results in decreased plasma leptin levels. Substitution of PUFA for saturated fatty acids in the diet causes a decrease in plasma leptin levels (Reseland *et al*., 2001; Duplus *et al*., 2000). The C20 and C22 n-6 and n-3 PUFA affect visual acuity and are required for optimal development of the brain.

The eicosanoids derived from AA and EPA and the docosanoids derived from DHA are involved in a variety of biological processes including modulating inflammation, platelet aggregation, immune response, cell growth and proliferation, and contraction and dilation of smooth muscle cells (Tables 3.6 and 3.7). The eicosanoids derived from EPA are generally less potent than those derived from AA. For example, prostaglandin PGE2 and thromboxane TXA2 derived from AA are produced in platelets and promote inflammation with potent chemo activity, serve as vasoconstrictors and stimulate platelet aggregation.

TABLE 3.6
Physiological actions of eicosanoids derived from arachidonic acid

Eicosanoid	Physiological action
PGE_2	pro-inflammatory, pro-aggregatory, suppress immune response, promote cell growth, proliferation, vasodilation, bronchoconstriction, mild anti-inflammatory (inhibits 5-LOX and so decreases inflammatory 4-series LT, induces 15-LOX which promotes formation of anti-inflammatory lipoxins)
PGI_2	anti-inflammatory, Inhibits platelet aggregation, potent vasodilator
TXA_2	potent platelet aggregation, potent vasoconstrictor,
PGD_2	inhibits platelet aggregation, vasodilation, promotion of sleep
$PGF_{2\alpha}$	induces smooth muscle contraction, uterine contraction
LTB_4	pro-inflammatory, causes neutrophil aggregation, neutrophil & eosinophil chemotaxis
LTC_4	pro-inflammatory, promote endothelial cell permeability, contracts smooth muscle cells, constricts peripheral airways
LTD_4	contracts smooth muscle cells, constricts peripheral airways
12-HETE	neutrophil chemotaxis, stimulates glucose-induced insulin secretion
15-HETE	inhibits 5- and 12-lipoxygenase
Lipoxin A	superoxide anion generation, chemotaxis
Lipoxin B	inhibits NK cell activity

Prostaglandins and thromboxanes from EPA act as vasodilators and anti-aggregators. Prostaglandin I2 (PGI2) derived from AA is also an inhibitor of platelet aggregation. An imbalance in the synthesis of eicosanoids in tissues can lead to development of certain pathological conditions, including thrombosis, kidney disease, inflammation, asthma, inflammatory bowel disease and several other inflammatory conditions (Calder, 2006). The role of n-6 and n-3 PUFA and the eicosanoids and docosanoids in inflammation, immune function and coronary heart disease are discussed in other chapters of this report.

TABLE 3.7
Physiological actions of eicosanoids derived from eicosapentaenoic acid (EPA) and docosanoids derived from docosahexaenoic acid (DHA)

Eicosanoid/Docasanoid	Physiological action
PGE_3	mild anti-aggregatory, vasodilation
PGI_3	mild anti-aggregatory
TXA_3	mild pro-aggregatory
EPA Resolvin E1	potent anti-inflammatory
DHA Resolvin D	potent anti-inflammatory
DHA Protectin D1	potent anti-inflammatory

The concentrations of eicosanoids and docosanoids synthesized in tissues are most likely related to dietary levels of n-6 and n-3 fatty acids. It may be possible that the risk of chronic diseases can be reduced by modulating eicosanoid formation through changes in the composition of dietary fatty acids. The competitive inhibition between n-3 and n-6 for desaturases and COX suggests that increasing n-3 PUFA, especially EPA and DHA, would reduce AA levels in tissue lipids and consequently, would decrease the formation of the potent inflammatory and pro-aggregatory eicosanoids derived from AA.

REFERENCES

Ackman, R.G. 2008a. Fatty acids in fish and shellfish. *In* Chow, C.K., ed., *Fatty Acids in Foods and Their Health Implications*, pp. 155-185. CRC Press, London, UK.

Ackman, R.G. 2008b. Application of gas-liquid chromatography to lipid separation and analysis: Qualitative and quantitative analysis. In Chow, C.K., ed., *Fatty Acids in Foods and Their Health Implications*, pp. 47-65. CRC Press, London, UK.

Agostoni, C., Riva, E., Giovannini, M., Pinto, F., Colombo, C., Risé, Galli, C. & Marangoni, F. 2008. Maternal smoking habits are associated with differences in infants' long-chain polyunsaturated fatty acids in whole blood: a case-control study. *Arch. Dis. Child*., 93: 414-418.

AOAC, 2005. AOAC Official Method 996.06. Fat (Total, Saturated, and Unsaturated) in foods, hydrolytic extraction gas chromatographic method, Revised 2001. *In* Horwitz, W., ed., *Official Methods of Analysis of AOAC International 18th Edition*, American Oil Chemists' Society. Gaithersburg, MD, USA

AOCS, 2005. AOCS Official Method Ce 1h-05. Determination of *cis*-, *trans*-, saturated, monounsaturated and polyunsaturated fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC method: *In Official Methods and Recommended Practices of the AOCS*, Fifth edition, American Oil Chemists' Society. Urbana, IL, USA.

Baer, D.J., Judd, J.T., Kris-Etherton, P.M., Zhao, G. & Emken, E.A. 2003. Stearic acid absorption and its metabolizable energy value are minimally lower than those of other fatty acids in healthy men fed mixed diets. *Am. J. Clin. Nutr.*, 133: 4129-4134.

Bassi, A., Avogaro, A., Crepaldi, C., Pavan, P., Zambon, S., Marin, R., Macdonald, I. & Manzato, E. 1996. Short-term diabetic ketosis alters n-6 polyunsaturated fatty acid content in plasma phospholipids. *J. Clin. Endocrinol. Metab.*, 81: 1650-1653.

Bazan, N.G. 2007. Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. *Curr. Opin. Clin. Nutr. Metab. Care*, 10: 136-141.

Blank, C., Neumann, M.A., Makrides, M. & Gibson, R.A. 2002. Optimizing DHA levels in by lowering the linoleic acid to α-linolenic acid ratio. *J. Lipid Res.*, 43: 1537-1543.

Bligh, E.G. & Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.*, 37: 911-917.

Bowen, R.A., Wierzbicki, A.A. & Clandinin, M.T. 1999. Does increasing dietary linolenic acid content increase the docosahexaenoic acid content of phospholipids in neuronal cells of neonatal rats. *Pediatr. Res.*, 45: 505-516.

Brenna, J.T. 2002. Efficiency of conversion alpha-linolenic acid to long chain n-3 fatty acids in man. *Curr. Opin. Clin. Nutr. Metab. Care*, 5: 127-132.

Burdge, G.C. & Calder, P.C. 2005. Conversion of α-linolenic acid to longer-chain polyunsaturated fatty acids in human adults. *Reprod. Nutr. Dev.*, 45: 581-597.

Burdge, G.C. & Wootton, S.A. 2002. Conversion of α-linolenic acid to eicosapentaenoic, docosapentaenoic and docosahexaenoic acids in young women. *Br. J. Nutr.*, 88: 411-420.

Burdge, G.C., Jones, A.E. & Wootton, S.A. 2002. Eicosapentaenoic and docosapentaenoic acids are the principal products of α-linolenic acid metabolism in young women. *Br. J. Nutr.*, 88: 355-363.

Calder, P.C. 2006. Polyunsaturated fatty acids and inflammation. *Pros. Leuk*. EFA, 75: 197-202.

Carrillo-Trip, M. & Feller, S.E. 2005. Evidence for a mechanism by which ω-3 polyunsaturated lipids may affect membrane protein function. *Biochem.*, 44: 10164-10169.

Christie, W.W. 2003. *Lipid Analysis. Isolation, separation, identification and structural analysis of Lipids*. 3rd Edition, The Oily Press, Bridgwater, UK.

Christie, W.W. 2008. Preparation of lipid extracts from tissues. *In The Lipid Library* (Accessed on August 7, 2008 as: www.lipidlibrary.co.uk/topics/extract2/index.htm)

Clemente, G., Mancini, M., Nazzaro, F., Lasorella, G., Rivieccio, A., Palumbo, A.M., Rivellese, A.A., Ferrara, L. & Giacco, R. 2003. Effects of different dairy products on postprandial lipemia. *Nutr. Metab. Cardiovasc. Dis.*, 13: 377-383.

Corey, E.J., Shih, C. & Cashman, J.K. 1983. Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis. *Proc. Natl. Acad. Sci.* USA, 80: 3581-3584.

Culp, B.R., Titus, B.G. & Lands, W.E.M. 1979. Inhibition of prostaglandin synthesis by eicosapentaenoic acid. *Prostagl. Leukot.* Med., 3: 269-278.

Craig-Schmidt, M.C. & Teodorescu, C.A. 2008. *Trans*-fatty acids in foods. *In* Chow, C.K., ed., *Fatty Acids in Foods and Their Health Implications.* pp. 377-437. CRC Press, London, UK.

D'Andrea, S., Guillou, H., Jan, S., Catheline, D., Thibault, J.-N.,Bouriel, M., Rioux, V. & Legrand, P. 2002. The same Δ6-desaturase not only acts on 18- but also on 24-carbon fatty acids in very-long-chain polyunsaturated fatty acid biosynthesis. *Biochem. J.*, 364: 49-55.

DeLany, J.P., Windhauser, M.M., Champagne, C.M. & Bray, G.A. 2000. Differential oxidation of individual dietary fatty acids in humans. Am. *J. Clin. Nutr.*, 72: 905-911.

De Caterina, R. & Libby, P. 1996. Control of endothelial leukocyte adhesion molecules by fatty acids. *Lipids*, 31: S57-S63.

Duplus, E. Glorian, M. & Forest, C. 2000. Fatty acid regulation of gene transcription. *J. Biol. Chem.*, 275: 30749-30752.

Ezaki, O., Takahashi, M., Shigematsu, T., Shimamura, K., Kimura, J., Ezaki, H. & Gotoh, T. 1999. Long term effects of dietary alpha-linolenic acid from perilla oil on serum fatty acid composition and on the risk factors of coronary heart disease in Japanese elderly subjects. *J. Nutr. Sci. Vitaminol. (Tokyo)*, 45: 759-762.

Fahy, E., Subramanium, S., Brown, A.H., Glass, C.K., Merril Jr., A.H., Murphy, R.C., Raetz, C.R.H., Russell, D.W., Seyama, Y., Shaw, W., Shimizu, T., Spener, F., van Meer, G., VanNieuwenhze, M.S., White, S.H., Witztum, J.L. & Dennis, E.A. 2005. A comprehensive classification system for lipids. *J. Lipid Res.*, 46: 839-861.

Folch, J., Lees, M. & Sloane-Stanley, G.H. 1957. A simple method for isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, 226: 497-509.

Francois, C.A., Connor, S.L., Bolewicz, L.C. & Connor, W.E. 2003. Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. *Am. J. Clin. Nutr.*, 77: 226-233.

Garaiova, I., Guschina, I.A., Plummer, S.F., Tang, J., Wang, D. & Plummer, N.T. 2007. A randomised cross-over trial in healthy adults indicating improved absorption of omega-3 fatty acids by pre-emulsification. *Nutr. J.*, 6: 4.

Garg, M.L., Snowswell, A.M. & Sabine, J.R. 1986. Influence of dietary cholesterol on desaturase enzymes of rat liver microsomes. *Prog. Lipid Res.*, 25: 639-644.

Gerster, H. 1998. Can adults adequately convert α-linolenic acid to eicosapentaenoic acid and docosahexaenoic acid? *Int. J. Vit. Nutr. Res.*, 68: 159-173.

Ghafoorunissa, A., Vanni, R., Laxmi, R. & Sesikeran, B. 2002. Effects of dietary alpha-linolenic acid from blended oils on biochemical indices of coronary heart diseases in Indians. *Lipids*, 37: 1007-1086.

Goyens, P.L.L., Spiker, M.E., Zock, P.L., Katan, M.B. & Mensink, R.P. 2006. Conversion of α-linolenic acid in humans is influenced by the absolute amounts of α-linolenic acid and linoleic acid in the diet and not by the ratio. *Am. J. Clin. Nutr.*, 84: 44-35.

Gunstone, F.D. 1999. Fatty acid structure. *In* F.D. Gunstone, J.L. Harwood and F.B. Padley, eds. *The Lipid Handbook*, pp. 1-19. Second Edition, Chapman and Hall, London, UK.

Gurr, M.L. & Harwood, J.L. 1991. *Lipid Biochemistry: An Introduction*, 4th ed. Chapman and Hall, London, UK.

Horrobin, D.F. 1987. Essential fatty acids, prostaglandins, and alcoholism: An overview. *Alcohol Clin*. Exp. Res., 11: 2-9.

Hu, F.B., Stampfer, M.J., Manson, J.E., Rimm, E.B., Wolk, A., Colfitz, G.A., Hennekens, C.H. & Willett, W.C. 1997. Dietary fat intake and risk of coronary heart disease in women. *N. Engl. J. Med.*, 337: 1491-1499.

Huang, Y.-S., Horrobin, D.F. & Manku, M.S. 1985. Short-term effect of dietary cholesterol on tissue n-6 fatty acids in fat-deficient rats. *Proc. Soc. Exp. Biol. Med*., 178: 209-214.

Huang, Y.-S., Mills, D.E., Ward, R. P., Simmons, V.A. & Horrobin, D.F. 1990. Stress modulates cholesterol induced changes in plasma and liver fatty acid composition in rats fed n-6 fatty acid-rich oils. *Proc. Soc. Exp. Biol. Med.*, 195: 136-141.

Huth, P.J. 2007. Ruminant *trans* fatty acids: composition and nutritional characteristics. In List, G.R., Kristchevsky, D. and W.M.N.Ratnayake, eds. *Trans fats in foods*, pp. 97-126. AOCS Press, Urbana, IL.

IUPAC-IUB Commission on Biochemical Nomenclature 1978. The nomenclature of lipids (Recommendations, 1976). *Biochem.* J., 171: 21-35.

Jones, D.B., Carter, R.D. & Mann, J.I. 1986. Indirect evidence of impairment of platelet desaturase enzymes in diabetic mellitus. *Hormone Metab. Res.*, 18: 3413-3444.

Jump, D.B. 2002. Dietary polyunsaturated fatty acids and regulation of gene transcription. *Curr. Opin. in Lipidol.*, 13: 155-164.

Jump, D.B. 2008. N-3 polyunsaturated fatty acid regulation of hepatic gene transcription. *Curr. Opin. in Lipidol.*, 19: 242-247.

Lands, B. 2008. A critique of paradoxes in current advice on dietary lipids. *Progress in Lipid Res.*, 47: 77-106.

Lee, J.Y. & Hwang, D.H. 2008. Dietary fatty acids and eicosanoids. *In* Chow, C.K., ed. *Fatty Acids in Foods and Their Health Implications*, pp. 713-739. CRC Press, London, UK.

Lehner, R. & Kuksis, A. 1996. Biosynthesis of triacylglycerols. *Prog. Lipid Res.*, 35: 169-201.

Leng, G.C., Smith, F.B., Fowkes F.G. *et al*. 1994. Relationship between plasma essential fatty acids and smoking, serum lipids, blood pressure and haemostatic and rhelogical factors. *Prostaglandins Leukot. Essent. Fatty Acids*, 51: 101-108.

Leyton, J., Drury, P.J. & Crawford, M.A. 1987. Differential oxidation of saturated and unsaturated fatty acids *in vivo* in the rat. *Br. J. Nutr.*, 57: 383-393.

Li, D., Sinclair, A., Wilson, A., Nakkote, S., Kelly, F., Abedin, L. & Watson, A. 1999. Effect of dietary alpha-linolenic acid on thrombotic risk factors in vegetarian men. *Am. J. Clin. Nutr.*, 69: 872-882.

Li, D., Bode O, Drummond, H. & Sinclair, A.J. 2002. Omega-3 (n-3) fatty acids. In Gunstone, F.D., ed. *Lipids for functional foods and nutraceuticals*, pp. 225-262, The Oily Press, UK.

Mantzioris, E., James, M.J., Gibson, R.A. & Cleland, L.G. 1994. Dietary substitution with an α-linolenic acid-rich vegetable oil increases eicosapentaenoic acid concentrations in tissues. *Am. J. Clin. Nutr.*, 59: 1304-1309.

Marangoni, F., Colombo, C. & Galli, C. 2004a. A method for the direct evaluation of the fatty acid status in a drop of blood from a fingertip in humans: applicability to nutritional and epidemiological studies. *Anal. Biochem.*, 326: 267-272.

Marangoni, F., Colombo, C., De Angelis, L., Gambaro, V., Agostoni, C., Giovannini, M. & Galli, C. 2004b. Cigarette smoke negatively and dose-dependently affects the biosynthetic pathway of the n-3 polyunsaturated fatty acid series in human mammary epithelial cells. *Lipids*, 39: 633-637.

Masood, S.S., Nasri, S. & Schechter, J. 2005. Leptonic CP violation in a two parameter model. *Physical Review***,** D71, Issue 9.

Moore, S.A., Hurt, E., Yoder, E., Sprecher, H. & Spector, A.A. 1995. Docosahexaenoic acid synthesis in human skin fibroblasts involves peroxisomal retroconversion of tetracosahexaenoic acid. *J. Lipid Res.*, 36: 2433-2443.

Mozaffarian, D., Ascherio, A., Hu, F., Stampfer, M.J., Willett, W.C., Siscovick, D.S. & Rimm, E.B. 2005. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in men. *Circulation*, 111: 157-164.

Nestel, P.J., Chronopulos, A. & Cehun, M. 2005. Dairy fat in cheese raises LDL cholesterol less than that in butter in mildly hypercholesterolaemic subjects. *EJCN*, 59: 1059-1063.

Nettleton, J.A. 1991. Omega-3 fatty acids: comparison of plant and seafood sources in human nutrition. *J. Am. Diet.* Assoc., 91: 331-337.

Nichols, B.W. 1963. Separation of the lipids of photosynthetic tissues: improvements in analysis by thin-layer chromatography. *Biochim. Biophys. Acta*, 70: 417-422.

Pawlosky, R.J., Hibbein. J.R., Novotny, J.A. & Salem Jr., N. 2001. Physiological compartmental analysis of α-linolenic acid metabolism in adult humans. *J. Lipid Res.*, 42: 1257-1265.

Pawlosky R.J. & Salem Jr., N. 2004. Perspectives on alcohol consumption: liver polyunsaturated fatty acids and essential fatty acid metabolism. *Alcohol*, 34: 27-33.

Ratnayake, W.M.N. 2004. Overview of methods for the determination of trans fatty acids by gas chromatography, silver-ion thin-layer chromatography, silver-ion liquid chromatography, and gas chromatography/mass spectrometry. *J. AOAC Int., 87: 523-529.*

Ratnayake, W.M.N., Hansen S.L. & Kennedy, M.P. 2006. Evaluation of the CP-Sil 88 and SP-2560 GC columns used in the recently approved AOCS Official Method Ce 1h-05: Determination of *cis*-, *trans*-, saturated, monounsaturated and polyunsaturated

fatty acids in vegetable or non-ruminant animal oils and fats by capillary GLC method. *J. Am. Oil Chem. Soc.*, 83: 475-488.

Reseland, J.E., Anderssen, S.A., Solvoll, K., Hjerman, J., Urdal, P., Holme, I. & Drevon, C.A. 2001. Effect of long term changes in diet and exer*cis*e on plasma leptin concentrations. *Am. J. Clin. Nutr.*, 73: 240-245.

Sampath, H. & Ntambi, J.M. 2005. Polyunsaturated fatty acid regulation of genes of lipid metabolism. *Ann. Rev. Nutr.*, 25: 317-340.

Santos, M.T., Valles, J., Aznar, J. et al. 1984. Effect of smoking on plasma fatty acid composition in middle-aged men. *Atherosclerosis*, 50: 53-62.

Samuelsson, B., Dahlen, S.E., Lindgren, J.A., Rouzer, C.A. & Serhan, C.N. 1987. Leukotrienes and lipoxins: structures, biosynthesis, and biological effects. *Science*, 237: 1171-1176.

Serhan, C.N., Clish, C.B., Brannon, J., Colgan, S.P., Chiang, N. & Kronert, K. 2000. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. *J. Exp. Med.*, 192: 1197-1204.

Serhan, C.N., Hong, S., Gronert, K., Colgan, S.P., Devch&, P.R., Mirick, G. & Moussignac, R.L. 2002. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J. Exp. Med.*, 196: 1025-1037.

Simon, J.A., Fong, J., Bernert, J.T. & Browner, W.S. 1996. Relation of smoking and alcohol consumption to serum fatty acids. *Am. J. Epidemiol.*, 144: 325-334.

Smith, W.L., Marnett, L.J. & DeWitt, D.L. 1991. Prostaglandin and thromboxanes biosynthesis. *Pharmacol. Ther.*, 49: 153-179.

Spiteller, G. 2005. Furan fatty acids: Occurrence, Synthesis, and Reactions. Are furan fatty acids responsible for the cardiopotective effects of a fish diet? *Lipids*, 40: 753-771.

Spener, F., Lagarde, M. & Géloën, A. 2003. What is lipidomics? *Eur. J. Lipid Sci.* Technol., 105: 481-482.

Sprecher, H. 2002. The roles of anabolic and catabolic reactions in the synthesis and recycling of polyunsaturated fatty acids. *Prostaglandins Leukot. Essent. Fatty Acids*, 67: 79-81.

Strawford, A., Antelo, F., Christiansen, M. & Hellerstein, M.K. 2004. Adipose tissue triglyceride turn over, *de novo* lipogenesis, and cell proliferation in humans measured with 2H2O. *Am. J. Physiol. Endocrinol. Metab.*, 286: E577-E588.

Thomas, L.H., Olpin, S.O., Scott, R.G. & Wilkins, M.P. 1987. Coronary heart disease and the composition of adipose tissue taken at biopsy. *Human Nutr.: Food Sci. Nutr.*, 41F: 167-172.

Tricon, S., Burge, G.C., Williams, C.M. & Calder, P.C. 2005. The effects of conjugated linoleic acid on human health-related outcomes. *Proc. Nutr. Soc.*, 64: 171-82.

Tsuzuki, T., Tokuyama, Y., Igarashi, M. & Miyazawa, T. 2004. Tumor growth suppression by α-eleostearic acid, a linolenic acid isomer with a conjugated triene system, via lipid peroxidation. *Carcinogenesis*, 25: 1417-1425.

Vemuri, M. & Kelley, D.S. 2008. The effects of dietary fatty acids on lipid metabolism. In Chow, C.K., ed. *Fatty Acids in Foods and their Health Implications*, pp. 591-630. CRC Press, London, UK.

Visioli, F., Risé, P., Barassi, M.C., Marangoni, F. & Galli, C. 2003. Dietary intake of fish vs. formulations leads to higher plasma concentrations of n-3 fatty acids. *Lipids*, 38: 415-418.

White, P.J. 2008. Fatty acids in oilseeds (vegetable oils). *In* Chow, K.C. ed. *Fatty Acids in Foods and their Health Implications*, pp. 227-262. CRC Press, New York, NY.

Wolf, C. & Quinn, P.J. 2008. Lipidomics: practical aspects and applications. *Prog. Lipid Res.*, 47: 15-36.

Wood, J.D., Enser, M., Richardson, R.I. & Whittington, F.M. 2008. Fatty acids in meat and meat products. In Chow, C.K., ed. *Fatty Acids in Foods and their Health Implications* pp. 87-107. CRC Press, London, UK.

Woods, J., Ward, G. & Salem Jr., N. 1996. Is docosahexaenoic acid necessary in infant formula? Evaluation of high linolenate diets in the neonatal rat. *Pediatr.* Res., 40: 687-694.

Chapter 4: Choice of DRI, criteria and types of evidence

Different types of criteria (outcomes), evidence (study designs) and dietary reference intakes (DRI) have been used to set fatty acid dietary guidelines, without consistency either within or between guideline reports (Table 4.1). The types of criteria and types of evidence used to set specific guidelines could not always be clearly discerned from the reports.

This chapter reviews the potential types of criteria, study designs that are relevant in selecting the best approach in setting DRI in order to provide guidance for fatty acid intake, including the strengths and limitations of each, to provide best appropriate evidence for setting dietary guidelines. It provides examples from newer dietary guidelines for fats and fatty acids established by other national and international organizations since 1994, to illustrate how such evidence has been used by others in the past.

Reports from Australia and New Zealand (National Health and Medical Research Council, 2003), China (Chinese Nutrition Society, 2008), Europe (Eurodiet Project, 2000), Germany, Austria and Switzerland (DACH, 2000), India (Indian Council of Medical Research, 1998), the International Society for the Study of Fatty Acids and Lipids (ISSFAL 2004), The Netherlands (Health Council of the Netherlands, 2001), and the United States (IOM, 2005) have been included in this report.

CHOICE OF DRI

Historically, nutrient reference values – more recently termed DRI – were developed to address acute or sub acute clinical deficiencies of vitamins, minerals, protein, and energy (calories). More recently, the use of DRI has been expanded to include other substances in foods, such as fats and fatty acids, and to address chronic diseases. Several different types of DRI exist (Table 4.2).

When applied to fatty acids, DRI have been used inconsistently among different countries and institutions. For example, in the US and Canadian guidelines, acceptable macronutrient distribution ranges (AMDR) refer to appropriate ranges of usual intakes of individuals, while in the 1993 expert consultation (FAO, 1994) an AMDR refers to a population mean intake goal (King *et al.*, 2007). In this review, the inconsistent use of DRI is obvious from the data presented in the Table 4.2. For instance, the IOM (2005) reported an AMDR for total fat in adults, but adequate intakes (AI) for total fat intake among infants; Eurodiet (2000) used population goals to set DRI; and the 1993 expert consultation used only a tolerable upper intake level (UL) for total fat intake (FAO, 1994).

Similarly, for linoleic acid (LA), several reports used AI, based on prevention of deficiency, or even UL. Use of AI or UL for LA would prevent higher intakes that may decrease the risk of chronic disease. For LA, as well as total fat, AMDR might be more appropriate. Thus, whereas DRI were developed with a focus on preventing deficiencies, their application to many fatty acid recommendations must be considered in the context of reducing risk of chronic disease, which may not be adequately captured in DRI. As discussed in using

TABLE 4.1

Summarized overview of stated criteria and evidence used to determine dietary guidelines for fatty acids

(Based on adverse effects on outcome, unless otherwise noted)

	Disease outcome (D)	Physiological measure (P)	Average intake
Total fat	CVD, RCT[5,6,]*, CO [6]*, EC[1]	LDL-C, EC[1], RCT[7] HDL-C, RCT[1]	Average intake from human milk (infants) [1,4,7] Gradual decrease of fat intake compared with human milk (infants 6–12 months)[4]
	Increased obesity, RCT[7,]**, A[1], EC[3]?[5]	Triglycerides NS[4]	
	No effect on obesity CS[7]	Dyslipoproteinaemia and atherosclerosis E?[5], O?[5] Postprandial lipids and blood coagulation factor VII concentration (used to set upper limit)*, RCT[4]	
	Colon cancer EC[1], CC[1], A[1], conference review[5], (not according to CO[1]) Colon cancer C[1], CC[1], A[1], conference review[5], (not according to CO[1]) Breast cancer EC[1] Prostate cancer CC[1] Diabetes O?[6], E?[6] *Favourable effects on*: Stroke CO[1], A[1], EC[1]	*Favourable effects on:* Triglycerides and HDL-C (used to set lower limit)*, RCT[4] Responses in postprandial glucose and insulin concentrations RCT[6], CO [6]	
Saturated fat	CVD, CO[7]	Serum total cholesterol, LDL-C, RCT[1,6,7], CO[6,7], EC[1], Serum triglycerides NS[5] Ratio total cholesterol: HDL-C, RCT[4]	Upper 10th percentile of national intake[4] Average intake from human milk (infants)[4]
Monounsaturated fatty acids	-	*Favourable effects on:* Serum total cholesterol RCT[1]	-
Polyunsaturated fatty acids	Cancer CO[4]	Favourable effects on: Serum total cholesterol RCT[1]	-
	Favourable effects on: CHD CO[4]	*Equilibrium maintenance (E):* Deposition of essential fatty acids in growing tissue of pregnant women[1] Composition of milk in omnivorous lactating women[1]	
Alpha-linolenic acid	*Favourable effects on:* CVD RCT[3,4], A[3], CO [4,9], CC[9], EC[3], CS[9]	*Favourable effects on:* Serum total cholesterol RCT[1,]?[7]	Median national intake[6,7,]***
	Myocardial Infarction RCT[1], EC[1], CS[1]	Platelet aggregation, adhesion of monocytes to vessel walls, vascular dilatation, blood pressure, inflammatory processes and immune reactions RCT[5]	
	Cardiac arrhythmias A[1]	Leukocyte function NS[5]	
	Colon Cancer NS[5]	Neural integrity, (infants) A[1]	
	Deficiency symptoms O?[4], A[4]	N-3 deficiency in pre and post natal nutrition of infants affects: Neural integrity, learning and visual abilities and depressed development of retinal function and visual acuity A[1]	

TABLE 4.1 (continued)

Summarized overview of stated criteria and evidence used to determine dietary guidelines for fatty acids

(Based on adverse effects on outcome, unless otherwise noted)

EPA/DHA	*Favourable effects on:* CHD, EC[1], CO [4,7] and [3,5] (fatal) RCT[4,7] Fatal MI RCT[3,5]	Hemorrhage risk functions of leucocytes and the immune system RCT[5] *Favourable effects on:* Serum trigylcerides, VLDL-C O?[1] Platelet aggregation, adhesion of monocytes to vessel walls, vascular dilatation, blood pressure, inflammatory processes and immune reactions RCT[5] Rod photoreceptor, visual acuity, neural function (infants) RCT[1]	Median national Intake[7] Average intake from human milk (infants) [1,4]
Linoleic acid	*Favourable effects on:* CVD mortality RCT[7], CO[7] Deficiency disease NS[4]	*Favourable effects on:* Serum total cholesterol, LDL-C, HDL-C NS[5], RCT[3,1] Platelet aggregation, adhesion of monocytes to vessel walls, vascular dilatation, blood pressure, inflammatory processes and immune reactions RCT[5]	Median intake [6,7,***] Average intake from human milk (infants) [4,6]
Trans fatty acids	Fatal CHD, fatal and non-fatal MI CO[1,3,4], CC[1], CS[1]	Serum HDL-C and triglycerides RCT[1,3, 5], CO[5] Serum LDL-C RCT[6], CO[6]	Upper 10th percentile of national intake [4,****] Average *trans* fat intake from natural occurring *trans* fat[5]
Cholesterol	-	Serum total cholesterol RCT[5,1] ,NS[6]	-

Dietary Guidelines Report:

1 FAO/WHO (FAO, 1994)
2 India (India, 1998)
3 Eurodiet (Eurodiet, 2000)
4 The Netherlands (Netherlands, 2001)
5 DACH (DACH, 2000)
6 United States/Canada (IOM, 2005)
7 Australia /New Zealand (AU/NZ, 2003)
8 China (China, 2008)
9 ISSFAL (ISSFAL, 2004)

Study design of provided evidence:

NS = not specified
Experimental Studies: (E? = not further specified)
RCT: Randomized Controlled Clinical Trial
A: Animal Study
Observational Studies: (O? = not further specified)
CO: Cohort Study.
CC: Case–control Study.
EC: Ecologic Study.
CS: Cross-Sectional (Prevalence Study) Study.

Notes:

* The association between total fat and serum cholesterol and risk of CVD is noted because high fat intake is associated with saturated fat intake, not because of a direct effect of high fat diets on risk of CHD.

** The association between total fat intake and obesity is based on the high energy density of fat. Energy density contributes to a higher intake of energy (a risk factor for obesity) and fat content is closely linked to energy density in the Australian diet (AU/NZ, 2003). No evidence for a direct causal effect between fat intake as percent of energy and obesity is provided by the NHMRC (2003).

*** Based on the highest median intakes of the gender-related age group taken from an analysis of the National Nutrition Survey of Australia.

**** Based on the 10th percentile of intake of naturally-occurring *trans* fats that varies, dependent on the age group, between 0.7 and 1.0 percent of total energy.

TABLE 4.2
Types of dietary reference intakes (DRIs)

Dietary reference intakes (DRIs)	Definition and description	Historic use for fats and fatty acids
Estimated Average Requirement (EAR)	Intake that meets the nutrient needs of half of the healthy individuals in a life stage or gender group. Reflects the estimated average requirement and is particularly appropriate for applications related to planning and assessing intakes for groups of persons.	Not traditionally used for fats and fatty acids.
Recommended Dietary Allowance (RDA)	A value (EAR + 2 SD) that covers the estimated nutrient requirements of most healthy individuals (commonly 97.5%) in a population, based on an accepted criteria relevant to nutrition or health of a population.	Not traditionally used for fats and fatty acids.
Tolerable Upper Intake Level (UL)	Intake that is likely to have no adverse effects on health or nutrition. In the absence of evidence of adverse effects it is commonly set at 10 × EAR.	Has been used for total fat, saturated fat, total polyunsaturated fat, ALA, EPA+DHA, and dietary cholesterol.
Adequate Intake (AI)	An intake range based on observed or experimentally determined estimates of nutrient intake by groups of people who are apparently healthy and considered to maintain an adequate nutritional state. Used when an EAR cannot be estimated.	Has been used for total fat, linoleic acid, a-linolenic acid, EPA and DHA.
Acceptable Macronutrient Distribution Range (AMDR)	An intake range for an energy source associated with reduced risk of chronic disease.	Has been used for total fat, linoleic acid, and a-linolenic acid.
Lower value of Acceptable Macronutrient Distribution Range (L-AMDR)	The lower portion of an intake range for an energy source associated with reduced risk of chronic disease.	Has been used for total fat, total PUFA and 3-n PUFA.
Upper value of Acceptable Macronutrient Distribution Range (U-AMDR)	The upper portion of an intake range for an energy source associated with reduced risk of chronic disease.	Has been used for total fat, SFA, total PUFA, and total LCPUFA.

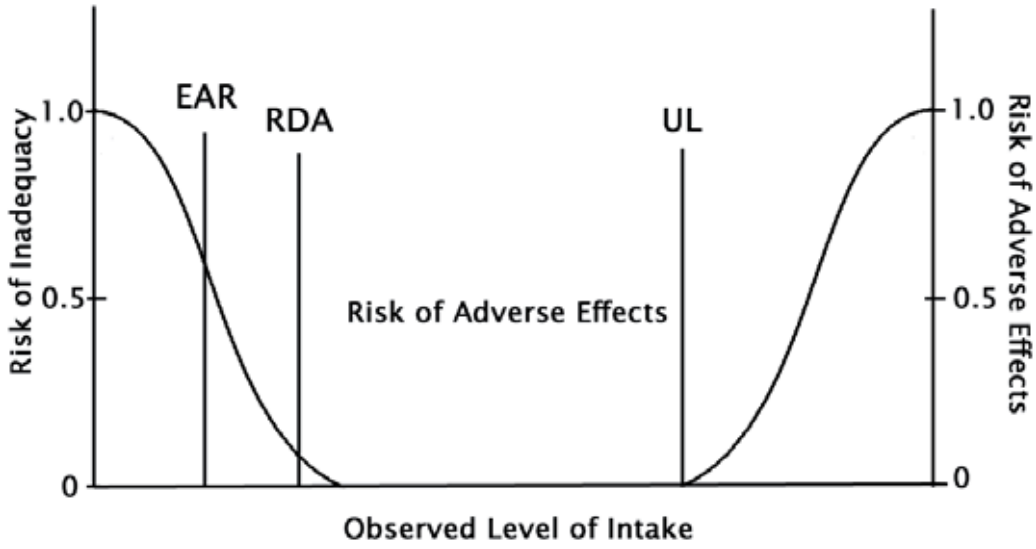
criteria to establish nutrient intake values (NIV), it is also possible to have multiple average nutrient requirements corresponding to different criteria, and have public health policy planners determine which value is appropriate for the population of interest (Yates, 2007).

For some fatty acids, such as *trans* fatty acids, there is no known value of inadequacy and even small incremental intakes of these fatty acids are associated with risk of chronic disease. Therefore traditional DRIs such as estimated average requirements (EAR) and recommended dietary allowances (RDA) are not adequately relevant to the health effects of these fatty acids. As shown in Figure 4.1, the EAR and RDA are based on a U-shaped association between intake of the nutrient and adverse effects (either inadequacy or other adverse effects). Therefore, for fatty acids, for which there is no risk of inadequacy, neither EAR nor RDA are appropriate. UL may also be problematic. For example, in the IOM report on DRI: "A UL is not set for *trans* fatty acids because any incremental increase in trans fatty acid intake increases CHD risk" (IOM, 2005). The UL is defined by a level at which intake does not pose a risk, which is not the case for *trans* fatty acids.

OVERVIEW OF PRIOR CRITERIA AND TYPES OF EVIDENCE

The choice of the criterion or functional outcome (indicator of adequacy) that is used to determine the recommend intake for fat and fatty acids is crucial. Depending on

FIGURE 4.1
Dietary reference intake distribution



Source: IOM, 2005

the criterion, recommended levels of intake may differ. For example, the level of n-3 intake necessary for prevention of deficiencies is lower than the level of intake that minimizes the risk of CHD. Table 4.1 summarizes the stated criteria provided by the dietary guideline reports and includes the types of evidence (study designs) stated to be used to estimate associations between intake of the fat or fatty acid and the indicator of adequacy (criterion).

CHOICE OF CRITERIA

Potential general criteria to define dietary requirements include the following aims:

- To prevent clinical deficiencies;
- To provide optimal health;
- To reduce the risk of developing chronic disease.

The most appropriate and practical criteria for setting most worldwide fatty acid recommendations should be to optimize health and reduce the development of common chronic diseases; such criteria would also in nearly all cases prevent clinical deficiencies. Specific chronic diseases of interest should be identified based on burdens of morbidity and early mortality in the population and on meaningful effects of dietary fatty acids on their development. In order to remain transparent about the development of dietary requirements, care should be taken to be explicit about the types of criteria used to set each dietary recommendation.

Chronic disease outcomes

Examples of chronic disease outcomes used as criterion for dietary recommendations for fatty acids include CHD, obesity, diabetes, and specific types of cancers (Table 4.1). The primary strength of using disease outcome as an indicator of adequacy or optimal intake is that it represents the most direct method to assess effects on health.

A drawback of using disease outcome is the absence of such data for many fatty acids, specific disease endpoints, and/or populations. However, given that many such studies are available, the more relevant drawback is often the failure to consider the different strengths and limitations of different study designs and specific studies when drawing inferences from the findings.

Examples of using disease outcome as criteria

Obesity. To assess the effects of total fat intake on obesity, reports have used evidence from animal, ecological, and cross-sectional studies, and short-term RCT on weight loss. As described in section "choosing the type of evidence", the results of animal, ecological, and cross-sectional studies should be considered hypothesis-generating and are not considered reliable or sufficient evidence for setting dietary guidelines. Observational studies of diet and body weight also have particular limitations relating to underreporting of calories or recall bias. Reverse causation is also highly problematic: small changes in body weight (or perceived body shape) can readily change individuals' diets and introduce bias in diet-weight associations. RCT are therefore superior for assessing diet-obesity effects. However, many RCT have been short-term and may not reflect long-term effects of the diet on weight.

Cardiovascular disease (CVD). To assess the effects of total fat intake on CVD, reports have used evidence from animal experiments and from retrospective case-control, ecological, and cross-sectional designs. As described previously, such designs are generally insufficient to set dietary guidelines. For example, in many rodent studies, a high-fat rat chow is compared to standard chow, but total energy is not controlled, biasing the association between total fat as percentage of energy and the outcomes. Additionally, to obtain CVD-susceptibility, animal experiments often use specific gene-knockout models, which (in addition to other species-specific differences) may greatly reduce relevance to humans.

Physiological measures

Examples of physiological measures used as criterion to set dietary recommendations for dietary fatty acids are serum cholesterol levels, triglyceride levels, and neural integrity. The strength of using physiological measures as indicators of adequacy is that they are quantifiable measures that can estimate disease risk before the occurrence of clinical disease and can often be assessed directly in controlled trials. The major drawback is that physiological measures are indirect measures of actual disease outcome: They reflect only certain pathways of risk and may not be valid surrogates for total effects of the dietary intervention on health, which might also be mediated by multiple other pathways.

Because physiological measures can be assessed relatively easily in controlled trials, most of the physiological criteria are based on evidence from RCT (Table 4.1). Although RCT allow direct control of diet and minimize confounding, often participants are relatively healthy and evaluated over relatively short-term time periods, limiting potential generalizability (see 'chronic disease outcomes').

Examples of using physiological measures as criteria

Among the most commonly used physiological criteria for setting dietary fatty acid guidelines are the effects of saturated fat intake on LDL-C (Table 4.1). RCT in humans have consistently demonstrated that saturated fat consumption raises LDL-C, and higher levels of LDL-C are a well-established risk factor for CHD. This evidence provides an excellent illustration of the strengths and limitations of using a physiological criterion. The strength is that the quantitative effects of saturated fat on LDL-C can be definitively established. However, potential limitations of this criterion include: (a) the lack of confirmation in RCT that diet-induced changes in LDL-C alter CVD event rates;

(b) the lack of consideration of effects of saturated fat on other pathways of risk, such as HDL-C, triglycerides, or other non-lipid risk factors; and (c) the possible qualitative or even quantitative differences in effects of saturated fat on some physiological risk factors (e.g. the total cholesterol:HDL-C ratio) in populations other than those tested in RCT, which have generally enrolled younger healthier men (rather than older adults or postmenopausal women who are at highest risk). For example, whereas saturated fat intake (compared with carbohydrate) raises LDL-C, it also raises HDL-C so the net effect on the total cholesterol:HDL-C ratio is neutral (or even unfavourable in postmenopausal women).

Deficiency symptoms and disease

Deficiency symptoms are most often studied in case series/reports, animal experiments, or short-term controlled feeding studies. A strength of using deficiency symptoms as a criterion is that deficiency symptoms for essential fatty acids can be clearly defined and studied in relatively small controlled trials. Drawbacks include strong ethical limitations of testing many deficiencies, which may have unacceptable long-term effects in humans. For this reason, there are few data for most nutrients concerning the level of intake at which symptoms occur. As discussed previously, an additional major drawback is the sometimes large difference between levels at which clinical deficiency occur versus levels that cause lowest risk of chronic diseases, such as cancer or CVD.

For instance, deficiency symptoms have been used as criteria for recommendations for essential polyunsaturated fatty acids such as LA and ALA. However, intakes that prevent deficiency symptoms or diseases do not appear to be optimal for preventing incidence of other chronic diseases. Specifically, levels of intakes that reduce chronic diseases (e.g. LA and ALA intakes to decrease risk of CVD) are much higher than those needed to prevent clinical deficiencies. Thus, using deficiency symptoms as the criterion will result in underestimates of recommended intake. In comparison, using disease outcome as the criterion to set dietary guidelines for essential fatty acids will inherently prevent clinical deficiency.

Average intakes in national survey studies

For some fatty acids and age groups, insufficient data are available to use disease or physiological criteria for setting dietary guidelines. In these cases, average national intakes have been used as a criterion when deficiencies are not present in the population. Guidelines can be set based on average intakes (e.g. median national intakes) or relative extremes of intake (e.g. the upper 10th percentile of national intake).

The strengths of this approach are that mean national intakes are relatively practical and easy to measure, and that recommendations based on average national intakes are unlikely to have large unexpected adverse consequences, given that much of the population is already at these levels. A major drawback is that such intakes may not be optimal for reducing disease risk, even though overt deficiencies are not present. For example, in populations without overt clinical deficiencies of n–3 fatty acids, higher intakes may nevertheless substantially reduce the risk of fatal CHD and sudden cardiac death. Such guidelines, based on average intakes in one population, may also be less appropriate for other populations or age groups.

