Effects of Fat and Fatty Acid Intake on Inflammatory and Immune Responses: A Critical Review

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Introduction

In addition to their role as major substrates for energy production and storage, fats as fatty acids (FA) are involved in the modulation of structural and functional properties at the cellular level. Functional roles of FA are largely and selectively attributable to the long-chain polyunsaturated fatty acids (LC PUFA) of the n–6 and n–3 series (described in 'Fat and Fatty Acid Terminology, Methods of Analysis and Fat Digestion and Metabolism: A Background Review Paper' by Ratnayake and Galli, pp 8–43 of this issue), which are quantitatively minor components of dietary fats. In addition to being directly ingested through the diet, these LC PUFA, can also, to some extent, be endogenously generated from their 18C essential precursors. In essence, the situation can be summarized as follows:

- Conversions of linoleic acid (LA) to arachidonic acid (AA) in the n–6 series and of α -linolenic acid (ALA) to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the n–3 series are based on sequential enzymatic steps (elongation, desaturation, and β -oxidation in the case of synthesis of 22C PUFA), and in these pathways desaturases appear to be major regulated steps, being affected by dietary, hormonal, pharmacological and toxicological factors.
- Conversion of LA to AA appears to be much more efficient than that of ALA to its LC derivatives, but the global conversion rates of LA to AA in relation to LA

intakes, and the relative relevance of direct AA intakes versus endogenous production, have not been fully assessed.

- Formation of LC n–3 FA, especially to DHA, from ALA appears to be rather inefficient, although somewhat higher conversion rates have been shown in premenopausal women than in men [Giltay et al., 2004].
- There is competition between the two metabolic pathways, especially at the desaturation steps, and at the cellular level competition may also take place for the esterification into the 2 position of glycerol of glycerolipids, depending on the relative abundance of the competing FA [Visioli et al., 2006].
- Since the production of LC PUFA from precursors and of the transporters (e.g. lipoproteins) from the site of synthesis to cells takes place mainly in the liver, hepatic metabolism in relation to physiological states (e.g. neonates, pregnancy, ageing) and to pathological conditions may affect the overall availability of LC PUFA to cellular compartments.

An additional relevant issue in the overall relationships between the intakes of LC PUFA, especially those that are present in very low amounts in the diet while being of unique nutritional importance, such as the LC n–3 PUFA, concerns their bioavailability in relation to the matrix (food vs. formulation). Available data indicate that elevation of circulating levels of these FA is greater when they are ingested as food components rather than as formu-

lated preparations (possibly due to various factors; for example, more effective activation of digestion/absorption processes in the intestine in the presence of food, localization of these FA mainly in position 2 of glycerolipids facilitating the subsequent incorporation in endogenous lipids, their facilitated absorption due to the lipid 'vehicle' in food and to the higher absorption surface in the intestine in relation to the large size of the portion versus the situation with, for example, encapsulated formulations). This issue is relevant also in consideration of the need to optimize the use of FA that are becoming progressively more scarce in nature. On the other hand, the preparation of n-3 FA formulations with improved bioavailability should be promoted, since this approach will be required for the supplementation of LC n-3 FA in subjects who may have some difficulty in increasing fish intake. In addition, although the bioavailability of formulated LC n-3 FA preparations may be lower, their sustained intakes and the incorporation of LC n-3 FA in rather stable lipid pools in the body will compensate for the difference.

Finally, the assessment of dietary intakes of quantitatively minor FA such as the LC n-3 PUFA, based on food composition data and dietary questionnaires, is rather unreliable, while on the other hand information on the FA status of individuals and populations is essential. Therefore, the assessment of the FA profiles in appropriate biological samples through direct analysis should be recommended, as discussed by Ratnayake and Galli (pp 8–43 of this issue). This type of information will allow correct estimations of FA intakes in populations, correlating FA levels and various FA indexes with lifestyles, dietary habits, physiological parameters, risk factors and clinical situations in pathological states, as well as providing the basis for recommendations.

Special Functional Roles of LC PUFA in Relation to Immunomodulation and Inflammation

LC PUFA are ultimately derived from the diet, either as their precursors (more important in the case of the LC n-6 PUFA), or as preformed compounds (more important in the case of the LC n-3 PUFA). LC PUFA are selectively incorporated in membrane lipids in various types of cells, especially in those endowed with specialized functions (e.g. cardiac, muscle, nerve and immune cells), and modulate the production of lipid mediators (eicosanoids and other oxygenated compounds) and lipid signalling molecules in cells (e.g. those involved in immune regulation and inflammation).

Particularly in the n-6 series, AA is a major precursor of eicosanoids (dihomo- γ -linolenic acid (DHGLA) 20:3n-6 being a minor precursor of prostaglandins of the 1 series), and in the n-3 series, eicosapentaenoic acid (EPA) is involved as a precursor of analogous compounds. DHA instead gives rise to docosanoids (see Ratnayake and Galli, pp 8–43 of this issue). Most of these compounds exert complex, diverse and somewhat contrasting effects on inflammation.

The Immune System

The immune response is a complex series of steps that includes an inflammatory component and is aimed at identifying, attacking and eliminating organisms and substances that invade our system or cause disease. The immune system is made up of a network of cells, tissues and organs cooperating in the protection of the body. The system, in addition to protect against environmental infective agents (bacteria, viruses, fungi and parasites), confers tolerance to autoantigens and benign environmental agents (food proteins and commensal gut bacteria). Both soluble factors (e.g. complement) and cells are involved. Immune cells are produced in bone marrow and are stored throughout the body in several locations, for example thymus, spleen, lymph nodes, and lymphoid tissues associated with various organs (e.g. the gut). When in the bloodstream, they are commonly termed white blood cells or leucocytes. These circulate through the body between organs and nodes through both the bloodstream and lymphatic vessels.

There are 3 types of immunity: innate, adaptive and auto [Janeway et al., 2001; Alberts et al., 2002].

Innate Immunity

Innate immunity is the first line of defence against infectious agents. It is a type of general protection that is based on immune cells recognizing and responding to pathogens in a generic way. In addition to physical barriers and the complement cascade, phagocytic cells are involved. Innate immunity is thought to represent an evolutionary more ancient defence strategy and is the dominant system found in plants, fungi, insects and primitive multicellular organisms.

This type of immunity, which has no memory and so is not influenced by previous exposure to infective agents, is based on the following steps [Janeway, 2001].

- (1) Recruitment of immune cells to the sites of infection and inflammation through the production of chemical factors including cytokines, which are specialized chemical mediators.
- (2) Activation of the complement cascade to identify bacteria, activate cells and promote clearance of dead cells and antibody complexes. The complement system is a biochemical cascade that 'complements' the ability of antibodies to clear pathogens or mark them for destruction by other cells. The cascade is composed of plasma proteins synthesized by hepatocytes. These proteins trigger the recruitment of inflammatory cells, tag the pathogens for destruction by other cells by opsonizing or coating the surface of pathogens, disrupt the plasma membranes of infected cells (cytolysis) causing pathogen death, and remove neutralized antigen-antibody complexes.
- (3) Identification and removal of foreign substances present in tissues, organs, blood and lymph, by specialized white cell phagocytosis.
- (4) Activation of the adaptive immune system through antigen presentation.

