CHAPTER 1

Review of Literature
Foods contain various substances that can control the physiological functions of the body. Modulating immune responses is one of the most important functions of foods. Immune functions are indispensable for defending the body against attack by pathogens or cancer cells, and thus play a pivotal role in the maintenance of health. However, the immune functions are disturbed by malnutrition, aging, physical and mental stress or undesirable lifestyle. Therefore, foods with immunemodulatory activities are considered to be efficient in prevention of declining immune functions and reduce the risk of infection. (Kaminogawaand Nanno 2004).

Nutrients that are required (in animals or humans) for the immune system to function efficiently include essential amino acids, essential fatty acids (EFA), vitamin A, folic acid, vitamin B6, vitamin B12, vitamin C, vitamin E, zinc (Zn), copper (Cu), iron (Fe) and selenium (Se) (Calder and Kew 2002). Immunity may be affected by deficiencies or excesses in one or more of these nutrients leaving the animal or individual susceptible to infections or over responsive to harmless encounters. Regulation of numerous immune responses by nutrients could be through its influence on inflammatory mediator production, signal transduction and expression of critical immunoregulatory genes (Meydani and Ansari 1998). A critical role of nutrients in immune dysregulations such as that of aging, inflammation and vascular diseases have also been demonstrated (Meydani 1998; Chandra 1993).

Dietary fats, especially the essential fatty acids (EFAs) present in it play an important role in immune health. In recent years, a large number of studies were conducted to investigate the relevance of certain fatty acids in alteration of immune system functions in both animals and humans. Early epidemiological findings established a strong link between fatty acids supplied in the diet (particularly long chain \( \omega-3 \) PUFA fatty acids contained in fish oil) and its effect on immune responses of Greenland Eskimos, indicating low prevalence of inflammatory disorders in this population (Horrobin 1987; Kromannand Green 1980). Subsequent experimental investigations determined the efficacy of several fatty acids in modulating the immune system, as well as the mechanism by which they offer alterations in the way immune system works. These studies have led to the understanding that EFAs in diet are precursors of inflammatory
lipid mediators that determine the fluidity of immune cell membranes, alter the expression of inflammatory gene and generation of protein inflammatory mediators. Therefore, optimal supply of EFA in diet is prime requisite of ideal immune health making cause-and-effect relationship between dietary fats and non-communicable diseases as one of the most interesting facet of nutrition research today.

Epidemics of non-communicable diseases (NCD) are presently emerging in most of the developing countries (Murray and Lopez 1996). Even as infections and nutritional deficiencies are receding as leading contributors to death and disability, cardiovascular diseases (CVDs), cancers, diabetes, neuropsychiatric ailments and other chronic diseases are becoming major contributors to the burden of disease. India too illustrates this health transition, which positions NCDs as a major public health challenge of growing magnitude in the twenty-first century. Several of these chronic diseases including CVDs, diabetes, cancer, and arthritis have their roots in disorders of the immune system and therefore the course of these diseases can be altered by nutritional interventions (Kelley and Bendich 1996).

**Immune system**

The immune system acts to protect the host from infectious agents that exist in the environment (bacteria, viruses, fungi, parasites) and from other noxious insults. It has two functional divisions such as; innate immune system and the acquired immune system. Both components of immunity involve various blood-borne factors (complement, antibodies, and cytokines) and cells. These cells are generally termed leucocytes. Leucocytes are of two types; phagocytes [granulocytes (neutrophils, basophils, and eosinophils), monocytes and macrophages] and lymphocytes. Lymphocytes are classified as T lymphocytes, B lymphocytes and natural killer cells. T lymphocytes are further divided into helper T cells (CD4 cells) and cytotoxic T cells (CD8 cells). All cells of the immune system originate from bone marrow. They are found circulating in the bloodstream, organized into lymphoid organs such as the thymus, spleen, lymph nodes and gut-associated lymphoid tissue, or dispersed in other locations around the body.
Innate and acquired immunity

Innate immunity is the first line of defense present before exposure to potential pathogens. This type of immunity consists of anatomic barriers such as intact skin and mucosal membranes that prevent entry of pathogens into the host. If entry is gained, granulocytes (neutrophils, eosinophils, basophils) and monocytes / macrophages can directly destroy pathogens by extracellular release of lysosomal enzymes and toxic chemicals (e.g. superoxide radicals and hydrogen peroxide, nitric oxide), or through engulfment and subsequent nonspecific killing accomplished largely by oxygen-dependent mechanisms (Collins1999). Plasma proteins like complement or toxic proteins released from natural killer (NK) cells also play a role in destruction of many pathogens (Abbas et al.2000; Calder2001).