Examples of using average national intake as criteria

The Health Council of the Netherlands (2001) used the upper 10th percentile of national intake of natural trans fatty acids (i.e. trans fatty acid intake from natural sources such as meats and dairy products) to determine the recommended upper intake level of total trans fat intake, which varies between 0.7% and 1.0% of total energy.

Because the intake of dairy products in the Netherlands is very high, this upper level of intake may be less appropriate for Asian countries, in which the intake of dairy products is much lower. Specifically, the goal of the Dutch guidelines was to limit intake of partially hydrogenated oils, but not natural *trans* fatty acids. If applied to an Asian country, this intake level could result in a much higher level of intake of *trans* fatty acids from partially hydrogenated vegetable oil than desirable.

Equilibrium maintenance

Equilibrium maintenance describes the balance of nutrient intake and loss, as measured by factorial estimation. The factorial method involves estimating the factors that determine the requirement, such as increased requirements for growth, pregnancy, and lactation, or losses via urine or feces. Examples of equilibrium maintenance used as criterion to set dietary recommendations are estimations in tissue deposition and milk secretion of LA and ALA during pregnancy and lactation (factorial methods).

A strength of using equilibrium maintenance as an indicator of adequacy is that the factorial method measures the actual losses of a fatty acid and estimates the required intake when other data are not available. The drawback of using these measures is that individual losses of fatty acids may vary to a great extent and estimations may not apply to all individuals. The intake required to maintain equilibrium depends on the level at which equilibrium is maintained, and thus the existing level of an individual or population may not be optimal. Importantly, such criteria may also have little relevance to the incidence of disease, the main endpoint of interest.

Animal models

In the international reports discussed, animal models of inadequacy were not used as primary criteria to set dietary recommendations for fats and fatty acids. However, animal experiments that evaluated disease outcome or physiological measures have been used as supporting evidence for recommendations. Animal studies are powerful for doing basic research and generating hypotheses, but the major limitations in generalizability to humans makes such evidence insufficient to set dietary recommendations.

CHOOSING THE TYPE OF EVIDENCE

Types of evidence generally used to establish dietary requirements have included the following:

- Animal studies;
- Ecological studies, prevalence studies;
- Retrospective case-control studies of disease outcomes;
- RCT of physiological measures;
- Prospective cohort studies of disease outcomes;
- RCT of disease outcomes.

Compared to information available about criteria and DRI, often discussion of the choice of evidence is missing in reports addressing dietary guidelines. Both the WHO/FAO and NHMRC have issued useful rankings of the criteria describing the strength of evidence (Tables 4.3 and 4.4). However, these rankings of evidence do not provide clear guidance for setting dietary recommendations, but rather discuss the general strength of evidence.

In this report, we propose a ranking system for evidence from studies that can be considered as a guideline to determine whether current data are sufficient to evaluate human requirements and set dietary recommendations (Figure 4.2), assuming of course that the study is well-conducted.

FIGURE 4.2
Ranking of validity of types of evidence for setting dietary fatty acid requirements

(Validity of type of study decreases from left to right)

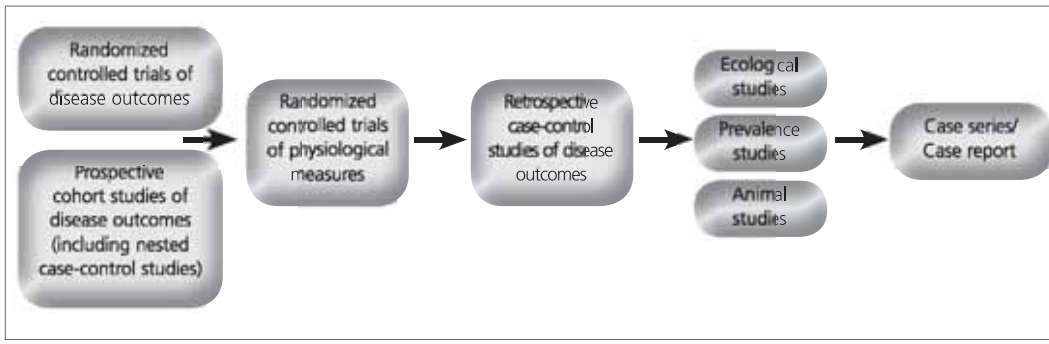


TABLE 4.3
WHO/FAO criteria used to describe the strength of evidence relating diet and NCD outcomes

Convincing evidence	Evidence is based on epidemiological studies showing consistent associations between exposure and disease, with little or no evidence to the contrary. The available evidence is based on a substantial number of studies including prospective observational studies and where relevant, randomized controlled trials of sufficient size, duration and quality showing consistent effects. The association should be biologically plausible.
Probable evidence	Evidence is base on epidemiological studies showing fairly consistent associations between exposure and disease, but where there are perceived shortcomings in the available evidence or some evidence to the contrary, precluding a more definite judgment. Shortcomings in the evidence may be any of the following: insufficient duration of trials (or studies); insufficient trials (or studies) available; inadequate sample sizes; and incomplete follow-up. Laboratory evidence is usually supportive. Again, the association should be biologically plausible.
Possible evidence	Evidence is based mainly on the findings from case-control and cross-sectional studies. Insufficient randomized controlled trials, observational studies or nonrandomized controlled trials are available. Evidence based on no epidemiological studies, such as clinical and laboratory investigations, is supportive. More trials are required to support the tentative associations, which should also be biologically plausible.
Insufficient evidence	Evidence is based on the findings of a few studies which are suggestive, but are insufficient to establish an association between exposure and disease. Limited or no evidence is available from randomized controlled trials. More well-designed research is required to support the tentative associations.

Source: WHO, 2003.

Optimally, evidence for setting dietary fatty acid requirements would be derived from concordant evidence from well-conducted RCT of incidence of disease outcomes, prospective cohort studies of incidence of disease outcomes (including nested case-control studies), and RCT of physiological measures, supported by findings from retrospective case-control studies, ecological studies, and animal experiments. For many fats and fatty acids, well-conducted RCT of disease outcomes with adequate power (sufficient number of subjects) are not available, especially for chronic diseases. When such evidence is not available, concordant evidence from well-conducted prospective cohort studies of disease outcomes and RCT of physiological measures are often sufficient to set dietary recommendations. Evidence from only RCT of physiological measures without additional concordant evidence from controlled trials or prospective cohort studies of disease outcomes, or from only retrospective case-control studies, ecological or cross-sectional studies, or animal experiments may be insufficient to set dietary recommendations, especially for chronic diseases. When evaluating studies as evidence for setting dietary recommendations, these strengths and limitations of each study design should be critically evaluated. The major strength of properly executed RCT is the minimization of confounding, but many other study design limitations can be present and limit the utility of the results.

Prospective cohort studies have many strengths, but the major potential limitation is the inability to definitively exclude residual confounding. A review of these strengths and limitations demonstrates the strong complementary nature of the strengths and limitations of RCT versus prospective cohorts. When RCT of disease outcomes are not available, RCT of physiological measures (intermediate endpoints or risk factors for disease) can provide concordant evidence for the effects on disease risk.

Retrospective case-control studies are efficient for evaluating rare diseases, but the limitations of recall bias, selection bias, and inability to include fatal cases render them suboptimal for studying other disease endpoints. Because dietary guidelines for the population should not be determined based on rare diseases, retrospective case-control studies are useful for generating hypotheses, but are usually insufficient for setting dietary guidelines.

Ecological, cross-sectional, or prevalence studies are very useful in providing an initial hypothesis that can be further tested in prospective cohort studies and clinical trials, but design limitations for assessing causality are too strong for such data to be sufficient for determining dietary recommendations.

Animal experiments are powerful study designs for evaluating mechanisms, assessing pathways, and providing concordant evidence to findings of human studies, but by themselves are insufficient to set dietary recommendations for fats and fatty acids in humans.

Case series or reports describe the manifestation, the course, or the prognosis of a condition. Due to lack of comparability, this type of evidence is generally insufficient for setting dietary recommendations, except perhaps for deficiency symptoms that are manifested in specific populations or during historical incidents.

This approach of ranking the validity of study designs based on the strengths and limitations of each study design allows for clear and explicit criteria, but does not take into account whether the data are available for each fatty acid and disease outcome. For some associations, availability of data or studies may be less than optimal, for example between nutrition and cancer. In such cases, dietary fatty acid requirements can be considered, but will require careful consideration of available data and, most importantly, transparency regarding the approach and the strength of evidence used to set the dietary requirements.

During the preparatory process for the Expert Consultation the participants agreed on the criteria that would be used to judge the levels and strength of evidence required to conclude that total fat and fatty acids affect major health and disease outcomes and to draw transparent conclusions from the scientific review of the totality of the evidence, including both RCT in humans and observational studies involving long-term follow-up of cohorts and experimental animal and laboratory studies when no other data were available. In doing so the participants recognized that ranking evidence does not provide clear guidance for setting dietary guidelines, but rather represents a gauge and ranking of the general strength of evidence.

It was decided to follow the criteria employed in the report *Diet, Nutrition, and the Prevention of Chronic Diseases; Report of a Joint WHO/FAO Expert Consultation* (WHO, 2003) and subsequent FAO and WHO scientific reviews and studies, which had based the criteria on a modified version of that used by the World Cancer Research Fund (Table 4.3). In doing so the experts acknowledged other equally valid criteria that exist, one being the NHMRC (Table 4.4).

Four levels of judgment were identified:

- Convincing
- Probable
- Possible
- Insufficient

TABLE 4.4
National Health and Medical Research Council levels of evidence

I	Evidence obtained from a systematic review of all relevant randomized controlled trials
II	Evidence obtained from at least one properly designed randomized controlled trial
III-1	Evidence obtained from well-designed pseudo-randomized controlled trials (alternate allocation or some other method)
III-2	Evidence obtained from comparative studies (including systematic reviews of such studies) with concurrent controls and allocation not randomized, cohort studies, case-control studies, or interrupted time series with a control group
III-3	Evidence obtained from comparative studies with historical control, two or more single-arm studies, or interrupted time series without a parallel control group
IV	Evidence obtained from case series, either post-test or pre-test/post-test

Source: NHMRC, 1999

Given the limited number of randomized controlled trials on dietary fat and chronic disease or death it was agreed that only evidence of sufficient strength to be "convincing" or "probable" would allow a dietary recommendation to be formulated.

The framework for DRI development background paper by Taylor suggests that even in the face of limited data, scientific judgment can be important (IOM, 2008). It advocates that science-based judgment is more useful than no recommendation at all. In that light, it might be useful to look at data not meeting the suggested optimal criteria for setting dietary fatty acid requirements (described previously), e.g. when RCT and prospective cohort studies of incidence of disease outcomes are not possible or available. In some limited cases, scientific judgment may be necessary to offer a reference value when only limited data are available (e.g. only ecological and animal studies), but action is necessary and there is insufficient time to wait for more data. In these cases, a "portfolio" or "mosaic" approach - in which all available types of studies, the biological plausibility, and data consistency (taking weight of study design into account) are considered - may be a useful approach when the linear approach based solely on study design described previously is not suitable. On the other hand, it must be remembered that reliance on scientific judgment, in the absence of optimal data, can lead to subjective and erroneous conclusions that can result in unhelpful or even harmful health consequences.

In order to remain transparent about the development of dietary requirements, the type of evidence selected to base the recommendation should be specified and ranked, particularly if according to higher ranking evidence it may be considered suboptimal.

REFERENCES

Chinese Nutrition Society. 2008. *Dietary Guidelines for the Chinese Population*. People's Publishing House. China, Tibet.

DACH. 2008. Deutsche Gesellschaft für Ernährung. *Die Referenzwerte für die Nährstoffzufuhr*. Neustadt an der Weinstarsse, Umschau.

Eurodiet. 2000. *Eurodiet core report. European dietary guidelines*. /eurodiet.med.uoc.gr/eurodietcorereport.pdf (accessed 1 October 2008).

FAO. 1994. *Fats and oils in human nutrition; Report of a joint FAO/WHO Expert Consultation*. FAO Food and Nutrition Paper 57, FAO, Rome.

Health Council of the Netherlands. 2001. *Dietary Reference Intakes: energy, proteins, fats and digestible carbohydrates*. The Hague.

Indian Council of Medical Research. 1998. *Dietary Guidelines for Indians – A Manual*. ICMR, New Delhi.

Institute of Medicine. 2005. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. The National Academies Press, Washington DC.

International Society for the Study of Fatty Acids and Lipids (ISSFAL). 2004. *Recommendations for Dietary Intake of Polyunsaturated Fatty Acids in Healthy Adults*. ISSFAL.

King, J., Vorster, H. & Tome, D. 2007. Nutrient intake values (NIVs): A recommended terminology and framework for the derivation of values. *Food Nutr. Bull.*, 28 (1 Suppl.): S16-26.

National Health and Medical Research Council (Dept. of Health and Ageing). 2006. *Nutrient Reference Values for Australia and New Zealand.* NHMRC, Canberra.

National Health and Medical Research Council. 2003. *Dietary Guidelines for Australian Adults*. NHMRC, Canberra.

National Health and Medical Research Council: 1999. *A Guide to the development evaluation and implementation of clinical practice guidelines.* NHMRC, Canberra..

Taylor, C. 2008. *Framework for DRI Development,* Institute of Medicine, Washington DC. www.iom.edu/Object.File/Master/54/358/DRI%20Framework.pdf.

WHO. 2003. *Diet, Nutrition and the Prevention of Chronic Diseases*. Report of the Joint WHO/FAO Expert Consultation on Diet Nutrition and the Prevention of Chronic Diseases. Technical Report Series 916: WHO, Geneva.

Yates, A.A. 2007. Using criteria to establish nutrient intake values (NIVs). *Food Nutr. Bull.*, 28(1 Suppl. International): S38-50.

Chapter 5: Fat and fatty acid requirements for adults

FAT AND FATTY ACID REQUIREMENTS FOR ADULTS

Fats enhance the taste and acceptability of foods; lipid components largely determine the texture, flavour and aroma of foods. In addition, fats slow gastric emptying and intestinal motility, thereby prolonging satiety. Dietary fats provide essential fatty acids (EFA) and facilitate the absorption of lipid-soluble vitamins. The Expert Consultation agreed that there was convincing evidence that energy balance and dietary patterns are critical to maintaining healthy body weight and ensuring optimal nutrient intakes, regardless of macronutrient distribution expressed in energy percentage (Elmadfa and Kornsteiner, 2009). The requirements for total fat and different fatty acid groups, as well as the evidence levels, are summarized in Table 5.1.

DIETARY RECOMMENDATIONS FOR TOTAL FAT INTAKE

The Expert Consultation considered that the acceptable macronutrient distribution range (AMDR) for total fat intake ranges between 20% and 35% of energy (E) (Elmadfa and Kornsteiner, 2009). Total fat intake should be greater than 15%E (lower value of acceptable macronutrient distribution range, L-AMDR) to ensure an adequate intake of essential fatty acids and energy and to facilitate the absorption of lipid soluble vitamins (Jequier, 1999). While for most individuals engaged in moderate physical activity 30%E is recommended, for those associated with a high physical activity level it can amount to 35%E. The upper value of acceptable macronutrient distribution range (U-AMDR 35%E) should consider energy balance and diet quality. However, high fat intakes are habitually accompanied by increased saturated fat, cholesterol and energy density (Eurodiet, 2008).

Moderate dietary fat intake, in addition to a diet rich in refined carbohydrates, can raise the risk of non-communicable diseases in a population with an habitually low fat ingestion (<20%E) (Bourne *et al*., 2002; Suh *et al*., 2001; Vorster *et al*., 2005). Therefore, an AMDR between 20% and 35% fat of total energy can only be considered under the condition that the energy balance is maintained and the anthropometrics are within the normal range, although more information is needed from populations in developing and transitional countries or in countries undergoing rapid food and nutrition transition. In severely malnourished populations, intake greater than 20% energy can help to increase the energy density and increase calories consumed, as well as maintain or improve the overall dietary pattern.

DIETARY RECOMMENDATIONS FOR SATURATED FATTY ACIDS (SFA)

Individual saturated fatty acids (SFA) have different effects on the concentration of plasma lipoprotein cholesterol fractions. For example, lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids increase LDL cholesterol whereas stearic (C18:0) has no effect.

TABLE 5.1
Recommended dietary intakes for total fat and fatty acid intake for adults

			Level of Evidence			
Fat/FA	Measure	Numeric amount	Convincing	Probable	Possible	Insufficient
Total fat	AMDR U-AMDR L-AMDR	20–35%E 35%E 15%E		No relation with CHD events, fatal CHD, total cancer, or cancer subtypes		Risk of diabetes, metabolic syndrome components, body weight/adiposity
SFA	U-AMDR	10%E	C12:0–16:0 ↑ LDL and total/HDL ratio in comparison to cis MUFA or PUFA; ↑ LDL but no effect on total/HDL in comparison to carbohydrate		↑ risk of diabetes	Risk of hypertension, body weight/adiposity
MUFA	AMDR	By difference [a], [b]	↓ LDL and total/HDL ratio when substituting SFA (C12:0–16:0)		↓ risk of metabolic syndrome components	Risk of diabetes, body weight/adiposity, CHD events, total cancer or cancer subtypes
Total PUFA	AMDR (LA + ALA + EPA + DHA) U-AMDR L-AMDR AI	6–11%E 11%E 6%E 2.5–3.5%E	See above, for exchange of SFA for PUFA Essential (LA, ALA) ↓ risk of CHD events when PUFA replace SFA		↓ risk of metabolic syndrome components, diabetes ↑ lipid peroxidation with high consumption, especially when tocopherol intake is low Specific minimum to prevent deficiency unclear	Risk of body weight/adiposity, total cancer or cancer subtypes
n-6 PUFA	AMDR (LA) EAR AI	2.5–9%E 2%E (SD of 0.5%) 2–3%E	See above, for exchange of SFA for PUFA Essential (LA)	↓ risk of metabolic syndrome components, diabetes	Specific minimum to prevent deficiency unclear	Risk of body weight/adiposity, total cancer or cancer subtypes
n-3 PUFA	AMDR (n-3[c]) L-AMDR (ALA) AMDR (EPA + DHA)	0.5–2%E > 0.5%E 0. 250–2* g/day	↓ risk of fatal CHD events (EPA+DHA) Essential (ALA)		↓ risk of total CHD events, stroke Specific minimum to prevent deficiency unclear	
TFA[d]	UL	<1%E	↓ HDL and ↑ total/HDL ratio in comparison to SFA (C12:0–C16:0), cis MUFA or PUFA ↑ risk of CHD events	↑ risk of fatal CHD and sudden cardiac death ↑ risk of metabolic syndrome components, diabetes		Risk of body weight/adiposity, diabetes, total cancer or cancer subtypes

[a] Total fat [%E] – SFA [%E] – PUFA [%E] – TFA [%E] [b] can be up to 15 – 20 %E, according to total fat intake [c] ALA + n-3 long-chain PUFA [d] total TFA from ruminant and industrially-produced sources

* for secondary prevention of CHD

(Explanations of the abbreviations are found in the list of acronyms and symbols)

There is convincing evidence that:

- Replacing SFA (C12:0–C16:0) with PUFA decreases LDL cholesterol concentration and the total/HDL cholesterol ratio. A similar but lesser effect is achieved by replacing these SFA with MUFA.
- Replacing dietary sources of SFA (C12:0–C16:0) with carbohydrates decreases both LDL-C and HDL-C concentration but does not change the total/HDL cholesterol ratio.
- Replacing SFA (C12:0–C16:0) with TFA decreases HDL cholesterol and increases the total /HDL cholesterol ratio.

Based on coronary heart disease (CHD) morbidity and mortality data from epidemiological studies and controlled clinical trials (using CHD events and death), it was also agreed that:

- There is convincing evidence that replacing SFA with PUFA decreases the risk of CHD.
- There is probable evidence that replacing SFA with largely refined carbohydrates has no benefit on CHD, and may even increase the risk of CHD and favour metabolic syndrome development. (Jakobsen *et al.*, 2009).

Reducing SFA by itself (reducing the amount of SFA or the % energy from SFA) has no effect on CHD and stroke (Siri-Tarino *et al.*, 2010). However, the methodology used by Siri-Tarino *et al*. in the pooling of these studies was questioned by Stamler (2010), who highlighted the important limitations of this pooled analysis. There is a possible positive relationship between SFA intake and increased risk of diabetes. There is insufficient evidence relating to the effect on the risk of CHD in replacing SFA with either MUFA or largely whole grain carbohydrates; however, based on indirect lines of evidence this could result in a reduced risk of CHD.

There is insufficient evidence that SFA affects the risk for alterations in indices related to the components of the metabolic syndrome. Based on cancer morbidity and mortality data, it was also agreed that there is insufficient evidence for establishing any relationship between SFA consumption and cancer. Therefore, it is recommended that SFA should be replaced with PUFA (n-3 and n-6) in the diet and the total intake of SFA should not exceed 10%E (Elmadfa and Kornsteiner, 2009).

CONCLUSIONS AND RECOMMENDED DIETARY REQUIREMENTS FOR MUFA

- There is convincing evidence that replacing carbohydrates with MUFA increases HDL cholesterol concentrations.
- There is convincing evidence that replacing SFA (C12:0–C16:0) with MUFA reduces LDL cholesterol concentration and total/HDL cholesterol ratio.
- There is possible evidence that replacing carbohydrates with MUFA improves insulin sensitivity.
- There is insufficient evidence for establishing a relationship between MUFA consumption and chronic disease end points such as CHD or cancer.
- There is insufficient evidence for establishing a relationship between MUFA consumption and body weight and percent adiposity.
- There is insufficient evidence for establishing a relationship between MUFA intake and risk of diabetes.

The determination of intake of MUFA is unique in that it is calculated by difference, i.e. MUFA=Total fat intake (%E) – SFA – PUFA – TFA . Therefore, the resulting MUFA intake may cover a wide range depending on the total fat intake and dietary fatty acid pattern (Elmadfa and Kornsteiner, 2009).

CONCLUSIONS AND RECOMMENDED DIETARY REQUIREMENTS FOR PUFA

- There is convincing evidence that LA and ALA are indispensable since they cannot be synthesized by humans.
- There is convincing evidence that replacing SFA with PUFA decreases the risk of CHD.
- There is convincing and sufficient evidence from experimental studies to set an acceptable intake to meet essential FA needs for LA and ALA consumption.
- There is possible evidence that PUFA affect the risk of alterations in indices related to the metabolic syndrome.
- There is possible evidence of a relationship between PUFA intake and reduced risk of diabetes.
- There is insufficient evidence for establishing any relationship between PUFA consumption and cancer.
- There is insufficient evidence for establishing relationships between PUFA consumption and body weight and percent adiposity.

The minimum intake levels for essential fatty acids to prevent deficiency symptoms are estimated at a convincing level to be 2.5%E LA plus 0.5%E ALA (DACH, 2000). Based on epidemiological studies and randomized controlled trials of CHD events, the minimum recommended level of total PUFA consumption for lowering LDL and total cholesterol concentrations, increasing HDL cholesterol concentrations and decreasing the risk of CHD events is 6%E. Based on experimental studies, risk of lipid peroxidation may increase with high (>11%E) PUFA consumption, particularly when tocopherol intake is low (Elmadfa and Schwalbe, 1989). This value is only slightly different from former recommendations (WHO, 2003). Therefore, the resulting acceptable range for total PUFA (n-6 and n-3 fatty acids) is between 6 and 11%E. The adequate intake to prevent deficiency is 2.5–3.5%E. Thus, the recommended range (AMDR) for PUFA is 6–11%E.

CONCLUSIONS AND RECOMMENDED DIETARY REQUIREMENTS FOR N-6 POLYUNSATURATED FATTY ACIDS

It is recognized that data for humans are sparse for establishing a precise quantitative estimate of the LA requirement to prevent deficiency; thus a range rather than an average LA requirement is recommended. Animal and human studies demonstrate that the prevention of deficiency signs (e.g. in rats reduced growth, scaliness of skin, necrotic tail) occurs when 1-2% of total energy is provided by LA (Anderson and Connor, 1989; Hansen *et al*., 1963; Holman, 1978, 1998; Mohrhauer and Holman, 1963; Strijbosch *et al*., 2008; Wollbeck *et al.*, 1981). Therefore, an estimated average requirement (EAR) for LA of 2%E and an adequate intake (AI) of 2–3%E are proposed (DACH, 2000). In accepting that the U-AMDR values of total PUFA and total n-3 fatty acids are 11%E and 2%E respectively, the resulting AMDR for n-6 fatty acids (LA) intake is 2.5–9%E. The lower value or AI (2.5–3.5%E for LA and ALA) corresponds to the prevention of deficiency symptoms, whereas the higher value represents part of a healthy diet contributing to long-term health by lowering LDL and total cholesterol levels and therefore lowering the risk for CHD (Elmadfa and Kornsteiner, 2009). For infants 6–12

months of age, an AI range of 3.0-4.5%E is recommended with a U-AMDR of <10%E. There is insufficient evidence for establishing any relationship between n-6 PUFA consumption and cancer. AA is not essential for a healthy person who gets enough LA (> 2.5%E) from the habitual diet, which can be well demonstrated in vegans who have negligible amounts of long-chain n-6 fatty acids in their diet (Kornsteiner *et al*., 2008). AA is not essential for a healthy adult whose habitual diet provides LA > 2.5%E. For infants 0-6 months of age AA should be supplied in the diet within the range of 0.2-0.3%E based on human milk composition as a criterion.

CONCLUSIONS AND RECOMMENDED DIETARY REQUIREMENTS FOR N-3 POLYUNSATURATED FATTY ACID INTAKE

The available evidence indicates that 0.5–0.6%E ALA per day corresponds with prevention of deficiency symptoms (Bjerve *et al*., 1989; DACH, 2000; Holman *et al*., 1982). The total n-3 fatty acid intake (ALA, EPA and DHA) can range between 0.5–2%E, whereas the minimum dietary requirement for ALA (>0.5%E) prevents deficiency symptoms in adults. The higher value of 2%E includes the recommendation for ALA and n-3 LCPUFA (AMDR for EPA and DHA 0.250 g–2.0 g) can be part of a healthy diet. While ALA may have specific properties, there is evidence that the n-3 LCPUFA can contribute to the prevention of CHD and possibly other degenerative diseases associated with aging. For adult males and non-pregnant/non-lactating adult females 0.250 g/day of EPA plus DHA is recommended, with insufficient evidence to set a specific minimum intake of either EPA or DHA alone; both should be consumed. For adult pregnant and lactating females, the minimum intake for optimal adult health and foetal and infant development is 0.3 g/d EPA+DHA, of which at least 0.2 g/d should be DHA.

The U-AMDR for EPA + DHA consumption is set at 2 g/d due to experimental evidence indicating that high supplement intakes of n-3 LCPUFA may increase lipid peroxidation and reduce cytokine production (Meydani, 2000; Sanders, 2009; Vedin *et al*., 2008). However, this consultation also acknowledged that higher consumption levels, up to 3 g/d, reduce other cardiovascular risk factors and have not had adverse effects in short- and intermediate-term randomized trials, and that some individuals in populations with high seafood consumption consume higher levels with no apparent evidence of harm. In this regard, the experts noted that the Australian and New Zealand reference value for the upper value of intake of EPA + DPA + DHA has been set at 3 g/d (NHMRC, 2006) and in 1997 the US Food and Drug Administration set a 'Generally Regarded as Safe' level of 3000 mg/day for n-3 LCPUFA (IOM, 2005). Following careful consideration and extensive debate, and considering the issue of sustainability of the supply of fish, the experts agreed on the value of 2 g/d as the U-AMDR for EPA plus DHA acknowledging that future RCT and other research may justify raising this figure in the future. It was decided not to include DPA in the recommendations because it is currently a research issue with limited evidence from RCT studies.

CONCLUSIONS AND RECOMMENDED DIETARY REQUIREMENTS FOR N-6 TO N-3 RATIO

Based on the evidence and conceptual limitation, there is no rational for a specific recommendation for n-6 to n-3 ratio, or LA to ALA ratio, if intakes of n-6 and n-3 fatty acids lie within the recommendation established in this report.

CONCLUSIONS AND RECOMMENDED DIETARY REQUIREMENTS FOR *TRANS*-FATTY ACID INTAKE

The consultation devoted substantial time and discussion to the issue of *trans*-fatty acid (TFA) intake, but in doing so drew heavily from the conclusions of the recently concluded and published reports of the WHO Scientific Update on TFA (Nishida and Uauy, 2009). There is convincing evidence that TFA from commercial partially hydrogenated vegetable oils (PHVO) increase CHD risk factors and CHD events – more so than had been thought in the past. There also is probable evidence of an increased risk of fatal CHD and sudden cardiac death in addition to an increased risk of metabolic syndrome components and diabetes. In promoting the removal of TFA, which are predominantly a by-product of industrial processing (partial hydrogenation), usually in the form of PHVO, particular attention must be given to what would replace them; this is a challenge for the food industry. It was noted that among adults, the estimated average daily ruminant TFA intake in most societies is low. The experts acknowledged the current recommendation of a mean population intake of TFA of less than 1%E might need to be revised in light of the fact that it does not fully take into account the distribution of intakes and thus the need to protect substantial subgroups from having dangerously high intakes. This could well lead to the need to remove partially hydrogenated fats and oils from human food supply.

In adults, the estimated average daily ruminant TFA intake in the US is about 1.5 g for men and 0.9 g for women. Average intake for both men and women, is 1.2 g, which corresponds to 0.5%E (Federal Register, 2003). If similar average intake values from industrially hydrogenated fat could be anticipated, then the TFA intake from all sources should be no more than 1%E.

CONSIDERATIONS FOR FOOD-BASED DIETARY GUIDELINES

The experts agreed that in addition to dietary requirements for total fat and fatty acids, food-based dietary guidelines are essential for promoting health and preventing disease. However, the consultation did not conduct a review of this subject. A general recommendation is to follow a dietary pattern predominantly based on whole foods (i.e., fruits and vegetables, whole grains, nuts, seeds, legumes, other dietary fibre sources, LCPUFA-rich seafood) with a relatively lower intake of energy-dense processed and fried foods, and sugar-sweetened beverages; and to avoid consumption of large portions. Moderate consumption of dairy products and lean meats and poultry can also be an important part of recommended food-based dietary guidelines. Maintaining recommended dietary patterns, appropriate energy intake, and adequate physical activity levels are critical to prevent unhealthy weight levels (e.g. overweight and obese) and to ensure optimal health for those predisposed to insulin resistance.

REFERENCES

Anderson, G.J. & Connor W.E. 1989. On the demonstration of omega-3 essential-fatty-acid deficiency in humans. *Am. J. Clin. Nutr.*, 49: 585-587.

Bjerve, K.S., Fischer, S., Wammer. F. & Egeland, T. 1989. Alpha-linolenic acid and long-chain omega-3 fatty acid supplementation in three patients with omega-3 fatty acid deficiency: effect on lymphocyte function, plasma and red cell lipids, and prostanoid formation. *Am. J. Clin. Nutr.*, 49: 290-300.

Bourne, L.T., Lambert, E.V. & Steyn, K. 2002. Where does the black population of South Africa stand on the nutrition transition? *Public Health Nutr.*, 5: 157-162.

DACH. 2000. Deutsche Gesellschaft für Ernährung (DGE), Österreichische Gesellschaft für Ernährung (ÖGE),Schweizerische Gesellschaft für Ernährung (SGE), Schweizerische Vereinigung für Ernährung (SVE): D-A-CH Referenzwerte für die Nährstoffzufuhr. Frankfurt/Main, Umschau Braus, 2000 [D-A-CH, German Nutrition Society (DGE), Austrian Nutrition Society (ÖGE), Swiss Society for Nutrition Research (SGE), Swiss Nutrition Association (SVE): Reference values for nutrient intake. Frankfurt/Main, Umschau Braus, 2002].

Elmadfa, I. & Kornsteiner, M. 2009. Fats and fatty acid requirements for adults. *Ann. Nutr. Metab*., 55: 56-75.

Elmadfa, I. & Schwalbe, P. 1989. Some Aspects of alpha-tocopherol bioavailability. *Fat Sci. Technol.*, 91: 402-407.

Eurodiet. 2008. *European Diet and Public Health: The Continuing Challenge.* Working Party 1: Final Report. www.eurodiet.med.uoc.gr/.

Federal Register. 2003. Part III. Department of Health and Human Services. Food and Drug Administration. 21 CFR Part 101. Food Labeling; *Trans* Fatty Acids in Nutrition Labeling; Consumer Research to Consider Nutrient Content and Health Claims and Possible Footnote or Disclosure Statements; Final Rule and Proposed Rule. *Federal Register*, 68: 41434-41506.

Hansen, A.E., Wiese, H.F., Boelsche, A.N., Haggard, M.E., Adam, D.J.D. & Davis, H. 1963. Role of linoleic acid in infant nutrition: Clinical and chemical study of 428 infants fed on milk mixtures varying in kind and amount of fat. *Pediatrics*, 31: 171.

Holman, R.T. 1978. How essential are essential fatty acids? *J. Am. Oil. Chem. Soc*., 55: 774A-781A.

Holman, R.T. 1998. The slow discovery of the importance of omega 3 essential fatty acids in human health. *J. Nutr*., 128: 427S-433S.

Holman, R.T., Johnson, S.B. & Hatch, T.F. 1982. A case of human linolenic acid deficiency involving neurological abnormalities. *Am. J. Clin. Nutr*., 35: 617-623.

Institute of Medicine. 2005. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. National Academies of Science, Washington DC.

Jakobsen, M.U., O'Reilly, E.J., Heitmann, B.L., Pereira, M.A., Bälter, K., Fraser, G.E., Goldbourt, U., Hallmans, G., Knekt, P., Liu, S., Pietinen, P., Spiegelman, D., Stevens, J., Virtamo, J., Willett, W.C. & Ascherio, A. 2009. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *Am. J. Clin. Nutr*., 89: 1425-1432.

Jequier, E. 1999. Response to and range of acceptable fat intake in adults. *Eur. J. Clin. Nutr*., 53 Suppl. 1: S84-88; discussion S88-93.

Kornsteiner, M., Singer, I. & Elmadfa, I. 2008. Very low n-3 long-chain polyunsaturated fatty acid status in Austrian vegetarians and vegans. *Ann. Nutr. Metab*., 52: 37-47.

Meydani, M. 2000. Omega-3 fatty acids alter soluble markers of endothelial function in coronary heart disease patients. *Nutr. Rev*., 58: 56-59.

Mohrhauer, H. & Holman, R.T. 1963. The effect of dose level of essential fatty acids upon fatty acid composition of the rat liver. *J. Lipid Res*., 4: 151-159.

National Health and Medical Research Council (Dept. of Health and Ageing). 2006. *Nutrient Reference Values for Australia and New Zealand*. NHMRC, Canberra.

Nishida, C. & Uauy, R. 2009. WHO Scientific Update on *trans* fatty acids. *EJCN*, 63: Suppl 2.

Sanders, T.A. 2009. Fat and fatty acid intake and metabolic effects in the human body. *Ann. Nutr. Metab*., 55: 162-172.

Siri-Tarino, P.W., Sun Q., Hu F.B., Krauss, R.M. 2010. Meta-analysis of prospective cohort studies evaluating the association of saturated fat with cardiovascular disease. *Am. J. Clin. Nutr.* 91:535-46.

Stamler, J. 2010. Diet-heart: a problematic revisit. *Am. J.Clin. Nutr*. 91 (3):497-9.

Strijbosch, R.A., Lee, S., Arsenault, D.A., Andersson, C., Gura, K.M., Bistrian ,B.R. & Puder, M. 2008. Fish oil prevents essential fatty acid deficiency and enhances growth: clinical and biochemical implications. *Metabolism*, 57: 698-707.

Suh, I., Oh, K.W., Lee, K.H., Psaty, B.M., Nam, C.M., Kim, S.I., Kang, H.G., Cho, S.Y. & Shim WH. 2001. Moderate dietary fat consumption as a risk factor for ischemic heart disease in a population with a low fat intake: a case-control study in Korean men. *Am. J. Clin. Nutr*., 73: 722-727.

Vedin, I., Cederholm, T., Freund Levi, Y., Basun, H., Garlind, A., Faxen Irving, G., Jonhagen, M.E., Vessby, B., Wahlund, L.O. & Palmblad, J. 2008. Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study. *Am. J. Clin. Nutr*., 87: 1616-1622.

Vorster, H.H., Venter, C.S., Wissing, M.P. & Margetts, B.M. 2005. The nutrition and health transition in the North West Province of South Africa: a review of the THUSA (Transition and Health during Urbanisation of South Africans) study. *Public Health Nutr*., 8: 480-490.

WHO. 2003. *Diet, Nutrition and the Prevention of Chronic Diseases*. Report of the WHO/FAO Expert Consultation Technical Report Series 916, WHO, Geneva.

Wollbeck, D., Kleist, E. & Elmadfa, I. 1981. Biochemical parameters to determine the minimum need of linoleic acid. *Fette Seifen Anstrichmittel*, 83: 317-323.

Chapter 6: Fat and fatty acid requirements and recommendations for infants of 0-2 years and children of 2-18 years

Fats have traditionally been considered a necessary part of the dietary energy supply. Until recently the main focus of research regarding infants and children was the total amount of fat that can be tolerated and digested, while the composition of dietary fat received relatively little attention. Interest in the quality of dietary lipid supply in early life as a major determinant of growth, infant development and long-term health is increasing, to the extent that selection of dietary fat and fatty acid sources during the first years of life is now considered to be of critical importance (Koletzko *et al*., 1997; Uauy *et al*., 2000a). Fats exhibit slow gastric emptying and intestinal motility, thereby prolonging satiety, which is particularly important for infants and children due to their small stomach size. Dietary fats provide essential fatty acids (EFA) and facilitate the absorption of lipid-soluble vitamins. Lipids are the main energy source in the infant diet and are therefore necessary for normal growth and physical activity. Fats normally provide around half of the energy in human milk (and in most artificial formulas). Fat also constitutes the major energy store in the body; the energy content of adipose tissue on a wet weight basis is 7 to 8-fold higher than that of tissue containing glycogen or protein.

BACKGROUND ON THE ROLE OF FATS AND FATTY ACIDS IN INFANT AND CHILD NUTRITION

Over recent decades interest in lipid nutrition has focused on the role of essential lipids in central nervous system development, and of specific fatty acids and cholesterol in lipoprotein metabolism. The impact of fats and fatty acids on the development of nutrition-related chronic diseases (NRCD) throughout the lifespan has also received considerable attention. Lipids are structural components of all tissues and are indispensable for the assembly of membranes of cells and cell organelles. The brain, retina and other neural tissues are particularly rich in LCPUFA. Some LCPUFA derived from the n-6 and n-3 EFA are precursors in eicosanoid and docosanoid production (prostaglandins, prostacyclins, thromboxanes, leukotrienes, resolvins and neuroprotectins). These autocrine and paracrine mediators are powerful regulators of physiological functions (such as thrombocyte aggregation, inflammatory responses, leukocyte migration, vasoconstriction and vasodilatation, blood pressure, bronchial constriction, uterine contractility, apoptosis and reperfusion oxidative damage).

Dietary lipids affect cholesterol metabolism at an early age, and can be associated with cardiovascular morbidity and mortality in later life. Lipid supply, particularly of EFA and LCPUFA, has also been shown to affect neural development and function (Uauy and Hoffman, 1991; Uauy *et al*., 2000c). Evidence indicates that specific fatty acids exert their effect by modifying the physical properties of membranes, including

membrane-related transport systems, ion channels, enzymatic activity, receptor function and various signal transduction pathways. More recently, specific fatty acids were reported to play a role in determining levels of gene expression for key transcription factors, peroxisome proliferator-activated receptors (PPAR) and retinoic acid receptors, leading to increased interest in better defining the role of these critical nutrients in the regulation of lipid metabolism, energy partitioning, insulin sensitivity, adipocyte development and neural function throughout the lifespan (Innis, 1991; Lauritzen *et al*., 2001).

BACKGROUND ON ESSENTIAL FATTY ACID DEFICIENCY

George and Mildred Burr (Burr and Burr, 1929) introduced the concept that specific components of fat may be necessary for the proper growth and development of animals, possibly including humans. They proposed that three specific fatty acids be considered as essential: linoleic acid (LA C18:2 n-6), arachidonic acid (AA C20:4 n-6) and α-linolenic acid (ALA C18:3 n-3). Despite this important early work, EFA were considered of only marginal nutritional importance for humans until the 1960s, when signs of clinical deficiency were first recorded in infants fed a skimmed milk based formula (Hansen *et al*., 1963) and in neonates given fat-free parenteral nutrition (Caldwell *et al*., 1972; Paulsrud *et al*., 1972). These seminal observations firmly established that LA is essential for normal infant nutrition. Hansen observed dryness, desquamation and thickening of the skin and growth faltering as frequent clinical manifestations of LA deficiency in young infants. The study included 428 infants fed cow milk based formulations with different types of fat providing a daily LA intake ranging from 10 mg/kg, when fed a fully skimmed milk based preparation, to 800 mg/kg when fed a corn and coconut oil based preparation. More subtle symptoms appear in n-3 EFA deficiency, including skin changes unresponsive to LA supplementation, abnormal visual function and peripheral neuropathy was reported in subjects receiving high n-6, low n-3 fat sources as part of their intravenous nutrition supply (Holman *et al*., 1982; Holman, 1998).

Human neonates as young as 28 weeks and weighing 900 g are able to synthesize LCPUFA from their precursors (Salem *et al*., 1996; Carnielli *et al*., 1996; Uauy *et al*., 2000b). However, this conversion is quite limited (3-5% of a tracer dose of labelled precursors was converted to LCPUFA over a 96 hour period (Uauy *et al*., 2000b), and the overall evidence indicates that in early life C18:n-3 precursors are not sufficiently converted to DHA to allow for biochemical and functional normalcy (Salem *et al*., 1996; Uauy *et al*., 2000b). Moreover, recent studies of genetic polymorphisms in genes responsible for fatty acid desaturation suggest that variability in biochemical responses and functional central nervous system effects following changes in diet are partly explained by single nucleotide polymorphisms (SNP) affecting a large proportion of the population (Schaeffer *et al*., 2006).

The uniqueness of the biological effects of feeding human milk on EFA metabolism is based on the direct supply of preformed LCPUFA, bypassing the regulatory step of both the Δ-6 and Δ-5 desaturases (Salem *et al*., 1996; Llanos *et al*., 2005). Excess dietary LA associated with some vegetable oils, particularly safflower, sunflower and corn oil, may decrease the formation of DHA from ALA because the Δ-6 desaturase is inhibited by excess n-6 substrates. In addition, on a relative conversion basis, AA formation is lower when excess LA is provided. The inhibitory effect of EPA on Δ-5 desaturase activity has been considered responsible for the lower membrane and plasma AA content observed when marine oil is consumed. Excess LA, as seen in infants receiving corn oil or safflower oil as the predominant fatty acid supply, will inhibit the elongation and desaturation of the parent EFA and thus lower the LCPUFA supply available for membrane synthesis.

Human milk and LCPUFA from dietary sources provide minimal preformed AA and substantial amounts of preformed n-3 LCPUFA such as DHA (Jensen, 1995; Jensen, 1996). LA and ALA should be considered indispensable since humans cannot synthesize them. While DHA and AA can be synthesized from ALA and LA respectively, they should be considered non-essential, although a dietary supply may be necessary for long-term health. However, given the limited and highly variable formation of DHA from ALA (1–5%), and because of their critical role in normal retinal and brain development in the human, they should be considered conditionally essential during early development. Similarly, they might be considered conditionally essential for life-long health considering intakes required for the prevention of cardiovascular disease (WHO, 2003).