Cells of the Innate Immune System

Natural killer (NK) cells, mast cells, eosinophils, basophils and the phagocytic cells including macrophages, neutrophils and dendritic cells constitute the cellular component of the innate immune system. Activated mast cells (which are also involved in allergy and anaphylaxis) produce histamine, leukotrienes, heparin, and cytokines. Phagocytes (macrophages, neutrophils and dendritic cells) engulf pathogens and particles that are then incorporated in endosomes and merge with lysosomes releasing lytic enzymes and acids that kill and digest pathogens/particles. Activation of phagocytosis follows the response to molecule signals (cytokines) produced by other cells and the expression of surface receptors specific for bacterial antigens. Phagocytosis of the pathogenic micro-organisms is followed by their destruction by complement or free radical production. Macrophages in tissues where the immune reaction (and inflammation) takes place derive from circulating monocytes and are very efficient phagocytes producing reactive oxygen species (ROS), cytokines and chemokines. Neutrophils (the major type of granulocyte) attack pathogens through respiratory burst (producing ROS including hydrogen peroxide hypochlorite). Dendritic cells are also phagocytes in tissues in contact with the external environment (skin, respiratory tract, gastrointestinal tract) and are important in antigen presentation. Basophils and neutrophils

release histamine (e.g. in allergic reactions and asthma), toxic proteins and free radicals. NK cells are endowed of surface receptors. They attack mainly tumour cells and virus-infected host cells by releasing cytotoxic proteins.

The cells forming the innate immune system are involved in inflammation.

Acquired Immunity

Acquired (or adaptive) immunity develops throughout our lives, is highly specific and involves T and B lymphocytes. It allows for a stronger and more specific immune response and for development of immunological memory. This type of response is antigen-specific and requires the recognition of specific non-self antigens during antigen presentation, allowing responses that are tailored to specific antigens or pathogen-infected cells. The cells involved are lymphocytes (B cells are involved in the humoral immune response and T cells in cell-mediated immune responses).

Both types of lymphocytes carry surface receptors for a specific antigen and following activation, their function persists for several days after removal of the initiating antigen. T cell recognition requires that non-self targets (e.g. pathogens) and antigens (fragments of pathogens) are processed and presented in combination with the major histocompatibility complex (MHC) molecules on antigen-presenting cells (dendritic cells, macrophages).

Immunological memory is therefore created, which is the basis for a stronger and more effective immune response in the case of a repeated exposure to the same pathogen (antigen-carrying source). Humoral immunity, that is acquired immunity involving antibodies, acts through B lymphocytes, which are able to produce antibodies (antigen-specific immunoglobulins) specific for the individual antigen. Antibodies prevent the attachment of micro-organisms to host cells, thus neutralizing their pathogenic potential, and can also activate plasma complement proteins, which in turn promote phagocyte-mediated destruction of bacteria.

If a pathogen (e.g. viruses or some bacteria) exert their infective activity by entering cells, thus escaping humoral immunity, a cell-mediated system involving T lymphocytes is activated. These cells express antigen-specific T cell receptors that are able to recognize antigens presented on a cell surface (the major difference from B lymphocytes and humoral immunity). Infected cells express on their surface peptide fragments that are derived from the

intracellular pathogen, and these fragments are recognized by T lymphocytes. A coordinated process is then activated resulting in transport of fragments to the surface of the infected cells and expression together with MHC proteins. The pathogen-derived fragments bound to MHC II are then recognized by T lymphocytes. Cytotoxic T lymphocytes are stimulated by intracellular pathogens to destroy the infected cells, while extracellular pathogens stimulate helper T cell-mediated responses. There are several other types of cells involved, which are discussed below.

Killer T Cells

Killer T cells are a subgroup of T cells that kill cells infected with viruses (and other pathogens) or are otherwise damaged or dysfunctional. As with B and other T cells, each type of T cell recognises a different antigen and is activated following the binding of the T cell receptor to this specific antigen in a complex with the MHC class I receptor of another cell. Recognition of this MHC/antigen complex involves the co-receptor CD8. The T cell then interacts with other cells where the MHC I receptors bear this antigen. Cytotoxins are then released, forming pores in the target cell's plasma membrane, allowing ions, water and toxins to enter. T cell activation is tightly controlled and generally requires a very strong MHC/antigen activation signal, or additional activation signals provided by 'helper' T cells.

Helper T Cells

These regulate both the innate and adaptive immune responses and help to determine which types of immune response the body will make to a particular pathogen. These cells have no cytotoxic activity and do not kill infected cells or clear pathogens directly; instead, they control the immune response by directing other cells to perform these tasks.

Helper T cells express T cell receptors that recognize antigens bound to class II MHC molecules. The MHC/antigen complex is also recognized by the helper cell's CD4 co-receptor, which recruits molecules inside the T cell (e.g. Lck) that are responsible for activating the T cell. The activation of a resting helper T cell causes it to release cytokines that influence the activity of many cell types. Cytokine signals produced by helper T cells enhance the microbicidal function of macrophages and the activity of killer T cells. In addition, helper T cell activation causes an up-regulation of molecules expressed on the T cell's surface, such as the CD40 ligand (also called CD154), which provide extra stimulatory

signals typically required to activate antibody-producing B cells.

Helper T lymphocytes produce various types of cytokines which act as signalling molecules in the inflammatory process.

Immune Cell Cytokines: General Considerations

Cytokine is a general name, and others include lymphokine (cytokines produced by lymphocytes), monokine (cytokines produced by monocytes), chemokines (cytokines with chemotactic activities) and interleukin (cytokines made by one leucocyte but acting on other leucocytes). Cytokines may act on the cells that secrete them (autocrine action), on nearby cells (paracrine action), or in some instances on distant cells (endocrine action).

Cytokines are small secreted proteins, produced *de novo* in response to an immune stimulus, which mediate and regulate immunity, inflammation and haematopoiesis. They generally (although not always) act over short distances and short time spans and at very low concentrations. Responses to cytokines include increasing or decreasing expression of membrane proteins (including cytokine receptors), proliferation, and secretion of effector molecules. It is common for different cell types to secrete the same cytokine, or for a single cytokine to act on several different cell types. Cytokines are redundant in their activity, meaning that similar functions can be stimulated by different cytokines. Cytokines are often produced in a cascade, as 1 cytokine stimulates its target cells to make additional cytokines. Cytokines can also act synergistically (2 or more cytokines acting together) or antagonistically (cytokines causing opposing activities).

The combination of short half-life, low plasma concentrations, pleiotropy, and redundancy complicates the isolation and characterization of cytokines. Searches for new cytokines are now often conducted at the DNA level, identifying genes similar to known cytokine genes.