Acquired immunity involves recognition of specific antigens or peptide components of invading pathogens. Recognition is accomplished either by antigen-specific antibodies produced by B-lymphocytes, termed the humoral response or by receptors on the surface of T-lymphocytes called the cell-mediated response. T lymphocytes are able to recognize foreign peptides presented by other host cells in conjunction with proteins which are termed as major histocompatibility complex (MHC). The MHC class I complex is present on the surface of all nucleated host cells. It is responsible for presenting peptides that originate from intracellular pathogens, like viruses and certain bacteria (Sharon 1998). MHC class I is specifically recognized by cytotoxic T lymphocytes that express the CD8 receptor; CD8+ T lymphocytes subsequently destroy the infected host cell and prevent further propagation of the pathogen. The MHC class II complex is only found on antigen-presenting cells such as macrophages, dendritic cells, and B-lymphocytes. The peptides presented by this complex are derived from extracellular pathogens that have been engulfed or endocytosed by antigen-presenting cells, and are recognized by helper T-lymphocytes expressing the CD4 receptor. Recognition of foreign antigen in the context of MHC class II stimulates CD4+ T lymphocytes to produce chemical messengers called cytokines, which activate or inhibit different cell types, depending on the type of pathogen (bacteria, viruses, fungi, or helminthic parasites) (Sharon 1998; Abbas et al. 2000). CD4+ helper T lymphocytes are categorized into two subtypes based on their
cytokine production. Type 1 helper (Th1) cells secrete interferon (IFN)-γ and interleukin (IL)-2 which facilitate cell-mediated immunity by activating macrophages, cytotoxic T-lymphocytes, and NK cells. Type 2 helper (Th2) cells secrete IL-4 and IL-10 which promote B-lymphocytes to produce antibodies. Th2 cells also secrete IL-5 which is an important eosinophil-activating factor.

Communication within the acquired immune system and between the innate and acquired immune systems is accomplished through binding adhesion molecules of adjacent cells and by production and release of chemical messengers (Figure-1) (Calder 2001). The most important of these chemical messengers include synthesized polypeptides called cytokines, and PUFA fatty acid metabolites called eicosanoids. There are a multitude of cytokines, many of which have multiple functions. However, a few of the important cytokines have been categorized based on their major function(s) on target cell (Collins 1999). The cytokines most important to the acquired immune response regulate lymphocyte and macrophage activation, proliferation, differentiation and inhibition. These cytokines include IL-2, IL-4, IL-10, IL-12, transforming growth factor (TGF)-β, and interferon (IFN)-γ. The cytokines most involved in natural immunity are tumor necrosis factor (TNF)-α, IL-1, and IL-6. These are synthesized and released by activated macrophages and promote neutrophil and macrophage-mediated killing of bacteria and increase expression of adhesion molecules on neutrophils and endothelial cells. IL-1 receptor antagonists have also been shown to inhibit the production of pro-inflammatory eicosanoids LTB₄ (Conti et al. 1993) and PGE₂ (Arend et al. 1990). TNF-α, IL-1, and IL-6 stimulate T- and B-lymphocyte proliferation and up regulate MHC, and are therefore an important link between natural and acquired immunity (Calder 2001). Macrophage secretion of IL-1 also stimulates T-lymphocyte synthesis of IL-2. IL-2, first described as a T-cell growth factor (Morgan et al. 1976), is an important autocrine and paracrine cytokine that promotes the proliferation of T lymphocytes, B-lymphocytes, and NK cells.
Inflammation

Inflammation is defined as ‘a pathologic process consisting of a dynamic complex of cytologic and histologic reactions that occur in the affected blood vessels and adjacent tissues in response to an injury or abnormal stimulation caused by physical, chemical, or biological agents. The “cardinal signs” of inflammation are rubor, redness; calor, heat; tumor, swelling; dolor, pain; and functio laesa, loss of function. These signs occur as a result of localized increased blood flow, increased vascular permeability which allows complement, antibodies, and cytokines to leave the bloodstream and increase the movement of circulating leukocytes to affected tissue (Collins 1999). To a large extent cytokines orchestrate the inflammatory response. They are major determinants of the make-up of the cellular infiltrate, the state of cellular activation, and the systemic responses to inflammation (Carol et al. 1997). Since cytokines control the generation of lipid derived mediators the latter also plays a very important role in inflammation.
Although inflammation is part of the normal, innate defense against pathogens, it can be harmful. Animals and humans can develop an excessive inflammatory reaction after an inappropriate immune response to benign environmental antigens, leading to food allergies, asthma, and atopy. In other diseases such as rheumatoid arthritis, systemic lupus erythematosus and inflammatory bowel disease (IBD), inflammatory reactions are directed at ‘self’ antigens rather than foreign pathogens, and often cause irreparable tissue damage. Lastly, the immune-mediated inflammatory response to some intracellular bacterial agents and fungi is so intense that it actually causes more host damage than the pathogen, as with *Mycobacterium tuberculosis* infections. Pro-inflammatory cytokines such as TNF-α have been identified as mediators of anorexia, weight loss and anemia associated with chronic infectious diseases (Darling et al. 1990).