BACKGROUND ON ENERGY SUPPLY FROM FAT AND EARLY GROWTH

The energy cost of growth is a major component of total energy requirements for the first 6 months of life (typically approximately 20–30% of total energy requirements), and this progressively drops in relative terms to <5% at 12 months of age (Uauy *et al*., 2000a). Weight gain is therefore a sensitive indicator of overall dietary energy adequacy for the first years of life (Torun *et al*., 1996; FAO, 2004). If the diet provides an adequate supply of energy and essential nutrients, there is no convincing evidence that a dietary fat intake of 30% of energy adversely affects the growth and development of healthy children living in a clean environment. A review of studies from Europe and North America also found little evidence of adverse effects of low dietary fat on growth of young children aged 6–36 months. Percentage of dietary fat was not correlated with energy intake, growth rate or energy density of the diet between ages 6 and 12 months, whereas energy density was positively associated with energy intake and weight gain (Fjeld *et al*., 1989; Butte, 1996; Torun *et al*., 1996; Muñoz *et al*., 1997; Nicklas *et al*., 1992; Shea *et al*., 1993). Dietary energy density, nutrient density and feeding frequency may be more important than dietary fat content in determining intake and growth of young children. No association between fat intake and growth was detected in infants aged 7–13 months or children aged 2–5 years or 3–5 years (Friedman *et al*., 1976; Lapinleimu *et al*., 1995; Michaelsen, 1997).

A number of studies have found that low-fat diets in the 25–30%E range result in lower energy intakes in children, with no measurable impact on growth performance provided overall energy intake is sufficient to support maintenance, normal activity and normal tissue accretion. If the diet records accurately reflect habitual intake, these findings raise the possibility of decreased physical activity in infants and young toddlers as a way to adjust to the low-fat diets. Some investigators reported lower vitamin and mineral intakes in association with low-fat diets (Reddy *et al*., 1980; Zlotkin, 1996). A cohort of 500 Canadian preschoolers was stratified according to: <30%, 30–40% or >40% of energy from fat between ages 3 and 6 years. Low-fat intake was associated with inadequate intake of fat-soluble vitamins. For children habitually on low-fat diets, the odds ratio for underweight 6 year olds was 2.3 (Gibson *et al*., 1993). There are clearly insufficient data to firmly establish a lower and an upper mean level for the population range for % energy intake from fat. These levels will clearly be context specific, depending on age, activity level, prevalence of diarrhoeal disease and other infectious morbidity.

The recommendations for total fat and fatty acids for infants (0-24 months) and children (2-18 years) are outlined in Table 6.1.

TABLE 6.1
Recommended dietary intakes for total fat and fatty acid: infants (0-24 months) and children (2-18 years)

Fat/FA	Age Group	Measure	Numeric Amount	Level of Evidence
Total fat	0-6 mo	AMDR	40-60%E	Convincing
		AI	based on composition % of total fat in HM,	Convincing
	6-24 mo	AMDR	gradual reduction, depending on physical activity, to 35%E[a]	Convincing
	2-18 yr	AMDR	25-35%E*	Probable
SFA	2-18 yr	U-AMDR	8%E* Children from families with evidence of familiar dyslipidemia (high LDL cholesterol) should receive lower SFA but not reduced total fat intake	Probable
MUFA	2-18 yr	AMDR	total fat [%E] - SFA [%E] - PUFA [%E] - TFA [%E]	Probable
Total PUFA	6-24 mo	U-AMDR	<15%E	Probable
	2-18 yr	U-AMDR	11%E	Probable
LA & ALA	0-24 mo	Comment	essential and indispensable	Convincing
n-6 PUFA				
AA	0-6 mo	AI	0.2-0.3%E[b]	Convincing
		U-AMDR	Based on HM composition as %E of total fat	Convincing
LA	0-6 mo	AI	HM composition as %E of total fat	Convincing
	6-12 mo	AI	3.0-4.5%E	Convincing
	6-12 mo	U-AMDR	<10%E	Probable
	12-24 mo	AI	3.0-4.5%E	Convincing
	12-24 mo	U-AMDR	<10%E	Probable
n-3 PUFA				
ALA	0-6 mo	AI	0.2-0.3%E[b]	Convincing
	6-24 mo	AI	0.4-0.6%E	Probable
	6-24 mo	U-AMDR	<3%E	Probable
DHA	0-6 mo	AI	0.1-0.18%E[b]	Convincing
	0-6 mo	U-AMDR	no upper value within the HM range up to 0.75%E	Convincing
	0-6 mo	Comment	conditionally essential due to limited synthesis from ALA	Probable
	6-24 mo	AI	10-12 mg/kg	Probable
	0-24 mo	Comment	critical role in retinal and brain development	Convincing
EPA+DHA	2-4 yr	AI	100-150 mg (age adjusted for chronic disease prevention)[c]	Probable
	4-6 yr	AI	150-200 mg (bridged from an infant value of 10 mg/kg)	Probable
	6-10 yr	AI	200-250 mg (to the adult value assigned at age 10 years)	Probable
TFA[d]	2-18 yr	UL	<1%E	Convincing

(Explanations of the abbreviations are found in the list of acronyms and symbols)

* Simell *et al.*, 2009

[a] For infants 6-12 mo, the proposed fat intake as a %E is lower than those recommended in the 1994 report. The primary reasons are the concern over increased obesity rates and the redefined growth standards based on human milk-fed infants, associated with leaner growth in later infancy (WHO 2006).

[b] The amounts are expressed as %E in order to be consistent with the other entries in the table. However based on human milk composition as is often the case when referring to infants of breast feeding age, the amounts for AA and ALA would be expressed as 0.4-0.6%FA and for DHA as 0.20-0.36%FA. This conversion assumes that half of the energy in human milk comes from fat. For children 6-24 months of age the estimation is based on provision of breast milk to meet half of the daily energy needs, the rest of the energy would come from non breast milk diet.

[c] Although there is no specific data from long term studies on the relationship between fatty acid intake and chronic disease prevention from children the assumption is that children also benefit from lower saturated fat and higher PUFA intakes.

[d] total TFA from ruminant and industrially-produced sources

RECOMMENDATIONS FOR TOTAL FAT INTAKE OF INFANTS 0-24 MONTHS

There is convincing evidence that during the first 6 months of life, dietary total fat should contribute 40–60%E to cover the energy needed for growth and the fat required for tissue deposition. There is convincing evidence that from age 6–24 months fat intake should be reduced gradually, depending on the physical activity of the child, to ~35% of energy, which is in line with the U-AMDR for adults.

RECOMMENDATIONS FOR FATTY ACID INTAKE OF INFANTS 0-24 MONTHS

There is convincing evidence that LA and ALA be considered essential and indispensable since they cannot be synthesized by humans and that DHA plays a critical role in normal retinal and brain development. There is probable evidence that although DHA can be synthesized from ALA given its limited and highly variable formation (1-5%) it should be considered conditionally essential for the first 6 months of life.

0-6 months

Fatty acid requirements for normal growth and development of this age group can be expressed as %E and when done so are consistent with the expressions of the other age groups. However, since the primary food source for this age group is human milk, it is conventional to base the amount on human milk composition and thus express the value as %FA. Since it is assumed that half of the energy in human milk comes from fat, the value expressed as %FA is double the value for %E. Both expressions are presented here. There is convincing evidence that the AI for DHA is 0.1–0.18%E or 0.2–0.36%FA and for AA and ALA is 0.2–0.3%E or 0.4–0.6%FA. However, because the DHA content of human milk approaches the level of 1.5%FA (or 0.75%E) there is no UL up to 1.5%FA if it is used at the criterion for setting the AI.

6-12 months

There is convincing evidence that the AI for the EFA for optimal growth and development of this age group are 3–4.5%E for LA and 0.4-0.6 %E for ALA. The U-AMDR for LA is < 10%E and for ALA is < 3%E at a probable level of evidence. The AI for DHA is 10-12 mg/kg at a probable level of evidence.

12-24 months

Due to limited data concerning this age group the experts decided to use the same recommendations as given for the age group 6-12 months.

COMPARISON WITH THE 1994 RECOMMENDATIONS AND THE PROPOSED VALUES

The proposed recommendations differ from those of the 1993 expert consultation (0 - 6 months, 50-60%E; 6 months - 3 years, 30-40E%; children >3 years, 30-40E%). The justification for the slight decrease in lower and upper values of the acceptable range is based on the need to control energy intake more diligently in order to retard and even prevent the progression of the obesity epidemic. This, at first, appears to contradict the existing evidence summarized in previous sections that indicates that percentage fat in the diet in early life is not associated with increased prevalence of overweight and obesity at later ages. However, the physiological standards for energy intake (FAO, 2004) and the acceptable weight for children 0-5 years (WHO/MGRS, 2006) have recently been significantly redefined (Uauy *et al*.,

2006). The new standards suggest that after completing the first 6 months of life, children should gain less weight and slightly more height than previously considered, since the new standard is derived from a prescriptive approach corresponding to predominantly breast-fed infants up to 6 months and non-smoking mothers, i.e. the new reference supports leaner and slightly taller children for the 0-5 yrs. The new energy recommendations based on measured energy expenditure rather than reported energy intakes indicate that children 0-24 months have energy needs that are 15-20% lower than previously recommended. For children 2-6 years there was also a significant overestimation. The new norms coupled with the epidemiological evidence of a significant progression of the obesity epidemic in young children support the need to restrict the percentage fat intake to facilitate the achievement of the energy balance without undue increase in body fat. Objective controlled studies of the impact of the percentage fat in the infant and child diet should be conducted in order to strengthen the evidence for these recommendations. However, the new normative standards limiting both the upper and lower ranges of the AMDR for fat are reasonable in light of the public health implications of the childhood obesity epidemic.

Health promotion efforts for the general population emphasize the importance of limiting the dietary intake of saturated and total fats to prevent NRCD. This has lead to a reduction in total lipid intakes in children of some populations, reaching average values as low as 28–30% energy after 6–12 months of age. Adverse effects of low-fat diets (<25% of energy) on weight gain and longitudinal growth in young children have been documented. Lowering saturated fat but not total fat intake may be considered exclusively in children from families with evidence of dyslipidemia due to high LDL cholesterol or elevated triglyceride levels.

The total diet should provide infants with at least 3–4.5%E from LA and 0.4-0.6%E from ALA to meet EFA requirements. Very high intakes of EFA confer no advantage and are associated with potential health risks. Intake of LA and other n-6 fatty acids should be limited to <10%E and intake of total polyunsaturated fatty acids should be limited to <15%E. After 2 years of age the composition of dietary fat should aim at reducing the risk of NRCD: saturated fatty acid intake should not exceed 8% total energy, *trans* fatty acids should be reduced to <1% of total fat, polyunsaturated fatty acids should contribute 6–10%E and the remaining fat energy should come from monounsaturated fatty acids.

The proposed values are more specific in terms of recommending maximum values for total PUFA intake and, this can be justified by the emerging information on the effect of excess n-6 PUFA on eicosanoid related functions and the implications for oxidative stress and chronic inflammation. The recommendation for slightly lower percentage energy from saturated fat is derived from the evidence of a beneficial effect of reduced saturated fat on LDL-C plasma levels in adults.

One practical approach to limiting saturated fat is to advise consumption of low-fat milk and dairy products. If this is done, appropriate sources of lipid soluble vitamins (A, E and D) should be provided. Processed foods rich in hydrogenated fats should be avoided to reduce *trans* fatty acid intake. Unless children are very active, total fat intake should be in the range of 30–35% of total energy.

There is some evidence of a requirement for preformed LCPUFA after weaning at age 6 months, even in infants fully breast-fed for the first 6 months of life and receive a variety of foods; the introduction of food sources of LCPUFA, such as eggs, liver and fish are currently being delayed due to concerns about allergies.

RECOMMENDATIONS FOR TOTAL FAT INTAKE FOR CHILDREN 2-18 YEARS

There is probable evidence that the AMDR should be 25–35%E. It is stressed that adverse effects of low-fat diets (<25% of energy) on weight gain and longitudinal growth in young children have been documented. Whereas children from families with evidence of familiar dyslipidemia (high LDL-C) should receive lower saturated fat, there should be no reduced total fat intake. Health promotion efforts for the general population emphasize the importance of healthy dietary patterns to prevent nutrition-related chronic diseases (NRCD).

RECOMMENDATIONS FOR FATTY ACID INTAKE FOR CHILDREN 2-18 YEARS

There is sufficient probable evidence to set the value of SFA intake at <8%E and the PUFA (n-6 plus n-3 intake) at 11%E. However, as in the case of adults, there is convincing evidence to limit (UL) TFA intake to <1%E. There is probable evidence to recommend an AI range of EPA + DHA intake targeted at preventing chronic disease (adjusted for age) of:

100-150 mg for 2-4 yrs
150-200 mg for 4-6 yrs
200-300 mg for 6-10 yrs

As is the case for adults, the amount of MUFA intake is based on the difference. The need to provide EFA to meet needs of children and maintain dietary fatty acid intake patterns that contribute to the prevention of chronic diseases is recognized at a possible level of evidence. Children aged 2-18 years form part of the household and could thus consume at least one to two meals of fatty fish per week as is recommended for the adult population. However, the currently available evidence does not permit defining an age-specific quantitative estimate of recommended dietary intake for EPA + DHA for children aged 2-18 years. Although there is a general concern that the dietary intakes of EPA and DHA among children in many Western and non-Western countries are lower than desirable there is currently insufficient evidence to link increased intake levels of DHA and/or EPA to improved physical or mental development or specific functional benefits in children 2-18 years of age.

HUMAN MILK AS A MODEL TO DEFINE ACCEPTABLE INTAKES (AI) FOR FATS AND FATTY ACIDS IN EARLY LIFE FOR NORMAL INFANTS (0 - 2 YEARS)

Human milk is the preferred infant food; the current recommendations are that term infants be exclusively breastfed for the first 6 months of life (FAO, 2004). The new WHO growth standards (WHO/MGRS, 2006) are also based on predominant breastfeeding for the first 6 months of life. Moreover, currently the evaluation of adequacy of artificial formula feeding is based on the capacity of formula to support growth, development and functional responses in a manner similar to that for human milk and thus the need to compare the biochemical, metabolic and functional responses of breast-fed infants with those given artificial nutrient formulations. The expert group addressing protein and amino acid requirements of children during the first 6 months of life recently used this same paradigm (WHO, 2008).

Mature human milk (after the first 2-3 weeks of life) provides a fat:energy ratio (FER) of 50%. Human milk provides mainly saturated (palmitic) and monounsaturated (oleic) fatty acids and a relatively high cholesterol intake of 100–150 mg/d (Jensen, 1996).

Formula-fed infants receive a similar FER, but in contrast have a much lower cholesterol intake, 25–60 mg/d. A mix of vegetable oils (corn, soy, safflower, olive or sunflower) is added to most formulas (Uauy *et al*., 2000a), resulting in the oleic acid and LA content depending on the oil source. The use of vegetable oils in the infant diet is based on availability, nutritional properties and relative costs of oil sources. However, the need to include LA, ALA and LCPUFA (>18 carbon chain length) in formulas is now well established (FAO, 1994; Uauy *et al*., 1999).

Human milk is a source of LA, ALA, DHA, AA, and other LCPUFA. The level of AA is relatively constant on a worldwide basis while the level of DHA is more variable and depends on maternal diet and lifestyle (Yuhas *et al.*, 2006; Marangoni *et al.*, 2002; Smit *et al.*, 2002; Agostoni *et.al.*, 1998; Agostoni *et al.*, 2003). Population means for AA in human milk range between 0.3-0.7 weight % of total fatty acids (Yuhas *et al.*, 2006; Marangoni *et al.*, 2002; Smit *et al.*, 2002), while mean values for DHA range from 0.2–1.0% FA (Yuhas *et al.*, 2006). Lactating women supplemented with DHA have increased milk DHA levels (Fidler *et al*., 2000; Jensen *et al.*, 2005). Gibson *et al*. (1997) reported a dose-dependent response between maternal DHA consumption and DHA levels in human milk, although human milk DHA levels above 0.8 %FA did little to increase the plasma or red blood cell DHA content of the infants studied. The content of human milk EFA and LCPUFA can serve to define AI values, taking into consideration the factors of expected volume of intake, the fat content of human milk and the range of compositions measured in different regions of the world where children grow well and develop normally.

RECOMMENDATIONS FOR DIETARY INTAKES OF SPECIFIC ESSENTIAL FATTY ACIDS FOR INFANTS AND CHILDREN

The suggested approach is to define an AI based on observed intakes of healthy populations. There is general concern that n-3 LCPUFA intakes in children and adolescents tend to be low on a unit body weight basis and as %E (Meyer *et al*., 2003). However, reliable and comparable data on dietary intake of n-3 fatty acids and on biochemical markers of status in different populations of children are scarce (Lambert *et al*., 2004). The available data are insufficient to assume that increasing n-3 LCPUFA intakes will improve physical or mental development or yield specific functional benefits relevant to the health and wellbeing of this age group.

RECOMMENDATIONS FOR DIETARY INTAKES OF SPECIAL GROUPS OF INFANTS AND CHILDREN

Preterm infants

This group of infants is particularly susceptible to EFA and LCPUFA deficiency since they have very limited fat stores and greater nutrient demand given their rapid growth rate. They are thus heavily dependent on dietary EFA and LCPUFA supply for tissue accretion (Uauy *et al*., 1990; Carlson *et al*., 1993; Dobbing, 1994). A recent Cochrane review indicates that LCPUFA supplementation appears safe in preterm infants when growth is used as the safety parameter. Four out of thirteen studies reported benefits of LCPUFA on growth of supplemented infants at different postnatal ages. Recent studies adding AA to the supplement have found no significant negative effect on growth. A variable of importance in studies on preterm infants is the medical complications and treatments associated with early delivery and thus most studies enrol only relatively healthy infants. The Cochrane review concludes that no clear long-term benefits for

visual or intellectual development have been demonstrated in trials providing n-3 LCPUFA to pre-term infants. Gibson *et al*. (2008) recently completed the largest clinical trial on preterm infants ever conducted (>1000 Australian infants). The data support a beneficial effect of higher DHA provision on visual acuity (in this study 0.3% DHA [as % total fat] was compared to 1.0%). In addition, improved mental development was noted, as assessed using the Bayley infant development scale. However, the justification to date for adding LCPUFA to formula is based on the need to mimic the composition of human milk and not on evidence of important clinical benefits. Another approach would be to mimic the fetal accretion rate. If this approach is taken, the need for DHA would be ~3 times the mean DHA content of term human milk (Lapillonne and Jensen 2009). A supplement containing a balance of n-3 and n-6 LCPUFA is unlikely to impair the growth of preterm infants. A supplement containing a balance of n-3 and n-6 LCPUFA is unlikely to impair the growth of preterm infants.

Further work is clearly necessary to determine the extent of the benefit of supplemental LCPUFA on the neurodevelopment and health outcomes of infants born preterm. Any benefit to neurodevelopment may be important to this group of infants because the mean score of the preterm infants included in these studies was one standard deviation lower than standardized norms.

SAFETY ISSUES WHEN CONSIDERING FOOD SOURCES OF FATS INTENDED FOR USE BY CHILDREN

The selection of fat sources for infant complementary foods must consider the safety aspects and not only the level of fat absorption. This is especially relevant for developing countries where fats included in foods given to young children are low-cost oils or by-products of industrial processing. Since fats are structural components of tissues, especially neural tissues, n-3 and n-6 essential fatty acids must be provided in the diet.

The European Union set an upper limit of 4%FA for the *trans* fatty acid content of foods for infants and young children. This may need to be reconsidered in view of the current limit of 2%FA set from the standpoint of cardiovascular disease prevention. If rapeseed oil is used, it should be derived from genetically low erucic acid varieties. All children should be given foods that meet acute and long-term safety standards. While the low price of food ingredients is desirable they should not be eaten at the expense of compromising long-term safety of the product.

STORAGE, PACKAGING AND DISTRIBUTION

Safety problems may also arise from the way oils are stored, distributed and/or dispensed, particularly regarding infants and children. Large tins or plastic barrels used in developing countries to reduce costs of distribution may facilitate adulteration of products and promote peroxidation given the large volumes and the long times needed to sell the products. A study in marasmic children demonstrated altered antioxidant defence systems and increased lipid peroxidation, suggesting an increased risk of oxidative damage in malnourished infants (Mansur *et al*., 2000). Bottled oil ready for consumer purchase is undoubtedly safer, but is also more expensive. Soft plastic containers made with phthalic acid as the plasticizer can also create safety problems because this agent is fat-soluble and a known carcinogen. Rigid plastics or glass bottles are preferable (Korhonen *et al*., 1983). Tetrapak containers have been introduced in some countries to package oils, preventing rancidity by limiting exposure to light and oxygen.

RESEARCH NEEDS FOR CHILDREN 2-18 YEARS

Further systematic research is needed to provide a sound scientific basis for formulating specific intake values for n-3 LCPUFA in children 2-18 years of age. Relevant public health outcomes that are likely to be linked to life-long intakes of EPA and DHA include future risk of CVD and metabolic syndrome, optimal mental development and behaviour, and immune response. Dietary studies should be carefully conducted and analyzed, using a specific and standardized methodology, and taking account of the substantial challenges in assessing individual intakes of EPA and DHA in children. However, because assessment of dietary intake is always inaccurate, age-specific information on fatty acid status based on biological markers is required. Cross sectional analyses from prospective birth and childhood cohort studies may provide valuable insights that can contribute to designing intervention trials. Age specific effects of different fatty acid intakes and dosages on relevant endpoints should be assessed in controlled intervention studies. The data obtained should aim at establishing the effect of different doses of individual fatty acids, and of different combinations and ratios of PUFA, on well-defined and quantifiable outcomes of public health significance. Potential adverse effects of recommending increased dietary intakes of EPA and DHA or of fatty fish, such as risk of contamination with environmental pollutants or increased bleeding risks, should also be carefully assessed (Innis *et al*., 2006). Future research should consider short and long term effects of genetic variation in fatty acid desaturase activities and the respective effect of LCPUFA intake prior to and during pregnancy, lactation and infancy. Studies addressing subgroups with potential specific needs and benefits are needed, including women with restricted dietary intakes, multiple or at risk pregnancies, or short time intervals between pregnancies. Supplementation studies should aim to examine growth, body composition and bone mineralization, visual and cognitive development, as well as effects on immune outcomes such as allergy and inflammatory disorders, and cardiovascular function. Studies evaluating different amounts of LCPUFA, and the specific effects of AA supply, with sufficient duration of intake, adequate sample sizes, and standardized methodology for outcome measurements need careful consideration. Dose response studies for LCPUFA intake during the second six months of life should be undertaken. Simplified measures of dietary supply and of LCPUFA status that permit evaluation of large population groups, including young children, should be developed and evaluated.

REFERENCES

Agostoni, C., Marangoni, F., Giovannini, M., Riva, E. & Galli, C. 1998. Long-chain polysaturated fatty acids, infant formula, and breastfeeding (Letter). *Lancet*, 352: 1703-1704.

Agostoni, C., Marangoni, F., Grandi, F., Lammardo, A.M., Giovannini, M., Riva, E. & Galli, C. 2003. Earlier smoking habits are associated with higher serum lipids and lower milk fat and polyunsaturated fatty acid content in the first six months of lactation. *EJCN*, 57: 1466-1472.

Burr, G.O. & Burr, M.M. 1929. A new deficiency disease produced by rigid exclusion of fat from the diet. *J. Biol. Chem*., 82: 345-367.

Butte, N.F. 1996. Energy requirements of infants. *EJCN*, 50: S24-S36.

Caldwell, M.D., Johnson, H.T. & Othersen, H.B. 1972. Essential fatty acid deficiency in an infant receiving prolonged parenteral alimentation. *J. Pediatr*., 81: 894-898.

Carlson, S.E., Werkman, S.H., Rhodes, P.G &, Tolley, E.A. 1993. Visual-acuity development in healthy preterm infants: of marine-oil supplementation. *Am. J. Clin. Nutr*., 58: 35– 42.

Carnielli, V.P., Wattimena, D.J.L., Luijendijk, I.H.T., Boerlage, A., Degenhart, H.J. & Sauer, P.J.J. 1996. The very low birth weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acids from linoleic and linolenic acids. *Pediatr. Res*., 40: 169.

Dobbing, J. 1994. The later development of the brain and its vulnerability. *In* Davis, J.A. and Dobbing, J., eds. *Scientific Foundations of Paediatrics*, pp. 565-577. William Heinemann Medical Books, Ltd., London.

FAO. 1994. *Fats and oils in human nutrition*: Report of a Joint FAO/WHO expert consultation. FAO Food and Nutrition Paper No. 57. FAO, Rome.

FAO. 2004. *Human Energy Requirements*. Report of a Joint FAO/WHO expert consultation FAO Food and Nutrition Technical Report Series No. 1. FAO, Rome.

Fidler, N., Sauerwald, T., Pohl, A., Demmelmair, H. & Koletzko, B. 2000. Docosahexaenoic acid transfer into human milk after dietary supplementation: a randomized clinical trial. *J. Lipid Res*., 41: 1376.

Fjeld, C.R., Schoeller, D.A., Brown, K.H. 1989. A new model for predicting energy requirements of children during catch-up growth developed using doubly labeled water. *Pediatr. Res*., 25: 503-508.

Friedman, G. & Goldberg, S.J. 1976. An evaluation of the safety of a low-saturated-fat, low-cholesterol diet beginning in infancy. *Pediatrics*, 58: 655-657.

Gibson, R.S., MacDonald, C.A., Smit Vanderkooy, P.D., McLennan, C.E. & Mercer, N.J. 1993. Dietary fat patterns of some Canadian preschool children in relation to indices of growth, iron, zinc, and dietary status. *J. Can. Diet. Assoc*., 54: 33-37.

Gibson, R.A., Neumann, M.A. & Makrides M. 1997. Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipids fatty acids and neural indices of exclusively breast fed infants. *EJCN*, 51: S78-S84.

Gibson, R.A., Smithers, L.G., McPhee, A. & Makrides, M. 2008. Effect of long-chain polyunsaturated fatty acid supplementation of preterm infants on disease risk and neurodevelopment: a systematic review of randomized controlled trials. *Am. J. Clin. Nutr*., 87: 912-20.

Hansen, A.E., Wiese, H.F., Boelsche, A.N., Haggard, M.E., Adam, D.J.D. & Davis, H. 1963. Role of linoleic acid in infant nutrition: clinical and chemical study of 428 infants fed on milk mixtures varying in kind and amount of fat. *Pediatrics*, 31: 171-192.

Holman, R. T., Johnson, S. B. & Hatch, T. F. 1982. A case of human linolenic acid deficiency involving neurological abnormalities. *Am. J. Clin. Nutr*., 35: 617–623.

Holman, R. T. 1998.The slow discovery of the importance of n-3 essential fatty acids in human health. *J. Nutr.*, 128: 427S–433S.

Innis, S.M. 1991. Essential fatty acids in growth and development. *Prog. Lipid Res.*, 30: 39-103.

Innis, S.M., Palaty, J., Vaghri, Z. & Lockitch, G. 2006. Increased levels of mercury associated with high fish intakes among children from Vancouver, Canada. *J. Pediatr.*, 148: 759-763.

Jensen, R.G. 1995. *Handbook of Milk Composition*. Academic Press, San Diego.

Jensen, R.G. 1996. The lipids in human milk. *Prog. Lipid Res.*, 35: 53-92.

Jensen, C. L., R. G. Voigt, *et al*. 2005. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. *Am. J. Clin. Nutr.*, 82: 125-32.

Koletzko, B., Tsang, R., Zlotkin, S.H., Nichols B. & Hansen J.W. 1997. Eds., Importance of dietary lipids. *In Nutrition during infancy. Principles and Practice*, pp. 123-153 (1st ed.), Digital Educational Publishing, Cincinnati.

Korhonen, A., Hemminki, K. & Vainio, H. 1983. Embryotoxic effects of phthalic acid derivatives, phosphates and aromatic oils used in the manufacturing of rubber on three day chicken embryos. *Drug Chem. Toxicol.*, 6: 191-207.

Lambert, J., Agostoni, C., Elmadfa, I., Hulshof, K., Krause, E., Livingstone, B., Socha, P., Pannemans, D. & Samartin, S. 2004. Dietary intake and nutritional status of children and adolescents in Europe. *Br. J. Nutr.*, 92 (Suppl 2): S147-S211.

Lapillonne, A. & Jensen, C.L. 2009. Reevaluation of the DHA requirement for the premature infant. Prostaglandins, Leukotrienes and Essential Fatty Acids,143-150.

Lapinleimu, H., Viikari J, Jokinen, E., Salo, P., Routi, T., Leino, A., Ronnemaa T., Seppanen, R., Valimaki, I. & Simell, O. 1995. Prospective randomised trial in 1062 infants of diet low in saturated fat and cholesterol. *Lancet*, 345: 471-476.

Lauritzen, L., Hansen, H.S., Jorgensen, M.H. & Michaelsen, K.F. 2001. The essentiality of long chain n-3 fatty acids in relation to development and function of the brain and retina. *Prog. Lipid Res.*, 40: 1-94.

Llanos, A., Li, Y., Mena, P., Salem, N. Jr. & Uauy, R. 2005. Infants with intrauterine growth restriction have impaired formation of docosahexaenoic acid in early neonatal life: a stable isotope study. *Pediatr. Res.*, 58: 735-40.

Mansur, M., Tatli, M.M., Vural, H., Koc A. & Kosecik, M. 2000. Altered anti-oxidant status and increased lipid peroxidation in marasmic children. *Pediatr. Int.*, 42: 289-291.

Marangoni, F., Agostini, C., Lammardo, A.M., Bonvissuto, M., Giovannini, M., Galli, C. & Riva, E. 2002. Polyunstaturated fatty acids in maternal plasma and in breast milk. *Prostaglandins Leukot. Essent. Fatty Acids*, 66: 535.

Meyer, B.J., Mann, N.J., Lewis, J.L., Milligan, G.C., Sinclair, A.J. & Howe, P.R. 2003. Dietary intakes and food sources of omega-6 and omega-3 polyunsaturated fatty acids. *Lipids*, 38: 391-398.

Michaelsen, K.F. 1997. Nutrition and growth during infancy. The Copenhagen Cohort Study. *Acta Paediatr.*, (Suppl) 420: 1-36.

Muñoz, K.A., Krebs-Smith, S.M., Ballard-Barbash, R. & Cleveland, L.E. 1997. Food intakes of US children and adolescents compared with recommendations. *Pediatrics*, 100: 323-329.

Nicklas, T.A., Webber, L.S., Koschak, M.L. & Berenson, G.S. 1992. Nutrient adequacy of low fat intakes for children: the Bogalusa Heart Study. *Pediatrics*, 89: 221-228.

Paulsrud, J. R., Pensler, L., Whitten, C. F. & Holman, R. T. 1972. Essential fatty acid deficiency in infants induced by fat-free intravenous feeding. *Am. J. Clin Nutr*., 25: 897–904.

Reddy, V. & Mohanran, M. 1980. Effect of dietary fat on absorption of beta-carotene from green leafy vegetables in children. *Ind. J. Med. Res*., 71: 53-56.

Salem, Jr N., Wegher, B., Mena, P. & Uauy, R. 1996. Arachidonic and docoahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. *Proc. Natl. Acad. Sci. USA*, 93: 49-54.

Schaeffer, L., Gohlke, H., Muller, M., Heid, I.M., Palmer, L.J., Kompauer, I., Demmelmair, H., Illig, T., Koletzko, B. & Heinrich, J. 2006. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Hum. Mol. Genet*., 15: 1745.

Shea, S., Basch, C.E., Stein, A.D., Contento, I.R., Irigoyen, M. & Zybert, P. 1993. Is there a relationship between dietary fat and stature or growth in children three to five years of age? *Pediatrics*, 92: 579-586.

Simell, O., Niinikoski, H., Rönnemaa, T., Raitakari, O.T., Lagström, H., Laurinen, M., Aromaa, M., Hakala, P., Jula, A., Jokinen, E., Välimäki, I. & Viikari, J.; STRIP Study Group. 2009. Cohort Profile: the STRIP Study (Special Turku Coronary Risk Factor Intervention Project), an Infancy-onset Dietary and Life-style Intervention Trial. *Int J Epidemiol*. Jun;38(3): 650-5.

Smit, E.N., Martini, I.A., Mulder, H., Boersma, E.R., Muskiet, F.E. 2002. Estimated biological variation of the mature human milk fatty acid composition. *Prostaglandins Leukot. Essent. Fatty Acids*, 66: 549.

Torun, B., Davies, P.S., Livingstone, M.B., Paolisso, M., Sackett, R. & Spurr, G.B. 1996. Energy requirements and dietary energy recommendations for children and adolescents 1 to 18 years old. *EJCN*, (Suppl 1): 50: S37-80.

Uauy, R.D., Birch, D.G., Birch, E.E., Tyson, J.E. & Hoffman, D.R. 1990. Effect of dietary omega-3 fatty acids on retinal function of very-low-birth-weight neonates. *Pediatr. Res*., 28: 485-492.

Uauy, R. & Hoffman, D.R. 1991. Essential fatty acid requirements for normal eye and brain development. *Semin. Perinatol*., 15: 449-455.

Uauy, R., Mena, P. & Valenzuela, A. 1999. Essential fatty acids as determinants of lipid requirements in infants, children and adults. *EJCN*, (Suppl. 1): 53 S66-S77.

Uauy, R., Mize, C. & Castillo-Durán, C. 2000a. Fat intake during childhood: metabolic responses and effects on growth. *Am. J. Clin Nutr*., (Suppl.) 72: 1354S-1360S.

Uauy, R., Mena, P., Wegher, B., Nieto, S., & Salem, N. Jr. 2000b. Long chain polyunsaturated fatty acid formation in neonates: effect of gestational age and intrauterine growth. *Pediatr. Res*., 47: 127.

Uauy, R., Mena, P. & Rojas, C. 2000c. Essential fatty acids in early life: structural and functional role. *Proc. Nutr. Soc*., 59: 3-15.

Uauy, R., Rojas, J., Corvalan, C., Lera, L. & Kain, J. 2006. Prevention and control of obesity in preschool children: importance of normative standards. *J. Pediatr. Gastroenterol. Nutr*., 43 S3: S26-37.

WHO/MGRS (Multicentre Growth Reference Study Group). 2006. WHO Child growth standards based on length/height, weight and age. *Acta Paediatr.*, (Suppl.) 450: 76.

WHO. 2003. Diet, nutrition and the prevention of chronic diseases: Report of a joint WHO/FAO expert consultation. WHO Technical Report Series, 916, WHO, Geneva.

WHO. 2007. *Protein and amino acid requirements in human nutrition*. *Report of a Joint WHO/FAO/UNU Expert Consultation* WHO Technical Report Series 935. WHO, Geneva.

Yuhas, R., Pramuk, K. & Lien, E.L. 2006. Human milk fatty acid composition from nine countries varies most in DHA. *Lipids*, 41(9): 851-858.

Zlotkin, S.H. 1996. A review of the Canadian "Nutrition recommendations update: dietary fat and children". *J. Nutr*., (4 Suppl.) 126: 1022S-1027S.

Chapter 7: Fat and fatty acid during pregnancy and lactation

DIETARY FAT INTAKE DURING PREGNANCY AND LACTATION

Fat is used for energy and as a critical building material for membranes. In this regard pregnancy and lactation impose special nutritional needs on the mother-foetus/infant. Most research since the last expert consultation regarding fat (FAO, 1994) on fat requirements has been on polyunsaturated fatty acids (PUFA) and of these, DHA and AA have received the most attention. Significant study has also been devoted to industrially produced unsaturated fatty acids in partially hydrogenated vegetable oil, collectively referred to as *trans* fatty acids or TFA.

The major functional outcomes that have been studied in the infant are visual and cognitive maturity, immune function and growth. For the mother, glucose tolerance, pre-eclampsia, and psychiatric health have been considered, and for the mother-offspring pair, maintenance of normal pregnancy to term has been of most interest with respect to fat and fatty acid intake.

Basic research has confirmed that long-chain PUFA (LCPUFA) are required components of the rapidly growing perinatal CNS. Since it is not known whether dietary LCPUFA (primarily DHA) is preferentially directed to the brain, its accumulation in organs other than the CNS should be taken into consideration when estimating requirements for preterm infants. Unlike some nutrients, such as folate, for which intake around the time of conception is crucial, it is likely that initiation of enhanced LCPUFA intake at any stage in pregnancy or lactation can at least partially make up for previously low intakes prior to conception and during the first weeks of gestation. Clinical studies have established widespread consensus that preterm infants require a supply of the LCPUFA DHA and AA to optimize visual and neural function, and numerous results suggest that there is a requirement for term infants.

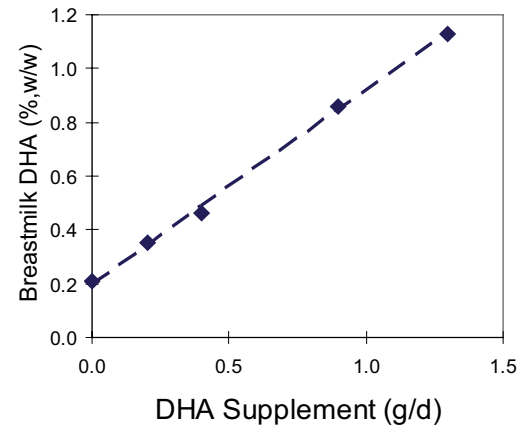
Typical diets supplied by industrial production rely on seed oils that provide surfeit amounts of the AA precursor LA. Consumption of these diets is long-established in developed countries, and as developing societies accumulate wealth, they also favour these oils over other fat sources with a more balanced LA to ALA ratio (Ghafoorunissa, 1996; Ghafoorunissa, 1998; Ghafoorunissa, 2005). LA and ALA apparently compete for the same enzyme systems for biosynthesis to LCPUFA and, importantly, for incorporation into membranes. High LA reduces tissue n-3 LCPUFA by both these mechanisms, and thus research on perinatal fatty acid requirements has focused mainly on n-3 sufficiency for both mother and infant. The emphasis has been on DHA, with some work on EPA. Research on dietary AA functional effects has primarily been as an adjuvant to DHA.

DHA and AA are the major LCPUFA components of breast milk, with much lower amounts of EPA, DPA, and other n-6 LCPUFA. AA levels are more conserved than DHA, which responds sensitively and predictably to dietary DHA (Figure 7.1). Incremental increases in dietary ALA increase EPA and DPA n-3 status, but do not increase blood or breast milk DHA in adults, although ALA does increase blood DHA in infants. Changes in vegetable cooking oils that simultaneously increase ALA, while

decreasing LA in the diet, increase blood DHA. These observations in humans are congruent with results from experiments with animals.

Perinatal health effects of LCPUFA have been most closely associated with (1) improvement of infant visual and cognitive function, (2) treatment and prevention of maternal depression, and (3) slight increases in gestational length to reduce the prevalence of prematurity. Long-term consequences of preformed DHA and AA intake for mothers and infants have also been found. In addition there is compelling evidence that shows that intake of DHA and EPA and of AA combined with DHA are not associated with toxicity for mothers, infants or children.

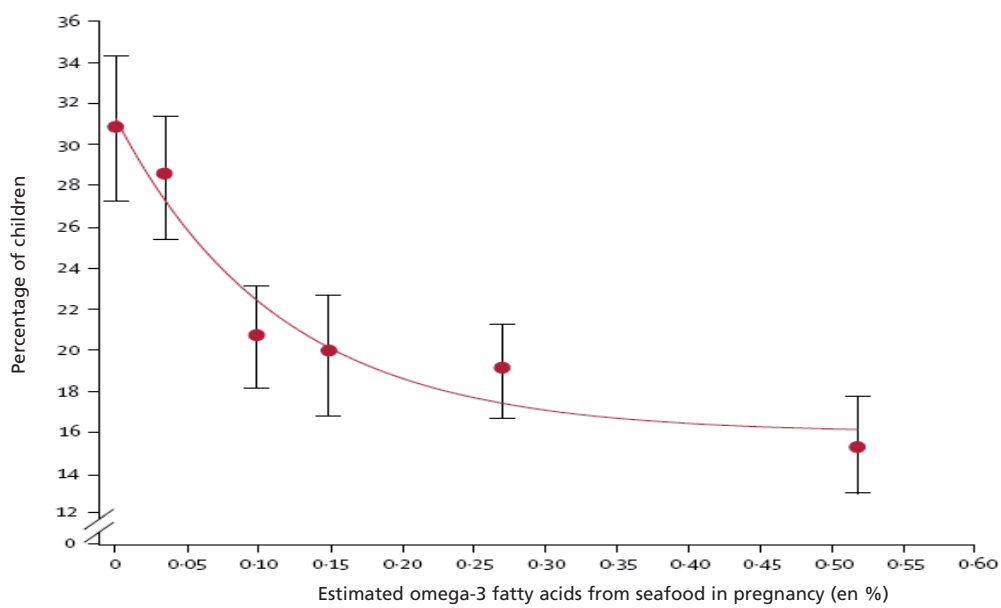
FIGURE 7.1
Regression analysis of breast milk DHA (B) concentration vs DHA intake (I). B=(0.72×I)+0.20 (r^2 = 0.998)



Source: Gibson et al., 1997

Many RCT and several meta-analyses have appeared on DHA and AA supplementation. Overwhelmingly, studies show either a neutral or positive effect on health outcome, with negative effects rare. Table 7.1 summarizes those studies related to LCPUFA supplementation and pregnancy outcome. Based on a 2006 meta-analysis (Freeman *et al.*, 2006), a US professional medical organization, the American

FIGURE 7.2
Dose response for prevalence of children in the lowest quartile for verbal IQ at age 8 based on maternal seafood consumption during pregnancy. At maternal seafood consumption corresponding to LCPUFA intake of 0.10 %E (about 300 mg/day), the reduction in risk for low verbal IQ drops from 31% (no seafood consumption) to about 20.5%. With 5-fold more seafood consumption, risk drops to about 15.5%



Source: Hibbeln et al., 2007

TABLE 7.1
Meta-analyses and systematic reviews of LCPUFA supplementation with pregnancy outcomes

Meta-analysis Study	Research studies reviewed	Purpose of review	Conclusions
Horvath *et al.*, 2007 (meta-analysis)	Moodley and Norman, 1989 Bulstra-Ramakers *et al.*, 1995 Onwude *et al.*, 1995 Olsen *et al.*, 2000	Effects of LCPUFA supplementation in high risk pregnancies on several parameters	LCPUFA supplementation reduced early preterm delivery
Szajewska *et al.*, 2006	Olsen *et al.*, 1992 Helland *et al.*, 2001 Malcolm *et al.*, 2003b Smuts *et al.*, 2003a Smuts *et al.*, 2003b Sanjurjo *et al.*, 2004	Evaluation of birth outcomes	There is a mild increase of length of pregnancy with marine oil supplementation
Makrides *et al.*, 2006 (Cochrane systematic review)	D'Almeida *et al.*, 1992 Olsen *et al.*, 1992 Bulstra-Ramakers *et al.*, 1995 Onwude *et al.*, 1995 Olsen *et al.*, 2000 Smuts *et al.*, 2003b	EPA or EPA and DHA on the risk of pre-eclampsia, preterm birth, low birth-weight, and small-for-gestational age	Analysis of a subset of three high quality trials concluded that women allocated to a marine oil supplement had a mean gestational period 2.6 days longer than those allocated to placebo or no treatment groups. However, the authors concluded that the evidence at that time was not sufficient to warrant routine use of marine oil to reduce the rate of those factors studied
Olsen *et al.*, 2007	Olsen *et al.*, 2000	Reassessment of data from 2000 study	Showed lengthening of gestation in a group of 495 women with a history of preterm delivery, IUGR, or pregnancy-induced hypertension by supplementation with 2.7 g EPA+DHA from week 30 of gestation. An effect was detected in low and moderate fish eaters and no effect was detected in high fish eaters

Psychiatric Association, concluded that there is sufficient evidence to recommend dietary preformed DHA for women, and the enhanced demand for DHA in pregnancy and lactation strongly implies a greater requirement. An independent meta-analysis of the same set of studies arrived at similar conclusions (Lin and Su, 2007). Other meta-analyses (Szajewska *et al.*, 2006; Makrides *et al.*, 2006; Olsen *et al.*, 2007) concluded that gestational length is increased significantly by a few days, though reductions in the prevalence of preterm birth were observed only in high risk pregnancy (Horvath *et al.*, 2007). Considering RCT and weighing the absence of toxicity and the potential for benefit, expert panels have recommended minima of 200–300 mg/d DHA for pregnant and lactating women.