Cytokine Activities. The largest group of cytokines stimulates immune cell proliferation and differentiation. This group includes interleukin 1 (IL-1), which activates T cells; IL-2, which stimulates proliferation of antigen-activated T and B cells; IL-4, IL-5, and IL-6, which stimulate proliferation and differentiation of B cells; IL-10 with an anti-inflammatory activity; interferon gamma, which activates macrophages, and IL-3, IL-7 and granulocyte macrophage colony-stimulating factor, which stimulate haematopoiesis. A list of some cytokines and their activities is shown in table 1.

Table 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 dendritic cells
IL-1 α ; IL-1 β	Monocytes; macrophages; B cells; dendritic cells	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; NK cells	Activation, growth and proliferation
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; B cells	Anti-inflammatory (e.g. decreases TNF- α synthesis)
IL-12	Dendritic cells; macrophages; B cells	Activated Tc cells	Differentiation into CTL (with IL-2)
		NK cells	Activation
IFN- α	Leucocytes	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
MIP-1 α	Macrophages	Monocytes; T cells	Chemotaxis
MIP-1 β	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	Adhesion molecule and cytokine expression
		Tumour cells	Death
TNF- β	Th1; CTL	Phagocytes	Phagocytosis, NO production
		Tumour cells	Death

CTL = Cytotoxic T lymphocytes; GM-CSF = granulocyte-macrophage colony-stimulating factor; IL = interleukin; IFN = interferon; MHC = major histocompatibility complex; MIP = macrophage inflammatory protein; TGF = transforming growth factor; TNF = tumour necrosis factor.

B Lymphocytes and Antibody Production

A B cell identifies pathogens when antibodies on its surface bind to a specific antigen. The complex is taken up by the B cell and processed by proteolysis into peptides. The B cell then displays these antigenic peptides on its surface MHC class II molecules. This combination of MHC and antigen attracts a matching helper T cell, which releases lymphokines and activates the B cell. As the activated B cell then begins to divide, its offspring (plasma cells) secrete millions of copies of the antibody that recognizes this antigen. These antibodies circulate in blood plasma and lymph, bind to pathogens expressing the antigen and mark them for destruction by complement activation or for uptake and destruction by phagocytes. Antibodies can also neutralize challenges directly by binding to bacterial toxins or by interfering with the receptors that viruses and bacteria use to infect cells.

Autoimmunity

Autoimmunity is the failure of an organism to recognize its own constituent parts as *self*, which results in an immune response against its own cells and tissues. Any disease that results from such an aberrant immune response is termed an autoimmune disease. Prominent examples include coeliac disease, diabetes mellitus type 1, systemic lupus erythematosus, Sjögren's syndrome, Churg-Strauss syndrome, multiple sclerosis, Hashimoto's thyroiditis, Graves' disease, idiopathic thrombocytopenic purpura and rheumatoid arthritis.

Several mechanisms are thought to be operative in the pathogenesis of autoimmune diseases, against a backdrop of genetic predisposition and environmental modulation.

T Cell Bypass. The requirement of a T cell can be bypassed in rare instances, such as infection by organisms producing super-antigens.

Molecular Mimicry. An exogenous antigen sharing structural similarities with certain host antigens and any antibody produced against this antigen (which mimics the self-antigens) can also bind to the host antigens and amplify the immune response. The most striking form of molecular mimicry is observed in group A β -haemolytic streptococci, which share antigens with human myocardium and are responsible for the cardiac manifestations of rheumatic fever.

Idiotypic Cross-Reaction. Idiotypes are antigenic epitopes found in the antigen-binding portion (Fab) of the immunoglobulin molecule. Autoimmunity can arise as a result of a cross-reaction between the idiotypic on an an-

tiviral antibody and a host-cell receptor for the virus in question. In this case, the host-cell receptor is envisioned as an internal image of the virus, and the anti-idiotypic antibodies can react with the host cells.

Cytokine Dysregulation. T cell-derived cytokines are divided into 2 groups according to the population of cells whose functions they promote: helper T cell type 1 or type 2. The second category of cytokines, which include IL-4, IL-10 and TGF- β (to name a few), seem to play a role in prevention of exaggerated pro-inflammatory immune responses.

Dendritic Cell Apoptosis. Dendritic cells bear antigens to active lymphocytes. Dendritic cells that are defective in apoptosis can lead to inappropriate systemic lymphocyte activation and consequent decline in self-tolerance.

Epitope Spreading/Drift. This is where the immune reaction changes from targeting the primary epitope to targeting other epitopes also. In contrast to molecular mimicry, the other epitopes need not be structurally similar to the primary one.

General Considerations

The immune system is essential to host defence and in the prevention of infectious diseases, but disorders of the system can lead to various pathological conditions: immunodeficiency disorders (primary or acquired), autoimmune disorders, allergic disorders (overreaction of response to an antigen) and cancers of the immune system.

Signalling Molecules in Cellular Responses

Several classes of molecules are involved in signalling in cellular responses. These are outlined below.

Nuclear Factor-Kappa B

Nuclear factor-kappa B (NF- κ B) is a transcription factor found in almost all animal cell types and involved in cellular responses to stimuli such as stress, cytokines, free radicals, ultraviolet irradiation, oxidized low-density lipoprotein, and bacterial or viral antigens. NF- κ B plays a key role in regulating the immune response to infection. Consistent with this role, incorrect regulation of NF- κ B has been linked to cancer, inflammatory and autoimmune diseases, septic shock, viral infections, and improper immune development. In addition, NF- κ B has been implicated in processes of synaptic plasticity and memory [Mattson et al., 2006].

Part of NF- κ B's importance in regulating cellular responses is that it belongs to the category of 'rapid-acting' primary transcription factors, that is, transcription factors that are present in cells in an inactive state and do not require new protein synthesis to be activated (other members of this family include transcription factors such as c-Jun, STATs, and nuclear hormone receptors). This allows NF- κ B to act as a 'first responder' to harmful cellular stimuli. Stimulation of a wide variety of cell surface receptors, such as RANK and TNFR, leads directly to NF- κ B activation and fairly rapid changes in gene expression [Gilmore, 2006].

Many bacterial products can activate NF- κ B. The identification of Toll-like receptors (TLRs) as specific pattern-recognition molecules and the finding that stimulation of TLRs leads to activation of NF- κ B improved our understanding of how different pathogens activate NF- κ B. For example, studies have identified TLR4 as the receptor for the lipopolysaccharide component of Gram-negative bacteria. TLRs are key regulators of both innate and adaptive immune responses [Doyle et al., 2006]. Activation of NF- κ B is initiated by the signal-induced degradation of I κ B proteins. This occurs primarily via activation of a kinase called I κ B kinase that phosphorylates 2 serine residues located in an I κ B regulatory domain [Perkins, 2007]. With the degradation of the I κ B inhibitor, the NF- κ B complex is then freed to enter the nucleus where it can activate the expression of specific genes that have DNA-binding sites for NF- κ B nearby. The activation of these genes by NF- κ B leads then to the given physiological response, for example an inflammatory or immune response, a cell survival response, or cellular proliferation. NF- κ B turns on the expression of its own repressor, I κ B α . The newly synthesized I κ B α thus forms an auto-feedback loop, which results in oscillating levels of NF- κ B activity.