**Modulation of immune function by dietary fatty acids**

At least four modes of action have been proposed to explain the potential action of fatty acids on the modulation of immune system in both animals and humans. Accordingly, immune system modulation by dietary lipids may be attributed to changes in the composition of membrane phospholipids, lipid mediator production, and alteration of gene expression leading to altered immune cell function (De Pablo et al. 2002). Altered fatty acid composition of membrane phospholipids might be expected to influence immune cell function for a variety of reasons, summarized in Figure-2. Firstly, the fluidity of the plasma membrane or composition of lipid rafts in the plasma membrane is important in the functioning of immune cells (Horejsi 2003). Moreover, cell culture experiments have demonstrated the efficacy of PUFAs to alter the fluidity of immune cell membrane.
Figure-2: Mechanisms where by PUFAs might exert effects on inflammation and immunity. (Adopted from Calder and Grimble 2002)

Secondly, a number of cell signaling molecules are generated from membrane phospholipids (inositol-1,4,5-trisphosphate, diacylglycerol, phosphatidic acid, choline, ceramide, platelet activating factor, AA) or phospholipid derived mediators such as diacylglycerol have important roles in regulating the activity of some proteins involved in cell signaling mechanisms within immune cells. Changing the fatty acid composition of phospholipids may change their affinity as substrates for the enzymes which generate the signaling molecules and so could alter immune cell responsiveness. Thirdly, AA is a substrate for synthesis of the family of bioactive mediators known as eicosanoids, and altering the availability of AA as a substrate alters the ability of cells to produce eicosanoids, and thus potentially alters a range of inflammatory and immune cell responses (Calder and Grimble 2002).
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Fatty acids

Dietary fats are composed of fatty acids of varying chain lengths and double bonds. They are classified as saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA). The principal fatty acids are mostly straight-chain aliphatic monocarboxylic acids with even number of carbon atoms in them. Fatty acids are carboxylic acids varying from 2 to 22 carbons in length. The final carbon opposite to carboxyl end, is called the “omega” carbon, and is designated by either the letter “ω” or “n”. Nomenclature for fatty acids is typically based on the chain length, the number of double bonds within the carbon chain, and the location of the first double bond with respect to the omega carbon (Reinhart, 1996). For example, α-linolenic acid is a 18-carbon chain with 3 double bonds, the first of which is between the 3rd and 4th carbon molecules as counted from the omega carbon. It is designated as 18:3, ω-3 or C18:3 (Figure-3). In the same fashion, the saturated fatty acid, palmitic acid is simply represented as C16:0, as it consists of a 16-carbon chain with no double bonds.

![α-linolenic acid (18:3ω-3). COOH is the carboxyl group on the opposite end from the omega carbon, CH3. Double bonds are represented by parallel lines.](image)

SFAs [palmitic acid (16:0), stearic acid (18:0)] and MUFAs[oleic acid (18:1),eicosanoic acid (20:1)] are not essential as these fatty acids can be synthesized in the body whereas PUFAs are essential dietary components for mammalian species. Therefore, PUFAs must be provided in the diet (Reinhart, 1996; Calder, 1997; Kelley, 2001). The de novo synthesis of PUFA from endogenously synthesized palmitic acid is limited due to lack of desaturase enzyme that can catalyze the insertion of double bond beyond 9th carbon atom from the carboxyl end. The functional attributes of PUFAs depends a lot on the double bonds beyond the 9th carbon atom in their structure. The essential fatty acids (EFA) belong to either ω-6 or ω-3 family, distinguished based on the position of double bonds in their structure (Figure-4).
Linoleic acid (18:2, ω-6) consumed in the diet can be converted via γ-linolenic (18:3, ω-6) and di homo-γ-linolenic (20:3, ω-6) acids to AA (20:4, ω-6) by the pathway outlined in Figure-5. Using the same pathway dietary ALA (18:3, ω-3) can be converted to EPA (20:5, ω-3), DPA (22:5, ω-3) and DHA (22:6, ω-3). The desaturase and elongase enzyme systems can also elongate and desaturate the monounsaturated oleic acid (18:1 ω-9) to produce mead acid (20:3 ω-9) but they have a substrate preference for 18 carbon ω-3 and ω-6 PUFA over 18:1 ω-9. Thus 20:3 ω-9 is normally not produced, and its presence in significant amounts in membranes is normally indicative of ω-3 and ω-6 PUFA dietary deficiency (Figure-5) (Hulbert et al. 2005). Fatty acids of different families (ω-3, ω-6 and ω-9) are elongated and / or desaturated to fatty acids of the same family but these classes of fatty acids are not interchangeable. The fatty acids of different families compete for the enzymes which metabolize them (Calder and Grimble 2002). Therefore a balance of these fatty acids is emphasized in the diet, especially the fatty acids of ω-6 and ω-3 family. Oleic acid can also be synthesized by elongation and desaturation of endogenous
palmitic acid by fatty acid synthase enzyme using acetyl CoA as substrate and therefore considered to be non essential fatty acid.