Minimal incremental maternal DHA requirements in the first six months of lactation can be estimated with confidence from biochemical data and human breast milk DHA measurements. Breast milk DHA cannot be increased with the addition of ALA or other DHA precursors to the diet, and in line with this observation, the breast milk of strict vegans contains among the lowest DHA amounts reported (Sanders and Reddy, 1992). Based on the global mean from available studies, mothers of exclusively breastfed infants transfer 110 mg/d DHA to the infant on average (Brenna *et al.*, 2007). Using this value, dose-response data can be used to estimate dietary DHA intake of at least 170 mg/d, and from that derive an AI value of 190–210 mg/d. This value ensures breast milk DHA at a level slightly above the global DHA levels, and provides benefit to the infant and maintenance of maternal DHA.

Maternal DHA requirements in pregnancy can be estimated using several methods but all converge on similar values. At term parturition, DHA lost from the mother to the conceptus is dominated by newborn DHA accretion. An average of 14 mg/d DHA is transferred to the foetus through 40 weeks of gestation, though most transfer is in the last 12 weeks during the brain growth spurt. Studies in humans and non-human primates convincingly demonstrate that the omega-3, PUFA, ALA and EPA do not serve as efficient precursors to DHA. However, tissue DHA increases in a saturable dose-response manner to dietary preformed DHA, and consumption of preformed DHA leads to tissue DHA not achieved by consumption of precursors.

RCT of DHA or DHA+EPA intake have shown positive effects on cognitive development in breastfed infants whose mothers had incremental intake increases as low as +100 mg/d (Colombo *et al.*, 2004). Furthermore, DHA supplements of 200–400 mg/d prevented signs of DHA deficiency based on the development of visual acuity (Innis and Friesen, 2008). Finally, a major prospective, observational study demonstrated that greater maternal intake of seafood was associated with lower risk of suboptimum verbal IQ. The dose-response curve indicates that most of the benefit to the child is obtained at about 300 mg/d (0.1%E) LCPUFA derived from seafood; about half of this is DHA (Hibbeln *et al.*, 2007) (Figure 7.2). Notably, further consideration of this study has led to an estimate that 445–830 mg/d EPA+DHA from seafood during pregnancy prevented risk of maternal depression and adverse neurodevelopmental outcomes for 98% of the population, consistent with recent results in a similarly sized study. A summary of the studies related to DHA supplementation not related to pregnancy outcome is provided in Table 7.3. Together these data support a DHA intake in pregnancy of average nutrient requirement (ANR) = 200 mg/d. There is no evidence for detrimental effects of high levels of dietary DHA, EPA, or AA in the diets. Studies are available to support U-AMDR (DHA) = 1.0 g/d, U-AMDR (EPA+DHA) = 2.7 g/d, and U-AMDR (AA) = 0.8 g/d.; all of these levels are NOAEL (no observable adverse effect level). A large methodologically strong randomized controlled trial of 800 mg DHA/100 mg EPA in pregnancy appeared as the consultancy report was going to press (Makrides *et al.*, 2010). A 14% reduction in depression in the DHA group was not significant ($p<0.09$) evaluated by intent-to-treat analysis. Notably, the actual control group depression rate (11.2%) was 33% lower than used for study powering (16.9%).

Preterm birth less than 34 weeks was significantly reduced in the DHA group (2.25% vs. 1.09%) and the rate of post-term medical/surgical delivery increased in the DHA group. Admissions to neonatal intensive care units were reduced (3.08% vs 1.75%, p<0.04), and neonatal deaths were non-significantly but suggestively reduced (1.00% vs 0.33%, p<0.06). The DHA group had fewer infants with delayed cognitive development, and girls, only, had a greater rate of delayed language, all assessed with Bayley scales once at 18 months.

TABLE 7.2
Recommended NIV in pregnancy and lactation

Type of fatty acid	Average nutrient requirement	Upper nutrient limits
	ANR	UNL
DHA	200 mg/d	1.0 g/d[a]
DHA+EPA	300 mg/d[b]	2.7 g/d[a]
AA		800 mg/d[a]
Industrial *trans* fatty acids		As low as practical

[a] NOAEL: No observed adverse effect level in RCT

[b] Based on minimum adult AMDR plus an increment for energy demands of pregnancy, as discussed in the text

As shown in Table 7.1, the L-AMDR lower limit for men and non-pregnant/non-lactating women is set at 250 mg/d EPA+DHA, based on benefit for prevention of cardiovascular disease. Although food sources of LCPUFA will, on average, provide similar amounts of EPA and DHA, strict interpretation of the L-AMDR allows for DHA to range between 0 and 250 mg/d. Women seeking to follow this guideline and allow an increment for additional energy demands of reproduction may consider an average increment of 300 kcal/d (1256 kJ/d) in pregnancy, and 500 kcal/d (2093 kJ/day) in lactation. Total energy requirements in pregnancy and lactation are then 2300 kcal/d (9630 kJ/d) and 2500 kcal/d (10467 kJ/day), respectively, which correspond to increments of 115% and 125% above the non-pregnant, non-lactating levels. These lead to total recommended EPA+DHA intakes of 288-313 mg/d. Rounding for ease of reference leads to the recommendation for an intake of at least 300 mg/d EPA+DHA, of which 200 mg/d are DHA. Refer to Table 7.2.

Trans fatty acids in partially hydrogenated vegetable oils (PHVO) are transmitted from mother to foetus during pregnancy and from mother to infant in breast milk (Koletzko and Muller 1990). They have been associated with several negative outcomes related to conception, foetal loss, and growth (Albuquerque *et al*., 2006; Morrison *et al*., 2008; Pisani *et al*., 2008a; Pisani *et al*., 2008b). The vulnerability of the mother-foetus/infant pair suggests that industrially-derived *trans* fatty acids should be as low as practical for pregnant and lactating women.

There is no evidence that the requirement for total fat, as a percentage of energy, is different in pregnancy or lactation. Similarly, there is no compelling evidence that requirements for saturated, monounsaturated, or total PUFA is different in pregnancy or lactation. Thus, no change in the AMDR for these nutrients is recommended.

TABLE 7.3
RCT of n-3 LCPUFA in pregnancy and lactation that report functional outcomes other than birth outcomes (gestational length, birth weight, birth length)

Study	Participants [a]	Test Dose	Dose	Duration	Primary Functional Outcome (n.s.) = not significant	Comments
Makrides (Makrides et al., 2010)	N=1197 (fish) N=1202 (contr)	Fish oil	800 mg DHA 100 mg EPA	Wk 19 to delivery	Maternal depression (n.s., p<0.09)	Very premature delivery (<34 weeks) reduced (2.25%% vs 1.09%) Greater rates of postterm medical/surgical induction, Lower risk of cognitive delay at 18 mo Girls only, delayed language at 18 mo
Innis (Innis and Friesen, 2008)	N=135	Algal oil	400 mg DHA	Wk 16 to delivery	Distribution of visual acuity suggestive of DHA deficiency in girls	Visual acuity at 60 d (n.s.) Study was explicitly not designed to detect group differences
Olsen (Olsen *et al*., 2008)	N=19 of 266	Fish oil	1.6 g EPA 1.1 g DHA or 4 g Olive oil	Wk 30 to delivery	Reduced asthma and allergic asthma at 16 y	Small subset of participants from Olsen 92 (Olsen *et al*., 1992)
Krauss-Etschmann (Krauss-Etschmann et al., 2008)	N=195	Fish oil	0.5 g DHA 0.15 g EPA	Wk 22 to delivery	Cord blood mRNA CCr4,IL-13,IL-4 lower and TGF-β higher	
Judge (Judge *et al*., 2007b)	N=29	Cereal-based bar with low EPA fish oil	0.214 g DHA	Wk 24 to delivery	Problem-solving improved in DHA group, age 9 m Fagan test of infant intelligence (n.s.)	Participants overlap in these studies Participants basal DHA intake averaged 80 mg/day
Judge (Judge *et al*., 2007a)	N=30	Cereal-based bar with low EPA fish oil	0.214 g DHA	Wk 24 to delivery	Visual acuity (Teller cards) improved at age 4 m; at age 6 m (n.s.)	

TABLE 7.3 (continued)
RCT of n-3 LCPUFA in pregnancy and lactation that report functional outcomes other than birth outcomes (gestational length, birth weight, birth length)

Study	Participants [a]	Test Dose	Dose	Duration	Primary Functional Outcome (n.s.) = not significant	Comments
Tofail (Tofail *et al*., 2006)	N=249	Fish oil	1.2 g DHA; 1.8 g EPA or 2.3 g LA; 0.27 g ALA	Wk 25 to delivery	Bayley MDI and PDI, age 10 m (n.s.)	PDI 95%CI (-4.3, 0.1) by multiple regression
Jensen (Jensen et al., 2005)	N~165	Algal oil	200 mg/d DHA	Postpartum 5-120 d	Bayley PDI greater at 30 mo VEP amplitude lower at 4, 8 mo Visual acuity (VEP, Teller cards) (n.s.) Several other neurodevelopmental outcomes (n.s.)	
Lauritzen (Lauritzen *et al*., 2004)	N=97	Fish oil	1 g DHA 0.5 g EPA	Postpartum 1 wk to 4 m	Visual acuity (VEP) (n.s.)	Infant RBC DHA correlated with visual acuity at 4 months
Lauritzen (Lauritzen *et al*., 2005b)	N=122	Fish oil	1 g DHA 0.5 g EPA	Postpartum 1 wk to 4 m	Problem solving at 9 m (n.s.) Passive vocabulary at 1 y lower in Fish, at 2 y (n.s.)	Word comprehension at age 1 y inversely correlated with 4 mo RBC-DHA
Lauritzen (Lauritzen *et al*., 2005a)*	N=72	Fish oil	1 g DHA 0.5 g EPA	Postpartum 1 wk to 4 m	Weight, height at 2.5 y (n.s.) BMI greater, head circumference greater at 2.5 y	BMI correlated with maternal DHA at 4 m postpartum
Lauritzen (Lauritzen *et al*., 2005c)	N=65	Fish oil	1 g DHA 0.5 g EPA	Postpartum 1 wk to 4 m	LPS stimulated IFNγ higher at 2.5 y	No difference in IL-10 mean.s.; difference in IL-10 distribution
Larnkjaer (Larnkjaer *et al*., 2006)	N=66	Fish oil	1 g DHA 0.5 g EPA	Postpartum 1 wk to 4 m	Blood pressure, electrocardiogram pulse wave velocity, heart rate, heart rate variability at 2.5 y (n.s.)	--
Columbo (Colombo *et al*., 2004)	N=70	Eggs	133 mg DHA Or 33 mg DHA	Wk 24-28 to delivery	Mental processing (look duration) improved with high DHA at ages 4 and 6 m; 8 m (n.s.) Increase in examining and less distractability between age 1 and 2 y; attentional disengagement (n.s.)	Subset of participants in (Smuts *et al.*, 2003b)
Dunstan (Dunstan *et al.*, 2003a)	N=83 of 98	Fish Oil	2.2 g DHA 1.1 g EPA	20 wk to delivery	Cord blood cytokine responses to cat allergen (n.s.); IL-10 response lower with fish oil	--
Dunstan (Dunstan *et al.*, 2003b)	N=83 of 98	Fish Oil	2.2 g DHA 1.1 g EPA	20 wk to delivery	Cord blood IL-4, IL-5, IL-6, IL-12 (n.s.) IL-13 lower with fish o	Less severe disease at age 1 y
Dunstan (Dunstan *et al.*, 2007)	N~60	Fish Oil	2.2 g DHA 1.1 g EPA	20 wk to delivery	Cognitive scores correlated with breast milk DHA and EPA at 2.5 y	
Dunstan (Dunstan *et al.*, 2008)	N = 72 of 98	Fish Oil	2.2 g DHA 1.1 g EPA	20 wk to delivery	Eye-hand coordination favored Fish at age 2.5 y	Cord blood RBC DHA and EPA correlated with eye-hand coordination and inversely with AA

TABLE 7.3 (continued)
RCT of n-3 LCPUFA in pregnancy and lactation that report functional outcomes other than birth outcomes (gestational length, birth weight, birth length)

Study	Participants[a]	Test Dose	Dose	Duration	Primary Functional outcome	Comments
Prescott (Prescott *et al.*, 2007)	N = 98	Fish oil	2.2g DHA 1.1 g EPA	week 20 to delivery	Cord blood neutrophil LTB4, IL-6, IL-10 stimulated production lower with fish oil	-
Malcolm (Malcolm *et al.*, 2003a; Malcolm *et al.*, 2003b)	N~25[b]/group	Fish oil blend 40% DHA 7% EPA 4% DPA(n-6)	200 mg/d fish oil or sunflower oil	15 wk to delivery	Visual acuity (VEP, ERG) (ns)	Sig correlations found for VEP and ERG with infant DHA status
Helland (Helland *et al.*, 2001)	N=341	Cod liver oil	10 g/da 1.18 g DHA 0.80 g EPA	18 wk of pregnancy to 13 wk postpartum	EEG (ns) Fagan (ns)	Sig correlation of EEG with umbilical plasma PL DHA
Helland (4 y) (Helland *et al.*, 2003)	N = 84	Cod liver oil	10 g/da 1.18 g DHA 0.80 g EPA	18 wk of pregnancy to 13 wk postpartum	IQ favoring cod liver oil at age 4 y	Sig correlation of IQ with maternal DHA intake
Helland (7 y) (Helland *et al.*, 2008)	N =142	Cod liver oil	10 g/da 1.18 g DHA 0.80 g EPA	18 wk of pregnancy to 13 wk postpartum	IQ (ns)	Sig correlation of IQ at age 7 with maternal PL DHA & ALA in late pregnancy
Gibson (Gibson *et al.*, 1997)	N=52	Algal oil	0, 0.2, 0.4, 0.9, 1.3 g DHA	Day 5 to wk 12	Bayley MDI correlated with 12 wk breast milk, infant RBC, plasma DHA at age 1 y but not 2 y. Visual acuity (VEP) at 12 and 16 wk (ns).	
Treatment for Depression						
Su (Su *et al.*, 2008)	N=24	Menhaden oil	1.2 g DHA 2.2 g EPA	Mid-gestation, duration 8 wks	Depressive symptoms reduced; higher response rate to treatment.	Strong responders to placebo ineligible for randomization to treatment
Freeman (Freeman *et al.*, 2008)	N=51	Fish oil	0.8 g DHA 1.1 g EPA	Mid-gestation, duration 8 wks	Depressive symptoms (ns)	
Rees (Rees *et al.*, 2008)	N=26	Fish oil	1.6g DHA 0.4 d EPA	6 weeks total in trial [Wk 28 to 6 m postpartum	Depressive symptoms (ns)	

n.s. = Not significant; MDI = Mental Development Index; PDI = Psychomotor Development Index; VEP = visuvisual evoked potential; ERG = electroretinography.

[a] Total subjects all groups unless otherwise indicated.

[b] N varied based on outcome.

REFERENCES

Albuquerque, K.T., Sardinha, F.L. ,Telles, M.M., Watanabe, R.L., Nascimento, C.M., Tavares do Carmo, M.G. & Ribeiro, E.B. 2006. Intake of *trans* fatty acid-rich hydrogenated fat during pregnancy and lactation inhibits the hypophagic effect of central insulin in the adult offspring. *Nutrition*, 22(7-8): 820-829.

Brenna, J.T., Varamini, B., Jensen, R.G., Diersen-Schade, D.A., Boettcher, J.A. & Arterburn, L.M. 2007. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. *Am J Clin Nutr* 85:1457-1464.

Bulstra-Ramakers, M.T., Huisjes, H.J. & Visser, G.H. 1995. The effects of 3g eicosapentaenoic acid daily on recurrence of intrauterine growth retardation and pregnancy induced hypertension. *Br. J. Obstet. Gynaecol*., 102(2): 123-126.

Colombo, J., Kannass, K.N., Shaddy, D.J., Kundurthi, S., Maikranz, J.M., Anderson, C.J., Blaga, O.M. & Carlson, S.E. 2004. Maternal DHA and the development of attention in infancy and toddlerhood. *Child Dev*., 75(4): 1254-1267.

D'Almeida, A., Carter, J.P., Anatol, A. & Prost, C. 1992. Effects of a combination of evening primrose oil (gamma linolenic acid) and fish oil (eicosapentaenoic + docahexaenoic acid) versus magnesium, and versus placebo in preventing pre-eclampsia. *Women's Health*, 19(2-3): 117-131.

Dunstan, J.A., Mitoulas, L.R., Dixon, G., Doherty, D.A., Hartmann, P.E., Simmer, K. & Prescott, S.L. 2007. The effects of fish oil supplementation in pregnancy on breast milk fatty acid composition over the course of lactation: A randomized controlled trial. *Pediatr. Res.*, 12: 12.

Dunstan, J.A., Mori, T.A. Barden, A., Beilin, L.J., Taylor, A.L., Holt, P.G. & Prescott, S.L. 2003a. Fish oil supplementation in pregnancy modifies neonatal allergen-specific immune responses and clinical outcomes in infants at high risk of atopy: a randomized controlled trial. *J. Allergy Clin. Immunol.*, 112(6): 1178-1184.

Dunstan, J.A., Mori, T.A., Barden, A., Beilin, L.J., Taylor, A.L., Holt, P.G. & Prescott, S.L. 2003b. Maternal fish oil supplementation in pregnancy reduces interleukin-13 levels in cord blood of infants at high risk of atopy. *Clin. Exp. Allergy*, 33(4): 442-448.

Dunstan, J.A., Simmer, K., Dixon, G. & Prescott, S.L. 2008. Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. *Arch. Dis. Child Fetal. Neonatal. Ed.*, 93(1): F45-50.

FAO. 1994. *Fats and oils in human nutrition; Report of a joint FAO/WHO expert consultation*. FAO Food and Nutrition Paper 57, FAO. Rome.

Freeman, M.P., Hibbeln, J.R., Wisner, K.L., Davis, J.M., Mischoulon, D., Peet, M., Keck Jr., P.E., Marangell, L.B., Richardson, A.J., Lake, J. & Stoll, A.L. 2006. Omega-3 fatty acids: evidence basis for treatment and future research in psychiatry. *J. Clin. Psychiatry*, 67(12): 1954-1967.

Freeman, M.P., Davis, M., Sinha, P., Wisner, K.L., Hibbeln, J.R., & Gelenberg, A.J. 2008. Omega-3 fatty acids and supportive psychotherapy for perinatal depression: A randomized placebo-controlled study. *J. Affect. Disord*., 110(1-2): 142-148.

Ghafoorunissa, S.A. 2005. Dietary Fat and Diet-Related Chronic Diseases: Indian Perspective. *In Touch. (Heinz Nutrition Foundation India)*, 7(2): 2-6.

Ghafoorunissa, S.A. 1996. Fats in Indian diets and their nutritional and health implications. *Lipids*, 31 (Suppl.): S287-291.

Ghafoorunissa, S.A. 1998. Requirements of dietary fats to meet nutritional needs & prevent the risk of atherosclerosis--an Indian perspective. *Indian J. Med. Res*., 108: 191-202.

Gibson, R.A., Neumann, M.A. & Makrides, M. 1997. Effect of increasing breast milk docosahexaenoic acid on plasma and erythrocyte phospholipid fatty acids and neural indices of exclusively breast fed infants. *EJCN*., 51(9): 578-584.

Helland, I.B., Saugstad, O.D., Smith, L., Saarem, K., Solvoll, K., Ganes, T. & Drevon, C.A. 2001. Similar effects on infants of n-3 and n-6 fatty acids supplementation to pregnant and lactating women. *Pediatrics*, 108(5): E82.

Helland, I.B., Smith, L., Blomen, B., Saarem, K., Saugstad, O.D. & Drevon, C.A. 2008. Effect of supplementing pregnant and lactating mothers with n-3 very-long-chain fatty acids on children's IQ and body mass index at 7 years of age. *Pediatrics*, 122(2): e472-479.

Helland, I.B., Smith, L., Saarem, K., Saugstad, O.D. & Drevon, C.A. 2003. Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics*, 111(1): e39-44.

Hibbeln, J.R., Davis, J.M., Steer, C., Emmett, P., Rogers, I., Williams, C. & Golding, J. 2007. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. *Lancet*, 369(9561): 578-585.

Horvath, A., Koletzko, B. & Szajewska, H. 2007. Effect of supplementation of women in high-risk pregnancies with long-chain polyunsaturated fatty acids on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. *Br. J. Nutr*., 98(2): 253-259.

Innis, S.M. & Friesen, R.W. 2008. Essential n-3 fatty acids in pregnant women and early visual acuity maturation in term infants. *Am. J. Clin. Nutr*., 87(3): 548-557.

Jensen, C.L., Voigt, R.G., Prager, T.C., Zou, Y.L., Fraley, J.K., Rozelle, J.C., Turcich, M.R., Llorente, A.M., Anderson, R.E. & Heird, W.C. 2005. Effects of maternal docosahexaenoic acid intake on visual function and neurodevelopment in breastfed term infants. *Am. J. Clin. Nutr*., 82(1): 125-132.

Judge, M.P., Harel, O. & Lammi-Keefe, C.J. 2007a. A docosahexaenoic acid-functional food during pregnancy benefits infant visual acuity at four but not six months of age. *Lipids*, 42(2): 117-122.

Judge, M.P., Harel, O. & Lammi-Keefe, C.J. 2007b. Maternal consumption of a docosahexaenoic acid-containing functional food during pregnancy: benefit for infant performance on problem-solving but not on recognition memory tasks at age 9 mo. *Am. J. Clin. Nutr*., 85(6): 1572-1577.

Koletzko, B. & Muller, J. 1990. Cis- and *trans*-isomeric fatty acids in plasma lipids of newborn infants and their mothers. *Biol. Neonate*, 57(3-4): 172-178.

Krauss-Etschmann, S., Hartl, D., Rzehak, P., Heinrich, J., Shadid, Del Carmen M., Ramirez-Tortosa, C., Campoy, S., Pardillo, D.J., Schendel, T., Decsi, H., Demmelmair & Koletzko, B.V. 2008. Decreased cord blood IL-4, IL-13, and CCR4 and increased TGF-beta levels after fish oil supplementation of pregnant women. *J. Allergy Clin. Immunol.*, 121(2): 464-470 e466.

Larnkjaer, A., Christensen, J.H., Michaelsen, K.F. & Lauritzen, L. 2006. Maternal fish oil supplementation during lactation does not affect blood pressure, pulse wave velocity, or heart rate variability in 2.5-y-old children. *J. Nutr.*, 136(6): 1539-1544.

Lauritzen, L., Hoppe, C., Straarup, E.M. & Michaelsen, K.F. 2005a. Maternal fish oil supplementation in lactation and growth during the first 2.5 years of life. *Pediatr Res* 58(2): 235-242.

Lauritzen, L., Jorgensen, M.H., Mikkelsen, T.B., Skovgaard, M. Straarup, E.M., Olsen, S.F., Hoy, C.E., Michaelsen, K.F. 2004. Maternal fish oil supplementation in lactation: effect on visual acuity and n-3 fatty acid content of infant erythrocytes. *Lipids*, 39: 195-206.

Lauritzen, L., Jorgensen, M.H., Olsen, S.F.. Straarup, E.M. & Michaelsen, K.F. 2005b. Maternal fish oil supplementation in lactation: effect on developmental outcome in breast-fed infants. Reprod. Nutr. Dev., 45(5): 535-547.

Lauritzen, L., Kjaer, T.M., Fruekilde, M.B., Michaelsen, K.F. & Frokiaer, H. 2005c. Fish oil supplementation of lactating mothers affects cytokine production in 2 1/2-year-old children. *Lipids*, 40(7): 669-676.

Lin, P.Y. & Su, K.P. 2007. A meta-analytic review of double-blind, placebo-controlled trials of antidepressant efficacy of omega-3 fatty acids. *J. Clin. Psychiatry*, 68(7): 1056-1061.

Makrides, M., Duley, L. & Olsen, S.F. 2006. Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction. *Cochrane Database Syst. Rev.*, 3(3): CD003402.

Makrides, M., Gibson, R.A., McPhee, A.J., Yelland, L., Quinlivan, J., Ryan, P. & the DOMInO Investigative Team. Effect of DHA Supplementation During Pregnancy on Maternal Depression and Neurodevelopment of Young Children: A Randomized Controlled Trial. 2010. *JAMA* 310(15): 1675-1683.

Malcolm, C.A., Hamilton, R., McCulloch, D.L., Montgomery, C. & Weaver, L.T. 2003a. Scotopic electroretinogram in term infants born of mothers supplemented with docosahexaenoic acid during pregnancy. *Invest. Ophthalmol. Vis. Sci.*, 44(8): 3685-3691.

Malcolm, C.A., McCulloch, D.L., Montgomery, C., Shepherd, A. & Weaver, L.T. 2003b. Maternal docosahexaenoic acid supplementation during pregnancy and visual evoked potential development in term infants: a double blind, prospective, randomised trial. *Arch. Dis. Child Fetal Neonatal Ed.*, 88(5): F383-390.

Moodley, J. & Norman, R.J. 1989. Attempts at dietary alteration of prostaglandin pathways in the management of pre-eclampsia. *Prostaglandins Leukot. Essent. Fatty Acids*, 37(3): 145-147.

Morrison, J.A., Glueck, C.J. & Wang, P. 2008. Dietary *trans* fatty acid intake is associated with increased fetal loss. *Fertil. Steril.*, 90(2): 385-390.

Olsen, S.F., Osterdal, M.L., Salvig, J.D., Mortensen, L.M., Rytter, D., Secher, N.J. & Henriksen, T.B. 2008. Fish oil intake compared with olive oil intake in late pregnancy and asthma in the offspring: 16 y of registry-based follow-up from a randomized controlled trial. *Am. J. Clin. Nutr.*, 88(1): 167-175.

Olsen, S.F., Osterdal, M.L., Salvig, J.D., Weber, T., Tabor, A. & Secher, N.J. 2007. Duration of pregnancy in relation to fish oil supplementation and habitual fish intake: a randomised clinical trial with fish oil. *EJCN.*, 61(8): 976-985.

Olsen, S.F., Secher, N.J. , Tabor, A., Weber, T., Walker, J.J. & Gluud, C. 2000. Randomised clinical trials of fish oil supplementation in high risk pregnancies. Fish Oil Trials In Pregnancy (FOTIP) Team. *BJOG*, 107(3): 382-395.

Olsen, S.F., Sorensen, J.D., Secher, N.J., Hedegaard, M., Henriksen, T.B., Hansen, H.S. & Grant, A. 1992. Randomised controlled trial of effect of fish-oil supplementation on pregnancy duration. *Lancet*, 339(8800): 1003-1007.

Onwude, J.L., Lilford, R.J., Hjartardottir, H., Staines, R.J. & Tuffnell, D. 1995. A randomised double blind placebo controlled trial of fish oil in high risk pregnancy. *Br. J. Obstet. Gynaecol.*, 102(2): 95-100.

Pisani, L.P., Oller do Nascimento, C.M., Bueno, C.M., Biz, C., Albuquerque, K.T., Ribeiro, E.B. & Oyama, L.M. 2008a. Hydrogenated fat diet intake during pregnancy and lactation modifies the PAI-1 gene expression in white adipose tissue of offspring in adult life. *Lipids Health Dis.*, 7: 13.

Pisani, L.P., Oyama, L.M., Bueno, A.A., Biz, C., Albuquerque, K.T., Ribeiro, E.B. & Oller do Nascimento, C.M. 2008b. Hydrogenated fat intake during pregnancy and lactation modifies serum lipid profile and adipokine mRNA in 21-day-old rats. *Nutrition*, 24(3): 255-261.

Prescott, S.L., Barden, A.E., Mori, T.A. & Dunstan, J.A. 2007. Maternal fish oil supplementation in pregnancy modifies neonatal leukotriene production by cord-blood-derived neutrophils. *Clin. Sci. (Lond)*, 113(10): 409-416.

Rees, A.M., Austin, M.P. & Parker, G.B. 2008. Omega-3 fatty acids as a treatment for perinatal depression: randomized double-blind placebo-controlled trial. *Aust. N. Z. J. Psychiatry*, 42(3): 199-205.

Sanders, T.A. & Reddy, S. 1992. The influence of a vegetarian diet on the fatty acid composition of human milk and the essential fatty acid status of the infant. *J. Pediatr.*, 120(4 Pt 2): S71-77.

Smuts, C.M., Borod, E., Peeples, J.M. & Carlson, S.E. 2003a. High-DHA eggs: feasibility as a means to enhance circulating DHA in mother and infant. *Lipids*, 38(4): 407-414.

Smuts, C.M., Huang, M., Mundy, D., Plasse, T., Major, S. & Carlson, S.E. 2003b. A randomized trial of docosahexaenoic acid supplementation during the third trimester of pregnancy. *Obstet. Gynecol.*, 101(3): 469-479.

Su, K.P., Huang, S.Y., Chiu, T.H., Huang, K.C., Huang, C.L., Chang, H.C. & Pariante, C.M. 2008. Omega-3 fatty acids for major depressive disorder during pregnancy: results from a randomized, double-blind, placebo-controlled trial. *J. Clin. Psychiatry*, 69(4): 644-651.

Szajewska, H., Horvath, A. & Koletzko, B. 2006. Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.*, 83(6): 1337-1344.

Tofail, F., Kabir, I., Hamadani, J.D., Chowdhury, F., Yesmin, F., Mehreen, F. & Huda, S.N. 2006. Supplementation of fish-oil and soy-oil during pregnancy and psychomotor development of infants. *J. Health Popul. Nutr.*, 24(1): 48-56.

Chapter 8: Fat and fatty acid intake and inflammatory and immune response

The immune system represents the body's defence against infectious organisms and other environmental disturbances. Its action is based on a complex series of steps (termed the immune response), which a) prevent entry of infectious organisms, b) identify infectious organisms if they do invade, c) eliminate invading infectious organisms, d) retain memory of the encounters. The immune system also ensures that the host is tolerant to its own macromolecules, cells and tissues and to benign environmental substances such as foods (i.e. it does not mount an active immune response against itself). It consists of cells that originate in the bone marrow and which are dispersed throughout the body, including in discrete lymphoid organs such as the thymus, spleen, lymph nodes and mucosal-associated lymphoid tissues. Cells of the immune system circulate in the bloodstream and in the lymph; those found in the bloodstream are collectively termed leukocytes or white blood cells. Each different cell type has a specific function, which contributes to the overall integrated response.

IMMUNITY

There are two types of immunity: innate and acquired, which are essentially functional divisions within the immune response.

Innate immunity

This provides general protection based on the "non-specific" recognition of, and response to, pathogens by immune cells and constitutes the first line of defence. Recognition is not based on specific antigens, but on general structural features of the pathogens. Innate immunity has no memory and so is not influenced by prior exposure to a particular organism. Cells of the innate immune system include phagocytic cells (neutrophils, macrophages, monocytes), natural killer cells, mast cells, eosinophils, and basophils. These cells destroy pathogens by different processes, including phagocytosis and production of toxins (e.g. reactive oxygen species). The inflammatory response represents part of innate immunity. This acts to create a hostile environment for pathogens and to aid movement of leukocytes to sites of infection. For example, chemical mediators produced as part of the inflammatory response induce fever, increase local blood flow, and enhance vascular permeability to allow leukocytes and plasma proteins to move from the bloodstream to extravascular compartments. These actions account for the typical signs of inflammation: redness, swelling, heat and pain.

Acquired (or adaptive) immunity

This type of immunity develops throughout life, and is highly specific. Specificity is induced because of the unique recognition of structures termed antigens on pathogens by antigen-specific host immune cells. Acquired immunity allows for a strong and

specific immune response and for immunological memory. The cells involved include antigen presenting cells (many cell types can present an antigen, but only some of them like dendritic cells are "professional" antigen presenting cells), T lymphocytes (peptide mediators termed cytokines produced by these cells regulate the activity of other immune cells; reactions involving T lymphocytes, or "T cells", are considered to constitute cell-mediated immunity) and B lymphocytes (these are the cells that produce antibodies; reactions involving B lymphocytes, or "B cells", are considered to constitute humoral immunity). A list of some cytokines and their activities is shown in Table 8.1. There are several subsets of T lymphocytes, including helper T cells, central to acquired immune responses, cytotoxic T cells that kill virally infected cells, and two more recently discovered types of regulatory T cells. T cells are also involved in inflammatory processes since they enhance the activity of inflammatory cells such as monocytes and macrophages.

Loss of tolerance can occur and appears to be due to loss of regulatory mechanisms. Loss of tolerance can result in autoimmune diseases, allergic reactions and conditions, or inflammatory bowel diseases. Despite the different stimuli and the different locations of the pathology, all conditions involving loss of tolerance have common elements, including the cells, mediators and signalling systems involved, and they are commonly referred to as "inflammatory conditions". This is because they typically involve movement of cells of the innate immune response to the site of inflammatory activity and the production of the standard profile of inflammatory mediators including peptide mediators (cytokines, chemokines, matrix metalloproteinases), lipid mediators (eicosanoids, platelet activating factor), and reactive oxygen derivatives (superoxide). While these mediators exert local inflammatory responses and damage, some of them spill over into the bloodstream from where they act to elicit systemic inflammatory responses like hepatic acute phase protein synthesis and mobilisation of fuels from adipose tissue and skeletal muscle.

FATTY ACIDS AND INFLAMMATION

Introduction

Studies on humans have largely focussed on the effects of LCPUFA on inflammation (Calder, 2006). This is mainly because lipid-derived mediators involved in the inflammatory response are produced from LCPUFA, mainly the n-6 PUFA AA and the n-3 PUFA EPA and DHA. It is now recognized that mediators produced from these fatty acids are involved in both the activation and the resolution of the inflammatory process.

Lipid mediators in inflammation

AA is quantitatively the most important fatty acid precursor of lipid mediators. Once released from the PL precursor, AA is converted into different members of the eicosanoid family (prostaglandins, thromboxanes, leukotrienes, lipoxins, hydroxy- and hydroperoxyeicosatetraenoic acids) by the sequential action of various enzymes, chief among which are the cyclooxygenases (COX) and lipoxygenases (LOX). These enzymes have different cellular distributions and are induced by different inflammatory stimuli. More recently, analogous mediators derived from EPA (eicosanoids, resolvins, docosanoids) and DHA (resolvins, protectins) have been identified. Increased consumption of EPA and DHA in the diet can decrease the levels of AA in cell membrane PL and can also inhibit AA metabolism. Thus, the relationships between the levels of substrate LCPUFA in inflammatory cell membranes and the production of the bioactive derivatives are quite complex and the production of mediators from LCPUFA depends on substrate PUFA level; the intensity, duration, and nature of the stimulus and the type of cell

TABLE 8.1
Selected cytokines and their activities

Cytokine	Principal producing cells	Main target cells	Function
GM-CSF	Th cells	Progenitor cells	Growth and differentiation of monocytes and DC
IL-1α; IL-1β	Monocytes; macrophages; B cells; DC	Th cells	Co-stimulation
		B cells	Maturation and proliferation
		NK cells	Activation
		Various	Inflammation, acute phase response, fever
IL-2	Th1 cells	Activated T and B cells	Activation, growth and proliferation
		NK cells	
IL-3	Th cells, NK cells	Stem cells	Growth and differentiation
		Mast cells	Growth and histamine release
IL-4	Th2 cells	Activated B cells	Proliferation and differentiation;
			IgG_1 and IgE synthesis
		Macrophages	MHC Class II expression
		T cells	Proliferation
IL-5	Th2 cells	Activated B cells	Proliferation and differentiation; IgG_1 and IgE synthesis
IL-6	Monocytes; macrophages; Th2 cells; stromal cells	Activated B cells	Differentiation into plasma cells
		Plasma cells	Antibody secretion
		Stem cells	Differentiation
		Various	Acute phase response
IL-7	Marrow stroma; thymus stroma	Stem cells	Differentiation into progenitor B and T cells
IL-8	Macrophages; endothelial cells	Neutrophils	Chemotaxis
IL-10	Monocytes; macrophages; Th2 cells	Macrophages;	Anti-inflammatory (e.g. decreases TNF-α synthesis)
		B cells	
IL-12	DC; macrophages; B cells	Activated Tc cells	Differentiation into CTL (with IL-2)
		NK cells	Activation
IFN-α	Leukocytes	Various	Inhibition of viral replication; MHC I expression
IFN-β	Fibroblasts	Various	Inhibition of viral replication; MHC I expression
IFN-γ	Th1 cells; CTL; NK cells	Various	Inhibition of viral replication;
		Macrophages	MHC expression
		Activated B cells	Ig class switch to IgG_{2a}
		Th2 cells	Inhibition of proliferation
		Macrophages	Pathogen elimination
MIP-1α	Macrophages	Monocytes; T cells	Chemotaxis
MIP-1β	Lymphocytes	Monocytes; T cells	Chemotaxis
TGF-β	T cells; monocytes	Monocytes; macrophages	Chemotaxis
		Activated macrophages	IL-1 synthesis
		Activated B cells	IgA synthesis
		Various	Inhibition of proliferation
TNF-α	Macrophages; mast cells; NK cells	Macrophages	Adehesion molecule and cytokine expression
		Tumour cells	Death
TNF-β	Th1; CTL	Phagocytes	Phagocytosis, NO production
		Tumour cells	Death

CTL: cytotoxic T lymphocytes; DC: dendritic cells; GM-CSF: Granulocyte-macrophage colony stimulating factor; IL: Interleukin; IFN: Interferon; MHC = major histocompatability complex; MIP = mcrophage inflammatory protein; TGF: Transforming growth factor; TNF: Tumour necrosis factor.

involved. Therefore, a mix of eicosanoids is usually generated and this mix will change with time after the initial stimulus. Sometimes the eicosanoids produced have opposing actions and so the overall physiological (or pathophysiological) outcome will depend on the timing of eicosanoid generation, the concentrations of the mediators present and the sensitivity of the target cells to the compounds. Most of the products of the AA cascade are pro-inflammatory in their effects (and their production is a recognized pharmacological target). However, it is now recognized that some (e.g. PGE2) have both pro-and anti-inflammatory effects, depending upon the timing of their production, while others (e.g. lipoxin A4) are clearly anti-inflammatory (Calder, 2009a).

The eicosanoid products generated from the n-3 PUFA EPA, are, generally speaking, less potent than those produced from AA. Recently a new family of mediators produced by complex metabolism on EPA or DHA, apparently involving both COX and LOX activity, has been described. These have been termed resolvins and protectins (Bazan, 2007; Serhan *et al*., 2008) (Figure 8.1). These compounds have been demonstrated in experimental systems to possess potent anti-inflammatory and inflammation resolving properties (King *et al*., 2006; Farooqui *et al*., 2007). These may explain many of the clinical effects of n-3 LCPUFA (see below). However, their role in human biology has not yet been demonstrated.

HUMAN STUDIES ON DIETARY FATS AND INFLAMMATION: N-3 PUFA

Introduction

Because of the early recognition that eicosanoids produced from AA are involved in many inflammatory conditions and the observations that n-3 LCPUFA decrease the production of eicosanoids from AA, most clinical studies have focussed on the use of n-3 LCPUFA, usually in the form of fish oil, as a potential therapeutic agent. These clinical studies have been supported by cell and animal studies investigating efficacy and mechanisms involved. n-3 LCPUFA exert several anti-inflammatory effects, but these are dose-dependent and may require quite high intakes. n-3 LCPUFA supplementation studies have been conducted for a number of inflammatory diseases, but the evidence of beneficial effects appears to be greater for some of them, e.g. asthma (in children rather than in adults), inflammatory bowel disease (Crohn's disease, ulcerative colitis) and rheumatoid arthritis (Calder, 2006).

Asthma

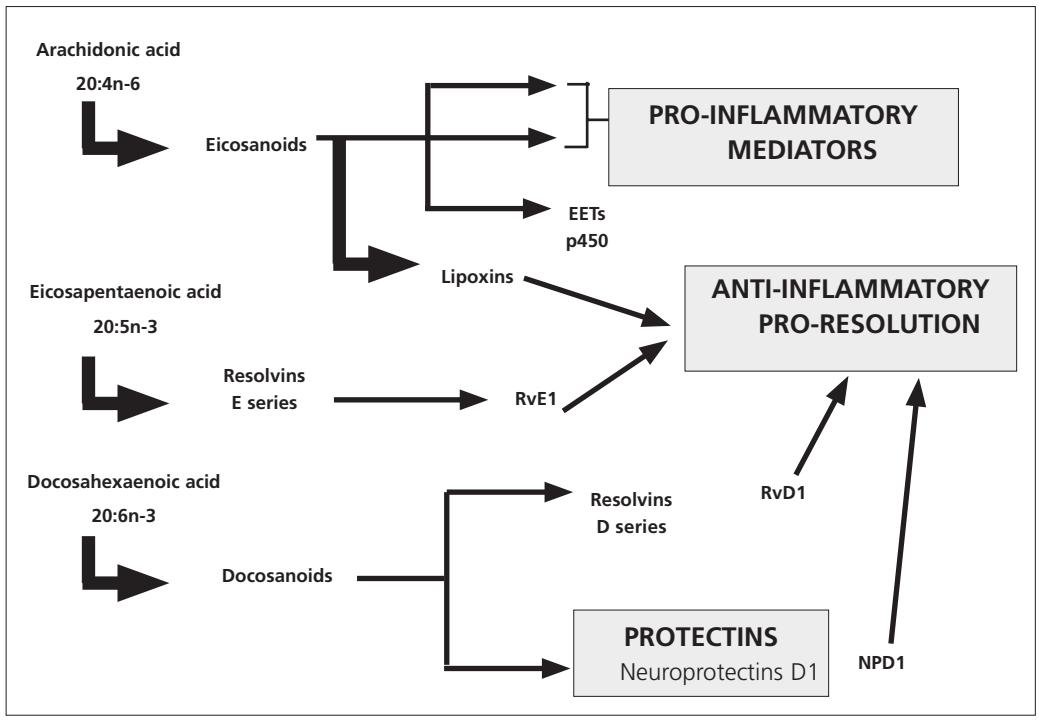
Studies have reported anti-inflammatory effects (reduction of 4-series LT and of leukocyte chemotaxis) of fish oils in asthmatic patients and several uncontrolled trials in adults have shown clinical benefits (Calder, 2006). There have been about 9 randomized, placebo controlled, double blind studies (Calder, 2006 for details). These have used between 1.2 and 5.4 g n-3 LCPUFA/day and lasted 4–52 weeks. Most of these studies have included adults and do not provide evidence of a strong clinical benefit (Schachter *et al*., 2004). One study in children reported a significant benefit of n-3 LCPUFA on lung function and on disease severity (Nagakawa *et al*., 2000), but another similar study in children did not find benefits (Hodge *et al*., 1998).

The overall conclusion is a possible benefit (with adequate dose) in children but with no evidence of benefit in adults.

Inflammatory bowel disease (IBD)

There are two main forms of IBD: Crohn's disease and ulcerative colitis. Beneficial effects n-3 LCPUFA have been demonstrated in animal models of IBD (Calder, 2009b). Dietary fish oils result in incorporation of n-3 LCPUFA into the intestinal mucosa of patients with IBD, and in anti-inflammatory effects, e.g. decreased inflammatory eicosanoid

FIGURE 8.1
Production pathways of mediators derived from LCPUFA



production (Calder, 2009b). There have been about 12 randomized, placebo controlled, double blind studies, which have used between 2.1 and 5.6 g (average about 3.3 g) n-3 LCPUFA/day and lasted 12–104 weeks. Some of these studies report a favourable effect on Crohn's disease, including improved gut histology and better maintenance in remission. However, studies in ulcerative colitis do not indicate any benefit. The overall conclusion is a possible benefit (with adequate dose) in Crohn's disease, but there is insufficient evidence of benefit in ulcerative colitis.