NF- κ B is widely used by eukaryotic cells as a regulator of genes that control cell proliferation and cell survival. Because NF- κ B controls many genes involved in inflammation, it is not surprising that NF- κ B is found to be chronically active in many inflammatory diseases, such as inflammatory bowel disease, arthritis, sepsis and asthma.

Mitogen-Activated Protein Kinases

Mitogen-activated protein (MAP) kinases are serine/threonine-specific protein kinases that respond to extracellular stimuli (mitogens) and regulate various cellular activities, such as gene expression, mitosis, differentiation and cell survival/apoptosis.

MAP kinases are involved in the action of most non-nuclear oncogenes. They are involved in cell responses to growth factors such as brain-derived neurotrophic factor or nerve growth factor. Extracellular stimuli lead to activation of a MAP kinase via a signalling cascade (the MAP kinase cascade) that is composed of MAP kinase, MAP kinase kinase (MMK, MEKK or MAP2K), and MAP kinase kinase kinase (MKKK or MAP3K, EC 2.7.11.25). The cascade is as follows: MAP kinase activation (mitogen) $\rightarrow$ MAP3K $\rightarrow$ MAP2K $\rightarrow$ MAP kinase $\rightarrow$ further signalling.

A MAP3K that is activated by extracellular stimuli phosphorylates a MAP2K on its serine and threonine residues, and then this MAP2K activates a MAP kinase through phosphorylation on its serine and tyrosine residues. This MAP kinase signalling cascade has been evolutionary well conserved from yeast to mammals.

To date, 6 distinct groups of MAP kinases have been characterized in mammals.

- Extracellular signal-regulated kinases (ERK1, ERK2). The ERK1/2 (also known as classical MAP kinases) signalling pathway is preferentially activated in response to growth factors and phorbol esters (a tumor promoter), and regulates cell proliferation and differentiation.
- c-Jun N-terminal kinases (MAPK8, MAPK9, MAPK10) are also known as stress-activated protein kinases (SAPKs).
- p38 isoforms [MAPK11, MAPK12 (ERK6), MAPK13, MAPK14]. Both c-Jun N-terminal kinases and p38 signalling pathways are responsive to stress stimuli, such as cytokines, ultraviolet irradiation, heat and osmotic shock, and are involved in cell differentiation and apoptosis.
- ERK5 (MAPK7), which has been discovered recently, is activated both by growth factors and by stress stimuli, and it participates in cell proliferation.
- ERK3 (MAPK6) and ERK4 (MAPK4) are structurally related atypical MAP kinases possessing SEG motifs in the activation loop and displaying major differences only in the C-terminal extension. ERK3 and ERK4 are primarily cytoplasmic proteins which bind, translocate and activate MK5 (PAK, MAP2K5). ERK3 is unstable, unlike ERK4 which is relatively stable.
- ERK7/8 (MAPK15) is the newest member of MAP kinases. It possesses a long C terminus similar to ERK3/4.

Peroxisome Proliferator-Activated Receptors

The peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function as transcription factors regulating the expression of genes [Michalik et al., 2006]. PPARs play essential roles in the regulation of cellular development, differentiation, and metabolism (carbohydrate, lipid and protein) of higher organisms [Berger and Moller, 2002; Feige et al., 2006].

Three types of PPARs have been identified [Berger and Moller, 2002]:

- α , expressed in liver, kidney, heart, muscle, adipose tissue and elsewhere;
- β/δ , expressed in many tissues but markedly in brain, adipose tissue and skin;
- γ , although transcribed by the same gene, this PPAR is expressed in 3 forms through alternative splicing: $\gamma 1$ is expressed in virtually all tissues, including heart, muscle, colon, kidney, pancreas and spleen; $\gamma 2$ is expressed mainly in adipose tissue; $\gamma 3$ is expressed in macrophages, large intestine and white adipose tissue.

FA control the expression of genes encoding regulatory proteins involved in their own metabolism. In particular, the PUFA derived from the diet appear to be involved in the regulation of gene expression especially through PPARs, in addition to other transcription factors, for example liver X receptors (α and β), hepatic nuclear factor-4 α and sterol regulatory element binding proteins 1 and 2 [Jump, 2002].

Free FA or their CoA derivatives seem to be the main signals involved in the transcriptional effect of LC FA. PPARs modulate the activation of immune and anti-inflammatory cells [Marx et al., 1998]. However, although there is a vast literature supporting the concept that PUFA act through PPARs, data have been mainly obtained in studies on cultured cells using relatively high concentrations of free LC n-3 FA (i.e., in the range of $\geq 50 \mu\text{M}$) and generally as pure compounds. However, in vivo free FA are present in very small concentrations in cells, the LC n-3 PUFA represent a small fraction of total free FA, and free FA are mainly bound to fatty acid binding proteins. It is therefore rather difficult to extrapolate the actual in vivo quantitative relevance of these pathways after dietary LC n-3 FA intakes.

Inflammation

Inflammation, part of the body response to injury or infection, involves changes in the circulation, for example increased blood flow and increased permeability to

large molecules and cells (leucocytes) across blood capillaries from the blood stream into the surrounding tissues. Redness, swelling, heat and pain are major symptoms of the process. The immunologic process behind inflammation is targeted to the elimination of pathogens and toxins, and to repair damaged tissues. The sequence of events involves movement of cells into the inflammatory site following up-regulation of adhesion molecules (intercellular adhesion molecule 1, ICAM-1), vascular cell adhesion molecule 1 (VCAM-1) and E-selectin on endothelial cells, resulting then in the binding and diapedesis of leucocytes (granulocytes, monocytes, macrophages and lymphocytes in a sequence). Among the exogenous triggers of these activities of monocytes and macrophages, an important factor is the component of cell walls of Gram-negative bacteria, the endotoxin known as lipopolysaccharide. Cytokines are then produced (TNF- α , IL-1, IL-6 and IL-8), together with nitric oxide (NO), matrix metalloproteinases that are deeply involved in the regulation of inflammatory processes, eicosanoids (prostaglandins and other compounds), and other products, for example ROS. Expression of adhesion molecules is also induced by lipopolysaccharide. On the other hand, the production of certain cytokines (e.g. IL-10) and of modulatory compounds (e.g. the receptor antagonist of IL-1) opposes the inflammatory signal.

The inflammatory mediators produced as part of the inflammatory response (cytokines, eicosanoids, NO, ROS, etc.) are responsible for local tissue damage, systemic effects on the central nervous system (fever, loss of appetite), skeletal muscle (proteolysis), adipose tissue (lipolysis), liver (synthesis of acute phase proteins), and pathogen destruction. Under controlled conditions following the repair of lesions, the acute phase of the inflammatory process undergoes resolution and recovery. These processes are modulated by various mediators, including compounds generated from FA precursors.