![Diagram of Biosynthesis of the ω-9, ω-6 and ω-3 families of PUFAs.](image)

Figure-5: Biosynthesis of the ω-9, ω-6 and ω-3 families of PUFAs. Each step is catalyzed by the microsomal chain elongation or desaturase system: 1; elongase, 2; Δ6 desaturase, 3; Δ5 desaturase, 4; Δ4 desaturase. (Θ inhibition)

Dietary fatty acids once absorbed have different fates. They can be metabolized and incorporated into membrane lipids, utilized information of storage lipids (triacylglycerols) and/or oxidized to CO$_2$, H$_2$O and energy. Oxidation of fatty acids is inversely related to chain length and directly related to degree of unsaturation (Delany et al. 2000) indicating that, majority of ingested essential fatty acids are probably utilized for synthesis of storage triglycerides or incorporated into membrane lipids.

**Lipid mediators and their role in inflammation**

Bio-membranes serve as cellular barrier, source of rapidly generated signaling molecules and precursor of structurally diverse intracellular and extracellular lipid-mediators (LM). Phospholipases (PLA$_2$, PLC, PLD, sphingomyelinase) are pivotal in generating these LM. Activation of these enzymes is accomplished by either specific cell surface (i.e., cytokine and/or 7 transmembrane spanning) receptors or by membrane perturbations such as trauma or reperfusion injury. The LM produced include free fatty acids, their oxygenated products called eicosanoids and intracellular signaling molecules such as lysophospholipids, platelet activating factor (PAF), diacylglycerol (DAG), phosphatidic
acid and ceramide which play an important role in response of immune cells to infection or injury.

**Arachidonic acid as a precursor for eicosanoids**

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Figure-6: Eicosanoids and their biosynthetic origins. (1=Cyclooxygenase, 2=Lipoxygenase)

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Whether derived directly from dietary sources or converted from EFAs like LA, AA is esterified into membrane phospholipids of leukocytes and other cells, particularly at the carbon 2 position of phosphatidylcholine, phosphatidylinositol, and phosphatidylethanolamine (Collins, 1999; Bochsler and Slauson, 2002). When appropriate leukocyte cell membrane receptors bind to extracellular ligands such as cytokines, growth factors, or chemotactic peptides, cytosolic phospholipases are indirectly activated. These
Phospholipases, principally phospholipase A₂ (PLA₂), cleave AA from its esterified position within the membrane phospholipids. This also occurs at intracellular membranes such as endoplasmic reticulum and the nuclear membrane (Evans et al., 2001). Free AA is then metabolized by one of two major pathways to form compounds called eicosanoids (Figure-6). Eicosanoids include prostaglandins (PG), thromboxanes (TX), leukotrienes (LT), lipoxins, hydroperoxyeicosatetraenoic acids (HPETE), and hydroxyeicosatetraenoic acids (HETE).

Metabolism of AA by cyclooxygenase (COX) enzymes gives rise to pro-inflammatory eicosanoids, specifically 2-series PGs and TXs. There are two isoforms of COX: COX-1 is a constitutive enzyme that is responsible for day-to-day AA metabolism; COX-2 is transcriptionally regulated and is induced in leukocytes as a result of receptor stimulation during inflammation. Hence, COX-2 induction is responsible for increased synthesis of prostaglandins during an inflammatory response. The proportion of the different prostaglandins produced varies among different leukocytes. Macrophages produce large quantities of PGE₂ and PGF₂α whereas neutrophils produce moderate amounts of PGE₂. PGD₂ is the predominant prostaglandin produced by mast cells. PGE₂, PGF₂α, and PGD₂ are in part responsible for the pain, redness, and swelling of inflamed tissues. In contrast to its local pro-inflammatory effects, PGE₂ also has immunosuppressive and/or anti-inflammatory effects (Tilley et al., 2001). PGE₂ has been found to suppress in vitro T-cell proliferation and IL-2 production (Gordon et al., 1976; Rappaport and Dodge, 1982), as well as NK cell activity (Brunda et al., 1980). AA can also be metabolized by an alternative pathway involving lipoxygenase (LO) enzymes, best characterized of these is 5-LO. Upon stimulation 5-LO interact with 5-lipoxygenase-activating protein (FLAP), this active enzyme complex then acts on AA to form 5-HPETE (Collins, 1999; Bochsler and Slauson, 2002). 5-HPETE is the precursor of leukocyte chemotactic agent 5-HETE and the 4-series leukotrienes. Leukotriene B₄ (LTB₄) is a potent pro-inflammatory mediator that acts as chemotactic agent for neutrophils. LTB₄ also recruits the immune cells at the site of injury and orchestrate the inflammatory events leading to tissue damage (Goodarzi et al. 2003). It also increases their adhesiveness to vessel endothelium, lysosomal enzyme release, and oxygen free radical generation. LTC₄, LTD₄, and LTE₄
collectively produce intense vasoconstriction, increased vascular permeability, and bronchospasm.