RHEUMATOID ARTHRITIS (RA)

Pharmacological inhibition of the COX pathway (i.e. AA metabolism) is beneficial in treatment of RA symptoms. Beneficial effects n-3 LCPUFA have been shown in animal models of RA and in a number of randomized, placebo controlled, double blind studies (Calder, 2009c). About 20 of the latter studies have been conducted. These have used between 2.1 and 7 g (average about 3.3 g) n-3 LC PUFA/day and their duration was 12–52 weeks. Almost all of these studies report a favourable effect, many reporting several favourable effects (e.g. reduced number of swollen or tender joints, decreased duration of morning stiffness, reduced use of anti-inflammatory medication). Meta-analyses confirm the benefit on these outcomes (Calder, 2009c) and there is convincing evidence of a benefit with an adequate dose.

Role of dietary ALA in modulating inflammation

ALA can exert anti-inflammatory effects but is much less potent than the n-3 LC PUFA (Burdge and Calder, 2006). It is likely that the effects of ALA involve its conversion to EPA and beyond. Few studies examining efficacy of ALA in inflammatory disease have been performed, but where it has been used (e.g. in RA) it has not been effective and there is insufficient evidence of any benefit.

Human studies on dietary fats and inflammation: other fatty acids

Olive oil has frequently been used as a placebo in randomized clinical trials of fish oil in inflammatory conditions (e.g. in RA). There is insufficient evidence that MUFA affect inflammatory processes. Cell culture studies indicate that trans isomers of linoleic acid (*trans*-C18:2) and oleic acid (*trans*-C18:1) may have stronger pro-inflammatory effects than palmitoleic acid (*trans*-C16:1), but there is little information in the human context and further research is needed. Cell culture studies suggest that SFA directly provoke inflammatory processes. Consumption of SFA in humans impairs the anti-inflammatory properties of HDL and endothelial functions, but there is little information about SFA on inflammatory outcomes in humans. There is insufficient evidence of the involvement of other fatty acids.

CONCLUSIONS

Dietary fats play a role in modulating immune functions and inflammatory processes. Most of the impact is attributed to the LCPUFA, with some opposing effects at the cellular levels of the n-6 and n-3 LCPUFA. Of these two families of fatty acids the actions of n-3 LC PUFA are most clearly described. Mechanistic, animal model and human studies provide evidence of anti-inflammatory efficacy of n-3 LCPUFA that is dose-dependent and involves a variety of mechanisms that target key inflammatory processes. n-3 LCPUFA have been examined in many randomized controlled trials investigating clinical outcomes that have typically used quite high intakes of n-3 LCPUFA. Evidence of a benefit is strong in rheumatoid arthritis but weaker in other conditions. However, there are no studies of prevention of inflammatory disease by n-3 LCPUFA (only their potential therapeutic effect has been studied) and there is no information on n-3 LCPUFA requirements in individuals affected by inflammatory disease and how this might change during the life cycle. Other FA may contribute to modulate inflammatory processes and may thereby affect pathophysiological states (e.g. CVD, obesity and related conditions) in which inflammation is involved, but the impact of these other FA has been little studied. More extensive and carefully planned research is required to define fully the overall impact of the whole FA spectrum in the diet on inflammation.

RECOMMENDATIONS

Recommendations for optimal fat intakes for prevention, and, to some extent, treatment of inflammatory processes, are rather similar to those that are applied for the optimization and maintenance of other aspects of human health (control of body weight, cardiovascular function, prevention of cancers). Intakes of around 3 g n-3 LCPUFA/day are recommended for some chronic diseases, especially rheumatoid arthritis. Recommendations should be tailored to individuals on the basis of the assessment of their FA status and general situation.

REFERENCES

Bazan, N.G. 2007. Omega-3 fatty acids, pro-inflammatory signaling and neuroprotection. *Curr. Opin. Clin. Nutr. Metab. Care*, 10: 136-141.

Burdge, G.C. & Calder, P.C. 2006. Dietary α-linolenic acid and health-related outcomes: a metabolic perspective. *Nutr. Res. Rev.*, 19: 26-53.

Calder, P.C. 2006. N-3 polyunsaturated fatty acids, inflammation and inflammatory diseases. *Am. J. Clin. Nutr.*, 83 (Suppl.): 1505S-1519S.

Calder, P.C. 2009a. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. *Biochimie*, 91: 791-795.

Calder, P.C. 2009b. Fatty acids and immune function: relevance to inflammatory bowel diseases. *Int. Rev. Immunol.*, 28: 506-534.

Calder, P.C. 2009c. Polyunsaturated fatty acids and inflammation: therapeutic potential in rheumatoid arthritis. *Curr. Rheumatol. Rev.*, 5, 214-225.

Farooqui, A.A., Horrocks, L.A. & Farooqui, T. 2007. Modulation of inflammation in brain: a matter of fat. *J. Neurochem.*, 1010: 577-599.

Hodge, L., Salome, C.M., Hughes, J.M. *et al.* 1998. Effects of dietary intake of omega-3 and omega-6 fatty acids on severity of asthma in children. *Eur. Respir. J.*, 11: 361-365.

King, V.R., Huang, W.L., Dyall, W.L., Curran, O.E., Priestly, J.V. & Michael Titus, A.T. 2006. Omega 3 fatty acids improve recovery, whereas omega 6 fatty acids worsen outcome, after spinal cord injury in the adult rat. *J. Neurosci.*, 26: 4672-4680.

Nagakura, T., Matsuda, S., Shichijyo, K., Sugimoto, H. & Hata, K. 2000. Dietary supplementation with fish oil rich in omega-3 polyunsaturated fatty acids in children with bronchial asthma. *Eur. Respir. J.*, 16: 861-865.

Schachter, H., Reisman, J., Tran, K. *et al.* 2004. Health effects of omega 3 fatty acids on asthma. Evidence report/technical assessment n. 91. AHRQ publication n. 04-E013-2. Agency for Healthcare Research and Quality, Rockville, MD, USA.

Serhan, C.N., Chiang, N. & Van Dyke, T.E. 2008. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nature Reviews/Immunology*, 8: 349-361.

Chapter 9: Total fat, fatty acid intake and cancers

The relationship between fat intake and cancer has been investigated extensively for more than two decades. However, it is still debated despite substantial increases in scientific studies and improvements made in food composition tables, epidemiological methodologies and statistical methods. Assuming that the highly multi-factorial character of cancers contributes to the complexity, two issues are crucial to understanding why it is difficult to arrive at a firm conclusion on the relationship between fat intake and cancer.

Is there convincing evidence that obesity increases the risks for colorectal cancer (CRC), endometrial cancer and postmenopausal breast cancer? Does total fat contribute to obesity and if so in what way? The Expert Consultation affirmed that there is no direct relationship between total fat and obesity, and that it is energy imbalance, the nutrients contributing to it, and life styles that are responsible for obesity.

Do fatty acids play a specific role in cancer development beyond their contribution to providing energy? This question is particularly relevant to PUFA, especially n-3, and *trans* FA, not only because their contribution to energy intake is low, but because they are endowed with specific functional properties. However, foods that contribute mainly to the intake of these FA provide special characteristics in their own right. For example, the source of n-3 LCPUFA is essentially fish, and fish is also a source of vitamin D and selenium, both being credited with a possible protective effect against some cancers. Thus, if a reduction in risk associated with fish consumption is observed, it could be entirely or partly due to these other nutrients. Moreover, if fish substitutes for meat, which is recognized as a risk factor for colorectal cancer, any observed reduction in risk may be erroneously attributed to fish and its nutrients. *Trans* FA, which are often found in processed and energy-dense foods, being part of the Western diet pattern, are suspected to be a risk factor associated with several cancers (Chajes *et al*., 2008; Liu *et al*., 2007; Chavarro *et al*. 2008).

Thus observational epidemiology alone may be unable to provide sufficient evidence to conclude with certainty whether or not the quantity or type of fat in the diet has any effect on the risk of developing any type of cancer. Experimental studies and biological plausibility may contribute complementary lines of evidence and rationale and could help to reach a conclusion in some situations. Therefore, a portfolio (or mosaic) approach has been used to estimate the strength of the evidence, based on studies selected on methodological criteria, starting from previous expert reports (AFSSA, 2003; WCRF/AICR, 2007) and updated to September 2008.

One can also ask if is it yet possible to give figures on fatty acid intake aimed at reducing cancer risk following a thorough literature review of the evidence for association of fatty acid intake and risk of cancer incidence. Since it has been proposed to use disease outcome as an indicator of adequacy or optimal intake, recommendations will be proposed. These recommendations are quantitative whenever judged possible. This exercise is constrained by several limitations. Many of the studies do not quantify the FA associated with cancer risk; a food frequency questionnaire, which is the main tool for exposure assessment, is subject to measurement error (Bingham *et al*., 2003; Kipnis and Freedman, 2008) and hence the exposure values are supposed to classify

the cases relatively to controls, and cannot be considered absolute. However, where several different studies result in comparable figures, one could tentatively suggest a range of values for recommendations.

TOTAL FAT AND ITS RELATIONSHIP WITH VARIOUS TYPES OF CANCER

Colorectal cancer

Because there is a strong correlation between energy and total fat intakes in high-income countries, where most of the relevant studies have been conducted (Astorg *et al*., 2004), energy may confound any effect of total fat. This is shown in two case-control studies on colorectal cancer (Gerber, 2009) where adjustment of energy using the residual method negated the increased risk associated with total fat intake (Theodoratou *et al*., 2007), whereas the increased risk persisted when an adjustment was made for total energy (Hu *et al*., 2007). Taking into account this evidence, the consultation concluded that it is very likely that total fat confounds the effect of energy and that total fat *per se* does not contribute to CRC risk.

Breast cancer

Not all study results are in agreement on the relationship between total fat intake and breast cancer (BC). A suggestive, but limited, relationship was reported by the WCRF/AICR (2007). However, meta-analysis of 22 case-control studies presented in the support resource of the systematic literature review shows a modest but significant increased risk (OR=1.03; CI: 1.02-1.04, for an increment of 20 g/day of total fat) and that of seven case-control studies showed an overall OR of 1.11 (CI: 1.03-1.06). In addition, the Women's Health Initiative study (Prentice *et al*., 2006) reported a risk reduction for BC following a low-fat diet, though of borderline significance. In addition, several lines of evidence tend to confirm this effect: 1) it was stronger in women with the highest baseline fat intake; 2) hormone concentrations decreased in the experimental group, but were not modified in the control group. This change in hormone concentration is observed in women treated with anti-aromatase, and adipose tissue aromatase is believed to be responsible for extragonadal hormone synthesis; 3) sex-hormone-binding-globuline (SHBG) decreased in the experimental group and was not modified in the control group. Decreased SHBG releases free testosterone, and to a lesser extent estradiol, which are risk factors for BC and 4) among the 17 baseline demographic, medical history, and health behaviour variables applied to an unweighted proportional hazards model stratified by age and randomization group, the test for interaction was statistically significant for elevated hypertension and leukocyte count (evoking possible inflammation), both of which occur in metabolic syndrome. In addition, one recent, large prospective study (Thiébaut *et al*., 2007) showed a moderate but significant increased risk of BC with high intake of total fat (Gerber, 2009).

Because of the contradictory results in prospective studies and because of the absence of a relationship between total fat and obesity, the Expert Consultation concluded that there was an absence of a specific effect of total fat on BC at the probable level.

Data from BC survivors suggest a favourable effect of a low total fat intake. Several studies (Borugian *et al*., 2004[1]; McEligot *et al*., 2006[2]) and one intervention assay

[1] Cohort of 603 patients with BC, 112 fatal events; nutritional assessment by FFQ Block questionnaire. RR: 4.8 (1.3-18.1) and T: 0.08 in premenopausal women.

[2] Cohort of 512 patients with BC. HR for mortality was 3.12 (1.79-5.44) and T: <0.05.

(Cheblowski *et al*., 2006) showed better survival in BC patients with lower intake of total fat. Although the data are consistent, the consultation believed that the evidence was insufficient to conclude a definitive effect.

Endometrial cancer

A meta-analysis of 9 case-control studies and 3 recent studies agree on an association of total fat intake with an increased risk, but 2 prospective studies did not show such an association (Gerber, 2009). The Expert Consultation concluded that although based on limited data, there is a probable relationship between total fat and endometrial cancer through constitution of body fatness.

Ovarian cancer

There are very few studies and only an intervention assay revealed clear, significant results (Prentice *et al.*, 2007). Thus the consultation concluded that the data are too limited to reach a conclusion.

ANIMAL FAT

Animal fat has been linked with colon-rectum, endometrial and ovarian cancers. For these three cancers, given that animal fat is most often a component of energy-dense food, its effect can be confounded by energy. In addition, for colorectal cancer it might be confounded by some characteristics of the meat (Theodoratou *et al*. 2008). Data are too scarce to reach a conclusion for ovarian and endometrial cancer, but the WCRF/AICR (2007) concluded that there is limited but suggestive evidence that foods containing animal fat, which are energy-dense, increase the risk of CRC.

SATURATED FAT

There is no relationship between CRC and SFA. SFA, especially myristic and palmitic acids, have been reported to increase the risk of prostate cancer, PC (Kurahashi *et al*., 2008) and of PC progression (Strom *et al*., 2008). However, data are insufficient to definitively conclude that a relationship exists. Two recent prospective datasets concerning BC (Thiébaut *et al*., 2007; Sieri *et al*. 2008) report a modest but significantly increased risk for an intake higher than 11% of total energy intake (TEI), supporting the conclusion of a possible increased risk for breast cancer associated with a high saturated fat intake.

MONOUNSATURATED FATTY ACID

There are no data on the specific effect of MUFA on CRC risk. However, olive oil has been associated with risk reduction in ecological (Stoneham *et al*., 2000; Siari *et al*., 2002) and case-control studies (Rouillier *et al*., 2005; Galeone *et al*., 2007). MUFA either are not associated with BC or present a risk comparable with that of total fat (Gerber, 2009). However, this is not observed in Mediterranean countries, where olive oil is the main source of MUFA and is the largest contributor to MUFA consumption. The beneficial effect of olive oil might be conjointly or individually attributed to 3 variables:1) the presence of oleuropeine, a phenolic compound capable of modulation of phase I and II enzymes, in olive oil (Gerber, 1997); 2) an effect of substitution of animal fat by vegetable fat: Rasmussen *et al*. (1996) showed that a test meal with butter is followed by a higher peak of insulinemia than a test meal with olive oil, and

similar observations are reported in "the relationship between dietary fat and fatty acid intake and body weight, diabetes and the metabolic syndrome"; 3) the context of the Mediterranean diet pattern might either contribute to or confound the effect. Again, similar observations are reported in "the relationship between dietary fat and fatty acid intake and body weight, diabetes and the metabolic syndrome".

The data collected about MUFA underline the importance of taking into consideration the source of FA and the global dietary pattern when judging beneficial or deleterious effects of these FA on cancers (Gerber, 2001; Fung *et al*., 2006). Thus, olive oil provides a source of microconstituents that might offer specific nutritional benefit, in addition to being a source of fat, without deleterious effects on the level of LDL-cholesterol, thereby decreasing the risk of heart disease.

ESSENTIAL FATTY ACIDS: N-6 FA: LINOLEIC ACID AND N-3 FA: A-LINOLENIC ACID

Most epidemiological studies do not show any association of LA and n-6 FA with CRC, PC and BC. This contrasts with the results from animal studies and might be explained by a difference in the proportion of FA in the diet or by the part played by the diversification of foods in human diets. Contradictory results are reported for ALA with regard to prostate and breast cancers. There are no data providing evidence of a link between essential fatty acids and cancer risk, and thus no recommendation can be made.

N-3 LCPUFA

Colorectal cancer

The WCRF/AICR report (2007) noted that there is limited evidence that eating fish protects against CRC. Since that report, four case-control studies (Wakai, *et al*. 2006; Siezen, *et al*. 2006; Kimura *et al*. 2007; Hu *et al*. 2007) reported no association whereas one (Theodoratou *et al*., 2007) reported a risk reduction. In the same period, the results of six cohort studies were published (English *et al*., 2004, Norat, *et al*. 2005; Larsson, *et al*. 2005; Luchtenborg, *et al*. 2005; Engeset, *et al*. 2007; Hall *et al*., 2008). Only two reports (Norat, *et al*. 2005; Hall *et al*., 2008) indicated a significant risk reduction). A meta-analysis, including the data from 14 studies, but not those from Hall *et al*. (2008), showed a borderline significant risk reduction for CRC incidence: 0.88, 95% CI:0.78-1.00 (Geelen *et al*., 2007).

Recent studies on the relationship of n-3 LCPUFA and CRC have introduced new data (Gerber, 2009). One case-control study (Theodoratou *et al*., 2007) and two cohort studies reported on n-3 LCPUFA intake according to results of a questionnaire (Oba *et al*., 2006, Hall *et al*., 2008) with two identifying a significant risk-reduction (Theodoratou *et al*., 2007, Hall *et al*., 2008). This effect is reinforced in subjects carrying the APC 1822 gene variant (Theodoratou *et al*., 2008). In addition, two cohort studies (Kojima *et al*., 2005; Hall *et al*., 2007) using biomarkers of n-3 LCPUFA intake, showed a significant reduction in risk of CRC in the quantile with the highest percentage of n-3 LCPUFA in the blood (Kojima *et al*., 2005) and for subjects not taking aspirin (Hall *et al*., 2007).

It has invariably been observed in animal models, that an n-3 LCPUFA-rich diet inhibits colon tumorigenesis compared with LA (Reddy, 1984) or with a lipid-rich western type diet (Rao *et al*., 2001). Two hypotheses support the biological plausibility of the risk-reducing effect of n-3 LCPUFA. One is the anti-inflammatory effect with inhibition of the COX 2 enzyme, and the other is the apoptotic effect as shown in animal models (Chang *et al*., 1998).

As mentioned earlier, fish intake is not equivalent to n-3 LCPUFA consumption, given that fish contains other nutrients associated with protection against cancer, including vitamin D and selenium. However, there is a positive correlation between blood levels of n-3 LCPUFA and fish intake (Gerber *et al*., 2000; Hall *et al*., 2008). In addition, since meat is a very probable risk factor for CRC, replacing meat by fish might confound the risk reduction associated with high intakes of fish. Nevertheless, recent studies strengthen the probability of a causal relationship between fish intake and CRC indicated by experimental models and biological plausibility. Thus, fish intake probably decreases CRC risk, and the limited data suggest a possible causal relationship between n-3 LCPUFA intake and colorectal cancer risk reduction.

Prostate cancer

Few studies report on the effect of fish and/or n-3 LCPUFA on PC risk except one prospective study using blood markers (Gerber, 2009). Overall the evidence of a protective effect of n-3 LCPUFA on PC is limited. The observed heterogeneity of results might result from the possible wide range of contaminants in fish.

Breast cancer

Previous reports were unable to reach a conclusion on this subject due to insufficient data. Since that time, two prospective (Stripp *et al*., 2003; Engeset *et al*., 2006) and three case-control studies (Hirose *et al*., 2003; Kuriki *et al*., 2007; Bessaoud *et al*., 2008) investigating the relationship between fish/seafood intake and BC have been published. In the Asia and southern France studies, fish consumption was associated with BC risk reduction, but only significantly in Hirose *et al*. (2003). In contrast, the two prospective studies conducted in northern Europe indicated increased risk associated with fatty fish consumption (Engeset *et al*., 2006) and total consumption in a Danish study (Stripp *et al*., 2003).

With regard to n-3 LCPUFA, there have been publications on three prospective studies (Gago-Dominguez *et al*., 2003; Wakai *et al*., 2005; Thiébaut *et al*., 2009), two case-control studies (Gerber *et al*., 2005; Kuriki *et al*., 2007) with questionnaires, two case-control studies and two prospective studies investigating the relationship between BC risk and EPA/DHA, either in sera or in erythrocytes membranes (Gerber *et al*., 2005, Kuriki *et al*., 2007; Wirfält *et al*., 2004, Shannon *et al*., 2007). They also reported a risk reduction in Asian countries and southern France (EPA), but no effect in Denmark, Sweden or France as a whole (Gerber, 2009).

In countries where the diet is recognized to be good, as in Asian and Mediterranean countries, the highest intake of fish or n-3 LCPUFA is associated with a possible reduction in risk of developing BC, whereas there is either no effect or increased risk in northern European countries. Thus, there is limited but suggestive evidence that high to moderate consumption of fish and n-3 LCPUFA as part of a good diet is associated with reduced BC risk. The increased risk recorded for some European countries might be related to a less favourable contextual food pattern and/or to possible endocrine disruptor pollutants known to be present in the seas around these countries (Hoyer *et al*., 1998).

N-6 PUFA/N-3 PUFA

Several studies report that a high n-6/n-3 FA ratio is associated with an increased risk of CRC, PC and BC. Since a risk associated with n-6 has not been demonstrated, it can be concluded that a low n-3 PUFA intake is responsible for the observation. Thus, it is an absolute amount of EPA and DHA intake that is recommended, rather than the ratio.

TRANS FA

There is not a large body of evidence to suggest either a deleterious or a beneficial effect of *trans* FA and CLA on cancers (three studies on PC and two on BC showed a deleterious effect). The studies on PC are interesting in that they suggest a mechanistic hypothesis based on an contrary effect of *trans* FA compared with n-3 LCPUFA. On the one hand, there is interference with the RNAse L polymorphism (the enzyme involved in a pro-apoptose) (Liu *et al*. 2007) and on the other hand, a different effect on subjects taking or not taking aspirin (Chavarro *et al*., 2007). However, there are insufficient data to provide a recommendation with regard to cancers.

DISCUSSION OF NUTRITIONAL AND GENETIC ASPECTS

Several studies pointed out the importance of the quality of FA, FA food sources, and the foods contributing to their major intake in different populations: e.g. animal versus vegetable (Rasmussen *et al*., 1996; Gerber, 1997) and processed versus non-processed (Thiébault *et al*., 2007; Chajès *et al*., 2008; Wang *et al*., 2008). Any recommendations made may need to take into consideration the context of the food that contains the FA in determining its role in a disease outcome (Gerber, 2001; Fung *et al*., 2006). This may lead to the need for a decision as to whether the recommendations should be dietary-based reference values and/or population reference intakes of FA.

Genetic polymorphisms in relation to carcinogenesis have been mainly described for enzymes involved in detoxication (phase 1 and 2 enzymes) or DNA repair. There are fewer examples of nutrigenomic mechanisms for cancer than for heart disease. Subjects carrying the homozygous APC variant at codon 1822 (valine/valine) were at lower risk of cancer if they consumed a low-fat diet (OR, 0.2; 95% CI, 0.1-0.5) relative to those who were homozygous wild type and ate a high-fat diet. This finding was specific to a low-fat diet and was unrelated to other dietary variables (Slattery *et al*., 2001). Theodoratou *et al*. (2008) reported the enhanced effect of n-3 LCPUFA in subjects carrying the homozygous APC variant 1822. However, neither Menendez *et al*. (2004) nor Tranah *et al*. (2007) observed this. The RNASEL R462Q polymorphism (QQ/RQ genotype variant with deficient pro-apoptotic activity) is associated with higher risk of PC than the wild allele when exposed to *trans* 18:1, *trans* 18:2 intake.

In addition to the direct effect of FA on a gene resulting in the modulation of cancer risk, polymorphism of enzymes involved in metabolic pathways of FA potentially involved in adverse reactions, such as inflammation, might play a role. The association of PC with n-3 LCPUFA intake, indicating a reduction in risk, has been reported in subjects carrying a mutation of the COX 2 gene, this enzyme being more evident in prostate cancer tissue (Hedelin *et al*., 2007).

An area where further study might prove beneficial is in determining the relationship between the polymorphism of genes coding for proteins involved in the metabolism of the methyl group and obesity, since hypermethylation could influence obesity development via epigenetic control of gene expression (Junien and Natahannielz, 2005).

TABLE 9.1
Summary of strength of evidence: Fat, fatty acids and cancers

Type of fat	CRC	PC	BC	EC	OC	Observation on quantities in studies associated with risk
Total fat	C NR	C NR	P NR	PS↑	I	
TFA	C NR	PS↑	I			PC: ↑ >1.8% TEI
SFA	C NR	I	PS↑			BC: ↑ > 11% TEI
Lauric						
Myristic		I				
Palmitic		I				
Stearic		I				
MUFA	P NR	P NR	P NR			
PUFA, n-6 PUFA,LA	C NR	C NR	C NR			
ALA	C NR	I	I			
EPA+ DHA	P↓	I	PS↓			CRC+BC: ↓ 500mg/d
DHA						

C = convincing, P = probable, PS = possible, I = Insufficient, NR = not related
↑ increased risk ↓ decreased risk

TABLE 9.2
Summary of strength of evidence: Food, diet and cancers

Type of fat	CRC	PC	BC	EC	OC	Observation on quantities in studies associated with risk
Fish	P↓	I	I			CRC: ↓ 2–3 portions/week
Food patterns						
Mediterranean/Asian	PS↓		PS↓			
Prudent/low fat/low animal fat	PS↓				PS↓	

C = convincing, P = probable, PS = possible, I = Insufficient, NR = not related
↑ increased risk ↓ decreased risk

RECOMMENDATIONS

A summary of the strength of evidence for recommendations is provided in Tables 9.1 and 9.2.

Total fat

Since total fat intake is not recognized as a factor in obesity as such, only contributing to an excess of energy intake, it is assumed that there is no convincing relationship between CRC and PC. Data are insufficient for drawing conclusions on ovarian cancers. A relationship is more convincing regarding BC, following publication of a new prospective study but the limited data available indicate that there is no increased risk for BC up to 30-33%E. There is possible evidence of an increased risk for endometrial cancer being causally related for total fat intake related to increased body fatness.

SFA
The limited data regarding BC suggest keeping SFA under 11%E.

MUFA
Data are contradictory, suggesting the influence of the contributing food (see below dietary based recommendations).

Essential fatty acids, LA and ALA
There is no relationship between CRC, PC and BC and LA (convincing).

EPA+DHA
There are suggestive data that EPA+DHA decrease colorectal cancer risk (probable). The evidence is possible for BC. Limited data indicate that 500mg/day intake possibly decreases CRC and BC.

TRANS FA
There is no convincing relationship regarding CRC. The data are insufficient for the other cancers except PC, where there is a possible increase associated with *trans* fat intake. The limited data indicate that there is no increased risk below 1.8%E.

FOOD AND DIETARY-BASE RECOMMENDATIONS

Fish
There is possible/probable evidence that 2–3 portions of fish per week decreases CRC. Data are insufficient regarding PC and BC.

Food patterns

Mediterranean/Asian
There is possible evidence for a protective effect of these diets regarding CRC and BC.

Prudent/low fat diet and low animal fat
There is possible evidence for a protective effect of these diets regarding CRC and ovarian cancers.

RECOMMENDATIONS FOR FUTURE RESEARCH

Trans fatty acid and saturated fatty acids should be investigated further with regard to PC. N-3 PUFA and fish should be investigated further with regard to CRC, PC and BC.

REFERENCES

AFSSA (Agence Française pour la securité sanitaire alimentaire: French Food Safety Agency). 2003. Acides gras alimentaires et cancers: état des connaissances et perspectives. AFSSA, Paris.

Astorg, P., Arnault, N., Czernichow, S., Noisette, N., Galan, P. & and Hercberg, S. 2004. Dietary intakes and food sources of n-6 and n-3 PUFA in French adult men and women. *Lipids.*, 39: 527-535.

Bessaoud, F., Daurès, J.P. & Gerber, M. 2008, Dietary factors and breast cancer risk: A case control study among a population in Southern France. *Nutr. Cancer*, 60: 177-87.

Bingham, S.A., Luben, R., Welch, A., Wareham, N., Khaw, K.T. & Day, N. 2003 Are imprecise methods obscuring a relation between fat and breast cancer? *Lancet*, 362: 212-214.

Borugian, M.J., Sheps, S.B., Kim-Sing, C., Van Patten, C., Potter, J.D., Dunn, B., Gallagher, R.P. & Hislop, T.G. 2004. Insulin, macronutrient intake, and physical activity: are potential indicators of insulin resistance associated with mortality from breast cancer? *Cancer Epidemiol. Biomarkers Prev*., 13(7): 1163-72.

Chajès, V., Thiébaut, A.C.M,. Rotival, M., Gauthier, E., Maillard, V., Boutron-Ruault, M.C., Joulin, V., Lenoir, G.M. & Clavel-Chapelon, F. 2008. Association between serum *trans*-monounsaturated fatty acids and breast cancer risk in the E3N-EPIC study. *Amer. J. Epidemiol*., 167: 1312-1320.

Chang, W.L., Chapkin, R.S. & Lupton, J.R. 1998. Fish oil blocks azoxymethane-induced rat colon tumorigenesis by increasing cell differentiation and apoptosis rather than decreasing cell proliferation. *J. Nut*., 128: 491-497.

Chavarro, J.E., Stampfer, M.J., Li, H., Campos, H., Kurth, T. & Ma, J. 2007. A prospective study of polyunsaturated fatty acid levels in blood and prostate cancer risk. *Cancer Epidemiol. Biomarkers Prev.,* 16(7): 1364-1370.

Chlebowski, R.T., Blackburn, G.L., Thomson, C.A., Nixon, D.W., Shapiro, A., Hoy, M.K., Goodman, M.T., Giuliano, A.E., Karanja, N., McAndrew, P., Hudis, C., Butler, J., Merkel, D., Kristal, A., Caan, B., Michaelson, R., Vinciguerra, V., Del Prete, S., Winkler, M., Hall, R., Simon, M., Winters, B.L. & Elashoff, R.M. 2006. Dietary fat reduction and breast cancer outcome: interim efficacy results from the Women's Intervention Nutrition Study. *J. Natl. Cancer Inst*., 98(24): 1767-1776.

Engeset, D., Andersen, V., Hjartaker, A. & Lund, E. 2007. Consumption of fish and risk of colon cancer in the Norwegian Women and Cancer (NOWAC) study. *Br. J. Nutr*., 98: 576-582.

English, D.R., MacInnis, R.J., Hodge, A.M., Hopper, J.L., Haydon, A.M. & Giles, G.G. 2004. Red meat, chicken, and fish consumption and risk of colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.*, 13(9): 1509-1514.

Fung, T.T., Hu, F.B., Holmes, M.D., Rosner, B.A., Hunter, D.J., Colditz, G.A. & Willett, W.C. 2005. Dietary patterns and the risk of postmenopausal breast cancer. *Int. J. Cancer*, 116: 116–121.

Gago-Dominguez, M., Yuan, J.M., Sun, C.L., Lee, H.P. & Yu, M.C. 2003. Opposing effects of dietary n-3 and n-6 fatty acids on mammary carcinogenesis: The Singapore Chinese Health Study. *Br. J. Cancer*, 89: 1686-1692.

Galeone, C., Talamini, R., Levi, F., Pelucchi, C., Negri, E., Giacosa, A., Montella, M., Franceschi, S. & La Vecchia, C. 2007. Fried foods, olive oil and colorectal cancer. *Ann. Oncol.*, 18(1): 36-39.

Geelen, A., Schouten, J.M., Kamphuis, C., Stam ,B.E., Burema, J., Renkema, J.M., Bakker, E.J., Van't Veer, P. & Kampman, E. 2007. Fish Consumption, n-3 fatty acids, and colorectal cancer: a meta-analysis of prospective cohort studies. *Am. J. Epidemiol.*, 166: 1116-1125.

Gerber, M. 1997. Olive oil, monounsaturated fatty acids and cancer. *Cancer Lett.*, 114: 91-92.

Gerber, M., Scali, J., Michaud, A., Durand, M., Astre, C., Dallongeville, J. & Romon, M. 2000. Profiles of a healthy diet and its relationship with biomarkers in a population sample from Mediterranean southern France. *J. Amer. Diet. Assoc.*, 100: 1164-1171.

Gerber, M. 2001. The comprehensive approach to diet: a critical review. *J. Nutr.*, 131: 3051S-3055S.

Gerber, M., Thiébaut, A, Astorg, P., Clavel-Chapelon, F. & Combe, N. 2005. Dietary fat, fatty acid composition and risk of cancer. *Eur. J. Lipid Sci. Technol.*, 107: 540-559.

Gerber, M. 2009. Background review paper on total fat, fatty acid intake and cancers. *In* Burlingame, B., Nishida, C., Uauy, R. & Weisell, R., eds. *Fats and fatty acids in human nutrition. Joint FAO/WHO Expert Consultation*, November 10-14, 2008, Geneva, Switzerland. *Ann. Nutr. Metab.*, 55 (1-3): 140-161.

Hall, M.N., Campos, H., Li, H., Sesso, H.D., Stampfer, M.J., Willett, W.C. & Ma, J. 2007. Blood levels of long-chain polyunsaturated fatty acids, aspirin, and the risk of colorectal cancer. *Cancer Epidemiol. Biomarkers Prev.*, 16: 314-321.

Hall, M.N., Chavarro, J.E., Lee, I.M., Willett, W.C. & Ma, J. 2008. A 22-year prospective study of fish, n-3 fatty acid intake, and colorectal cancer risk in men. *Cancer Epidemiol. Biomarkers Prev.*, 17(5): 1136-1143.

Hedelin, M., Chang, E.T., Wiklund, F., Bellocco, R., Klint, A., Adolfsson, J., Shahedi, K., Xu, J., Adami, H.O., Gronberg, H. & Balter, K.A. 2007. Association of frequent consumption of fatty fish with prostate cancer risk is modified by COX-2 polymorphism. *Int. J. Cancer*, 120: 398-405.

Hirose, K., Takezaki, T., Hamajima, N., Miura, S. & Tajima, K. 2003. Dietary factors protective against breast cancer in Japanese premenopausal and postmenopausal women. *Int. J. Cancer*, 107 (2): 276-282.

Hoyer, A.P., Grandjean, P., Jorgensen, T., Brock, J.W. & Hartvig, H.B. 1998. Organochlorine exposure and risk of breast cancer. *Lancet*, 352: 1816-1820.

Hu, J., Mery, L., Desmeules, M. & Macleod, M. 2007. Diet and vitamin or mineral supplementation and risk of rectal cancer in Canada. *Acta Oncol.*, 46: 342-354.

Junien, C. & Natahannielz, P. 2005. Report of the IASO Stock conference 2006. Early and lifelong environmental epigenomic programming of metabolic syndrome, obesity and type 2 diabetes. *Obes. Rev.*, 8: 487-502.

Kimura, Y., Kono, S., Toyomura, K., Nagano, J., Mizoue, T., Moore, M.A., Mibu, R., Tanaka, M., Kakeji, Y., Maehara, Y., Okamura, T., Ikejiri, K., Futami, K., Yasunami, Y., Maekawa, T., Takenaka, K., Ichimiya, H. & Imaizumi, N. 2007.

Meat, fish and fat intake in relation to subsite specific risk of colorectal cancer. *Cancer Sci*., 98: 590-597.

Kipnis, V. & Freedman, L. 2008. Impact of measurement error in nutritional epidemiology. *J. Natl. Cancer Inst*., 100: 1658-1659.

Kojima, M., Wakai, K., Tokudome, S., Suzuki, K., Tamakoshi, K., Watanabe, Y., Kawado, M., Hashimoto, S., Hayakawa, N., Ozasa, K., Toyoshima, H., Suzuki, S., Ito, Y. & Tamakoshi, A. 2005. Serum levels of polyunsaturated fatty acids and risk of colorectal cancer: a prospective study. *Am. J. Epidemiol*., 161: 462-471.

Kurahashi, N., Inoue, M., Iwasaki, M., Sasazuki, S. & Tsugane, A.S. 2008. Japan Public Health Center-Based Prospective Study Group. Dairy product, saturated fatty acid, and calcium intake and prostate cancer in a prospective cohort of Japanese men. *Cancer Epidemiol. Biomarkers Prev*., 17(4): 930-937.

Kuriki, K., Hirose, K., Wakai, K., Matsuo, K., Ito, H., Suzuki, T., Hiraki, A., Saito, T., Iwata, H., Tatematsu, M. & Tajima, K. 2007. Breast cancer risk and erythrocyte compositions of n-3 highly unsaturated fatty acids. *Japanese Int. J. Cancer*, 21:377-85.

Larsson, S.C., Rafter, J., Holmberg, L., Bergkvist, L. & Wolk, A. 2005. Red meat consumption and risk of cancers of the proximal colon, distal colon and rectum: the Swedish Mammography Cohort. *Int. J. Cancer*, 113: 829-834.

Liu, X., Schumacher, F.R., Plummer, S.J., Jorgenson, E., Casey, G. & Witte, J.S. 2007. *Trans* fatty acid intake and increased risk of advanced prostate cancer: modification by RNASEL R462Q variant. *Carcinogenesis*, 28: 1232–1236.

Luchtenborg, M., Weijenberg, M.P., de Goeij, A.F., Wark, P.A., Brink, M., Roemen, G.M., Lentjes, M.H., de Bruine, A.P., Goldbohm, R.A., van't Veer, P. & van den Brandt, P.A. 2005. Meat and fish consumption, APC gene mutations and hMLH1 expression in colon and rectal cancer: a prospective cohort study (The Netherlands). *Cancer Causes Control*, 16: 1041-1054.

McEligot, A.J., Largent, J., Ziogas, A., Peel, D. & Anton-Culver, H. 2006. Dietary fat, fiber, vegetable, and micronutrients are associated with overall survival in postmenopausal women diagnosed with breast cancer. *Nutr. Cancer*, 55(2): 132-40.

Menendez, M., Gonzalez, S., Blanco, I., Guino, E., Peris, M., Peinado, M.A., Capella, G., & Moreno, V. 2004. Colorectal cancer risk and the APC D1822V variant. *Int. J. Cancer*, 112: 161-163.

Norat, T., Bingham, S., Ferrari, P., Slimani, N., Jenab, M., Mazuir, M., Overvad, K., Olsen, A., Tjonneland, A., Clavel, F., Boutron-Ruault, M.C., Kesse, E., Boeing, H., Bergmann, M.M., Nieters, A., Linseisen, J., Trichopoulou, A., Trichopoulos, D., Tountas, Y., Berrino, F., Palli, D., Panico, S., Tumino, R., Vineis, P., Bueno-de-Mesquita, H.B., Peeters, P.H., Engeset, D., Lund, E., Skeie, G., Ardanaz, E., Gonzalez, C., Navarro, C., Quiros, J.R., Sanchez, M.J., Berglund, G., Mattisson, I., Hallmans, G., Palmqvist, R., Day, N.E., Khaw, K.T., Key, T.J., San Joaquin, M., Hemon, B., Saracci, R., Kaaks, R., & Riboli, E. 2005. Meat, fish, and colorectal cancer risk: the European Prospective Investigation into cancer and nutrition. *J. Natl. Cancer Inst*., 97: 906-916.

Oba, S., Shimizu, N., Nagata, C., Shimizu, H., Kametani, M., Takeyama, N., Ohnuma, T. & Matsushita, S. 2006. The relationship between the consumption of meat, fat, and coffee and the risk of colon cancer: a prospective study in Japan. *Cancer Lett*., 244: 260-267.

Prentice, R.L., Caan, B., Chlebowski, R.T., Patterson, R., Kuller, L.H., Ockene, J.K., Margolis, K.L., Limacher, M.C., Manson, J.E., Parker, L.M., Paskett, E., Phillips, L., Robbins, J., Rossouw, J.E., Sarto, G.E., Shikany, J.M., Stefanick, M.L., Thomson, C.A., Van Horn, L., Vitolins, M.Z., Wactawski-Wende, J., Wallace, R.B., Wassertheil-Smoller, S., Whitlock, E., Yano, K., Adams-Campbell, L., Anderson, G.L., Assaf, A.R., Beresford, S.A., Black, H.R., Brunner, R.L., Brzyski, R.G., Ford, L., Gass, M., Hays, J., Heber, D., Heiss, G., Hendrix, S.L., Hsia, J., Hubbell, F.A., Jackson, R.D., Johnson, K.C., Kotchen, J.M., LaCroix, A.Z., Lane, D.S., Langer, R.D., Lasser, N.L. & Henderson, M.M. 2006. Low-fat dietary pattern and risk of invasive breast cancer: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. *JAMA*, 295(6): 629-642.

Prentice, R.L., Thomson, C.A., Caan, B., Hubbell, F.A., Anderson, G.L., Beresford, S.A., Pettinger, M., Lane, D.S., Lessin, L., Yasmeen, S., Singh, B., Khandekar, J., Shikany, J.M., Satterfield, S. & Chlebowski, R.T. 2007. Low-fat dietary pattern and cancer incidence in the Women's Health Initiative Dietary Modification Randomized Controlled Trial. *J. Natl. Cancer Inst*., 99: 1534-43.

Rao, C.V., Hirose, Y., Indranie, C. & Reddy, B.S. 2001. Modulation of experimental colon tumorigenesis by types and amount s of dietary fatty acids. *Cancer Res*., 61: 1927-1933.

Rasmussen, O., Lauszus, F.F., Christiansen, C., Thomsen, C. & Hermansen, K. 1996. Differential effects of saturated and monounsaturated fat on blood glucose and insulin responses in subjects with non-insulin-dependent diabetes mellitus. *Am. J. Clin. Nutr*., 63: 249-253.

Reddy, B.S. & Meura, Y. 1984. Tumor promotion by dietary fat in azoxymethane-induced colon carcinogenesis in female F344 rats: influence of amount and source of dietary fat. *J. Natl. Cancer Inst*., 72: 745-750.

Rouillier, P., Senesse, P., Cottet, V., Valléau, A., Faivre, J. & Boutron-Ruault, M.C. 2005. Dietary patterns and the adenomacarcinoma sequence of colorectal cancer. *Eur. J. Nutr*., 44(5): 311-318.

Shannon, J., King, I.B., Moshofsky, R., Lampe, J.W., Gao, D.L., Ray, R.M. & Thomas, D.B. 2007. Erythrocyte fatty acids and breast cancer risk: a case-control study in Shanghai, China. *Am. J. Clin. Nutr.*, 85: 1090-1097.

Siari, S., Scali, J., Richard, A., Tretarre, B., Daures, J.P., Padilla, M., Grosclaude, P. & Gerber, M. 2002. Subregional variations of dietary consumption and incidences of cancer in Southern France. *In* Riboli and Lambert, eds. *Nutrition and Lifestyle: opportunities for cancer prevention*, N°156–153. IARC Scientific publications, Lyon.

Sieri, S., Krogh, V., Ferrari, P., Berrino, F., Pala, V., Thiébaut, A.C.M., Tjønneland, A., Olsen, A., Overvad, K., Uhre Jakobsen, M., Clavel-Chapelon, F., Chajes, V., Boutron-Ruault, M.C., Kaaks, R., Linseisen, J., Boeing, H., Nöthlings, U., Trichopoulou, A., Nasak, A., Lagiou, P., Panico, S., Palli, D., Vineis, P., Tumino, R., Lund, E., Kumle, M., Skeie, G., Gonzalez, C.A., Ardanaz, E., Amiano, P., Tormo, M.J., Martínez-García, C., Quiros, J.R., Berglund, G., Gullberg, B., Hallmans, G., Lenner, P., Bueno-

de-Mesquita, H.B., van Duijnhoven, F.J.B., Peeters, P.H., van Gils, C.H., Key, T.J., Crowe, F.L., Bingham, S., Khaw, K.T., Rinaldi, S., Slimani, N., Jenab, M., Norat, T. & Riboli, E. 2008. Dietary fat and breast cancer risk in the European Prospective Investigation into Cancer and Nutrition. *Amer. J. Clin. Nut*., 88: 1304-1312.

Slattery, M.L., Samowitz, W., Ballard, L., Schaffer, D., Leppert, M. & Potter, J.D. 2001. A molecular variant of the APC gene at codon 1822: its association with diet, lifestyle, and risk of colon cancer. *Cancer Res*., 61: 1000-1004.

Stoneham, M., Goldacre, M., Seagroatt, V. & Gill, L. 2000. Olive oil, diet and colorectal cancer: an ecological study and a hypothesis. *J. Epidemiol. Community Health*, 54(10): 756-760.

Stripp, C., Overvad, K., Christensen, J., Thomsen, B.L., Olsen, A., Møller, S. & Tjønneland, A. 2003. Fish intake is positively associated with breast cancer incidence. *J. Nutr*., 133: 3664-3669.