FA and Inflammation

Most of the evidence concerning the relationships between dietary fats, and hence cell and tissue FA, and inflammation is attributed to the roles of LC PUFA. It is of particular relevance that although the role and involvement of lipid mediators generated from LC PUFA (AA in the n-6 series and EPA and DHA in the n-3 series) in the activation/resolution of the inflammatory process have long been established, they are currently being further elucidated.

Fig. 1. Production pathways of mediators derived from LC PUFA.

Lipid Mediators in Inflammation

A variety of lipid mediators, generated from complex cell lipids, are involved in cell signalling processes. They are derived from membrane lipids, mainly glycerophospholipids and sphingolipids, following activation of their enzymatic cleavage, resulting in the generation of breakdown products that are then enzymatically converted to a variety of bioactive molecules. The overall patterns of lipid-derived compounds involved in intracellular signalling and transcellular amplification of responses, and which are modulated by dietary fats, are at the core of the 'lipidomic' approach.

AA, the major n-6 LC PUFA in immune and inflammatory cell membranes, has been identified as the most relevant FA precursor of lipid mediators: the eicosanoids produced through a series of enzymatic reactions. More recently, analogous mediators derived from EPA and DHA have been identified (resolvins and protectins). The relationships between these LC PUFA and the production of the derivatives are, however, quite complex for the reasons outlined below.

- Cellular levels of specific LC PUFA precursors are modulated by dietary intakes through complex processes (conversion from precursors, mainly in the case of the n-6 LC PUFA, and also intake of preformed compounds, especially in the case of the LC n-3 PUFA). The effectiveness of the endogenous pathway and the balance between the 2 sources have not been fully elucidated under different physiological and dietary conditions.

- Efficiency and extent of incorporation into different cell types and into different classes of membrane lipids differ between FA families. Interestingly, AA levels are higher in cells specialized in eicosanoid production [Ris et al., 2007].
- Mechanisms of LC PUFA release from different phospholipid classes may be activated through different processes involving different enzymes (e.g. phospholipase A₂ or phospholipase C followed by a lipase).
- AA is released from cells more effectively than the LC n-3 PUFA (e.g. DHA).

The complexity of the processes clearly indicates that the overall production of eicosanoids and related compounds may not be directly related to the amounts of the LC PUFA precursors in tissues and that the whole sequence of processes should be considered. In essence, the production of mediators from LC PUFA depends to a large extent upon the intensity of the stimuli activating the whole process and upon their duration, which, in the case of the inflammatory process, may sequentially activate the production of pro- and anti-inflammatory compounds (resolvins).

The overall production of mediators derived from LC PUFA of the n-6 (mainly AA) and n-3 (EPA and DHA) series is described in 'Fat and Fatty Acid Terminology, Methods of Analysis and Fat Digestion and Metabolism: A Background Review Paper' by Ratnayake and Galli (pp 8-43 of this issue), and the underlying pathways are presented in figure 1.

While most of the products of the AA cascade are endowed with pro-inflammatory activities, some have opposite activities (e.g. lipoxins are anti-inflammatory) and others have contrasting activities (e.g. prostaglandin E₂ has both pro- and anti-inflammatory actions).

In addition, the generation of cyclooxygenase (COX) and lipoxygenase (LOX) products from EPA, generally endowed with lower bioactivity than those derived from AA, is less efficient than in the case of AA-derived compounds. In addition, LC n-3 PUFA may interact with the activation of the AA cascade through different mechanisms (replacement of AA in cell membranes, interference with the conversion of LA to AA, competitive inhibition of phospholipases) and they have recently been shown to give rise to a new class of bioactive mediators, resolvins and docosanoids.

Generally speaking, the much higher levels of AA than EPA or DHA in most cells would imply that the formation of AA-derived eicosanoids will prevail. However, as previously mentioned, the relationships between dietary n-6 FA (mainly LA), the levels of cell and tissue AA, the production of AA-derived mediators, and the relationships with inflammatory processes are rather complex and not easily predictable.

Contrasting Effects of AA and LC n-3 FA and Optimal n-6/n-3 Ratio in the Diet

The different and somewhat contrasting effects of AA and of EPA and DHA on various cell functions, and particularly in cells involved in immunomodulation and inflammation, has raised the issue whether the ratio of n-6/n-3 LC PUFA in the body should be controlled through nutritional strategies. The topic has been extensively discussed, but no definite conclusion has been reached for humans due to the complexity of the issue. However, several points have been raised.

- Rather than the ratio between n-6 and n-3 FA in the diet, and especially in cells and tissues, the absolute levels, especially of the LC n-3 PUFA, are more relevant [Stanley et al., 2007]. In fact, a low ratio but with very low levels of n-3 PUFA would not allow the expression of the activities of the n-3 FA. In addition, several studies have shown biological effects following the intakes of few grams per day LC n-3 PUFA, in spite and independently of the much greater intakes of total fats (>100 g/day) and of n-6 FA (LA >10 g/day vs. <0.5 g EPA + DHA).
- As already mentioned, levels of AA in cells and tissues are not directly correlated with changes of LA (the major n-6 FA in the diet) intakes, and n-6 and n-3 FA are incorporated in different lipid pools in plasma and

cells. These different distributions will have an impact on the significance of the n-6/n-3 ratio.

In conclusion, the general thought is that the above ratio does not have an absolute value, and that, since the n-3 FA play relevant roles in biological systems, adequate intakes (to be more carefully defined in relation to background diet, age groups, physiological conditions, etc.) need to be satisfied. On the other hand, it appears that there is no need to maintain high levels of LA intakes above the requirements to prevent deficiency, considering that through the food chain (animal fat enriched with this FA and high use of seed oils), LA intakes in populations have grown markedly during the last decades, and that there are large deposits of LA in whole body fats (especially adipose tissue).

Human Studies on Dietary Fats and Inflammation: LC n-3 PUFA

The evidence emerging from clinical studies of the anti-inflammatory effects of LC n-3 PUFA [Calder, 2006], the appreciation and understanding of the underlying mechanisms [Calder, 2003], obtained through in vitro and animal studies, and the availability of formulated preparations allowing simplified approaches in the design of clinical studies have greatly stimulated research mainly on these compounds. However, inflammatory processes, especially in locations such as the gastrointestinal (GI) and cardiovascular (CV) systems, could be modulated through less specific mechanisms by other major components [saturated fatty acids (SFA), monounsaturated fatty acids (MUFA)] of dietary fats as well as by the total amounts especially in the postprandial phase [Burdge and Calder, 2005].

The major types of diseases and conditions in which inflammation is involved and which may be beneficially affected by LC n-3 FA are listed in table 2 [see Calder, 2006].

Although supplementation studies have been conducted for most of these diseases, some have been the subject of a larger number of trials, and the evidence of beneficial effects appear to be greater, for example for asthma (childhood and adult), inflammatory bowel disease (Crohn's disease, ulcerative colitis) and rheumatoid arthritis.