**Omega-3 fatty acid as an alternative source of eicosanoid synthesis**

Omega-3 fatty acids, EPA and DHA can be derived directly from dietary sources such as fish oil, or they can be synthesized in vivo from precursor ω-3 fatty acids such as ALA. EPA and DHA directly inhibit the metabolism and biological effects of AA by at least four different mechanisms. First, these long chain ω-3 fatty acids can be esterified into cell membrane phospholipids, resulting in decreased esterification of AA. Second, phospholipase-induced release of AA from the cell membrane is inhibited by EPA and DHA (Lands et al., 1973). Third, EPA released from the cell membrane competes with AA as substrate for COX and 5-LO enzymes (Reinhart, 1996). Finally, EPA-derived eicosanoids will competitively antagonize the action of AA-derived eicosanoids assuming they bind and initiate activity through the same target cell receptors (Calder, 2001). Thus EPA and DHA to some extent, and their metabolites, are able to displace AA. This prevents the synthesis and activity of the pro-inflammatory AA metabolites, principally the 2-series prostaglandins and the 4-series leukotrienes. EPA metabolism by COX and 5-LO enzymes gives rise to 3-series prostaglandins and thromboxanes and 5-series leukotrienes, respectively (Figure-6). In contrast to their AA-derived counterparts, EPA-derived eicosanoids are less inflammatory, cause less vasodilation, decrease platelet aggregation, and are less immunosuppressive (Reinhart, 1996). For example, thromboxane A₃ (TXA₃) derived from EPA attenuates pro-aggregatory action on human platelets when compared to TXA₂ (Whitaker et al., 1979). EPA-derived PGE₃ appears to be a less potent inhibitor of lymphocyte proliferation than PGE₂ (Calder et al., 1992). Lastly, leukotriene B₅ (LTB₅) has markedly reduced potency in chemotactic and vasoconstriction activity when compared to the AA-derived LTB₄ (Lee et al., 1988). Therefore, EPA is anti-inflammatory not only due to its competitive inhibition of AA metabolism. More importantly, EPA-derived eicosanoids are less potent promoters of the inflammatory responses. Unlike other EPA-derived eicosanoids, PGI₃ retains the biologic properties of its AA derived counterpart PGI₂. Both of these prostaglandins inhibit platelet aggregation and relax vascular smooth muscles in vitro (Fischer and
Weber, 1984). In addition, PGI$_2$ production is maintained during periods of EPA dietary supplementation (Fischer and Weber, 1984). At least partial inhibition of platelet aggregation would therefore be expected in animals and humans supplemented with ω-3 PUFAs due to the combination of increased PGI$_3$ production, maintained PGI$_2$ production, and decreased production of pro-aggregatory TXA$_2$ (Lee and Austen, 1986).

The outcome of acute inflammation could be resolution, chronicity and/or fibrosis. This may be influenced by many factors, such as nature and intensity of the injury, location of injury and the over-responsiveness of the host, which is broadly controlled by both genetic and nutritional elements. The return from inflammation to normal homeostasis (complete resolution) is an actively regulated programme at the tissue level, called as catabasis (Bannenberg et al. 2005). Specific prostaglandins (PGE$_2$ and PGD$_2$) and LTB$_4$ are involved in the initiation and amplification of acute inflammation. Alternatively, chronic inflammation can result from excessive and/or unresolved inflammatory responses and can lead to chronic disorders (Figure-7).

Figure-7: Inflammatory process and PUFA regulation (Adopted from Serhan et al. 2008)
AA derived lipid mediators such as pro-inflammatory prostaglandins and leukotrienes can amplify this process. Fibrosis can occur when inflammatory injury causes substantial tissue destruction, connective tissue replacement which results in loss of tissue function.