Strom, S.S., Yamamura, Y., Forman, M.R., Pettaway, C.A., Barrera, S.L. & DiGiovanni, J. 2008. Saturated fat intake predicts biochemical failure after prostatectomy. *Int. J. Cancer*, 122(11): 2581-2585.

Theodoratou, E., McNeill, G., Cetnarskyj, R., Farrington, S.M., Tenesa, A., Barnetson, R., Porteous, M., Dunlop, M. & Campbell, H. 2007. Dietary fatty acids and colorectal cancer: a case-control study. *Am. J. Epidemiol*., 166(2): 181-195.

Theodoratou, E., Campbell, H., Tenesa, A., McNeill, G., Cetnarskyj, R., Barnetson, R.A., Porteous, M.E., Dunlop, M.G. & Farrington, S.M. 2008. Modification of the associations between lifestyle, dietary factors and colorectal cancer risk by APC variants. *Carcinogenesis on line*, Mar 28.

Thiébaut, A.C.M., Kipnis, V., Chang, S-C., Subar, A.F., Thompson, F.E., Rosenberg, P.S., Hollenbeck, A.R., Leitzmann, M. & Schatzkin, A. 2007. Dietary fat and postmenopausal invasive breast cancer in the National Institutes of Health–AARP Diet and Health Study Cohort *J. Natl. Cancer Inst*., 99(6): 451-462.

Thiébaut, A.C.M., Chajès, V., Gerber, M., Boutron-Ruault, M.C., Joulin, V., Lenoir, G., Berrino, F., Riboli, E., Bénichou, J. & Clavel-Chapelon, F. 2009. Dietary intakes of ω-6 and ω-3 polyunsaturated fatty acids and the risk of breast cancer. *Int. J. Cancer*, 124: 924-931.

Tranah, G.J., Giovannucci, E., Ma, J., Fuchs, C. & Hunter, D.J. 2007. APC Asp1822Val and Gly2502Ser polymorphisms and risk of colorectal cancer and adenoma. *Cancer Epidemiol. Biomarkers Prev*., 14: 863-870.

Wakai, K., Tamakoshi, K., Date, C., Fukui, M., Suzuki, S., Lin, Y., Niwa, Y., Nishio, K., Yatsuya, H., Kondo, T., Tokudome, S., Yamamoto, A., Toyoshima, H. & Tamakoshi, A. 2005. JACC Study Group. Dietary intakes of fat and fatty acids and risk of breast cancer: a prospective study in Japan. *Cancer Sci*., 96(9): 590-599.

Wakai, K., Hirose, K., Matsuo, K., Ito, H., Kuriki, K., Suzuki, T., Kato, T., Hirai, T., Kanemitsu, Y. & Tajima, K. 2006. Dietary risk factors for colon and rectal cancers: a comparative case-control study. *J. Epidemiol*., 16: 125-135.

Wang, J., John, E.M., Horn-Ross, P.L. & Ingles, S.A. 2008. Dietary fat, cooking fat and breast cancer risk in a multiethnic population. *Nut. Cancer*, 60: 492-504.

Wirfält, E., Vessby, B., Mattisson, I., Gullberg, B., Olsson, H. & Berglund, G. 2004. No relations between breast cancer risk and fatty acids of erythrocyte membranes in postmenopausal women of the Malmö Diet Cancer cohort (Sweden). *EJCN*, 58: 761–770.

WCRF/AICR (World Cancer Research Fund/American Institute for Cancer Research). 2007. *Food nutrition, Physical activity and the prevention of cancer: a global perspective*. AICR, Washington DC.

Chapter 10: Fat and fatty acid intake and metabolic effects in the human body

SUMMARY

Differences in plasma total cholesterol (TC) concentrations and blood pressure (BP) are powerful predictors of risk of cardiovascular disease (CVD) and contribute largely to the variation in CVD risk among different countries and populations. More recently other metabolic factors associated with CVD risk have been identified, including specific lipoproteins, the metabolic syndrome (insulin resistance syndrome), postprandial lipaemia, indices of inflammation and haemostasis, arterial stiffness and endothelial function. The effect of dietary fat and fatty acids on these CVD risk factors is reviewed and the evidence is summarized in Table 10.1.

TABLE 10.1
Change in serum lipids (mmol/L with 95% CI) predicted from replacing 1% energy by individual fatty acids for carbohydrate based on meta-analysis[1] and changes from increasing intake of dietary cholesterol by 100mg[2]

Fatty acid	Total cholesterol	LDL cholesterol	HDL cholesterol	Total:HDL cholesterol
Lauric acid (12:0)	+0.069 (0.040 to 0.097)	+0.052 (0.026 to 0.078)	+0.027 (0.021 to 0.033)	-0.037 (-0.057 to -0.017)
Myristic acid (14:0)	+0.059 (0.036 to 0.082)	+0.048 (0.027 to 0.069)	+0.018 (0.013 to 0.023	-0.003 (-0.026 to 0.021)
Palmitic acid (16:0)	+0.041 (0.028 to 0.054)	+0.039 (0.027 to 0.051)	+0.010 (0.007 to 0.013)	+0.005 (-0.008 to 0.019)
Stearic acid (18:0)	-0.010 (-0.026 to 0.006)	-0.004 (-0.019 to 0.011)	+0.002 (-0.001 to 0.006)	-0.013 (-0.030 to 0.003
Elaidic (18:1 trans)	+0.031 (0.020 to 0.042)	+0.040 (0.020 to 0.060)	0.000 (-0.007 to 0.006)	+0.022 (0.005 to 0.038)
Oleic acid (18:1 cis)	-0.006 (0.020 to 0.042)	-0.009 (-0.014 to -0.003	+0.008 (0.005 to 0.011	-0.026 (-0.035 to -0.017)
PUFA	-0.021 (0.020 to 0.042)	-0.019 (0.020 to 0.060)	+0.006 (0.007 to 0.006)	-0.032 (0.005 to 0.038)
Dietary cholesterol 100mg/d	+0.056 (0.046 to 0.065)	+0.050 (0.042 to 0.058)	+0.008 (0.042 to 0.058)	+0.020 (0.010 to 0.030)

[1] Adapted from EFSA, 2004

[2] Based on analysis of Weggemans *et al*., 2001

FASTING PLASMA LIPIDS AND LIPOPROTEINS

TC concentration shows a continuous association with CVD risk, without a threshold but with the absolute risk increasing with age, smoking habit and raised BP (Lewington *et al*., 2007). Reductions in TC and low density lipoprotein (LDL) concentrations with statin therapy convincingly lower CVD risk, but the effects on CVD risk reductions using other agents (drug or diet) are less well-established. Elevated plasma lipoprotein Lp(a) is linked with increased CVD risk especially when it is associated with elevated plasma LDL concentrations (Seed *et al*., 1990). The relationship between fasting plasma triacylglycerol (TG) concentration and CVD risk is more complex because it can be transiently changed by diet, alcohol intake and physical activity. However, prolonged elevation of plasma TG, which is often associated with the insulin-resistance syndrome and increased very low density lipoprotein (VLDL) synthesis, generates small dense LDL particles (which are rich in apolipoprotein B relative to cholesterol) and causes a fall in high density lipoprotein (HDL; measured as apolipoprotein A1 or HDL-C). This atherogenic dyslipidaemia (high TG, small dense LDL and low HDL-C) confers a substantial increase in risk of CVD (NCEP-3 2001). The ratio of TC:HDL-C, which indicates the ratio of apolipoprotein B:apolipoprotein A, is twice as informative (Lewington *et al*., 2007) of individual CVD risk than TC or LDL-C, and differences in this ratio within and among populations are predominantly due to lifestyle factors (diet, physical activity, obesity, alcohol use). Thus, the ratio of TC:HDL-C is probably the most robust lipid metric to estimate lifestyle-factor-related CVD risk.

Variation across population groups in plasma lipids has traditionally been due to differences in TC and LDL cholesterol concentrations, although with the worldwide obesity pandemic, atherogenic dyslipidaemia is increasingly prevalent. The equations developed by Keys and Hegsted in the 1960s can be used to predict changes in total cholesterol between diets (Keys and Parlin, 1966):

$$\Delta\text{serum cholesterol mg/dl} = 2.3(\Delta S) - \Delta P + 1.5\ (\sqrt{\Delta C})$$

(ΔS is the difference in % energy from saturated fatty acids excluding stearic acid, ΔP is the difference in % energy from polyunsaturated fatty acid and ΔC is the difference in cholesterol content in mg /1000kcal; to convert to mmol/L divide by 38.5)

More recent studies have focused on changes within the different lipoprotein fractions. These provide convincing evidence that saturated fatty acids (C12-C16) elevate TC, LDL-C and HDL-C compared with carbohydrates. The replacement of myristic (14:0) and palmitic (16:0) acids with carbohydrates results in little net change in the TC:HDL-C ratio. Lauric acid (12:0) acid raises LDL and HDL and decreases the TC:HDL-C by -0.037 for each 1% energy when it replaces carbohydrates (Mensink *et al*., 2003). Stearic acid (18:0) does not have any significant effects on TC or LDL-C or the TC:HDL-C ratio compared with carbohydrates and its effects are not statistically significantly different from those of oleic acid (18:1n-9). There is possible evidence to suggest that the TC and LDL-C raising effects of palmitic acid are lower for vegetable than animal sources because it is present predominantly in the sn-1 and sn-3 position as opposed to sn-2 position as in animal fats such as lard (Ng *et al*.; 1992; Choudhury *et al*., 1995; Zhang *et al*., 1997). There is evidence that dietary cholesterol, which is found in animal fats, raises TC and LDL-C and the TC:HDL-C ratio by 0.02 for each 100 mg consumed (Weggemans *et al*., 2001). The evidence is convincing that plant sterols and stanols lower TC and LDL-C and the TC:HDL-C ratio independent of changes in fatty acid composition, but these effects are only significant following the consumption of food products fortified with plant sterols/stanols (Law, 2000).

Compared with carbohydrates, the major monounsaturated fatty acid, oleic acid (18:1n-9), has a neutral effect on plasma LDL-C and PUFA (mainly linoleic acid) have a slight lowering effect on total and LDL-C (Mensink *et al*., 2003; Mozaffarian and Clarke, 2009). Compared with oleic acid, saturated fatty acids increase HDL-C and intakes of linoleic acid above 12% energy lower HDL-C. There is convincing evidence that the replacement of saturated fatty acids with unhydrogenated vegetable oils rich in *cis*-unsaturated fatty acids results in a reduction in the TC:HDL-C ratio. The ratio is lowered by approximately 0.029 and by 0.035 for each 1% energy of saturated fatty acids replaced with oleic acid and linoleic acid, respectively.

Compared with carbohydrate, *trans* isomeric fatty acids (TFA) raise LDL-C but have a similar effect on HDL-C to carbohydrate. Replacing 1% energy TFA by carbohydrate, oleic acid or linoleic acid lowers the TC:HDL-C ratio by 0.022, 0.054 and 0.067 respectively (Mozaffarian and Clarke, 2009). There is evidence to indicate that TFA from natural sources have similar effects on the TC:HDL-C ratio to those from industrial sources (Chardigny *et al*., 2008; Motard-Belanger *et al*., 2008; Brouwer *et al*., 2010).

There is convincing evidence that replacement of saturated or C18 *cis* unsaturated fats with carbohydrate increases fasting TG, and that replacement of *trans* fats with carbohydrate has little effect on fasting TG (Mensink *et al*., 2003). Previous research indicated that *trans* fatty acids increased Lp(a) concentrations (Nestel *et al*., 1992; Almendingen *et al*., 1995). However, it now appears that plasma Lp(a) concentrations are increased by the consumption of fats with a higher proportion of C18 fatty acids (*cis* or *trans*) compared with those dominated by C16 fatty acids (Sanders *et al*., 1997; Sundram *et al*., 1997).

n-3 LCPUFA (mainly eicosapentaenoic acid 20:5n-3, EPA and docosahexaenoic acid 22:6n-3, DHA) as supplied in the diet by oily fish, on average have no effect on total cholesterol concentrations (Bays, 2006) but lower plasma TG, VLDL cholesterol and raise LDL cholesterol concentrations in amounts exceeding 0.7g/d (~0.3% energy) (Caslake *et al*., 2008; Theobald *et al*., 2004). Dietary supplements providing usually in excess of 3g n-3 LCPUFA/d lower plasma TG on average by 27%, but have variable effects on LDL-C and HDL-C depending on the dose, type of fatty acid and lipoprotein phenotype: on average they increase both LDL-C (6%) and HDL-C (1.4%) concentrations (Balk *et al*., 2008), but also LDL and HDL particle size (Minihane *et al*., 2000; Griffin *et al*., 2006; Kelley *et al*., 2007). DHA from algal sources in the range of 0.7–1.5 g/d raises total and LDL-C between 6-12%, but has little influence on the ratio of TC:HDL-C ratio (Geppert *et al*., 2006; Sanders *et al*., 2006a; Theobald *et al*., 2004). Linolenic acid does not share the effects shown by n-3 LCPUFA and does not influence plasma lipid concentrations within the range of intakes likely to be encountered in human diets (Balk *et al*., 2006).

There is convincing evidence that individuals who maintain a healthy weight are less likely to develop a raised TC:HDL-C ratio (Whitlock *et al*., 2009). Furthermore, weight loss in overweight or obese subjects results in improvements in circulating lipid concentrations, including raising HDL-C and lowering TG and TC and improving the TC:HDL-C ratio (Yu-Poth *et al*., 1999).

Despite the global increase in obesity, serum total and LDL-C concentrations have fallen in several economically developed countries (Carroll *et al*., 2005; Evans *et al*., 2001; Vartiainen *et al*., 2000) where the fat supply has changed from predominantly animal fats (dairy fats, lard, lamb and beef fat), rich in saturated fatty acids, to vegetable oils rich in *cis*-unsaturated fatty acids. In contrast, there is evidence to suggest that TC and LDL-C are increasing in some emerging economies such as China (Critchley *et al*., 2004) and that this is accompanied by an increase in total and saturated fat from both animal and vegetable sources.

POSTPRANDIAL LIPIDS

Meals high in fat result in postprandial lipaemia. Elevated postprandial lipid concentrations are associated with progression of atherosclerosis and increased risk of thrombosis. Impaired postprandial lipaemia is associated with obesity, insulin-resistance and type 2 diabetes. Compared with meals low in fat and high in carbohydrate, meals high in long-chain fatty acids (C14-18) result in substantial lipaemia. Short and medium chain fatty acids (C2-C12) do not result in substantial lipaemia (Oakley *et al*., 1998; Sanders *et al*., 2000; Sanders *et al*., 2001). Stearic-rich fats result in variable effects on postprandial lipaemia according to the physical properties of the fat (Berry *et al*., 2007a; Sanders *et al*., 2000; Sanders *et al*., 2001; Sanders *et al*., 2003a; Tholstrup *et al*., 2001). *Trans* isomeric fatty acids have similar effects to *cis*-isomeric fatty acids (Sanders *et al*., 2000; Sanders *et al*., 2003c; Tholstrup *et al*., 2001). Intakes in excess of 1.5g n-3 LCPUFA result in a reduction in the elevation of postprandial lipaemia both acutely and chronically (Harris and Muzio, 1993; Zampelas *et al*., 1994; Finnegan *et al*., 2003; Griffin *et al*., 2006). There is consistent evidence that prolonged elevations of plasma TG concentrations result in an increased proportion of small dense LDL particles that are associated with increased progression of atherosclerosis and increased risk of coronary heart disease (Kwiterovich Jr., 2002). Diets containing a higher proportion of carbohydrate in place of fat result in an increase in plasma TG concentrations in the fasting state, but lower plasma TG concentration in the postprandial state (Mensink *et al*., 2003). While a decrease in adiposity is accompanied by a reduction in the proportion of small dense LDL (Siri-Tarino *et al*., 2009), there is no clear evidence to show replacement of energy in the diet derived from fat with that from carbohydrate has this effect.

INSULIN-SENSITIVITY

There is convincing evidence that regular physical activity and weight loss in overweight or obese subjects improve insulin sensitivity (Costacou and Mayer-Davis, 2003; Roumen *et al*., 2008). Animal studies indicate that diets rich in saturated fatty acids impair insulin sensitivity and that n-3 LCPUFA improve insulin sensitivity. There is limited evidence that replacing SFA from animal sources with monounsaturated fatty acids from plant sources improves insulin sensitivity and glycaemic control in type 2 diabetes (Garg, 1998). However, randomized controlled trials have generally failed to provide any consistent effect of changing either the level of fat or type of fat on insulin sensitivity when changes in weight or physical activity are taken into account (Griffin *et al*., 2006; Tardy *et al*., 2009; Vessby *et al*., 2001; Jebb *et al*., 2010). Where a reduction in the dietary intake of fat is accompanied by a reduction of energy intake and weight loss, an improvement in insulin sensitivity is likely (Tuomilehto *et al*., 2001; Orchard *et al*., 2005; Roumen *et al*., 2008).

INDICES OF OXIDATIVE STRESS

There is convincing mechanistic evidence to implicate lipoprotein oxidation in the pathogenesis of atherosclerosis (Griendling and FitzGerald, 2003), but the benefits in human studies of altering lipoprotein oxidation are not well-established. A number of biomarkers of oxidative damage are available but none are strongly predictive of risk of CVD and there is no convincing evidence to demonstrate that modifying the composition of dietary fat has a significant impact on the process of lipoprotein oxidation *in vivo*.

INFLAMMATORY MARKERS

Chronic inflammation results in the elevation of acute phase proteins including fibrinogen and C-reactive protein and is believed to be mediated by elevated production of cytokines, particularly IL-6. Chronic inflammation increases CVD risk especially if the TC:HDL-C ratio is high (Ridker, 2001). Obesity may directly contribute to increased production of IL-6 from adipose tissue. Postprandial lipaemia may also modify the production of cytokines involved in regulating inflammation and vessel remodeling (Grainger *et al*., 2000; Erridge *et al*., 2007). High intakes of n-3 LCPUFA (>3 g/d) in the form of dietary supplement decrease cytokine production (Meydani, 2000; Vedin *et al*., 2008) and probably decrease inflammatory markers, but randomized controlled trials using lower intakes, as may habitually be consumed in usual diets, have failed to demonstrate any clear effects (Balk *et al*., 2006; Blok *et al*., 1997; Theobald *et al*., 2007). There is possible evidence that *trans* fatty acids increase systemic inflammation (Baer *et al*., 2004), but not all studies (Motard-Belanger *et al*., 2008) have consistently shown such effects.

PRO-COAGULANT AND FIBRINOLYTIC ACTIVITY

Elevated procoagulant FVII and fibrinogen and decreased indices of fibrinolytic activity (as assessed by measures of clot lysis time or elevated plasminogen activator inhibitor PAI-1 activity) are associated with increased risk of athero-thrombosis (Folsom *et al*., 2001; Heinrich *et al*., 1994; Meade *et al*., 1993). Hyperlipidaemia is associated with elevated FVII and fibrinogen and insulin resistance syndrome is associated with elevated PAI-1. Treatment of hyperlipidaemia by weight loss with a diet with reduced total fat and saturated intake results in falls in FVIIc and an improvement in fibrinolytic activity (Hamalainen *et al*., 2005). There is possible evidence that n-3 LCPUFA, provided as dietary supplements (Sanders *et al*., 2006a), increase FVIIc but not with oily fish consumption (Sanders *et al*., 2006b). There is convincing evidence that meals high in fat compared to meals high in carbohydrate acutely increase the concentration of FVIIa (Oakley *et al*., 1998; Sanders *et al*., 1999; Sanders *et al*., 2000; Sanders *et al*., 2001; Sanders *et al*., 2003b; Sanders *et al*., 2006b; Sanders and Berry, 2005; Tholstrup *et al*., 2003). There is probable evidence that the increase in FVIIa is greater following meals rich in the monounsaturated fatty acids (oleic acid) than for some sources of saturated fatty acids (Sanders *et al*., 2000; Tholstrup *et al*., 2003; Berry *et al*., 2007a; Berry *et al*., 2007b). There is insufficient evidence to demonstrate chronic effects of different types of fatty acids on fibrinogen or fibrinolytic activity (Miller, 2005; Sanders *et al*., 2006b).

BLOOD PRESSURE AND ARTERIAL STIFFNESS

Both systolic and diastolic BP increase with age in economically developed communities and show a continuous association with risk of CVD without a threshold (Lewington and Clarke, 2005). Elevated BP is a self-amplifying condition and is strongly associated with body mass index. There is also a strong association between the development of hypertension and hyperlipidaemia. There is convincing evidence that weight loss results in a fall in BP (Neter *et al*., 2003). There is convincing evidence for a BP lowering effect of combining replacement of saturated fatty acids with monounsaturated fatty acids as part of a healthy lifestyle diet (DASH/OMNIHEART) that includes an increased proportion of fruit and vegetables, whole-grains and reduced salt intake (Appel *et al*., 2003; Appel *et al*., 2005). There is insufficient evidence that replacement of saturated with monounsaturated fatty acid alone has a significant effect on BP (Shah *et al*.,

2007). There is possible evidence that linoleic acid may contribute to the prevention of raised BP (Miura *et al*., 2008). High intakes (>2 g/d) of n-3 LCPUFA convincingly lower BP (Geleijnse *et al*., 2002), and there is possible evidence that habitual intakes at lower levels have the same effect (Ueshima *et al*., 2007). Over the age of sixty systolic BP increases more than diastolic BP and this is likely in part to be a consequence of arterial stiffening. Arterial stiffness is emerging as a strong predictor of CVD risk in the elderly (Terai *et al*., 2008; Anderson *et al*., 2009). There is possible evidence that n-3 LCPUFA may decrease arterial stiffening (Hamazaki *et al*., 1988; Yamada *et al*., 2000; Tomiyama *et al*., 2005).

ENDOTHELIAL FUNCTION

Impaired endothelial function as measured by the flow mediated dilatation technique is associated with increased CVD risk (Yeboah *et al*., 2007). Hyperlipidaemia and hyperglycaemia are two factors known to impair endothelial function. Meals high in long-chain fatty acids that induce substantial lipaemia, compared with meals low in fat but high in carbohydrate, result in an impairment of endothelial function in the postprandial period in healthy subjects (Vogel *et al*., 1997; Ong *et al*., 1999; Vogel *et al*., 2000; Bae *et al*., 2001; Cortes *et al*., 2006). There is possible evidence that n-3 LCPUFA may improve (Engler *et al*., 2004; Goodfellow *et al*., 2000; Leeson *et al*., 2002) and *trans* fatty acids may impair (de Roos *et al*., 2001) endothelial function. There is insufficient evidence to conclude that there are any other differences between monounsaturated, polyunsaturated and SFA (Hall, 2009).

DIETARY INTERACTIONS WITH GENOTYPE

Several gene polymorphisms for lipid and haemostatic risk factors have been identified that may have interactions with dietary fat intake. Subjects who carry the ε4 allele for apolipoprotein have higher total and LDL-C concentrations compared with those carrying the common ε3 allele. These ε4 carriers appear to show greater absolute falls in total and LDL-C compared with ε3 carriers when they decrease their intakes of saturated fatty acids and cholesterol (Lefevre *et al*., 1997; Sarkkinen *et al*., 1998). Subjects who are homozygous for the ε2 allele do not show increase in serum cholesterol in response to dietary cholesterol, but this genotype is associated with an increased prevalence of WHO Type II hyperlipoproteinaemia, which responds to a low fat diet.

About 1:500 people carry mutations for the LDL receptor. These individuals have higher TC and LDL-C concentrations and a 25-fold increased risk of developing premature cardiovascular disease. Plasma total and LDL-C concentrations in individuals who carry this mutation are relatively unresponsive to changes in the level or type of dietary fat (Poustie and Rutherford, 2001).

The interactions between genes and environmental factors require further elucidation. However, the current state of knowledge provides convincing evidence that the major determinants of differences in metabolic risk factors within and across populations primarily are due to behavioral and lifestyle factors (diet, physical activity, obesity, smoking, alcohol use), rather than genetic differences (Wu *et al*., 2007; Ordovas, 2009).

REFERENCES

Almendingen, K., Jordal, O., Kierulf, P., Sandstad, B. & Pedersen, J.I. 1995. 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.

Anderson, S.G., Sanders, T.A. & Cruickshank, J.K. 2009. Plasma fatty acid composition as a predictor of arterial stiffness and mortality. *Hypertension*, 53: 839-845.

Appel, L.J., Champagne, C.M., Harsha, D.W., Cooper, L.S., Obarzanek, E., Elmer, P.J., Stevens, V.J., Vollmer, W.M., Lin, P.H., Svetkey, L.P., Stedman, S.W. & Young, D.R. 2003. Effects of comprehensive lifestyle modification on blood pressure control: main results of the PREMIER clinical trial. *JAMA*, 289: 2083-2093.

Appel, L.J., Sacks, F.M., Carey, V.J., Obarzanek, E., Swain, J.F., Miller, E.R., Conlin, P.R., Erlinger, T.P., Rosner, B.A., Laranjo, N.M., Charleston, J., McCarron, P. & Bishop, L.M. 2005. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. *JAMA*, 294: 2455-2464.

Bae, J.H., Bassenge, E., Kim, K.B., Kim, Y.N., Kim, K.S., Lee, H.J., Moon, K.C., Lee, M.S., Park, K.Y. & Schwemmer, M. 2001. Postprandial hypertriglyceridemia impairs endothelial function by enhanced oxidant stress. *Atherosclerosis*, 155: 517-523.

Baer, D.J., Judd, J.T., Clevidence, B.A. & Tracy, R.P. 2004. Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. *Am. J. Clin. Nutr*., 79: 969-973.

Balk, E.M., Lichtenstein, A.H., Chung, M., Kupelnick, B., Chew, P. & Lau, J. 2006. Effects of omega-3 fatty acids on serum markers of cardiovascular disease risk: a systematic review. *Atherosclerosis*, 189: 19-30.

Bays, H. 2006. Clinical overview of Omacor: a concentrated formulation of omega-3 polyunsaturated fatty acids. *Am. J. Cardiol*., 98: 71i-76i.

Berry, S.E., Miller, G.J. & Sanders, T.A. 2007a. The solid fat content of stearic acid-rich fats determines their postprandial effects. *Am. J. Clin. Nutr*., 85: 1486-1494.

Berry, S.E., Woodward, R., Yeoh, C., Miller, G.J. & Sanders, T.A. 2007b. Effect of interesterification of palmitic acid-rich triacylglycerol on postprandial lipid and factor VII response. *Lipids*, 42: 315-323.

Blok, W.L., Deslypere, J.P., Demacker, P.N., van d, V, Hectors, M.P., van der Meer, J.W. & Katan, M.B. 1997. Pro- and anti-inflammatory cytokines in healthy volunteers fed various doses of fish oil for 1 year. *Eur. J. Clin. Invest*., 27: 1003-1008.

Brouwer, I.A., Wanders, A.J. & Katan, M.B. 2010. Effect of animal and trans fatty acids on HDL and LDL cholesterol levels in humans – a quantitative review. *PLoS One*, 2:5(3):e9434.

Carroll, M.D., Lacher, D.A., Sorlie, P.D., Cleeman, J.I., Gordon, D.J., Wolz, M., Grundy, S.M. & Johnson, C.L. 2005. Trends in serum lipids and lipoproteins of adults, 1960-2002. *JAMA*, 294: 1773-1781.

Caslake, M.J., Miles, E.A., Kofler, B.M., Lietz, G., Curtis, P., Armah, C.K., Kimber, A.C., Grew, J.P., Farrell, L., Stannard, J., Napper, F.L., Sala-Vila, A., West, A.L., Mathers, J.C., Packard, C., Williams, C.M., Calder, P.C. & Minihane, A.M. 2008. Effect of sex and genotype on cardiovascular biomarker response to fish oils: the FINGEN Study. *Am. J. Clin. Nutr*., 88: 618-629.

Chardigny, J.M., Destaillats, F., Malpuech-Brugere, C., Moulin, J., Bauman, D.E., Lock, A.L., Barbano, D.M., Mensink, R.P., Bezelgues, J.B., Chaumont, P., Combe, N., Cristiani, I., Joffre, F., German, J.B., Dionisi, F., Boirie, Y. & Sebedio, J.L. 2008. Do trans fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the trans Fatty Acids Collaboration (TRANSFACT) study. *Am. J. Clin. Nutr*., 87: 558-566.

Choudhury, N., Tan, L. & Truswell, A.S. 1995. Comparison of palmolein and olive oil: effects on plasma lipids and vitamin E in young adults. *Am. J. Clin. Nutr*., 61: 1043-1051.

Cortes, B., Nunez, I., Cofan, M., Gilabert, R., Perez-Heras, A., Casals, E., Deulofeu, R. & Ros, E. 2006. Acute effects of high-fat meals enriched with walnuts or olive oil on postprandial endothelial function. *J. Am. Coll. Cardiol*., 48: 1666-1671.

Costacou, T. & Mayer-Davis, E.J. 2003. Nutrition and prevention of type 2 diabetes. *Ann. Rev. Nutr*., 23: 147-170.

Critchley, J., Liu, J., Zhao, D., Wei, W. & Capewell, S. 2004. Explaining the increase in coronary heart disease mortality in Beijing between 1984 and 1999. *Circulation*, 110: 1236-1244.

de Roos, N.M., Bots, M.L. & Katan, M.B. 2001. Replacement of dietary saturated fatty acids by trans fatty acids lowers serum HDL cholesterol and impairs endothelial function in healthy men and women. *Arterioscler. Thromb. Vasc. Biol*., 21: 1233-1237.

EFSA (European Food Safety Authority). 2004. Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the presence of *trans* fatty acids in foods and the effect on human health of the consumption of *trans* fatty acids. *EFSA J*., 81: 1-49.

Engler, M.M., Engler, M.B., Malloy, M., Chiu, E., Besio, D., Paul, S., Stuehlinger, M., Morrow, J., Ridker, P., Rifai, N. & Mietus-Snyder, M. 2004. Docosahexaenoic acid restores endothelial function in children with hyperlipidemia: results from the EARLY study. *Int. J. Clin. Pharmacol. Ther*., 42: 672-679.

Erridge, C., Attina, T., Spickett, C.M. & Webb, D.J. 2007. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *Am. J. Clin. Nutr*., 86: 1286-1292.

Evans, A., Tolonen, H., Hense, H.W., Ferrario, M., Sans, S. & Kuulasmaa, K. 2001. Trends in coronary risk factors in the WHO MONICA project. *Int. J. Epidemiol*., 30 (Suppl. 1): S35-S40.

Finnegan, Y.E., Minihane, A.M., Leigh-Firbank, E.C., Kew, S., Meijer, G.W., Muggli, R., Calder, P.C. & Williams, C.M. 2003. Plant- and marine-derived n-3 polyunsaturated fatty acids have differential effects on fasting and postprandial blood lipid concentrations

and on the susceptibility of LDL to oxidative modification in moderately hyperlipidemic subjects. *Am. J. Clin. Nutr.,* 77: 783-795.

Folsom, A.R., Aleksic, N., Park, E., Salomaa, V., Juneja, H. & Wu, K.K. 2001. Prospective study of fibrinolytic factors and incident coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler. Thromb. Vasc. Biol*., 21: 611-617.

Garg, A. 1998. High-monounsaturated-fat diets for patients with diabetes mellitus: a meta-analysis. *Am. J. Clin. Nutr*., 67: 577S-582S.

Geleijnse, J.M., Giltay, E.J., Grobbee, D.E., Donders, A.R. & Kok, F.J. 2002. Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials. *J. Hypertens*., 20: 1493-1499.

Geppert, J., Kraft, V., Demmelmair, H. & Koletzko, B. 2006. Microalgal docosahexaenoic acid decreases plasma triacylglycerol in normolipidaemic vegetarians: a randomised trial. *Br. J. Nutr*., 95: 779-786.

Goodfellow, J., Bellamy, M.F., Ramsey, M.W., Jones, C.J. & Lewis, M.J. 2000. Dietary supplementation with marine omega-3 fatty acids improve systemic large artery endothelial function in subjects with hypercholesterolemia. *J. Am. Coll. Cardiol*., 35: 265-270.

Grainger, D.J., Mosedale, D.E., Metcalfe, J.C. & Bottinger, E.P. 2000. Dietary fat and reduced levels of TGFbeta1 act synergistically to promote activation of the vascular endothelium and formation of lipid lesions. *J. Cell Sci*., 113 (Pt 13): 2355-2361.

Griendling, K.K. & FitzGerald, G.A. 2003. Oxidative stress and cardiovascular injury: Part II: animal and human studies. *Circulation*, 108: 2034-2040.

Griffin, M.D., Sanders, T.A., Davies, I.G., Morgan, L.M., Millward, D.J., Lewis, F., Slaughter, S., Cooper, J.A., Miller, G.J. & Griffin, B.A. 2006. Effects of altering the ratio of dietary n-6 to n-3 fatty acids on insulin sensitivity, lipoprotein size, and postprandial lipemia in men and postmenopausal women aged 45-70 y: the OPTILIP Study. *Am. J. Clin. Nutr*., 84: 1290-1298.

Hall, W.L. 2009. Dietary saturated and unsaturated fats as determinants of blood pressure and vascular function. *Nutr. Res. Rev*., 22: 18-38.

Hamalainen, H., Ronnemaa, T., Virtanen, A., Lindstromm J., Erikssonm J.G., Valle, T.T., Ilanne-Parikka, P., Keinanen-Kiukaanniemi, S., Rastas, M., Aunola, S., Uusitupa, M. & Tuomilehto, J. 2005. Improved fibrinolysis by an intensive lifestyle intervention in subjects with impaired glucose tolerance. The Finnish Diabetes Prevention Study. *Diabetologia*, 48: 2248-2253.

Hamazaki, T., Urakaze, M., Sawazaki, S., Yamazaki, K., Taki, H. & Yano, S. 1988. Comparison of pulse wave velocity of the aorta between inhabitants of fishing and farming villages in Japan. *Atherosclerosis*, 73: 157-160.

Harris, W.S. & Muzio, F. 1993. Fish oil reduces postprandial triglyceride concentrations without accelerating lipid-emulsion removal rates. *Am. J. Clin. Nutr*., 58: 68-74.

Heinrich, J., Balleisen, L., Schulte, H., Assmann, G. & van de Loo, J. 1994. Fibrinogen and factor VII in the prediction of coronary risk. Results from the PROCAM study in healthy men. *Arterioscler. Thromb.*, 14: 54-59.

Jebb, S.A., Lovegrove, J.A., Griffin, B.A., Frost, G.S., Moore, C.S., Chatfield, M.D., Bluck, L.J., Williams, C.M. & Sanders T.A.; RISCK Study Group 2010. Effect of changing the amount and type of fat and carbohydrate on insulin sensitivity and cardiovascular risk: the RISCK (Reading, Imperial, Surrey, Cambridge, and Kings) trial. *Am. J. Clin. Nutr.*, 92(4): 748-758.

Kelley, D.S., Siegel, D., Vemuri, M. & Mackey, B.E. 2007. Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men. *Am. J. Clin. Nutr.,* 86: 324-333.

Keys, A.N.C.E. & Parlin R.W. 1966. Serum Cholesterol Response to Changes in Dietary Lipids. *Am. J. Clin. Nutr.,* 19: 175-181.

Kwiterovich, P.O., Jr. 2002. Clinical relevance of the biochemical, metabolic, and genetic factors that influence low-density lipoprotein heterogeneity. *Am. J. Cardiol.*, 90: 30i-47i.

Law, M. 2000. Plant sterol and stanol margarines and health. *Brit. Med. J.*, 320: 861-864.

Leeson, C.P., Mann, A., Kattenhorn, M., Deanfield, J.E., Lucas, A. & Muller, D.P. 2002. Relationship between circulating n-3 fatty acid concentrations and endothelial function in early adulthood. *Eur. Heart. J.*, 23: 216-222.

Lefevre, M., Ginsberg, H.N., Kris-Etherton, P.M., Elmer, P.J., Stewart, P.W., Ershow, A., Pearson, T.A., Roheim, P.S., Ramakrishnan, R., Derr, J., Gordon, D.J. & Reed, R. 1997. ApoE genotype does not predict lipid response to changes in dietary saturated fatty acids in a heterogeneous normolipidemic population. The DELTA Research Group. Dietary Effects on Lipoproteins and Thrombogenic Activity. *Arterioscler. Thromb. Vasc. Biol.*, 17: 2914-2923.

Lewington, S. & Clarke, R. 2005. Combined effects of systolic blood pressure and total cholesterol on cardiovascular disease risk. *Circulation*, 112: 3373-3374.

Lewington, S., Whitlock, G., Clarke, R., Sherliker, P., Emberson, J., Halsey, J., Qizilbash, N., Peto, R. & Collins, R. 2007. Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet*, 370: 1829-1839.

Meade, T.W., Ruddock, V., Stirling, Y., Chakrabarti, R. & Miller, G.J. 1993. Fibrinolytic activity, clotting factors, and long-term incidence of ischaemic heart disease in the Northwick Park Heart Study (see comments). *Lancet*, 342: 1076-1079.

Mensink, R.P., Zock, P.L., Kester, A.D. & Katan, M.B. 2003. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am. J. Clin. Nutr.,* 77: 1146-1155.

Meydani, M. 2000. Omega-3 fatty acids alter soluble markers of endothelial function in coronary heart disease patients. *Nutr. Rev.*, 58: 56-59.

Miller, G.J. 2005. Dietary fatty acids and the haemostatic system. *Atherosclerosis*, 179: 213-227.

Minihane, A.M., Khan, S., Leigh-Firbank, E.C., Talmud, P., Wright, J.W., Murphy, M.C., Griffin, B.A. & Williams, C.M. 2000. ApoE polymorphism and fish oil supplementation in subjects with an atherogenic lipoprotein phenotype. *Arterioscler. Thromb. Vasc. Biol*., 20: 1990-1997.

Miura, K., Stamler, J., Nakagawa, H., Elliott, P., Ueshima, H., Chan, Q., Brown, I.J., Tzoulaki, I., Saitoh, S., Dyer, A.R., Daviglus, M.L., Kesteloot, H., Okayama, A., Curb, J.D., Rodriguez, B.L., Elmer, P.J., Steffen, L.M., Robertson, C. & Zhao, L. 2008. Relationship of dietary linoleic acid to blood pressure. The International Study of Macro-Micronutrients and Blood Pressure Study (corrected). *Hypertension*, 52: 408-414.

Motard-Belanger, A., Charest, A., Grenier, G., Paquin, P., Chouinard, Y., Lemieux, S., Couture, P. & Lamarche, B. 2008. Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. *Am. J. Clin. Nutr.,* 87: 593-599.

Mozaffarian, D. & Clarke, R. 2009. Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. *EJCN*, 63 (Suppl. 2): S22-S33.

NCEP-3. 2001. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *JAMA,* 285: 2486-2497.

Nestel, P., Noakes, M., Belling, B., McArthur, R., Clifton, P., Janus, E. & Abbey, M. 1992. Plasma lipoprotein lipid and Lp(a) changes with substitution of elaidic acid for oleic acid in the diet. *J. Lipid Res*., 33: 1029-1036.

Neter, J.E., Stam, B.E., Kok, F.J., Grobbee, D.E. & Geleijnse, J.M. 2003. Influence of weight reduction on blood pressure: a meta-analysis of randomized controlled trials. *Hypertension,* 42: 878-884.

Ng, T.K., Hayes, K.C., DeWitt, G.F., Jegathesan, M., Satgunasingam, N., Ong, A.S. & Tan, D. 1992. Dietary palmitic and oleic acids exert similar effects on serum cholesterol and lipoprotein profiles in normocholesterolemic men and women. *J. Am. Coll. Nutr*., 11: 383-390.

Oakley, F.R., Sanders, T.A. & Miller, G.J. 1998. Postprandial effects of an oleic acid-rich oil compared with butter on clotting factor VII and fibrinolysis in healthy men. *Am. J. Clin. Nutr.,* 68: 1202-1207.

Ong, P.J., Dean, T.S., Hayward, C.S., la Monica, P.L., Sanders, T.A. & Collins, P. 1999. Effect of fat and carbohydrate consumption on endothelial function. *Lancet,* 354: 2134.

Orchard, T.J., Temprosa, M., Goldberg, R., Haffner, S., Ratner, R., Marcovina, S. & Fowler, S. 2005. The effect of metformin and intensive lifestyle intervention on the metabolic syndrome: the Diabetes Prevention Program randomized trial. *Ann. Intern. Med*., 142: 611-619.

Ordovas, J.M. 2009. Genetic influences on blood lipids and cardiovascular disease risk: tools for primary prevention. *Am. J. Clin. Nutr.,* 89: 1509S-17S.

Poustie, V.J. & Rutherford, P. 2001. Dietary treatment for familial hypercholesterolaemia. *Cochrane Database Syst. Rev*., CD001918.

Ridker, P.M. 2001. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation*, 103: 1813-1818.

Roumen, C., Corpeleijn, E., Feskens, E.J., Mensink, M., Saris, W.H. & Blaak, E.E. 2008. Impact of 3-year lifestyle intervention on postprandial glucose metabolism: the SLIM study. *Diabet. Med*., 25: 597-605.

Sanders, T.A. & Berry, S.E. 2005. Influence of stearic acid on postprandial lipemia and hemostatic function. *Lipids*, 40: 1221-1227.

Sanders, T.A., Berry, S.E. & Miller, G.J. 2003a. Influence of triacylglycerol structure on the postprandial response of factor VII to stearic acid-rich fats. *Am. J. Clin. Nutr.,* 77: 777-782.

Sanders, T.A., de Grassi, T., Miller, G.J. & Humphries, S.E. 1999. Dietary oleic and palmitic acids and postprandial factor VII in middle-aged men heterozygous and homozygous for factor VII R353Q polymorphism. *Am. J. Clin. Nutr.,* 69: 220-225.

Sanders, T.A., de Grassi, T., Miller, G.J. & Morrissey, J.H. 2000. Influence of fatty acid chain length and cis/trans isomerization on postprandial lipemia and factor VII in healthy subjects (postprandial lipids and factor VII). *Atherosclerosis*, 149: 413-420.

Sanders, T.A., Gleason, K., Griffin, B. & Miller, G.J. 2006a. Influence of an algal triacylglycerol containing docosahexaenoic acid (22 : 6n-3) and docosapentaenoic acid (22 : 5n-6) on cardiovascular risk factors in healthy men and women. *Br. J. Nutr.,* 95: 525-531.

Sanders, T.A., Lewis, F., Slaughter, S., Griffin, B.A., Griffin, M., Davies, I., Millward, D.J., Cooper, J.A. & Miller, G.J. 2006b. Effect of varying the ratio of n-6 to n-3 fatty acids by increasing the dietary intake of alpha-linolenic acid, eicosapentaenoic and docosahexaenoic acid, or both on fibrinogen and clotting factors VII and XII in persons aged 45-70 y: the OPTILIP study. *Am. J. Clin. Nutr.,* 84: 513-522.

Sanders, T.A., Oakley, F.R., Cooper, J.A. & Miller, G.J. 2001. Influence of a stearic acid-rich structured triacylglycerol on postprandial lipemia, factor VII concentrations, and fibrinolytic activity in healthy subjects. *Am. J. Clin. Nutr.,* 73: 715-721.

Sanders, T.A., Oakley, F.R., Crook, D., Cooper, J.A. & Miller, G.J. 2003b. High intakes of trans monounsaturated fatty acids taken for 2 weeks do not influence procoagulant and fibrinolytic risk markers for CHD in young healthy men. *Br. J. Nutr.,* 89: 767-776.

Sanders, T.A., Oakley, F.R., Crook, D., Cooper, J.A. & Miller, G.J. 2003c. High intakes of trans monounsaturated fatty acids taken for 2 weeks do not influence procoagulant and fibrinolytic risk markers for CHD in young healthy men. *Br. J. Nutr.,* 89: 767-776.

Sanders, T.A., Oakley, F.R., Miller, G.J., Mitropoulos, K.A., Crook, D. & Oliver, M.F. 1997. Influence of n-6 versus n-3 polyunsaturated fatty acids in diets low in saturated

fatty acids on plasma lipoproteins and hemostatic factors. *Arterioscler. Thromb. Vasc. Biol*., 17: 3449-3460.