Asthma

AA-derived eicosanoids, especially prostaglandin D₂, and the 5-lipoxygenase products LTC₄, LTD₄ and LTE₄ (detected in blood, bronchoalveolar lavage fluids and in urine of asthmatic patients) [Henderson, 1994] are pro-

Table 2. A list of some of the diseases and conditions with an inflammatory component that may be beneficially affected by LC n-3 PUFA

Acute cardiovascular events
Acute respiratory distress syndrome
Allergic disease
<i>Asthma (childhood and adult)</i>
Atherosclerosis
Cancer cachexia
Chronic obstructive pulmonary disease
Cystic fibrosis
<i>Inflammatory bowel disease (Crohn's disease, ulcerative colitis)</i>
Lupus
Multiple sclerosis
Neurodegenerative disease of ageing
Obesity
Psoriasis
<i>Rheumatoid arthritis</i>
Systemic inflammatory response to surgery, trauma, and critical illness
Type 1 diabetes
Type 2 diabetes

Diseases are listed in alphabetical order. Italics indicate diseases and conditions where the evidence of benefit from n-3 PUFA administration is greater.

Adapted from Calder [2006].

duced by inflammatory cells (e.g. mast cells) in pulmonary inflammation, contributing to bronchoconstriction. In addition, the COX product prostaglandin E₂ predisposes to allergic inflammation by regulating the development of the Th2 cells [Miles et al., 2003] and the formation of IgE by B lymphocytes [Roper and Phipps, 1994]. It has been hypothesized that elevated intakes of n-6 FA and especially low intakes of n-3 FA play a causal role in increasing asthma incidence [Hodge et al., 1994; Black and Sharp, 1997], on the basis of epidemiological evidence [Hodge et al., 1994; Dunder et al., 2001] and estimates of FA intakes. It should be noted, however, that assessment of LA intakes through questionnaires and food composition tables is more reliable than that of n-3 FA (low amounts in the diet, and variability of levels in major food sources and in procedures for food preparation). Various studies have reported anti-inflammatory effects (reduction of 4-series LT and of leucocyte chemotaxis) of fish oils in asthmatic patients, and various uncontrolled fish oil trials have also shown clinical benefits [Payan et al., 1986; Arm et al., 1988; Kirsch et al., 1988]. However, a more recent report covering a large number of studies (randomized placebo-controlled and others)

concluded that 'no definite conclusion can yet be drawn regarding the efficacy of n-3 FA supplementation as a treatment for asthma in children and adults' [Schachter et al., 2004], although there may be subgroups of patients that benefit greatly from LC n-3 PUFA [Broughton et al., 1997; Nagakura et al., 2000].

Conclusions. There is possible benefit (with adequate dose) in children, but no evidence of benefit in adults.

Inflammatory Bowel Disease

The GI system is subjected to sustained exposure to ingested foods throughout the whole life, and this gives rise to interactions between food components and GI mucosal cells. Interactions with transporters and transcription factors contribute to the modulation of various responses, including those of cells involved in inflammatory processes (see later). There are, however, specific chronic diseases of the alimentary tract that are based on inflammatory processes and hence are bound to be susceptible to modulation by dietary FA.

In Crohn's disease the mucosa of the whole alimentary tract from the mouth to the anus can be affected, with maximal manifestation in the ileum and colon, while in ulcerative colitis, the mucosa of the colon is mainly affected. Inflammatory cytokines and eicosanoids (LTB₄) are actively produced in situ [Sharon and Stenson, 1984]. A multivariate analysis suggested that the recently increased consumption of n-6 FA in Japan, resulting in an increased ratio of n-6 to n-3 PUFA and possibly also elevated AA levels, may be responsible for the increased incidence of Crohn's disease in this country [Shoda et al., 1996]. On the other hand, dietary fish oils, which exert beneficial effects in animal models of colitis, have been shown to result in the incorporation of LC n-3 PUFA into the intestinal mucosa of patients with inflammatory bowel disease (IBD), in anti-inflammatory effects (e.g. eicosanoid reduction) as well as in some clinical benefits as demonstrated in small open-label or pilot studies [reviewed by Calder, 2008a]. Out of several randomized, placebo-controlled, double-blind studies on the effects of fish oils (2.7–5.6 g/d) in IBD, some have reported benefits, including improved clinical scores, mucosal histology, sigmoidoscopic score and especially lower rates of relapse [Belluzzi et al., 1996]. Reviews of fish oil trials in IBD [Belluzzi, 2002] generally conclude that there is some benefit in the treatment, and a meta-analysis [MacLean et al., 2004] identified 13 studies reporting outcomes related to clinical score, remission and relapse. Relapse appears to be the most consistent outcome (5 studies) in the case of ulcerative colitis following fish oil treatments.

Although several studies have reported favourable effects of fish oils in IBD, the overall view [Calder, 2008a] is that the evidence of clinical benefits is rather limited, except for the potential of the LC n-3 PUFA to retain the patients in remission [Belluzzi et al., 1996]. However, this latter observation was not confirmed in a recent larger study using a similar protocol [Feagan et al., 2008].

Conclusions. Possible benefit (with adequate dose) in Crohn's disease; insufficient evidence of benefit in ulcerative colitis.

Rheumatoid Arthritis

This chronic inflammatory disease is characterized by joint inflammation, swelling, pain, impaired function, stiffness, osteoporosis, muscle wasting, and the participation of inflammatory cells (macrophages, T lymphocytes, plasma cells infiltrating the synovium). All the mediators of inflammation (cytokines, interleukins and typical pro-inflammatory factors and proteins) are actively produced by synovial cells. COX-2 is over-expressed in the synovium of patients, and its eicosanoid products, in addition to those of the 5-LOX, are found in the synovial fluids. The relevance of the pro-inflammatory COX pathway is also underlined by the efficacy of the pharmacological inhibition of this pathway (e.g. by non steroidal anti-inflammatory drugs). The beneficial effects of fish oil administration have been shown in several studies on animal models of arthritis [see Calder, 2008b]. Human studies in patients have shown that fish oils reduce the production of inflammatory mediators (LTB₄ by neutrophils, and monocytes, IL-1 by monocytes) as well as the plasma concentrations of IL-1 β and C-reactive protein, and normalize neutrophil function. A number of randomized, placebo-controlled, double-blind studies of fish oil treatments (on average with 3.5 g/day EPA + DHA) have been carried out and most of them reported some benefit [Calder, 2008b]. Clinical symptoms were improved, including reduced duration of morning stiffness, number of tender or swollen joints, joint pain, time of fatigue and increased grip strength [Calder, 2008b]. Of particular relevance was the reduction of the use of anti-inflammatory drugs. The effects of LC n-3 PUFA are attributable to their anti-inflammatory properties based on the previously discussed quantitative and qualitative modulation of the eicosanoid pathway. It is of interest that also reducing n-6 FA intakes (especially AA) down to less than 90 mg/day through an anti-inflammatory lacto-vegetarian (vs. normal Western) diet improved clinical symptoms, and the effects are substantially further improved by the intake of fish oil. In addition, the

requirement for non-steroidal anti-inflammatory drugs declined in patients receiving fish oil versus those on the Western diet, especially after 6–8 months on the fish oil diet [Adam et al., 2003]. This study shows also that intakes of preformed AA, in addition to formation from the precursor LA, may be a factor in the modulation of AA levels in the body, and since LA and AA are provided by quite different sources (seed oils vs. lean meat), this should be taken into consideration in the evaluation of strategies for optimal n-3 FA intakes.