**Influence of ω-3 PUFAs on inflammatory mediators**

Antagonizing AA metabolism is a key anti-inflammatory effect of ω-3 PUFAs. They exert a number of other anti-inflammatory effects which might occur downstream of altered eicosanoids production or might be independent of this activity. Moreover, inflammatory lipid mediators are also involved in mediating the cardinal signs of acute and chronic inflammation mainly through cytokines and nitric oxide.

**Cytokines**

Cytokines can be categorized in to two main groups, namely cytokines of acute inflammation (IL-1, TNF-α, IL-6, IL-11 and IL-8) and cytokines of either humeral responses of chronic inflammation (IL-4, IL-5, IL-6, IL-7, and IL-13) or cellular responses of chronic inflammation (IL-1, IL-2, IL-3, IL-4, IL-7, IL-9, IL-10, IL-12, IFNs, TGF-β and TNF-α and TNF- β). Most of these cytokines are multifunctional. They are pleotropic molecules that elicit effects locally or systemically in an autocrine or paracrine manner. Cytokines are involved in extensive networks that involve synergistic as well as antagonistic interactions and exhibit both negative and positive regulatory effects on various target cells (Feghali and Wright 1997).

Activated macrophages and lymphocytes secrete pro-inflammatory cytokines, including interleukin IL-6, IL-1, and TNF-α. These cytokines activate the endothelial expression of cell adhesion molecules and mediate a series of inflammatory responses such as the up-regulation of acute phase protein expression (Figure-8). Among all the cytokines IL-1 and TNF-α are chief orchestrators of acute as well as chronic inflammation and therefore implicated in a number of pathologies. The systemic and local effects of IL-1, IL-6 and TNF-α are shown in Figure-8 (Feghali and Wright 1997). These cytokines are the main pharmacological targets of rheumatic diseases, inflammatory bowel diseases and other dysregulated inflammatory conditions (Hanauer et al. 2006).
Inflammatory cytokine production is regulated by AA derived eicosanoids (Rolapleszczynski and Stankova 1992). Since ω-3 PUFA supplementation affect eicosanoid biosynthesis it might alter the synthesis and secretion of cytokines as well. Production of important inflammatory cytokines (TNF, IL-1, and IL-6) have shown to be suppressed by ω-3 PUFA in activated rodent macrophages (Billiar et al 1988, Reiner et al 1993, Yqoob and Calder 1995, Wallace et al 2000). Further, administering ω-3 PUFAs to healthy volunteers is shown to decrease bacterial lipopolysaccharide (LPS) induced production of the pro-inflammatory cytokines IL-1 and TNF-α in PBMNCs (Meydani et al. 1991; James et al. 2000; Caughey et al. 1996; Kelly et al. 1999). ALA, the precursor of LC PUFA can also inhibit IL-6, IL-1β, and TNF-α production in cultured PBMNCs from hypercholestrolemic subjects (Zhao et al. 2007). PUFAs of ω-3 family may bring about the suppression of cytokines production by suppressing their respective genes (Curtis et al. 2000; Chandrashekar and Fernandis 1994; Bouwens et al. 2009). In general, pro-inflammatory genes are suppressed by ω-3 PUFAs (left column of the Table-1), while genes critical for lipid peroxidation, energy utilization, and lipid homeostasis are increased by ω-3 PUFAs (right column of Table-1) (Deckelbaum et al. 2006).
Table 1: Genes influenced by \( \omega-3 \) PUFAs

<table>
<thead>
<tr>
<th>Inflammatory proteins</th>
<th>Energy/ Lipid metabolism</th>
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<tbody>
<tr>
<td>NF( \kappa )B</td>
<td>PPAR-( \alpha )</td>
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<td>IKK</td>
<td>PPAR-( \delta )</td>
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<tr>
<td>iNOS</td>
<td>PPAR-( \gamma )</td>
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<tr>
<td>IFN( \gamma )</td>
<td>SREBP( s )</td>
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<td>ACO</td>
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<td>TNF-( \alpha )</td>
<td>ABCA1</td>
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<td>COX-2</td>
<td>LpL</td>
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<td>LXR-( \alpha )</td>
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<td>Apo-E</td>
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Nuclear factor kappa B (NFkB) is involved in induction of inflammatory genes including COX-2, ICAM-1, VCAM-1, E-selectin, TNF-\( \alpha \), IL-1\( \beta \), IL-6, inducible NO synthase.
(iNOS), acute-phase proteins and matrix metalloproteinases in response to inflammatory stimuli (Christman et al. 1998). The emerging explanation is, ω-3 PUFA might exert their effects through direct actions on the intracellular signaling pathways leading to activation of NFκB (Xi et al. 2001; Chen & Zhao 2001; Ross et al. 1999). Moreover, activators of both PPARα and PPARγ have been shown to inhibit the activation of inflammatory genes, including TNF-α, IL-1β, IL-6, IL-8, COX-2, VCAM-1, iNOS, matrix metalloproteinases and acute-phase proteins (Jiang et al. 1998; Wang et al. 2001). PPAR dimerise with the retinoid-X-receptor (RXR) to regulate gene expression, and they can bind, and appear to be regulated by PUFA and eicosanoids (Kliewer et al. 1995; Devchand et al. 1996). Therefore, PPAR might stimulate the breakdown of inflammatory eicosanoids through induction of peroxisomal β-oxidation and might interfere with or antagonize the activation of other transcription factors, including NFκB (Mishra et al. 2004).