Sarkkinen, E., Korhonen, M., Erkkila, A., Ebeling, T. & Uusitupa, M. 1998. Effect of apolipoprotein E polymorphism on serum lipid response to the separate modification of dietary fat and dietary cholesterol. *Am. J. Clin. Nutr.,* 68: 1215-1222.

Seed, M., Hoppichler, F., Reaveley, D., McCarthy, S., Thompson, G.R., Boerwinkle, E. & Utermann, G. 1990. Relation of serum lipoprotein(a) concentration and apolipoprotein(a) phenotype to coronary heart disease in patients with familial hypercholesterolemia. *N. Engl. J. Med*., 322: 1494-1499.

Shah, M., dams-Huet, B. & Garg, A. 2007. Effect of high-carbohydrate or high-cis-monounsaturated fat diets on blood pressure: a meta-analysis of intervention trials. *Am. J. Clin. Nutr.,* 85: 1251-1256.

Siri-Tarino, P.W., Williams, P.T., Fernstrom, H.S., Rawlings, R.S. & Krauss, R.M. 2009. Reversal of small, dense LDL subclass phenotype by normalization of adiposity. *Obesity*, E-pub ahead of print.

Sundram, K., Ismail, A., Hayes, K.C., Jeyamalar, R. & Pathmanathan, R. 1997. Trans (elaidic) fatty acids adversely affect the lipoprotein profile relative to specific saturated fatty acids in humans. *J. Nutr*. 127: 514S-520S.

Tardy, A.L., Lambert-Porcheron, S., Malpuech-Brugere, C., Giraudet, C., Rigaudiere, J.P., Laillet, B., Leruyet, P., Peyraud, J.L., Boirie, Y., Laville, M., Michalski, M.C., Chardigny, J.M. & Morio, B. 2009. Dairy and industrial sources of trans fat do not impair peripheral insulin sensitivity in overweight women. *Am. J. Clin. Nutr.,* 90: 88-94.

Terai, M., Ohishi, M., Ito, N., Takagi, T., Tatara, Y., Kaibe, M., Komai, N., Rakugi, H. & Ogihara, T. 2008. Comparison of arterial functional evaluations as a predictor of cardiovascular events in hypertensive patients: the Non-Invasive Atherosclerotic Evaluation in Hypertension, (NOAH) study. *Hypertens. Res*., 31: 1135-1145.

Theobald, H.E., Chowienczyk, P.J., Whittall, R., Humphries, S.E. & Sanders, T.A. 2004. LDL cholesterol-raising effect of low-dose docosahexaenoic acid in middle-aged men and women. *Am. J. Clin. Nutr.,* 79: 558-563.

Theobald, H.E., Goodall, A.H., Sattar, N., Talbot, D.C., Chowienczyk, P.J. & Sanders, T.A. 2007. Low-dose docosahexaenoic acid lowers diastolic blood pressure in middle-aged men and women. *J. Nutr*., 137: 973-978.

Tholstrup, T., Miller, G.J., Bysted, A. & Sandstrom, B. 2003. Effect of individual dietary fatty acids on postprandial activation of blood coagulation factor VII and fibrinolysis in healthy young men. *Am. J. Clin. Nutr.,* 77: 1125-1132.

Tholstrup, T., Sandstrom, B., Bysted, A. & Holmer, G. 2001. Effect of 6 dietary fatty acids on the postprandial lipid profile, plasma fatty acids, lipoprotein lipase, and cholesterol ester transfer activities in healthy young men. *Am. J. Clin. Nutr.,* 73: 198-208.

Tomiyama, H., Takazawa, K., Osa, S., Hirose, K., Hirai, A., Iketani, T., Monden, M., Sanoyama, K. & Yamashina, A. 2005. Do eicosapentaenoic acid supplements attenuate age-related increases in arterial stiffness in patients with dyslipidemia?: A preliminary study. *Hypertens. Res*., 28: 651-655.

Tuomilehto, J., Lindstrom, J., Eriksson, J.G., Valle, T.T., Hamalainen, H., Ilanne-Parikka, P., Keinanen-Kiukaanniemi, S., Laakso, M., Louheranta, A., Rastas, M., Salminen, V. & Uusitupa, M. 2001. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N. Engl. J. Med*., 344: 1343-1350.

Ueshima, H., Stamler, J., Elliott, P., Chan, Q., Brown, I.J., Carnethon, M.R., Daviglus, M.L., He, K., Moag-Stahlberg, A., Rodriguez, B.L., Steffen, L.M., Van, H.L., Yarnell, J. & Zhou, B. 2007. Food omega-3 fatty acid intake of individuals (total, linolenic acid, long-chain) and their blood pressure: INTERMAP study. *Hypertension,* 50: 313-319.

Vartiainen, E., Jousilahti, P., Alfthan, G., Sundvall, J., Pietinen, P. & Puska, P. 2000. Cardiovascular risk factor changes in Finland, 1972-1997. *Int. J. Epidemiol*., 29: 49-56.

Vedin, I., Cederholm, T., Freund, L.Y., Basun, H., Garlind, A., Faxen, I.G., Jonhagen, M.E., Vessby, B., Wahlund, L.O. & Palmblad, J. 2008. Effects of docosahexaenoic acid-rich n-3 fatty acid supplementation on cytokine release from blood mononuclear leukocytes: the OmegAD study. *Am. J. Clin. Nutr.,* 87: 1616-1622.

Vessby, B., Unsitupa, M., Hermansen, K., Riccardi, G., Rivellese, A.A., Tapsell, L.C., Nalsen, C., Berglund, L., Louheranta, A., Rasmussen, B.M., Calvert, G.D., Maffetone, A., Pedersen, E., Gustafsson, I.B. & Storlien, L.H. 2001. Substituting dietary saturated for monounsaturated fat impairs insulin sensitivity in healthy men and women: The KANWU Study. *Diabetologia*, 44: 312-319.

Vogel, R.A., Corretti, M.C. & Plotnick, G.D. 1997. Effect of a single high-fat meal on endothelial function in healthy subjects. *Am. J. Cardiol*., 79: 350-354.

Vogel, R.A., Corretti, M.C. & Plotnick, G.D. 2000. The postprandial effect of components of the Mediterranean diet on endothelial function. *J. Am. Coll. Cardiol*., 36: 1455-1460.

Weggemans, R.M., Zock, P.L., Katan, M.B. 2001. Dietary cholesterol from eggs increases the ratio of total cholesterol to high-density lipoprotein cholesterol in humans: a meta-analysis. *Am. J. Clin. Nutr.,* 73:885-91.

Whitlock, G., Lewington, S., Sherliker, P., Clarke, R., Emberson, J., Halsey, J., Qizilbash, N., Collins, R. & Peto, R. 2009. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet,* 373: 1083-1096.

Wu, K., Bowman, R., Welch, A.A., Luben, R.N., Wareham, N., Khaw, K.T. & Bingham, S.A. 2007. Apolipoprotein E polymorphisms, dietary fat and fibre, and serum lipids: the EPIC Norfolk study. *Eur. Heart. J*., 28: 2930-2936.

Yamada, T., Strong, J.P., Ishii, T., Ueno, T., Koyama, M., Wagayama, H., Shimizu, A., Sakai, T., Malcom, G.T. & Guzman, M.A. 2000. Atherosclerosis and omega-3 fatty

acids in the populations of a fishing village and a farming village in Japan. *Atherosclerosis*, 153: 469-481.

Yeboah, J., Crouse, J.R., Hsu, F.C., Burke, G.L. & Herrington, D.M. 2007. Brachial flow-mediated dilation predicts incident cardiovascular events in older adults: the Cardiovascular Health Study. *Circulation*, 115: 2390-2397.

Yu-Poth, S., Zhao, G., Etherton, T., Naglak, M., Jonnalagadda, S. & Kris-Etherton, P.M. 1999. Effects of the National Cholesterol Education Program's Step I and Step II dietary intervention programs on cardiovascular disease risk factors: a meta-analysis. *Am. J. Clin. Nutr.,* 69: 632-646.

Zampelas, A., Peel, A.S., Gould, B.J., Wright, J. & Williams, C.M. 1994. Polyunsaturated fatty acids of the n-6 and n-3 series: effects on postprandial lipid and apolipoprotein levels in healthy men. *EJCN*, 48: 842-848.

Zhang, J., Ping, W., Chunrong, W., Shou, C.X. & Keyou, G. 1997. Nonhypercholesterolemic effects of a palm oil diet in Chinese adults. *J. Nutr*., 127: 509S-513S.\

Chapter 11: Dietary fat and coronary heart disease

Ecological studies that compare differences in CHD rates with mean intakes of fatty acids in different populations are uniquely informative because such associations are virtually unaffected by regression dilution bias. The best known ecological study of diet and CHD is the Seven Countries Study, which consisted of 16 cohorts in 7 different countries involving a total of 12,763 middle-aged men that were examined between 1958 and 1964 (Keys, 1980). The results showed that a substantial proportion of the variation in CHD death rates between geographical regions was explained by differences in intake of SFA and MUFA (Keys *et al*., 1986). Moreover, the Seven Countries study also demonstrated strong associations between mean intakes of SFA and mean levels of total cholesterol (Keys, 1980). The Seven Countries study prompted the "Diet Heart" hypothesis that high intakes of SFA and cholesterol and low intakes of PUFA increase the level of total cholesterol and ultimately result in the development of CHD.

The results of dietary feeding trials (or "metabolic ward" studies) which measured blood lipids in healthy volunteers after administration of controlled diets with varying intakes of fats were concordant with the findings of the associations observed between intakes of different fatty acids and changes in blood cholesterol levels observed in the ecological studies. In particular, Keys *et al*. (1965) and Hegsted *et al*. (1965) demonstrated that average change in serum cholesterol concentrations could be predicted as equations for the changes in intake of SFA and PUFA and dietary cholesterol. The concordance of the results of the ecological and the metabolic ward studies probably relate to the limited amount of measurement error in both study designs. In view of these findings, some investigators have concluded that use of cholesterol as an intermediary factor is the most rational way of studying the associations between diet and CHD, with appropriate correction for measurement error in both study designs. Nevertheless, many investigators have examined the associations of differences in intake of fatty acids directly with CHD risk within populations. This review summarizes the evidence from the cohort studies and dietary intervention trials that examined the effects of differences in diet (or exchanges of particular fats by another or by carbohydrate) on risk of CHD.

Few within-population studies have been able to demonstrate consistent associations between CHD risk and any specific dietary lipids, with the exception of *trans* fats and n-3 fatty acids. The available evidence from cohort studies and randomized controlled trials on which to make judgement and substantiate the effects of dietary fat on risk of coronary heart disease is unsatisfactory and unreliable. The null results of the observational and interventional studies of dietary lipids and CHD do not negate the importance of the underlying associations, but reflect the combined effects of limitations of dietary assessment methods, inadequate number of participants studied, narrow range of fat intakes, and the prolonged follow-up of individuals without repeat dietary assessment. Furthermore, the evidence from cohort studies of dietary intake of fats and CHD is mostly unreliable (with a few exceptions) because most studies have ignored the effects of measurement error and regression dilution bias. Few studies attempted to measure the within-person variability or reproducibility of

the categorizations of dietary fat when assessing these associations. Hence, the null results are very likely to result from regression dilution bias and confounding of one nutrient by another.

The body of evidence from prospective cohort studies of n-3 LCPUFA intake or fish consumption and risk of fatal CHD is comprehensive in a number of studies, duration of follow-up, number of participants and CHD events, geographic location of study populations, homogeneity of association between trials, and absence of evidence for publication bias. The observational evidence is convincing that an inverse association exists between n-3 LCPUFA or fish intake and risk of CHD. The evidence from randomized controlled trials is concordant, particularly when two trials with methodological concerns (Singh *et al*., 1997; Burr *et al*. 2003) are excluded from consideration. However, the clinical trial evidence for prevention of CHD death rests largely on the results from two trials GISSI (GISSI-Prevenzione Investigators, 1999) and DART I (Burr *et al*., 1989).

The observational evidence that TFA are independently associated with increased risk of CHD events is convincing, though based on a more limited body of evidence. The evidence of an association with fatal CHD is not as comprehensive. In view of the consistency and strength of the observational evidence, the absence of evidence from randomized controlled trials should not preclude a judgement of convincing.

There is probably no direct relation between total fat intake and risk of coronary heart disease. The strongest evidence in support of this judgement comes from the Women's Health Initiative that showed that CHD risk was not reduced after 8 years of a low-fat diet (Howard *et al*., 2006). The observational evidence, summarized in the meta-analysis, showed no association between total fat intake and CHD risk, although there was heterogeneity between the study results. The observational evidence for an association between dietary PUFA or MUFA and CHD risk is limited, inconsistent and unreliable.

The body of evidence from clinical trials of fat modified diets is limited, excluding n-3 LCPUFA and fish interventions. The ten or so published trials are heterogeneous in the nature of the dietary intervention and many of the trials are based on a small number of CHD deaths or events; nevertheless, taken together, there were slightly more than 600 CHD deaths and 3,700 CHD events in the intervention trials. The heterogeneous nature of the interventions and lack of compliance may undermine the validity of the summary estimates of risk obtained through meta-analysis of the trial results, as does the small number of trials.

Clinical trials of fat-modified diets, in particular low-fat or high P/S diets, and coronary disease are rarely single factor interventions. Substitution of one type of fat for another or reducing total fat intake, invariably results in a range of food substitutions such that intake of other macro and micronutrients is altered. Many of the early fat intervention trials of CHD required participants to follow a diet lower in cholesterol but with a higher P/S ratio (PUFA/SFA) without a reduction in total fat intake. Furthermore, many trials of advice to modify dietary intake of fat have included one or more other elements of dietary and non-dietary advice, examples include advice to: increase fibre intake, reduce meat consumption, reduce body weight, stop smoking, reduce salt intake, increase fruit and vegetable consumption, increase physical activity, or reduce alcohol consumption. The multifactorial nature of the dietary interventions and accompanying changes in dietary patterns makes it difficult to disentangle the specific effects of dietary fat from other components of the diet. In effect, the dietary interventions are not homogeneous and the results of the meta-analysis should be interpreted with caution. The meta-analysis of clinical trials in which serum cholesterol concentrations in the high P/S diet group were significantly lower at follow-up than in the control group, revealed that a diet higher in PUFA and lower in SFA decreased the risk of fatal CHD.

A pooled analysis of eleven cohort studies of dietary fat and coronary disease was presented to the Expert Consultation and the manuscript was published shortly thereafter in May 2009 (Jakobsen *et al.*, 2009). In the judgement of the Expert Consultation, the results of the "Pooling Project of Cohort Studies on Diet and Coronary Disease" were a significant advance in quality on the update, undertaken by the consultation, of the published meta-analyses of observational trials. The Pooling Project combined the results from 11 cohort studies – each meeting criteria for quality of dietary assessment, years of follow-up, and ascertainment of events – to examine the effect on CHD death and CHD events of replacing SFA with MUFA, PUFA or carbohydrate. The main finding was a significantly decreased risk of CHD death and CHD events when PUFA replace SFA. The multivariate-adjusted hazard ratio for CHD death per 5% total energy incremental substitution of PUFA for SFA was 0.87 (95%CI: 0.77–0.97); for CHD events, the hazard ratio for the same fat substitution was 0.74 (95%CI: 0.61–0.89). This result from the pooling of observational studies, along with supportive evidence from clinical trials of lower CHD risk in high P/S diets, and the effects of PUFA to lower LDL cholesterol and the total / HDL ratio, led the consultation to conclude there was convincing evidence of lower CHD risk when PUFA replace SFA. The comprehensive conclusions of the effect of dietary fat on both fatal CHD and CHD events are summarized in Table 11.1.

TABLE 11.1
Summary judgement of the epidemiological evidence for dietary fat and coronary heart disease

Type of fat	Fatal CHD	CHD events
Total fat	C-NR	C-NR
TFA	P↑	C↑
SFA for CHO	P-NR	P-NR
MUFA for SFA		
PUFA for SFA	C↓	C↓
linoleic		
α linolenic		
n-3 LCPUFA	P↓	C↓

C↑: convincing increase risk
C↓: convincing decrease risk
C-NR: convincing, no relation
P↑: probable increase risk
P↓: probable decrease risk
P-NR: probable no relation

REFERENCES

Burr, M.L., Ashfield-Watt, P.A. *et al*. 2003. Lack of benefit of dietary advice to men with angina: results of a controlled trial. *EJCN*, 57: 193-200.

Burr, M.L., Fehily, A.M., Gilbert, J.F., Rogers, S., Holliday, R.M., Sweetnam, P.M., Elwood, P.C. & Deadman, N.M. 1989. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet*, 2(8666): 757-761.

GISSI-Prevenzione Investigators. 1999. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. *Lancet*, 354: 447-455.

Hegsted, D. M., McGandy, R. B., Myers, M. L.& Stare, F. J. 1965. Quantitative effects of dietary fat on serum cholesterol in man. *Am. J. Clin. Nutr.*,17:281-295.

Howard, B.V., Van Horn, L., Hsia, J., Manson, J.E., Stefanick, M.L., Wassertheil-Smoller, S., Kuller, L.H., LaCroix, A.Z., Langer, R.D., Lasser, N.L., Lewis, C.E., Limacher, M.C., Margolis, K.L., Mysiw, W.J., Ockene, J.K., Parker, L.M., Perri, M.G., Phillips, L., Prentice. R.L., Robbins. J., Rossouw. J.E., Sarto, G.E., Schatz, I,J., Snetselaar, L.G., Stevens, V.J., Tinker, L.F., Trevisan, M., Vitolins, M.Z., Anderson, G.L., Assaf, A.R., Bassford, T., Beresford, S.A., Black, H.R., Brunner, R.L., Brzyski, R.G., Caan, B., Chlebowski, R.T., Gass, M., Granek, I., Greenland, P., Hays, J., Heber, D., Heiss, G., Hendrix, S.L., Hubbell, F.A., Johnson, K.C. & Kotchen, J.M. 2006 Low-fat dietary pattern and risk of cardiovascular disease: the Women's Health Initiative Randomized Controlled Dietary Modification Trial. *JAMA* Feb 8;295(6):655-66.

Jakobsen, M.U., *et al*. 2009. Major types of dietary fat and risk of coronary heart disease: a pooled analysis of 11 cohort studies. *Am. J. Clin. Nutr.*, 89: 1425-1432.

Keys, A. 1980. Coronary heart disease in seven countries. *Circulation*, 41: 1-211.

Keys, A., Anderson, J.T. & Grande, F. 1965. Serum cholesterol response to changes in the diet. IV. Particular saturated fatty acids in the diet. *Metabolism*, 14: 776-787.

Keys, A., Mienott, A., Karvonen, M.J., Aravanis, C., Blackburn, H., Buzina, R., Djordjevic, B.S., Dontas, A.S., Fidanza, F., Keys, M.H., Kromhout, D., Nedeljkovic, S., Punsar, S., Eccareccia, F. & Toshima, H.1986. The diet and 15-year death rate in the seven countries study. *Am. J. Epidemiol.*, 124: 903-915.

Singh, R.B., Niaz, M.A., Sharma, J.P., Kumar, R., Rastogi, V. & M. & Moshiri, M. 1997. Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the Indian experiment of infarct survival--4. *Cardiovascular Drugs & Therapy*, 11: 485-491.

Chapter 12: Fat intake and CNS functioning: Ageing and disease

ASSUMPTIONS AND LIMITATIONS

Brain disorders and mental ill-health

The cost of brain disorders and mental ill-health has been rising sharply and now exceeds all other costs of ill health. In the 25 European Union member states it cost €386 billion in 2004 (Andlin-Sobocki *et al*., 2005). In the UK in 2007 the cost was £77 billion and was estimated to become one of the top three burdens of ill health worldwide by 2020.

Docosahexaenoic acid (DHA) has been the only n-3 fatty acid used as a major structural and functional constituent of the photoreceptor, neurons and their signaling synapses throughout the 600 million years of animal evolution. This is despite there being similar molecules, such as docosapentaenoic acid (DPA), differing by only one double bond. This is one of many compelling reasons for the absolute necessity of DHA for the human brain.

The question arises as to how the requirement for DHA in the brain can be met. DHA can be synthesized from α-linolenic acid (Brenna *et al*., 2009), but the process appears to be very inefficient. Data from primate and rodent/animal experiments demonstrate dietary DHA is used with an order of magnitude greater efficiency for brain growth compared with its endogenous synthesis from ALA (Crawford *et al*., 1976), which is likely to represent an advantage during growth and maintenance.

It is logical to assume that the priority in human development concerns the brain. Based on brain composition of some 30 mammalian species (Crawford *et al*., 1976), one can argue that the target balance of n-6 to n-3 PUFA in the diet should be between 2:1 and 1:1.

The neural system develops extensively during the prenatal period and the first years of life (Dobbing, 1972) and is influenced by multi-generational considerations. There is convincing evidence that neural developmental milestones determine long-term brain functional capacity. Once brain milestones are passed it may be too late to intervene with LCPUFA in neurological/neuropsychological disorders such as depression and bipolar disorder, mood and cognition, Alzheimer's disease (AD), age-related macular degeneration, schizophrenia and Huntington's disease. However, this does not mean PUFA do not help stabilize or even partially reverse such conditions (Freeman *et al*., 2006). There is a need for well designed trials with sufficient power, and supplements that are relevant to supporting the neurovascular systems. Factors that might influence the delivery of energy to the brain cells need to be researched in view of the extraordinarily high energy requirement and dependence of the brain. In addition, a potential role is acknowledged for EPA in these conditions due to its influence on improving vascular function and the resulting effect on the delivery of glucose to the brain.

The ability to conduct RCT on the role of AA and DHA in brain development in humans during the perinatal period is likely to be limited by ethical considerations.

In adult brain disorders, any RCT will face the difficulty of addressing a system in which the origin of the disorder is likely to have a life history, possibly including the developmental period.

In view of the rising burden represented by brain disorders, there is a need to target food production to be in line with requirements of the brain and vascular system and for general good health. The future requirements of the increasing human population cannot be met by a diminishing fisheries catch. Furthermore, the requirement is unlikely to be met by terrestrial products because they do not have the full complement of essential nutrients found in seafood (iodine, n-3 FA, Se etc). It is recommended to expand both fresh water and marine aquaculture by applying the use of agricultural principles to expand productivity of the oceans.

In developing countries where children may be in energy deficit, and where it is planned to increase energy density of the diet with fats and oils, every encouragement should be given to development of indigenous oils that are more physiologically balanced in terms of linoleic and a-linolenic acids rather than importing linoleic acid rich oils which dominate the Western markets. Similarly, developing countries need to guard against importing food products that are rich in atherogenic and thrombogenic fats and do not provide a balance of essential fatty acids.

Limitations of current studies on brain research in humans:

- Studies thus far being done are in too short-term and too few.
- Epidemiological evidence on benefits attributed to n-3 fatty acids is associated with fish and seafood and not solely fish fat.
- Seafood and fish are not just oils. They are particularly rich in iodine, selenium, copper, zinc and manganese as well as a variety of anti-oxidants.

There is evidence that single nutrients do not have the same effect as the integral food or even nutrient clusters (Elvevoll *et al*., 2006). Interactions between the different macro- and micro-nutrients should be recognized and encouraged as a specific research theme (Haider and Bhutta, 2006).

SUMMARY OF REQUIREMENTS

Daily requirement of adult brain for PUFA

Limited data from one human study reveal that there is a requirement (based on turnover of labelled fatty acids) of approximately 18 mg of AA per adult brain/day and 5 mg DHA/brain/day, as free fatty acid (FFA) in the plasma compartment. More research is required to translate this figure into a daily dietary intake of AA and DHA, particularly as both AA and DHA are compartmentalized into different phosphoglycerides, triglycerides and cholesterol ester molecules and taken up by cell membrane phosphoglycerides in all organs.

No studies have been conducted on other plasma lipids or red blood cells, which are potentially rich sources of LCPUFA for the brain. There is some evidence (in rats) that plasma lysoPC-DHA could be a carrier of DHA to brain. The concentrations of arachidonic acid and DHA are high in the vascular endothelium and the brain, but the proportions in the free fatty acid fractions are very low, suggesting that factors other than FFA may be responsible for the biomagnifications. As with the placenta, it is plausible that phospholipids are used with selective sn-2 incorporation accounting for the biomagnifications across the cell membranes. More basic research is required on the turnover from sources other than the FFA fraction in plasma.

n-3 LCPUFA and depression and bipolar disorder

Encouraging data have been obtained from some epidemiological and intervention studies in this area. Doses used in intervention studies have ranged from 0.6–6 g/day. Future directions in this area should involve studies with purified preparations of n-3 LCPUFA (alone and in combination), attention to mode of delivery, dose response studies, and studies on the duration required for greatest benefit. Studies are also needed to delineate the importance of n-3 LCPUFA as monotherapy or adjunct therapy, with identification of the mechanism(s) of action of the PUFA in depression and bipolar disorder. The evidence suggests that there are more consistent benefits with the use of EPA and/or fish oil at a level of 1-2 g/day. The strength of the evidence is regarded as probable for relief of depression. In the case of bipolar disorder, where there have been fewer studies, the strength of the evidence is possible**.**

Cognitive decline

There is limited evidence to support the relationship in adults between n-3 LCPUFA intake/status and altered cognition, although there is support from observational studies. Future directions should involve thorough intervention studies in appropriate subjects using sufficiently sensitive tests designed to measure effects in mood and cognition. The strength of the evidence is regarded as possible**.**

Aggression, hostility and antisocial behaviour

Epidemiological studies have suggested a link between poor EFA status and aggression, hostility and anti-social behaviour. The results of intervention studies with n-3 LCPUFA plus other ingredients have been equivocal. The study populations have been heterogeneous, sometimes with only a small number of subjects. Despite this, there are some encouraging data emerging. Studies in prisoners in the USA have provided some support regarding micronutrients. A recent RCT in the UK brought about a >30% reduction in violence amongst young violent offenders in prison. A 24 hour video surveillance, as employed for legal purposes in the prisons, was used as the outcome measure. The intervention was a combination of EFA and micronutrients on the grounds of their interdependence. The study is being replicated on a larger scale. This is clearly an area where more research is required, particularly in defined populations with larger numbers of subjects. The strength of the evidence is regarded as possible.

Age-related maculopathy (ARM)

Epidemiological and observational data are strongly suggestive of a 30–40% reduction of risk for ARM among regular fish eaters. On this basis, several interventional studies are currently ongoing, examining the potential benefit of supplementation with n-3 LCPUFA for the prevention of late ARM, but none is published yet. There is also a lack of observational data with blood measurement of fatty acids, which could confirm the dietary data. The strength of the evidence is regarded as possible.

Alzheimer's disease

Epidemiological studies examining n-3 LCPUFA intake or blood levels support a role of DHA in the prevention of AD. Cell culture and animal models show promising mechanistic support for DHA in AD. Data from clinical trials are limited, but show some evidence that DHA may be of benefit to patients with milder forms of AD. Larger, randomized clinical trials in the prevention and treatment of AD are required. The strength of the evidence is regarded as insufficient to date.

Schizophrenia

Results from five clinical trials have produced inconsistent results with small effect sizes, which may be of little clinical significance. The strength of the evidence is regarded as insufficient to date.

Huntington's disease

Results from animal studies and several small-scale human studies report some beneficial effects in some of the studies with pure ethyl EPA. The strength of the evidence is regarded as insufficient to date.

TABLE 12.1
Current level of evidence for long-chain n-3 fatty acids in relation to CNS functioning

Condition	Evidence strength
Depression	Probable
Bipolar disorder	Possible
Cognitive decline	Possible
Aggression, hostility and antisocial behaviour	Possible
Age-related macular degeneration	Possible
Alzheimer's disease	Insufficient evidence to date
Schizophrenia	Insufficient evidence to date
Huntington's disease	Insufficient evidence to date

CONCLUSIONS FOR ADULTS CENTRAL NERVOUS SYSTEM (CNS) FUNCTION

Probable

1. Supplementation with n-3 LCPUFA as treatment for depression. Dose, treatment time, preferred n-3 PUFA (EPA, DHA or both), adjunct or monotherapy yet to be defined.

Possible

2. Supplementation with n-3 LCPUFA as treatment for bipolar disorder. Dose, treatment time, preferred n-3 PUFA , adjunct or monotherapy yet to be defined.
3. Supplementation with n-3 LCPUFA in aggression, hostility and antisocial behaviour. Dose, preferred n-3 PUFA, adjunct or monotherapy yet to be defined.
4. Supplementation with n-3 LCPUFA in age-related macular degeneration. Dose and preferred n-3 PUFA is yet to be defined.
5. Supplementation with n-3 LCPUFA in improvements in cognitive decline. Dose and preferred n-3 PUFA is yet to be defined.

Insufficient evidence to date

6. Supplementation with n-3 LCPUFA as treatment for Alzheimer's disease.
7. Supplementation with n-3 LCPUFA as treatment for schizophrenia.
8. Supplementation with n-3 LCPUFA as treatment for Huntington's disease.

REMARKS

There can be little doubt about the essentiality of DHA and AA for the brain. The rise in brain disorders is the most disturbing feature of the changing panorama of disease and disorder. There is a need to address the potential role of the food system as the root

cause of globalization of mental ill health. Based on the epidemiology and supported by basic science, there should be better use made of fresh water and marine food webs, and attention needs to be paid to the ways and means of restoring healthy rivers, estuaries, coastlines and all aspects of marine productivity. At the same time, the distortions of food and animal production that have amplified the non-essential, atherogenic and obesigenic fats at the expense of the fats essential to the vascular and immune systems and brain development, need to be corrected. The 1977 expert consultation on dietary fats and oils specifically commented, but the situation has worsened since then (FAO, 1978).

The Japanese have the lowest levels of depression, cardio-vascular disease, and breast and colon cancer of the industrialized nations. Any assessment for optimal intakes and balance of fatty acids required should take advantage of the amounts eaten in the traditional Japanese diets. However, the presence of competing fatty acids and the requirement for adequate anti-oxidants in the diet also need to be taken into account.

The conclusions outlined here emphasize the need for more research in:

1. Defining the requirement of the adult brain for a continuous supply of AA and DHA from the plasma for optimal neural functioning.
2. Defining the requirement in adults and children for the optimal development of the neuro-vascular system in the next generation, with the inclusion of epigenetic studies.
3. The role of PUFA in a variety of neural disorders including depressive illness, age-related macular degeneration, aggression, hostility and anti-social behaviour, Alzheimer's disease, schizophrenia and Huntington's disease.
4. Arachidonic acid and its companion LCPUFA.
5. Cost-benefit analysis to assess the potential contribution of an optimal intake of AA and DHA on health status and healthcare costs.

REFERENCES

Andlin-Sobocki, P., Jonsson, B., Wittchen, H.U. & Olesen, J. 2005. Cost of disorders of the brain in Europe. *Eur. J. Neurol.*, 12 (Suppl.) 1: 1-27.

Brenna, J.T., Salem, N. Jr., Sinclair, A.J. & Cunnane, S.C. 2009. International Society for the Study of Fatty Acids and Lipids, ISSFAL. Alpha-linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins Leukot. Essent. Fatty Acids*, 80(2-3): 85-91.

Crawford, M.A., Casperd, N.M. & Sinclair, A.J. 1976. The long chain metabolites of linoleic and linolenic acids in liver and brain in herbivores and carnivores. *Comp. Biochem. Physiol.*, 54B: 395-401.

Dobbing, J. 1972. Vulnerable Periods of Brain Development. *In* K. Elliott and J. Knights, eds. *Lipids, malnutrition and the developing brain,* pp. 1-7. Ciba. Foundation Symposium, Elsevier, North Holland.

FAO. 1978. Dietary fats and oils in human nutrition; a joint FAO/WHO report. FAO Food and Nutrition Paper 3, FAO. Rome.

FAO. 1994. *Fats and oils in human nutrition; Report of a joint FAO/WHO expert consultation*. FAO Food and Nutrition Paper 57, FAO. Rome.

Freeman, M.P., Hibbeln, J.R., Wisner, K.L., Davis, J.M., Mischoulon, D., Peet, M., Keck, P.E. Jr., Marangell, L.B., Richardson, A.J., Lake, J. & Stoll, A.L. 2006. N-3 fatty acids: evidence basis for treatment and future research in psychiatry. *J. Clin. Psychiatry*, 67(12): 1954-1967.

Elvevoll, E.O., Barstad, H., Breimo, E.S., Brox, J., Eilertsen, K.E., Lund, T., Olsen, J.O. & Osterud, B. 2006. Enhanced incorporation of n-3 fatty acids from fish compared with fish oils. *Lipids*, 41(12): 1109-1114.

Haider, B.A. & Bhutta, Z.A. 2006. Multiple-micronutrient supplementation for women during pregnancy. *Cochrane Database Syst. Rev*., 18 (4): CD004905.

Chapter 13: Global trends in production, intake and food composition

Vegetable oils and animal fats are the main sources of fat in the human diet. Other sources include nuts, cereals and legumes. Trends in the production of food sources of fat have a global impact on the availability of fat for human consumption. Global data on fat supply, as well as individual food intake data, contribute to an understanding of the relationship between fat intake patterns and health outcomes. In order to translate food intake data into fatty acids consumed, information from food composition databases is required. This information on the fatty acid composition of foods also contributes to monitoring trends in changes in the fatty acid composition of foods.

PRODUCTION OF VEGETABLE OILS AND ANIMAL SOURCE FOODS

The Food Balance Sheets (FBS) of the Food and Agriculture Organization of the United Nations (FAO), provide valuable information on the production (domestic supply) of food commodities (FAOSTAT/FBS, 2006). Production at the household level is not taken into account, but supply data at the national level are used and production figures could therefore underestimate the actual production of vegetable oils and animal fat sources.

PRODUCTION OF VEGETABLE OILS

The global production (domestic supply) of vegetable oils increased significantly between 1961-1963 and 2001-2003. Global trends in the production of specific vegetable oils between 1995-1997 and 2001-2003 are shown in Table 13.1. Soybean oil and palm oil were the main oils produced and production increased by 42.8% and 51.3%, respectively, during this period. At the same time the production of sunflower oil (5.4%) decreased.

Developing countries produced more vegetable oil than developed countries in 2001-2003, 68.8% and 31.2%, respectively. During this period Asia was the main producer of palm, rape and mustard, groundnut, coconut, cottonseed and palm kernel oils. South America was the main producer of soybean oil and Europe was the main producer of sunflower and olive oil.

Factors that influence the production of vegetable oils include population expansion and the per capita consumption of vegetable oils (Broeska, 2007). Globalisation can contribute to an increase in the availability and consumption of vegetable oil (Hawkes, 2006). New government policies in Brazil, in connection with the production and export of soybean oil, have contributed to an increase in the availability of soybean oil in countries such as China and India (Hawkes, 2006).

Vegetable oil prices are influenced by an increase in the demand for vegetable oils from countries such as China and India (Paton, 2008). The price of vegetable oil is also influenced by the demand for the production of biofuel and markets for vegetable oil move in tandem with crude oil prices (Thoenes, 2006; Business-standard.com, 2009).

TABLE 13.1
Global trends in the production (domestic supply) of vegetable oils in 1995-1997, 1998-2000 and 2001-2003

Vegetable oils	1995-1997[a]	1998-2000[a]	2001-2003[a]	% increase[b]
		1 000 metric tonnes		
All vegetable oils	80 777	91 120	101 722	25.9
Soybean	20 108	24 531	28 722	42.8
Palm	17 069	20 295	25 819	51.3
Rape and mustard	11 147	12 664	12 353	10.8
Sunflower	9 099	9 533	8 612	-5.4
Groundnut	4 885	4 975	5 353	9.6
Cottonseed	3 817	3 718	3 824	0.2
Coconut	3 357	3 186	3 416	1.8
Olive	2 477	2 659	3 024	22.1
Palm kernel	2 232	2 586	3 215	44.0
Sesame seed	713	726	827	16.0

[a] Values represent the means of each 3-year period

[b] Difference between the periods 1995-1997 and 2001-2003

Source: FAOSTAT/FBS, 2006

TABLE 13.2
Vegetable oils produced in different regions of the world (mean 2001-2003)

Vegetable oils	Asia	Africa	Europe	Americaa	Americab	Oceania
			1000 metric tonnes			
Soybean	6 902	176	3 279	8 925	9 433	7
Palm	22 231	1 858	0	422	984	324
Rape and mustard	6 130	15	4 394	1 638	30	145
Sunflower	1 534	360	4 920	281	1 491	25
Groundnut	3 777	1 296	78	118	83	2
Cottonseed	2 643	338	123	411	265	44
Coconut	3 050	107	35	141	16	68
Olive	326	229	2 457	2	11	0
Palm kernel	2 556	421	0	59	154	25

[a] North-and-Central America

[b] South America

Source: FAOSTAT/FBS, 2006

PRODUCTION OF ANIMAL SOURCE FAT

Milk production (excluding butter) increased in both developed and developing countries between 1962-1964 and 2001-2003, but the increase was larger in developing countries. Cheese is an important commodity in developed countries and in 2001-2003, the production was about six times higher than in developing countries (FAOSTAT/FBS, 2006). Market forces that impact on the production of dairy products are government policies regarding the dairy industry, quota restrictions and subsidies (Mitchell, 2001), which influence the production and export of dairy products in countries where dairying is a main agricultural activity. Weather conditions impact on the production of milk. Drought in New Zealand and Australia has led to a decline in milk production during recent years (FAO, 2008a).

The global production of beef, pork, poultry, as well as mutton and goat, increased significantly between 1962-1964 and 2001-2003. The global production of pork has exceeded the production of beef since 1980-1982 and was 57.6% higher than that of beef in 2001-2003. The global production and consumption of meat will probably continue to increase and it is suggested that by 2020 it will be 300 million metric tonnes (MT) compared to 233 million MT in 2000 (Speedy, 2003). Meat consumption is influenced by wealth and in general increases with an increase in the gross domestic product (GDP) of a country (Speedy, 2003). There are, however, exceptions such as the Latin American countries where the consumption of meat is high in relation to the GDP (Speedy, 2003).

Poultry production was about 7.7 times higher in 2001-2003 than in 1962-1964 and in 2001-2003, the production was higher than that of beef or mutton and goat. Poultry production costs increased significantly between 2000 and 2008 as a result of an increase in the cost of the feed (FAO, 2008b). In 2001-2003 the production of eggs was more than twice as high in developing countries (about 41 million MT) as in developed countries (about 19 million MT) (FAOSTAT/FBS, 2006). Less land is required to produce poultry and eggs (7.3 m^2 year kg^{-1} and 3.5 m^2 year kg^{-1}, respectively) than to produce other animal source foods such as beef (20.9 m^2 year kg^{-1}) (Gerbens-Leenes and Nonhebel, 2002).

PRODUCTION OF FISH OIL AND FISH

The total production of fish oils is about 1 million MT per year, and it seems to have stabilised at this level (FAO/Fisheries and Aquaculture Information and Statistics Service, 2007). In 2006, it was estimated that 87% of all fish oil was used by the aquaculture industry to produce feed, and salmon farming alone used approximately 33% of all fish oil produced. The remaining 13% was processed into products for human consumption, mainly as fish oil capsules.

There has been a steady increase in the production (capture and aquaculture) of fish since 1950, but a sharp decrease in production was recorded in 1998 (Figure 13.1). In 2003 the total world production of fish (capture and aquaculture combined) was 132.5 million MT (weight of fish and shellfish at capture or harvest - freshwater, brackish water and marine species of fish, crustaceans, molluscs and other aquatic organisms) and of this 104.2 million MT were available for human consumption, 24.4 million MT in developed countries and 79.8 million MT in developing countries (Food and Agriculture Statistics, 2005).

FAT SUPPLY AND INTAKE DATA

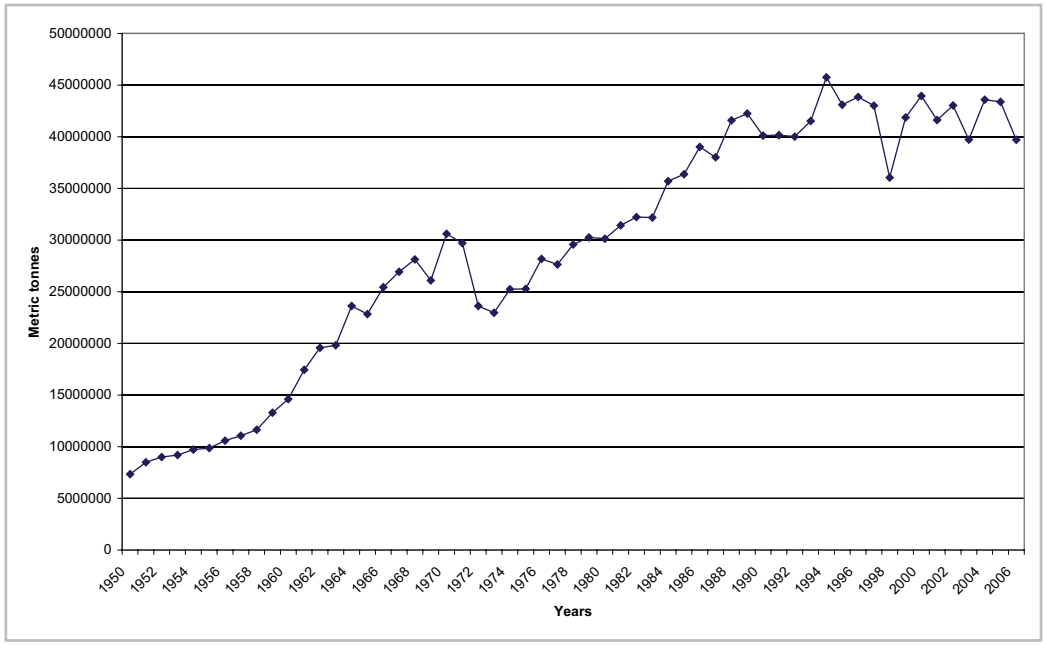
Energy and fat supply data from Food Balance Sheets

FBS do not provide information on actual consumption within communities or within households, but provide data on the per capita supply per day of energy (kilocalorie; kcal, one kilocalorie = 4.186 kilojoules; kj), protein (g) and total fat (g) (FAOSTAT/FBS, 2006).

Energy

Information from FAO FBS showed a global increase between 1995-1997 and 2001-2003 in the per capita supply per day of energy. In developed countries it was 663 kcal (2 774 kJ) higher than in developing countries, Africa having the lowest value (2 427 kcal/10 255 kJ). Large variations (1 521 - 3 346 kcal/6 364 - 14 000 kJ) were, however, recorded within Africa (FAOSTAT/FBS, 2006).

FIGURE 13.1
Total production (capture and aquaculture) of fish between 1950 and 2006 (fish included in total production: salmon, trout, smelt, herring, sardine, anchovy, tuna, bonito and billfish)



Source: FISHSTAT Plus, 2008

Percentage of energy from fat

FBS data showed that between 1995-1997 and 2001-2003 the percentage of energy from fat remained above 30%E in developed countries, while in developing countries it was below 23%E. Developed countries did not meet the recommendation to consume <30%E from fat (WHO, 2003). In Africa the mean percentage of energy from fat was about 20%E and in 12 of the 51 countries with FBS data, the per capita supply per day of fat was less than 15%E in 2001-2003 (FAOSTAT/FBS, 2006). In those countries the lower limit of fat intake recommended by the expert consultation in 2003, i.e. 15%E was not met (WHO, 2003). Guidelines on fat intake should therefore not only concentrate on the upper limit of fat intake, but should also address the inadequate intakes of fat in some groups and countries.

Per capita supply per day of total fat

Vegetable products made the biggest contribution to the per capita supply per day of total fat (g) globally and in developing countries in 2001-2003, but in developed countries it was animal products. Vegetable oils contributed to 39.2% of the per capita supply per day of fat in developed countries and to 38.8% in developing countries. Sharp increases in the per capita supply per day of fat from vegetable oils were observed in developed (112%) as well as developing (191%) countries between 1961-1963 and 2001-2003. At the same time the per capita supply per day of fat from animal fats decreased by 26% in developed countries and increased by 109% in developing countries. The per capita supply per day of fat from vegetable oils and animal fats was, however, respectively about 1.9 times and 3.4 times higher in developed than in developing countries.