The conclusions on the effects of fish oil treatments in rheumatoid arthritis patients outlined in several reviews are that the evidence of benefits is robust, and an editorial comment on the use of fish oil in this disease concluded that 'dietary fish oil supplements should be regarded as part of a standard therapy for rheumatoid arthritis' [Cleland and James, 2000].

Of 3 meta-analyses, 1 that included data from 9 published trials (1985–1992) concluded that dietary fish oil supplementation for 3 months reduces joint symptoms and morning stiffness [Fortin et al., 1995], but the second [MacLean et al., 2004], considered data from 10 trials (1985–2002, including 1 with flaxseed oil rich in ALA, 1 without the use of a control, and another 1 based on transdermal administration of n-3 PUFA) and concluded that fish oil supplementation has no major effect on clinical symptoms. Finally, a meta-analysis of 17 randomized controlled trials, conducted with Cochrane Review Manager for 6 separate outcomes, assessed the pain-relieving effects of n-3 PUFA in patients with rheumatoid arthritis or joint pain secondary to IBD and dysmenorrhoea. It showed that supplementation for 3–4 months reduces patient-reported joint pain intensity, minutes of morning stiffness and number of painful and/or tender joints. However, significant effects were not detected for physician-assessed pain at 3–4 months. The results suggest that n-3 PUFA are an attractive adjunctive treatment for joint pain associated with rheumatoid arthritis as well as for IBD and dysmenorrhoea [Goldberg and Katz, 2007].

Conclusions. Convincing evidence of benefit (with adequate dose).

Conclusions on LC n-3 PUFA for Inflammatory Processes

Taken together, there is fairly strong evidence that increasing the intakes of LC n-3 PUFA results in favourable effects on inflammatory processes [Stamp et al., 2005]. There are, however, various general points that should be taken into consideration.

- LC n-3 PUFA are essential components of cell membranes, and play relevant regulatory roles in the modulation of cell functions. Adequate intakes therefore should be provided throughout the whole life – from intrauterine life to advanced age – in order to allow optimal physiological development of organs and systems. Therefore, interventions only after certain disturbances (e.g. enhanced inflammation) have occurred, will be too late and will not allow the ‘prevention’ of the process but will just operate as a ‘pharmacological treatment’. Under these conditions the doses of LC n-3 PUFA administration (usually relatively high amounts compared to standard daily intakes) and the duration of treatment will be major factors.
- The requirements at the various stages of life, also in relation to physiological conditions and lifestyles, have not been fully assessed.
- Assessment of the FA profiles in populations is essential in order to define the overall status with respect to the most relevant FA, and to detect individuals with a ‘deficient’ profile who may profit from a modification in dietary fat intakes.
- The increments of the 2 major LC n-3 PUFA after administration follow different trends: in plasma lipids, increments of EPA are greater and more rapid, but levels return to basal more rapidly after suspension of administration, while increments of DHA are slower, smaller but remain elevated for longer periods after the administration is ended. The different behaviour of the 2 major LC n-3 PUFA may lead to different impacts (and different dose/response correlations) with respect to the effects.
- The relative roles of EPA and DHA (and possibly also of DPA) in the modulation of inflammatory processes still need to be adequately defined. In general, EPA would appear to exert a greater modulation of lipid mediators (eicosanoids) due to more direct interactions with AA (structural analogy, similar distribution in lipid pools) and therefore, it may exert more potent anti-inflammatory activities versus DHA. DHA, in contrast, might modulate more specifically functional processes (e.g. ion channels, neurotransmitters) in specialized membranes (retina, synapses). This issue needs further investigation.

The Role of Dietary ALA in Modulating Inflammation

ALA is the typical n-3 FA in terrestrial plants [Harris, 2005], and is generally consumed in higher amounts than the LC n-3 PUFA. Few studies have investigated the ef-

fects of this FA on inflammatory outcomes in humans. Few of them observed some effect on pro-inflammatory markers at rather high levels of intake, that is 13.4 g/day for 4 weeks [Caughey et al., 1996] or 6.5% of energy (i.e., in the range of about 14 g/day for 2,000 kcal intakes) for 6 weeks [Zhao et al., 2004]. The effects thus were obtained at much higher doses (and were less pronounced) than those required for LC n-3 PUFA. In addition, several studies using lower intakes of ALA (<9 g/day) did not find significant effects on inflammatory markers [reviewed by Calder, 2006]. In conclusion, ALA may reduce inflammatory processes at high doses and with more modest effects in comparison with LC n-3 compounds. The effect of ALA may relate to its conversion to EPA [Zhao et al., 2004].

Monounsaturated Fatty Acids

MUFA, especially the major MUFA oleic acid (C18:1n-9), are the most common FA in biological systems and are present in relatively high amounts in various types of oils and in particular in olive oil. While increments of their intakes at the expense of SFA have been shown to exert some beneficial effect on CV risk markers, e.g. cholesterol levels and lipoprotein profiles, there is little evidence that MUFA affect inflammatory processes. However, some studies have shown that the administration of a high-fat meal rich in oleic acid, in the form of refined olive oil, versus a high palmitic acid meal to healthy and hypertriglyceridemic subjects resulted in a reduction of postprandial levels of the inflammatory adhesion molecules sICAM-1 and sVCAM-1 [Pacheco et al., 2008]. A cross-sectional epidemiological study carried out in the Japanese population correlating serum levels of high-sensitivity C-reactive protein with intakes of 7 specific FA assessed through a validated dietary history questionnaire showed that C-reactive protein was significantly and inversely related to the intakes of oleic acid, LA and ALA, especially when the intake of LC n-3 PUFA is at a moderate level in Japanese [Yoneyama et al., 2007]. The mechanisms of these effects have not been elucidated and may be attributed to an indirect effect of oleic acid on postprandial lipemia. Concerning the specific use of oils very rich in oleic acid such as olive oil, a typical component of the Mediterranean diet, it should be mentioned that this oil, depending upon the cultivar, the climate, the preparation procedure, and especially in the case of the so-called extra virgin oil, contains appreciable levels of antioxidant compounds (e.g. the typical phenolic components oleuropein and hydroxytyrosol). The intake of an olive oil (extra virgin) rich in phenolics exerts significant

favourable effects on serum levels of inflammatory markers (LTB₄) and thrombotic indexes (TxB₂) especially in the postprandial phase, when compared to the intake of a refined olive oil (low in phenolics) or corn oil [Bogani et al., 2007].