**Nitric oxide**

Nitric oxide (NO) is an important inflammatory mediator gained importance in recent years. It is produced by nitric oxide synthase (NOS) using L-arginine as substrate. Three isoforms of NOS have been described: Endothelial (NOS1) and neuronal (NOS3) isoforms are constitutively expressed and therefore termed as cNOS. NOS2 is an inducible isoform designated as inducible NOS or iNOS. The cNOS produces picomolar amounts of NO for short periods, operating through a calcium dependent mechanism, whereas iNOS produces large and sustained amounts of NO after cell activation by inflammatory stimuli. Production of NO via cNOS has been linked to homeostasis, for instance, the regulation of arterial blood pressure, whereas NO produced after iNOS induction appears to be involved in pathophysiological phenomena (Moncada et al. 1991).

Nitric oxide (NO) and reactive nitrogen species (RNS) derived by the interaction of NO with oxygen and/or reactive oxygen species is reported to participate in the development of oxidative tissue/cellular damage, which has become an established mechanism for tissue damage and therefore received intense human-health interest (Burney et al. 1999). iNOS is induced by inflammatory stimuli in various cells such as macrophages,
fibroblasts, smooth-muscle cells and hepatocytes, and participate in host immune defense system directed specifically against exogenous pathogens (Mayer and Hemmens 1997). Excess NO production by iNOS accelerate RNS formation leading to damage of cellular macromolecules such as proteins, DNA, and lipids, and trigger numerous detrimental cellular responses (Radi et al. 1991a; Radi et al. 1991b; Vermilov et al. 1995). Further, NO is known to intensify the extent of inflammatory response by activating COX-2 and raising the levels of its product, PGE$_2$ at the site of inflammation or injury. This makes the iNOS enzyme an important target to attenuate excessive PGE$_2$ synthesis and peroxynitrates. In fact, a number of conditions including sepsis, cancer, diabetes, renal disease and atherosclerosis are characterized by abnormally high iNOS expression and high NO production. (Yu et al. 1994; Beckman and Koppenol 1996; Cooke and Dzau 1997). Elimination of NO both by NO-scavengers or iNOS inhibitors aids to ameliorate such injury (Matheis et al. 1992; Hooper et al. 1999; Menezes et al. 1999). Therefore, it would appear likely that the scavenging of NO or the suppressing of iNOS by certain food items would serve as a promising indicator for the beneficial “health effects” of such food.

Among ω-3 PUFAs, DHA (22:6) is shown to inhibit NO production and iNOS expression in RAW264 macrophages and mouse peritoneal macrophages in a dose-dependent manner accompanied by inhibition of NFκB activation (Komatsu et al. 2003). A recent in vitro study has also shown significant decrease in NO production and iNOS expression by murine macrophage cell lines treated with ω-3 PUFA emulsion and an opposite effect by ω-6 lipid emulsions in culture medium (Aldridge et al. 2008). Further, supplementation of fish oil to rats is shown to diminish the ex vivo release of NO in resident peritoneal macrophages (Boutard et al. 1994; Joe and Lokesh 1994). These observations give an indication that ω-3 PUFAs can modulate the expression of inducible NOS and NO production by macrophages and therefore may be of clinical importance in alleviating the immunopathologies. Effect of ALA on NO synthesis is not studied in humans and animals, therefore it would be interesting to see whether ALA rich oil supplementation can modulate the NO production by immunocytes.
**Effect of ω-3 PUFAs on cells of immune system**

Assessments of cell functions in *ex vivo* are important biomarkers of immune function. Functions of immune cells in *ex vivo* include, measurement of phagocytosis and respiratory burst (superoxide generation) by neutrophils, monocytes and macrophages which can be coupled with measures of bacterial killing. Measurement of NK cell activity (measured as killing of tumor cells known to be specific targets for NK cells), cytotoxic T lymphocyte activity (measured as killing of virally infected cells known to be specific targets for cytotoxic T cells), lymphocyte proliferation, production specific immunoglobulin (Ig) by lymphocytes and cell surface expression of molecules involved in antigen presentation (e.g. MHC) are some of the important functions of lymphocytes that can be assessed *ex vivo* (Calder and Kew 2002). A large amount of literature asserts that, dietary PUFA modulates each of the above mentioned activities of immune cells (Calder 1998; Calder et al 2002).