INDIVIDUAL DIETARY SURVEYS

Individual Dietary Surveys (IDS) showed high total fat intakes in Europe, the United States, South Africa, Kenya, China and India (Elmadfa *et al.*, 2004; Wright *et al.*,

2004; Labadarios *et al.*, 2005; MacIntyre *et al*., 2002; Fu *et al*., 2006; Shetty, 2002). Differences in total fat intakes were, however, also observed within countries. In China mean total fat intakes increased from 18.1%E in 1982 to 22%E in 1992 and to 29.6%E in 2002 (Chen, 1986; Zhai *et al*., 1996; He *et al*., 2005; Deng *et al*., 2008). Large variations in total fat intakes were observed in India with high fat intakes in high income urban groups (33.1%E) and very low fat intakes among slum dwellers (16.7%E) (Shetty, 2002). High total fat intakes have been reported in urban upper middle-income men (32%E) and women (33%E) from South India (Ghafoorunissa *et al*., 2002). Urbanisation plays an important role in the increase of fat intake as shown by studies from South Africa, China and India (Labadarios *et al.*, 2005; MacIntyre *et al*., 2002; Fu *et al*., 2006; Shetty, 2002).

There are indications that the increase in the prevalence of obesity is taking place at a much faster rate in developing countries than in high-income countries (Popkin and Gordon-Larsen, 2004). Although an increase in total fat intake is observed with urbanisation in developing countries, this is only one aspect of the nutrition transition. Other changes in diet and lifestyle, e.g. an increase in refined carbohydrate intake and a decrease in physical activity are also observed (Popkin and Gordon-Larsen, 2004). More research is warranted to understand the effect of an increase in the intake of total fat on the incidence of non-communicable diseases in developing countries (Popkin, 2002).

FATTY ACID COMPOSITION OF FOOD

Data on the nutrient composition of foods are available in various food composition databases and information on the nutrient composition of specific foods could differ as factors such as climate, soil, plant varieties and animal husbandry influence the nutrient composition of foods (Greenfield and Southgate, 2003).

Vegetable oils

The different vegetable oils available on the market for human consumption differ in fatty acid composition. Coconut oil and palm kernel oil are high in lauric (C12:0), about 45 g/100 g oil, and also contains a significant amount of myristic (C14:0) and palmitic acid (C16:0). Palm oil is high in saturated fatty acids (SFA), about 50%[1], while soybean oil contains about 50% linoleic acid (LA). Sunflower oil is high in LA (about 66% LA), but a high oleic acid sunflower oil (about 83% oleic acid) is also available on the market. Olive oil is high in oleic acid. Canola refers to *Brassica napus* and *B. campestris* lines of rapeseed and contains small amounts of erucic acid (C22:1n-9), less than 2% of the total fatty acids (Food Standards Australia New Zealand, 2003).

Margarine

The term margarine is only used when the product contains at least 80% fat. Bread spreads have a lower fat content than margarine and reduced fat spreads contain 60-70% fat, low-fat spreads 40% fat and very low fat spreads 3-25% fat (Henry, 2009). One of the main health concerns with the production of margarine and fat spreads is the use of partially hydrogenated vegetable oil as this increases the *trans* fatty acid content of the product (Tarrago-Trani *et al*., 2006). Health concerns about the effect of *trans* fatty acids led to changes in the methodology used by the food industry for the production of margarine (Lemaitre *et al*., 2006). As different methodologies are available for lowering the *trans* fatty acid content of fats used in the

[1] % refers to g/100 g oil and not to per 100 g fatty acids

production of margarine, one could expect differences in the fatty acid composition of the margarines/spreads between manufacturers, and thus also between countries (Karabulut and Turan, 2006; Kandhro *et al*., 2008).

Nuts

Nuts are high in energy and fat, but are also a good source of protein and fibre. In most nuts the predominant fatty acid is MUFA. Macadamia, pecan and hazelnuts contain more than 40 g of oleic acid per 100 g of nuts, but macadamia and Brazil nuts are also relatively high in SFA, 11.9 g/100 g and 15.1 g/100 g, respectively (USDA, 2008). Walnuts are high in PUFA (about 47 g/100 g) and have a P/S ratio of 7.7 and an LA/ALA ratio of 4.2 and as a result of this PUFA profile get rancid very quickly (calculated from, USDA, 2007).

Dairy products

Whole milk is not only a source of fat, but also of other important nutrients such as protein, calcium, and folic acid. Human milk contains about 4.4 g fat per 100 g. The total fat content of sheep milk (7.0 g/100 g), Indian buffalo milk (6.9 g/100 g) and goat milk (4.1 g/100 g) are higher than that of cow milk (3.3 g/100 g) (USDA, 2008). The SFA that predominates in milk fat is palmitic acid and the short chain SFA butyric acid (C4:0) and caproic acid (C6:0) are also present (USDA, 2008). The total fat content of hard-type cheeses such as cheddar is high, about 36%, while low-fat cottage cheese, a soft type of cheese in tubs, has a total fat content of about 1% (USDA, 2008). Milk is the best source of CLA in the diet and cheese also contains CLA (Khanal and Olson, 2004).

Livestock

Red meat

Changes in animal husbandry have been responsible for the changes in fat content of meat over the years. In the USA cattle were typically slaughtered before 1850 at 4-5 years, but as a result of the practice of fattening cattle in feedlots, it became possible to produce a steer for slaughter (545kg) with marbled fat in 24 months (Cordain *et al*., 2005). Feeding grain to animals in feedlots became common practice (Cordain *et al*., 2005). There are differences in the fatty acid composition of fat from feedlot cattle compared to pasture-fed cattle, and in the former the absolute amounts of SFA, MUFA and PUFA are higher, while the absolute amount of n-3 PUFA is lower (Cordain *et al*., 2002). Game meat has a lower absolute SFA content and a higher absolute n-3 PUFA content than either grain-fed or pasture-fed beef (Cordain *et al*., 2002). Health concerns about the impact of total fat intake and the composition of dietary fat have resulted in efforts by the food industry to change meat quality (Scollan *et al*., 2006).

The animal (genetics), nutrition (grain-fed or pasture-grazed), meat cut and fat trimming influence the total fat content of beef and mutton portions (Schönfeldt and Welgemoed, 1996; Droulez *et al*., 2006; Scollan *et al*., 2006; Van Heerden *et al*., 2007). In beef, the fat is present as membrane fat (phospholipids), intermuscular fat and as subcutaneous fat, while marbling refers to the adipose tissue between the bundles of muscle fibres (Scollan *et al*., 2006). Marbling is closely linked to the intermuscular fat (IMF) content of meat (Scollan *et al*., 2006). The amount of IMF determines the fat content of the meat and lean beef has a low IMF content, about 2-5% (Scollan *et al*., 2006). High fat meat cuts such as brisket can contain as much as 34% fat (Schönfeldt and Welgemoed, 1996).

Oleic acid is the predominant fatty acid in the muscle and adipose tissue of pigs, sheep and cattle, while palmitic acid is the main SFA (Droulez *et al*., 2006; Scollan *et al*.,

2006; Wood *et al*., 2008). There are small amounts of EPA and DPA present in red meat (Droulez *et al*., 2006). In cattle the long-chain n-6 and n-3 PUFA are found in muscle phospholipids, but not in adipose tissue or muscle neutral lipids. In pigs and sheep the long-chain n-6 and n-3 PUFA are, in addition to their presence in phospholipids, also found in muscle neutral lipids and adipose tissue (Wood *et al*., 2008). Higher levels of EPA and DPA could be expected where pasture-grazing instead of grain-feeding is practiced as the ALA content of grass is about 60%, while grains have a high LA content (Droulez *et al*., 2006). Feeding fresh grazed grass rather than silage grass or concentrate resulted in higher proportions of ALA in the fatty acids of subcutaneous adipose tissue in steers (Wood *et al*., 2008). The P/S ratio of beef is typically about 0.1 (Scollan *et al*., 2006). The P/S ratio of beef decreases with an increase of fatness of the meat (Scollan *et al*., 2006).

Meat is a source of CLA (18:2*cis*-9, *trans*-11) and is formed in the adipose tissue of ruminants from 18:1 *trans* vaccenic acid, a biohydrogenation product of C18:2*cis*-6 (Wood *et al*., 2008). Small amounts of CLA are also formed in the rumen. The CLA are present in higher concentrations in the adipose tissue than in the muscle (Droulez *et al*., 2006; Wood *et al*., 2008).

Poultry

Since the middle of the twentieth century chickens have been selected either to lay a large number of eggs or to produce meat. The time it now takes before slaughter weight is reached, is about half the time it took fifty years ago (Hall and Sandilands, not dated). In the mid 1960s it took about 68 days for a broiler chicken to reach a slaughter weight of 2 kg, but by 1987 it took about 45 days (Jones, 1986). Carcass composition of broilers is determined by genetics and dietary manipulations (Jones, 1986). High-energy diets fed to broilers to reach slaughter weight earlier increase the fat content of the edible portion significantly (Jones, 1986).

Broiler meat produced under the free-range system is not necessarily of higher nutritional value than meat produced under the conventional fast-growing system (Ponte *et al*., 2008). A higher SFA and MUFA content and lower PUFA content were found in breast meat of free-range broilers (slaughter at 81 days) compared with broilers produced from the conventional system and slaughtered between 35 and 42 days (Ponte *et al*., 2008). Higher levels of PUFA, n-3 PUFA and a higher P/S ratio, but a lower DHA level, were observed in the conventional broilers compared to the meat from broilers produced under the free-range system (Ponte *et al*., 2008).

The skin of the chicken is high in fat, >40% fat, dark meat is about 10% fat and white meat about 4% fat (Sayed *et al*., 1999). Chicken fat contains 30% SFA, 45% MUFA and 21% PUFA (USDA, 2008).

Designer eggs

Eggs are not high in total fat, but are an important source of cholesterol in the diet (about 210 mg per 50 g egg). Today designer eggs can be produced in which the n-3 PUFA content is increased by feeding fish oil (increase EPA and DHA) or flaxseed (increase ALA and DHA) to the chickens (Oh *et al*., 1991; Ferrier *et al*., 1995).

Fish

Oily marine fish are the most important source of the LCPUFA, EPA and DHA. In Table 13.3 the total fat, EPA and DHA content of some common fish species are shown. A daily intake of 500 mg EPA plus DHA per day is recommended for the primary prevention of coronary heart disease (ISSFAL, 2004). In order to meet this recommendation at least two portions (90 g each) of oily fish, such as salmon and herring, will have to be consumed per week. Two portions (90 g each) of cod per day, a low fat fish, will provide about 284 mg of EPA plus DHA per day.

TABLE 13.3
Total fat, EPA and DHA content of different fish species

Species	Total fat	EPA	DHA	EPA + DHA[a]
		g/100g		
Salmon, Atlantic, farmed	12.4	0.690	1.457	2.147
Anchovy, European, canned in oil[b]	9.7	0.763	1.292	2.055
Herring, Atlantic, cooked	11.6	0.909	1.105	2.014
Salmon, Atlantic, wild, cooked	8.1	0.411	1.429	1.840
Salmon, Chinook, cooked	13.4	1.010	0.727	1.737
Tuna, Bluefin, fresh, cooked	6.3	0.363	1.141	1.504
Sardine, Pacific, canned in tomb,[c]	10.5	0.532	0.865	1.397
Salmon, Sockeye, cooked	11.0	0.530	0.700	1.230
Mackerel, Atlantic, cooked	17.8	0.504	0.699	1.203
Halibut, Greenland, cooked	17.7	0.674	0.504	1.178
Trout, Rainbow, farmed, cooked	7.2	0.334	0.820	1.154
Trout, Rainbow, wild, cooked	5.8	0.468	0.520	0.988
Swordfish, cooked	5.1	0.138	0.681	0.819
Halibut, Atlantic and Pacific, cooked	2.9	0.091	0.374	0.465
Shrimp, mixed species, cooked	1.1	0.171	0.144	0.315
Tuna, light, canned in water	0.8	0.047	0.223	0.270
Grouper, mixed species, cooked	1.3	0.035	0.213	0.248
Haddock, cooked	0.9	0.076	0.162	0.238
Catfish, Channel, wild, cooked	2.9	0.100	0.137	0.237
Catfish, Channel, farmed, cooked	8.0	0.049	0.128	0.177
Cod, Atlantic, cooked	0.9	0.004	0.154	0.158

[a] Ranked from highest to lowest EPA + DHA value

a Drained solids

b Tomato sauce

Source: Lee *et al.*, 2008; USDA, 2007

As a result of the danger of mercury poisoning, the consumption of king mackerel (mercury concentration 0.730 ppm), shark (0.988 ppm), swordfish (0.976 ppm) and tile fish (1.450 ppm) is not recommended for young children, pregnant and lactating women (FDA/EPA, 2004; US FDA, 2006).

Fast foods

Fast food consumption contributes to an increase in energy and total fat intake and also has a negative effect on dietary quality (Paeratakul *et al.*, 2003). In addition, fast foods are also a source of *trans* fatty acids in the diet. One of the critical issues in studying the association between *trans* fatty acids and adverse effects on health is a lack of detailed information on the *trans* fatty acid content of food in food composition databases. There are large variations in the *trans* fatty acid content of snack and convenience foods (Innis *et al.*, 1999; Stender *et al.*, 2006). Different fats and oils are sometimes mixed, e.g. partially hydrogenated and non-hydrogenated vegetable oils such as coconut oil and palm kernel oil for preparation purposes and this determines the *trans* fatty acid content of the product (Innis *et al.*, 1999).

CONCLUSIONS

A global increase in total fat supply and total fat intake is evident. The significant increase in production and per capita supply per day of fat from vegetable oils, especially in developing countries, are probably contributing to the increase in total fat intake. Fat intakes remain high in developed countries, but the increase in total fat intake in developing countries is of concern as it may be a factor contributing to the increase in non-communicable diseases. Guidelines for fat intake should not only concentrate on high fat intakes, but also ensure that enough fat is provided in the diet to meet essential fatty acids and energy requirements. The type of fat consumed is of special importance. Expanding information in country-specific food composition databases on the fatty acid composition of food is essential for studying the relationship between fat, especially the type of fat, intake and health and disease, and to monitor changes over time in the fatty acid composition of food.

REFERENCES

Broeska, R. 2007. The global oils & fats market – current drivers, future prospects. OFI Middle East Conference, Cairo: 20-21 March. (www.iasc-oils.org/word%20docs/The%20global%20oils%20%20fats%20market.doc [Accessed on 24/08/2008]).

Business-standard.com. 2009. India: Veg oil prices to rise 20% in '09. (www.freshplaza.com/news_detail.asp?id=35927 [Accessed on 05/01/2009]).

Chen, C.M. 1986. The national nutrition survey in China, 1982: summary results. *Food Nutr.*, 12(1): 58-60.

Cordain, L., Eaton, S.B., Sebastian, A., Mann, N., Lindeberg, S., Watkins, B.A, O'Keefe, J.H. & Brand-Miller, J. 2005. Origins and evolution of the Western diet: health implications for the 21 st century. *Am. J. Clin. Nutr.*, 81(2): 341-354.

Cordain, L., Watkins, B.A., Florant, G.L., Kelher, M., Rogers, L. & Li, Y. 2002. Fatty acid analysis of wild ruminant tissues: evolutionary implications for reducing diet-related chronic disease. *EJCN*, 56(3): 181-191.

Deng, Z.Y., Zhou, X.Q., Huang, Y.H. & Liu, D.M. 2008. Investigation of dietary fatty acids intakes of Chinese people during twenty years. *J. Food Sci. Biotech.*, 27(1): 7-19.

Droulez, V., Williams, P.G., Levy, G., Strobaus, T. & Sinclair, A. 2006. Composition of Australian red meat 2002. 2. Fatty acid profile. *Food Australia*, 58(7): 335-341. (www.ro.uow.edu.au/hbspapers/1 [Accessed on 9/09/2008]).

Elmadfa, I., Weichselbaum, E., Koenig, J., Remaut de Winter, A-M., Trolle, E., Haapala, I., Uusitalo, U., Mennen, L., Hercberg, S., Wolfram, G., Tricholopoulou, A., Naska, A., Vassiliki, B., Dritsellis, E., Rodler, I., Zajkás, G., Branca, F., D'Acapito, P., Klepp, K-I., Ali-Madar, A., De Almeida, M.D.V., Alves, E., Rodrigues, S., Serra-Majem, L., Roman, B., Sjöström, M., Poortvliet, E. & Margetts, B. 2004. European Nutrition and Health Report. 2004 *Ann. Nutr. Metab.*, 48 (Suppl. 2): 1-16.

FAO/Fisheries and Aquaculture Information and Statistics Service. 2007. Fisheries and Aquaculture Information and Statistics Service. 2007. Fisheries commodities production

and trade 1976-2005. FISHSTAT Plus - Universal software for fishery statistical time series [online or CD-ROM]. Rome. (www.fao.org/fi/statist/FISOFT/FISHPLUS.asp).

FAO. 2008a. Global Market Analysis. Milk and milk products. *Food Outlook.* (www.fao.org/docrep/010/ai466e/ai466e09.htm [Accessed 20/10/2008]).

FAO. 2008b. Global Market Analysis. Meat and meat products. *Food Outlook* (www.fao.org/docrep/010/ai466e/ai466e08.htm [Accessed 20/10/2008]).

FDA/EPA (Environmental Protection Agency). 2004. What you need to know about mercury in fish and shellfish. (www.fda.gov/food/foodsafety/product-specificinformation/seafood/foodbornepathogencontaminants/methylmercury/ucm115662.htm [Accessed on 22/08/2008]).

FAOSTAT/FBS. 2006. Food Balance Sheets, last updated 3 March 2006. CD-Rom.

Ferrier, L.K., Caston, L.J., Leeson, S., Squires, J., Weaver, B.J. & Holub, B.J. 1995. α-Linolenic acid- and docosahexaenoic acid-enriched eggs from hens fed flaxseed: influence on blood lipids and platelet phospholipid fatty acids in humans. *Am. J. Clin. Nutr.*, 62(1): 81-86.

FISHSTAT Plus. 2008. Total fishery production 1950-2006. FISHSTAT Plus - Universal software for fishery statistical time series [online or CD-ROM]. Fisheries and Aquaculture Information and Statistics Service. FAO. (www.fao.org/fi/statist/FISOFT/FISHPLUS.asp).

Food and Agriculture Statistics. 2005. Summary of World Food and Agricultural Statistics 2005. (www.env-edu.gr?Documents/World%20Food%and%20Agricultural%20Statistics%20-%202005.pdf. [Accessed 27/08/2008]).

Food Standards Australia New Zealand. 2003. Erucic acid in Food. A toxicological review and risk assessment. Technical report series no 21. (www.foodstandards.gov.au/_srcfiles/Erucic%20acid%20monograph.pdf [Accessed 22/10/2008]).

Fu, J., Wang, Z. & Wang, B. 2006. Transitions of food groups and nutrients in the northeast of China: A 3-year-interval's follow-up study. *The Internet Journal of Nutrition and Wellness™*, 2(2) pp. 11.

Ghafoorunissa, Vani, A., Laxmi, R & Sesikeran, B. 2002. Effects of dietary α-linolenic acid from blended oils on biochemical indices of coronary heart disease in Indians. *Lipids*, 37(11): 1077-1086.

Gerbens-Leenes, P.W. & Nonhebel, S. 2002. Consumption patterns and their effects on land required for food. *Ecol. Econ.*, 42(1): 185-199.

Greenfield, H. & Southgate, D.A.T. 2003. *Food Composition Data. Production, Management and Use*. 2nd Edition. FAO, Rome.

Hall, C. & Sandilands, V. Not dated. Public Attitudes to the welfare of broiler chickens. Land Economy Working Paper Series. Number 13. (www.ageconsearch.umn.edu/bitstream/45998/2/Work13Hall.pdf; [Accessed 13/01/2008].

Hawkes, C. 2006. Uneven dietary development: linking the policies and processes of globalization with the nutrition transition, obesity and diet-related chronic diseases. *Globalization and Health,* 2 (4): 1-18 doi:10.1186/1744-8603-2-4 (www.globalizationandhealth.com/content/2/1/4 [Accessed 26/01/2009]).

He, Y.N., Zhai, F.Y., Wang, Z.H., Wang, H.J., Hu, Y.S. & Yang, X.G. 2005. The status and trend for dietary pattern of energy, protein and fat in Chinese residents. *Acta Nutrimenta Sinica*, 27(5): 358-365.

Henry, J. 2009. Processing, manufacturing, uses and labelling of fats in the food supply. *Ann. Nutr. Metab.* 55(1-3): 273-300.

Innis, S.M., Green, T.J. & Halsey, T.K. 1999. Variability in the *trans* fatty acid content of foods within a food category: Implications for estimation of dietary *trans* fatty acid intakes. *J. Am. Coll. Nutr*., 18(3): 255-260.

ISSFAL(International Society for the Study of Fatty Acids and Lipids). 2004. Recommendations for intake of Polyunsaturated Fatty Acids in Healthy Adults. ISSFAL 2004, UK (www.issfal.org.uk/) [Accessed 25/07/2008]).

Jones, R.L. 1986. Nutritional influences on carcass composition in the broiler chicken. *Proc. Nutr. Soc*., 45: 27-32.

Kandhro, A., Sherazi, S.T.H., Mahesar, S.A., Bhanger, M.I., Talpur, M.Y. & Rauf, A. 2008. GC-MS quantification of fatty acid profile including *trans* FA in the locally manufactured margarines of Pakistan. *Food Chem*., 109(1): 207-211.

Karabulut, I. & Turan, S. 2006. Some properties of margarines and shortenings marketed in Turkey. *J. Food Compos. Anal*., 19(1): 55-58.

Khanal, R.C. & Olson, K.C. 2004. Factors affecting conjugated linoleic acid (CLA) content in milk, meat, and egg: A Review. *Pakistan J. Nutr*., 3(2): 82-98.

Labadarios, D., Steyn, N.P., Maunder, E., MacIntyre, U., Gericke, G., Swart, R., Huskisson, J., Dannhauser, A., Vorster, H.H., Nesmvuni, A.E. & Nel, J.H. 2005. The National Food Consumption Survey (NFCS): South Africa, 1999. *Public Health Nutr*., 8(5): 533-543.

Lee, J.H., O'Keefe, J.H., Lavie, C.J., Marchioli, R. & Harris, W.S. 2008. Omega-3 fatty acids for cardioprotection. *Mayo Clin. Proc*., 83(3): 324-332.

Lemaitre, R.N., King, I.B., Mozaffarian, D., Sotoodehnia, N., Rea, T.D., Kuller, L.H., Tracy, R.P. & Siscovick, D.S. 2006. Plasma phospholipid trans fatty acids, fatal ischemic heart disease, and sudden cardiac death in older adults: the cardiovascular health study. *Circulation*, 114(3): 209-215.

MacIntyre, U.E., Kruger, H.S., Venter, C.S. & Vorster, H.H. 2002. Dietary intakes of an African population in different stages of transition in the North West Province, South Africa: the THUSA study. *Nutr. Res*., 22(3): 239-256.

Mitchell, N. 2001. Trade in livestock products. Presentation to the International Agricultural Trade Symposium. Auckland, New Zealand, 19-20 January. (www.ageconsearch.umn.edu/bitstream/14554/1/cp01mi01.pdf [Accessed on 03/09/2008]).

Oh, S.Y., Ryue, J., Hsieh, C-H. & Bell, D.E. 1991. Eggs enriched in ω-3 fatty acids and alterations in lipid concentrations in plasma and lipoproteins and in blood pressure. *Am. J. Clin. Nutr.*, 54(4): 689-695.

Paeratakul, S., Ferdinand, D.P., Champagne, C.M., Ryan, D.H. & Bray, G.A. 2003. Fast-food consumption among US adults and children: Dietary and nutrient intake profile. *J. Am. Diet. Assoc.*, 103(10): 1332-1338.

Paton, D. 2008. Vegetable oil prices set to stay high, US warns. (www.foodnavigator.com/Financial-Industry/Vegetable-oil-prices-set-to-stay-high-US-warns [Accessed on 12/08/2008]).

Ponte, P.I.P., Alves, S.P., Bessa, R.J.B., Ferreira, M.A., Gama, L.T., Brás, J.L.A., Fontes, C.M.G.A. & Prates, J.A.M. 2008. Influence of pasture intake on the fatty acid composition, and cholesterol, tocopherols, and tocotrienols content in meat from free-range broilers. *Poult. Sci.*, 87(1): 80-88.

Popkin, B.M. 2002. Part II. What is unique about the experience in lower- and middle-income less-industrialised countries compared with the very-high-income industrialised countries? The shift in stages of the nutrition transition in the developing world differs from past experiences? *Public Health Nutr.*, 5(1A): 205-214.

Popkin, B.M. & Gordon-Larsen, P. 2004. The nutrition transition: worldwide obesity dynamics and their determinants. *Int. J. Obesity*, 28: S2-S9.

Sayed, N., Frans, Y. & Schönfeldt, H.C. 1999. Composition of South African Foods. Milk and milk products, Eggs, Meat and meat products. Supplement to the MRC Food Composition Tables 1991. Parow Valley, Cape Town: South African Medical Research Council.

Scollan, N., Hocquette, J-F., Nuernberg, K., Dannenberger, D., Richardson, I. & Moloney, A. 2006. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Sci.*, 74(1): 17-33.

Schönfeldt, H.C. & Welgemoed, C. 1996. Composition of South African Beef. South African Meat Board, Pretoria.

Shetty, P.S. 2002. Nutrition transition in India. *Public Health Nutr.*, 5(1A): 175-182.

Speedy, A.W. 2003. Global production and consumption of animal source foods. *J. Nutr.*, 133(11): 4048S-4053S.

Stender, S., Dyerberg, J., Bysted, A., Leth, T. & Astrup, A. 2006. A trans world journey. *Atherosclerosis Supp.*, 7: 47-52.

Tarrago-Trani, M.T., Phillips, K.M., Lemar, L.E. & Holden, J.M. 2006. New and Existing Oils and Fats used in products with reduced *trans*-fatty acid content. *J. Am. Diet. Assoc.*, 106(6): 867-880.

Thoenes, P. 2006. Biofuels and commodity markets – Palm oil focus. FAO, Commodities and Trade Division. (www.rlc.fao.org/es/esc/common/ecg/629/en/full_paper_English.pdf [Accessed on 05/01/2009]).

USDA (United States Department of Agriculture). 2007. Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 20. Nutrient Data Laboratory Home Page, www.ars.usda.gov/nutrientdata.

USDA. 2008. Agricultural Research Service. USDA National Nutrient Database for Standard Reference, Release 21. Nutrient Data Laboratory Home Page, www.ars.usda.gov/nutrientdata.

US FDA. 2006. Mercury levels in commercial fish and shellfish. (www.cfsan.fda.gov/~frf/sea-mehg.html [Accessed on 22/08/2008]).

Van Heerden, S.M., Schönfeldt, H.C., Kruger, R. & Smit, M.F. 2007. The nutrient composition of South African lamb (A2 grade). *J. Food Compos. Anal*., 20 (8): 671-680.

WHO. 2003. *Diet, Nutrition and the prevention of chronic diseases.* Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series 916, WHO, Geneva.

Wood, J.D., Enser, M., Fisher, A.V., Nute, G.R., Sheard, P.R., Richardson, R.I., Hughes, S.I. & Whittington, F.M. 2008. Fat deposition, fatty acid composition and meat quality: A review. *Meat Sci.*, 78(4): 343-358.

Wright, J.D., Kennedy-Stephenson, J., Wang, C.Y., McDowell, M.A. & Johnson, C.L. 2004. Trends in intake of energy and macronutrients - United States, 1971--2000. *MMWR*., 53(04): 80-82.

Zhai, F.Y., Ge, K.Y., Jin, S.G. 1996. Summary report of China Health and Nutrition Survey. *J Hyg. Res*., 22:16–25.

Chapter 14: Processing, manufacturing, uses and labelling of fats in the food supply

Oils and fats occur naturally in a range of plant and animal sources. Whilst there are innumerable seeds and nuts that are sources of oils, globally only about 30 vegetable oils and animal fats have been commercially exploited. Of these, roughly a dozen oils are of worldwide importance. The four main large-scale commercially produced oils are soy, palm, rapeseed and sunflower, which represent about 80% of global production. Village level and small-scale processing of oil has a long history and shares common methodologies irrespective of the country of production. The generalised process can be summarised as consisting of five processes: raw material preparation, extraction, clarification, packaging and storage.

MANIPULATION OF PHYSIOCHEMICAL PROPERTIES OF OILS AND FATS

Three processes are used to manipulate the physico-chemical properties of food lipids: hydrogenation, interesterification and fractionation.

Hydrogenation

This is the addition of hydrogen to a fat in the presence of a catalyst in order to obtain different degrees of hardness. Hydrogenation is used to reduce the level of unsaturation in oils and thereby increase the solid fat content and stability. The formation of *trans* fatty acids during hydrogenation has compelled consumers, health authorities and manufacturers to reconsider the process as *trans* fatty acids are known to be a health risk.

Interesterification

Interesterification involves rearrangement or randomization of acyl residues in triacylglycerols with the fats and oils taking on new properties. 'Tailored fats' (fats with specific nutritional or textural properties) are easily obtained during this phase. The raw materials and processing conditions can be controlled or manipulated to produce a fat that has specific desired characteristics. The most widely used class of interesterification in the food industry is *trans*-esterification, where the ester bonds linking the fatty acids to the glycerol molecule are broken to release the fatty acids. The liberated fatty acids are then randomly shuffled in a fatty acid pool and re-esterified in new positions, either in the same or in a different glycerol molecule.

Fractionation

Fat fractionation involves the separation of fat into different fractions depending on the melting point, molecular structure, size and solubility in different solvents. The simplest method used for fractionation is controlled cooling. The melted fat is slowly cooled until the high melting triacylglycerols selectively crystallise. The separated crystals are then removed by filtration. In the processing step of "Winterisation" of

rapeseed (Canola), cottonseed or sunflower oil, small amounts of higher melting triacylglycerols or waxes are removed that would otherwise cause turbidity during refrigeration.

MARGARINE - PROCESSING

There is currently a large variety of margarine products available that are milk-free and made using sophisticated flavours such as lactones. Margarine manufacture essentially consists of three continuous basic steps: the emulsification of water within the continuous oil phase, chilling and mechanical handling of the emulsion, and crystallization, preserving the type of water/oil emulsion by efficient removal of released heat of crystallization. There are five types of margarines and spreads - table margarine, industrial margarines for baking, reduced fat spreads, low fat spreads and very low fat spreads.

STRUCTURED LIPIDS

Structured lipids (SL) or structured triacylglycerols (ST) may be broadly defined as triacylglycerols that have been altered or restructured using natural oils and fats. The earliest example of ST is the development of medium chain triglycerides (MCT). Using coconut and palm kernel oil, caprylic acid C8:0 and capric C10:0 are liberated. MCT are produced by esterification of these fatty acids with glycerol. The most widely available MCT have a C8:0:C10:0 ratio of 10:30. MCT also have the trade name Captrin®. One of the earliest uses of SL was in enteral and parenteral nutrition, followed by its application in a range of clinical settings including prevention of thrombosis, improved nitrogen balance, and enhanced immune function.

Fat replacers

Consumer demand for reduced-fat food products with the appearance, texture and flavour of full-fat counterparts has generated considerable interest in the development of fat replacers. Approaches to reduce the high-energy properties of fats in foods are based on one or more of the following principles:

Replace fats with combinations of water and surface-active lipids or non-lipid additives with smaller energy contributions such as proteins and/or polysaccharides, utilize compounds such as medium-chain triacylglycerols that contribute less energy per gram, and replace fats with compounds that significantly differ in structure to triacylglycerols.

Fat Substitutes

These are macromolecules that physically and chemically resemble triacylglycerols, and can replace oils and fats on a gram-to-gram basis. Basic strategies for developing this group of fat replacers are essentially based on one of the following approaches:

Sucrose polyesters (SPE)

This is a mixture of hexa-, hepta-, and octaesters of sucrose with long-chain fatty acids isolated from edible fats and oils, and is now recognized as Olestra® or Olean®. The lipases in the human body are unable to metabolize SPE and hence it provides no calories (Gerstoff, 1995; Kinsella, 1988). The FDA has concluded the following with respect to Olestra i) it is not toxic, carcinogenic, genotoxic or teratogenic, ii) all safety issues have been addressed, and iii) there is reasonable certainty that no harm will result from the use of Olestra® in savoury snacks. However, Olestra may need to

be used in small amounts as excessive consumption can lead to diarrhoea and the leaching of certain fat-soluble vitamins from the body.

Structured medium chain triacylglycerols

These are available under the brand name 'Salatrim®' developed by Nabisco Foods Group. 'Caprenin® was developed by Proctor and Gamble. Salatrim® is an acronym for short- and long-chain acid triacylglycerol molecules. This is a structured triacylglycerol exhibiting the physical properties of fat, but providing only a fraction of its energy content (5 kcal [21 kg])/g). Unlike polyol esters, it can be included in low moisture foods. The principles behind the properties of Salatrim® are that stearic acid is only partially absorbed in the body and short-chain fatty acids provide relatively fewer calories. It is produced by replacing the long-chain fatty acids in hydrogenated oils with short chains (acetic, butyric, propionic) and redistributing fatty acids in the glycerol molecule. Caprenin® is made up of behenic, caprylic and capric acids. It is recommended for use as a cocoa butter substitute. Similarly to Salatrim®, the behenic acid in Caprenin® is only partially absorbed by the body while the medium-chain fatty acids are metabolized in a similar manner to carbohydrates. Salatrim® and Carpenin® cannot be used for frying due to the generation of intense off-flavours.

OTHER APPROACHES (MULTIPLE EMULSIONS)

These involve replacing some of the fat inside fat globules (in an oil/fat emulsion) with water droplets. As a result, the fat content and the subsequent energy density are reduced. The physico-chemical properties of a multiple emulsion are expected to be similar to those of a normal oil-in-water emulsion. However, maintaining the stability of multiple emulsions over long periods of time has proven difficult and therefore is not widely used.

Reduced *trans* fatty acids (TFA)

In 1993, Walter Willet produced a paper that drew critical attention to the negative nutritional effect of consuming TFA (Willet, 1993). In the intervening years, numerous fat-containing foods have been developed called "virtually *trans* free", suggesting a level of TFA less than 1% in the lipid phase. Hydrogenated vegetable oils remain the most important source of TFA in our diet. Hydrogenated vegetable oils and fats from ruminant animals may contain up to 20 *trans* and *cis* positional isomers. TFA are mainly produced during hydrogenation of vegetable oils.

Manufacture of *trans*-free lipids

Many of the *trans*-free lipids are made into spreads, margarine, shortening and frying oils. There are numerous ways of producing these trans-free lipids, summarized in Table 14.1.

Processing losses

Oils from nuts and seeds represent a very concentrated form of energy, high in calories and very nutritious. Nuts and seeds also contain a substantial amount of protein. The nutritional value of an oil is directly related to its fatty acid content. High LA content decreases the shelf life of oils. From a nutritional viewpoint, high LA content is desirable due to it being an essential fatty acid. There is currently no evidence to demonstrate that the degree of roasting of the seeds has any effect on an oil's nutritional value. However, the amount of heat applied when roasting has a substantial effect on the oil's antioxidant content. Heat reduces the anti-oxidant content in the roasted seeds and nuts by up to 25%. While oils are usually not recognized as an important source

TABLE 14.1
Methods for manufacturing trans-free/low-trans fatty acids products

Food system	Method
Frying oil	Interesterification and fractionation to obtain olein fraction
Margarine	Trans esterification of stearic fats with vegetable oils using lipase.
	Interesterification of palm fats with high palmitic acid with hard lauric fats
Margarine – hard stocks	
Spread	Blend of interesterified hard stock and vegetable oil
Shortening products	Mixture of vegetable oils rich in stearine fraction combined with diglycerides
Confectionery fat	Fractionation of high stearic soy oil

of vitamins and minerals in the diet, the exceptions are that they are rich sources of tocopherol and carotene. Blending of vegetable oils to provide higher thermal stability has now been recognized. The concept is based on using naturally occurring antioxidants in oils to minimize fat oxidation.

In recent years the non-glyceride components of vegetable oils have received considerable attention as they contribute to shelf life and thermal stability at frying temperatures and they lower cholesterol and/or confer antioxidant effects.

The contribution of phytosterols from natural food fats and vegetable oils, sesamin (sesame seed and oil), and oryzanol (rice bran oil) in lowering serum cholesterol has been well documented. Sesame ligands, oryzanol, and phenolic compounds (olive oil) contribute to increasing the antioxidant potential of food or the diet (Hamalatha and Ghafoorunissa, 2007).

Frying oils

Since the quality of the oil used for frying has a large impact on fat absorption, it is recommended to use fresh oil as far as possible. Fresh frying oil is almost pure triglycerides, but its chemical structure alters with repeated usage. The physicochemical changes depend upon numerous factors including the type and volume of oil used, the food product being fried, the amount of food being produced, the temperature at which the fryer is being operated, the presence of trace elements and degree of exposure to air.

Some evidence suggests that highly oxidized and heated oils may have some carcinogenic properties. Most studies have concluded that deep-fried foods are not harmful unless foods are fried in extensively abused oils or if people consume excessive amount of fried foods. Nevertheless, it is recommended that the consumption of foods cooked in reused frying oil be kept to a minimum. As PUFA are lost during the frying process, this affects the nutritional value of a frying oil rich in PUFA.

FAT-CARBOHYDRATE INTERACTIONS IN FOOD SYSTEMS

Starch-lipid interactions

It is well recognized that the amylopectin fraction of starch is largely involved in swelling and hydration, leading to the thickening properties of cooked starch. The presence of fats and oils inhibits hydration, leading to a reduced viscosity and improved mouth feel. Glycaemic index measures the effect of a carbohydrate-based food on blood sugar. A high glycaemic index food will raise blood sugar quickly and higher than a low glycaemic food. Moreover, the blood sugar value is likely to fall to or below the baseline value much faster in subjects consuming high-GI food. Complexes

formed between amylose and long-chain saturated monoglycerides are generally more resistant to in vitro digestion than complexes with shorter chains or more unsaturated monoglycerides (Guraya *et al*., 1997).

Role of fats and oils in infant feeding

In many developing countries, weaning foods are starch based and are characterized by a low energy density and an unpalatable viscosity. Oils and fats can play a critical role in reducing the viscosity and improving the energy density of weaning foods. Human milk contains 40-55% of its energy in the form of fat. Energy density can be defined as the amount of energy stored in a specific food per unit volume or mass (usually in 100 g).

Energy density and viscosity of foods

One way to increase energy density without increasing viscosity is to add non-gelatinous carbohydrates such as simple sugars or fats to the diet. The addition of one tablespoon of vegetable oil to a typical weaning food (100 g) would increase the energy density from 0.30 to 0.70 kcal/g, but decrease the percentage as protein by 5% (Table 14.2). If this weaning food (pap) were consumed at sufficient levels to satisfy the children's energy requirements, it would not meet the children's protein requirements. Table 14.2 provides an example of how the energy density and protein density are altered by the simple addition of oil.

TABLE 14.2
Effects of added oil on energy, protein and iron density of maize

	Traditional maize pap	Oil-fortified maize pap
Amount of cereal (g/100 g)	8	8
Amount of oil (g/100 g)	0	5
Energy density (Kcal/g)	0.32	0.77
Protein density (% energy)	9.0	3.4

Oils and fats play a major role in meeting the energy requirement of infants, children and adults living in the developing world. Carbohydrate rich foods in many developing countries are difficult to consume in large quantities due to their bulkiness, thick viscous texture, poor palatability and low energy density. The addition of a small amount of oil or fat to carbohydrate rich foods not only improves palatability but also reduces its bulkiness and enhances its energy density.

LABELLING

Since the publication of the last expert consultation report on dietary fats (FAO, 1994), no significant changes in the recommendations on labelling have emerged except for the labelling of *trans* fatty acids. National legislation in the USA on *trans* fatty acids declarations varies considerably as shown in Table 14.3.

The FDA requires that the amount of *trans* fat in a serving is listed on a separate line under saturated fats on the nutritional facts panel. However, *trans* fats need not be listed if the total fat in the food is less than 0.5 g in one serving. In contrast, in Denmark from 2003 the content of *trans* fatty acids should not exceed 2 g^{-1} per 100 g^{-1} of oil or fat. In products that claim "free of *trans* fatty acids" the content of *trans* fatty acids should be less than 1 g^{-1} per 100 g^{-1} of the oil or fat.

TABLE 14.3
Dietary recommendations for trans fatty acids

US organizations	*Trans* fatty acids
American Heart Association	< 1% Energy (population)
Adult Treatment Panel III of the National Cholesterol Education program	Keep intake low
Health and Human Services/U.S Department of Agriculture	
Institute of Medicine of the National Academy of Sciences	Low as possible
	Low as possible

Adapted from Hunter, 2008

GENERAL CONCLUSIONS

Public concerns about obesity and cardiovascular disease have increased our interest in minimizing the consumption of saturated fats and *trans* fats. These concerns have been a driving force in the lipid industry to develop fats and fat-based ingredients with improved nutritional properties. New processing technologies, along with the creative use of newly discovered functional properties of triglycerides, have been the hallmark of innovation during the past decade. Using techniques such as interesterification, hydrogenation and fractionation, new and novel fat and sugar-based ingredients have been developed. These include zero calorie olestra®, low calorie salatrim® and diacylglycerols that have a lower net energy.

Future areas of research include the role that fats and fatty acids play in carbohydrate metabolism, notably glucose homeostasis. There is mounting evidence that the lipid-starch complex may play a significant role in reducing the glycaemic response of carbohydrate foods. The greatest global challenge is to find methods to reduce fat intake in peoples living in the Western hemisphere with the concomitant drive to increase fat consumption modestly in the developing world. Weaning foods consumed by children living in Asia, Africa and South America are of very low energy density. Moreover, carbohydrate-rich foods in these countries are a poor source of energy-rich foods. The addition of oils and fats to such foods will make these foods more palatable and enable the consumer to meet energy requirements more effectively. Finding innovative methods to increase the energy density of foods consumed in the developing world with the use of fats and oils remains a challenging area for research.

REFERENCES

Gershoff, S.N. 1995. Nutritional Evaluation of Dietary Fat Substitutes. *Nutr. Reviews*, 53: 305-313.

Guraya, H.S., Kadan, R.S. & Champagne, E.T. 1997. Effect of rice starch-lipid complexes on in vitro digestibility, complexing index, and viscosity. *Cereal Chem*., 74: 561-565.

Hamalatha, S. & Ghafoorunissa, C. 2007. Sesame ligands enhance the thermal stability of edible vegetable oil. *Food Chem*., 105: 1076-1085.

Hunter, J.E. 2008. Safety and health effects of *trans* fatty acids. *In* Chow, C.K., ed. *Fatty Acids in Foods and Their Health Implications* (3rd ed), pp. 757-790. London, CRC Press (Taylor Francis Group).

Kinsella, J.E. 1988. Food lipids and fatty acids: importance in food quality, nutrition, and health. *Food Technol*., 42(10): 124.

Willet, W.C., Stampfer, M.F., Manson, J.E., Colditz, G., Speizer, F.E., Posser, B.A., Sampson, I. & Hennekew, C.H. 1993. Intake of *trans* fatty acid and risk of coronary heart disease among women. *Lancet.*, 341: 58-85.

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Fats and fatty acids in human nutrition – Report of an expert consultation

Knowledge of the role of fatty acids in determining health and nutritional well-being has expanded dramatically in the past 15 years. In November 2008, an international consultation of experts was convened to consider recent scientific developments, particularly with respect to the role of fatty acids in neonatal and infant growth and development, health maintenance, the prevention of cardiovascular disease, diabetes, cancers and age-related functional decline. This report will be a useful reference for nutrition scientists, medical researchers, designers of public health interventions and food producers.

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