Conclusions. There is insufficient evidence of involvement of MUFA.

Trans FA

Consumption of *trans* fatty acids (TFA), produced through industrial hydrogenation of unsaturated FA and especially PUFA in seed oils, is associated with coronary heart disease and related pathologies. These associations appear to be greater than would be predicted by the effects on serum lipoproteins alone. The involvement of systemic inflammation and endothelial dysfunction in the pathogenesis of atherosclerosis and CV disease in general, and the evidence from both observational and experimental studies that TFA are pro-inflammatory provide an explanation. *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 further research is needed [Mozaffarian et al., 2004; Mozaffarian, 2006]. TFA also appear to induce endothelial dysfunction. The mechanisms of these effects are not well established, but may involve TFA incorporation into cell membranes (affecting membrane signalling pathway relating to inflammation) or ligand-dependent effects on PPARs or RXR (retinoid X receptor) pathways. Activation of inflammatory responses and endothelial dysfunction may represent important mediating pathways between TFA consumption and risk of coronary heart disease, sudden death and diabetes. Further studies are required to define these effects of TFA, the possible underlying mechanisms and the implications of such effects not only for CV health but also for related processes (e.g. inflammation). In fact, the impact of TFA on classical inflammatory diseases has not been adequately studied. Due to the overall negative effects of TFA on the CV system and health in general, it has been recommended to decrease their intakes (<1% of energy) by reducing the utilization of hydrogenated fats in the preparation and use of food products. In general, a decline of TFA consumption in most countries has taken place and some of the epidemiological evidence of their negative effects on, for example the CV system is related to the impact of relatively high (>2–3% of energy) intakes that took place in the last decades in certain countries [Hulshof et al., 1999]. The situation should be different in the years to come.

A distinct type of TFA that may be present in natural foods is represented by the class of conjugated LA compounds, produced in ruminants through the physiological process of biohydrogenation. Claims have been made concerning favourable properties of these compounds on some metabolic dysfunction (obesity) or disease (experimental cancer models), but the effects in humans are still somewhat controversial and under investigation. Their involvement in the modulation of inflammatory processes is unknown.

Conclusions. There is a possible pro-inflammatory role for TFA, but limited evidence prevents a clear conclusion. More research in this area is needed.

Other Types of Relationships between Dietary Fats and Inflammation

Inflammatory processes occurring in diseases that take place in body compartments (e.g. atherosclerosis in the CV system) that are not typical sites of primary inflammatory processes may also be affected by the intake of FA that are not directly involved in the modulation of inflammation. In fact, SFA, by affecting the levels of serum cholesterol and lipoprotein profiles, contribute to the onset and progression of atherosclerosis and hence to the activation of inflammation. It has been shown, for example, that the consumption of SFA in humans impairs the anti-inflammatory properties of high-density lipoprotein and endothelial functions [Nicholls et al., 2007]. Obviously, however, LC n–3 FA also exert favourable activities on CV inflammation.

Additional Sites of Inflammatory Processes Affected by Dietary Fats

The GI system is exposed to sustained interactions with foods at each meal throughout the whole life. The following digestive processes, sustained by lipases with the intervention also of the intestinal microbial flora, micro- and macronutrients and their modified derivatives, interact with the intestinal mucosa and, more specifically, with specific transporters and several transcription factors, thus activating and modulating local cellular processes, possibly including inflammation. These processes are evidently maximized during the postprandial state, a phase that is characterized by enhanced lipemia. Inflammatory processes of the GI tract, initiated and modulated by dietary factors, may then be transferred to the CV system, thus contributing to CV diseases. There is in fact growing evidence of an association between chronic GI inflammation and chronic heart disease [Krack et al., 2005]. In addition, various studies have

shown the rise of inflammatory markers during the post-prandial phase in normal subjects and in CV patients [Erridge et al., 2007; Kalsch et al., 2008] is induced by high-fat meals. The intake of LC n-3 PUFA has been shown to exert protective effects also on GI inflammation (see section on LC n-3 and IBD).

Conclusions

Dietary fats (to some extent the amounts but especially their composition) play a role in modulating immune functions and inflammatory processes. Most of these activities can be attributed to the PUFAs, with some opposing effects at the cellular levels of the n-6, especially AA, which are partly derived as such from the dietary intake but mainly derived from the conversion of LA, and of the n-3 FA, especially of the LC compounds EPA and DHA. Most of the attention of researchers has therefore been devoted to the roles of these FA and to their effects on processes taking place in immune and inflammatory cells, on the basis of the established understanding and knowledge of the underlying mechanisms (cell signalling and lipid mediators, expression of pro- and anti-inflammatory compounds, etc.), emerging from basic research and animal experiments. Other FA may also more or less indirectly contribute to modulate inflammatory processes by affecting pathophysiological states (e.g. CV diseases) and altering metabolism (obesity and related conditions) in which inflammation is involved. More extensive and carefully planned research is required to fully define the overall impact of the whole FA spectrum in the diet.

Recommendations

Recommendations for optimal fat intakes in relation to prevention, in the first place and, to some extent, treatment of inflammatory processes can be super-imposed on those that should be applied for the control of other aspects of human health (control of body weight, CV function, prevention of cancers) and for brain maturation and functions (neonatal development, ageing).

They can be summarized as follows:

- Total fat consumption should not exceed 35% of energy, possibly less than 30%, but special attention should be given to total energy intakes, in relation also to anthropometric characteristics (age group, life-styles, BMI).

- The intakes of the various FA classes should be optimized with special emphasis on reduction of SFA (<10 % of energy) versus the present intake levels in most countries.
- Adequate intakes of PUFA. Total PUFA intakes should be formulated specially to provide adequate amounts of n-3 PUFA; however, they need to be tailored to the FA status of the subjects. The general recommendations for these FA are: ALA 1–2 g/day and EPA + DHA in the order of at least 0.3 g/day (weight values are preferable to % of energy values since these FA are not involved in energy production), but requirements may differ depending upon other parameters (e.g. elevated serum TG levels), in addition to the general anthropometric ones. As to the n-6 FA, LA in the first place, it appears that, due to the present relatively high levels of these FA in our diet, there is no need to further increase their intakes. Intakes of the order of 2–3% of energy for LA are considered to be adequate, although requirements may be somewhat higher in neonates and children. Also, in the early stages of life, the intake of preformed AA, together with that of LC n-3 PUFA, may be important due to the high requirements by rapidly developing organs and systems and to the rather low efficiency of AA synthesis from LA. The association of adequate intakes of antioxidants in relation to PUFA (especially n-3) may be considered.
- The intakes of MUFA, especially oleic acid, should be adjusted on the basis of the reduction of SFA and of the recommended intakes of PUFA, with a resulting value of up to 15% of energy.

Again, it must be emphasized that recommendations should be tailored to individuals and that the general requirements for certain groups, such as children and aged subjects, have not yet been adequately established.

Disclosure Statement

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