Most studied biomarker of immune function as affected by the dietary fatty acids is lymphocyte proliferation. Experimentally, lymphocyte proliferation is achieved by mitogenicsignal. Commonly used mitogens are concanavalin A (Con A) and phytohaemagglutinin (PHA) which stimulate T lymphocytes, bacterial lipopolysaccharide (LPS) which stimulates B lymphocytes, and pokeweed (Phytolacca americana) mitogen which stimulates both T and B lymphocytes. The proliferative response of lymphocytes may be more conveniently achieved, by either measuring the incorporation of \[^3\text{H}\]thymidine into the DNA of the cells or measurement of violet color of formazan crystals formed in metabolically active lymphocytes after internalizing the yellow tetrazolium MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide).

*In vitro* studies have demonstrated that EPA and DHA decrease T-cell proliferation and the production of helper T-cell 1-type cytokines such as IL-2 (Calder and Newsholme 1992; Calder et al. 1992; Calder et al. 1994). Feeding studies in rodents and supplementation studies in human subjects have also shown that fish oil decreases T-cell proliferation (Yaqoob et al. 1994; Yaqoob and Calder 1995; Jolly et al. 1997; Peterson et al 1998; Meydani et al. 1991; Trebble et al. 2003) and production of helper T-cell cytokines such as IL-2 (Calder et al. 1994; Jolly et al. 1997; Meydani et al. 1991; Trebble...
et al. 2003) and IFN-γ (Calder et al. 1994; Treble et al. 2003). Studies on dietary supplementation of ALA to experimental animals have shown to suppress lymphocyte proliferation and other lymphocyte functions (Jeffery et al. 1996) in comparison to animals fed with lard or corn oil or sunflower oil which are rich in LA (Jeffery et al 1996, Fritsche et al 1991, Jeffery et al 1997). The precise effect of ALA on lymphocyte functions appears to depend on the level of LA and the total PUFA content of the diet (Jeffery et al. 1997). These effects of ALA have been confirmed in human subjects as well (Kelley et al. 1991).

Omega-3 PUFAs provided to spleen lymphocytes either in vitro or in the diet result in decreased cytokine production especially, Th1 cytokines. These effects occur at both the mRNA and the secreted protein levels. The decreased induction of mRNA for all three cytokines studied suggests that FO feeding results in a defect early in the signaling pathway which links cellular activation to cytokine gene induction. Con A binds to the T cell receptor/CD3 complex (Licastro et al. 1993) activating lymphocytes largely via phospholipase C-mediated events (Berry and Nishizuka 1990). Subsequently, the second messengers inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) are generated leading to an increase of intracellular calcium concentrations and activation of protein kinase C (Berry and Nishizuka1990). Fish oil feeding has shown to inhibit the activation of phospholipase C-γ1 in rat lymphocytes with a concomitant decrease in IP3 generation (Sanderson and Calder1998) and decrease in the generation of diacylglycerol and ceramide in Con A-stimulated mouse lymphocytes (Jolly et al. 1997). These actions would serve to diminish the elevation in intracellular free calcium concentrations and the activation of protein kinase C, thereby decreasing cellular responses. In addition, EPA and DHA can directly inhibit the activity of spleen lymphocyte protein kinase C (May et al. 1993). Thus, there are several potential early sites at which the inhibitory effects of the components of fish oil could be exerted.

Evidence suggests that effects of PUFAs on lymphocyte proliferation is independent of its effect on eicosanoid synthesis and lipid peroxidation (Santoli et al. 1990; Calder et al. 1992). The suppressive effects of long chain ω-3 PUFAs on lymphocyte proliferation (EPA+DHA) could be due to inhibition of synthesis of IL-2 (T-cell growth factor) and its
receptor at gene level (Jolly et al. 1998). Changes in membrane phospholipid fatty acid composition results in altered membrane fluidity which is shown to affect lymphocyte functions in vitro (Calder et al. 1994). Further, ω-3 PUFA can alter the fatty acyl composition of specific detergent resistant domains in the exoplasmic leaflet of T-cell membrane called “lipid rafts” (Fan et al. 2003). Rafts are composed mainly of cholesterol and sphingolipids and therefore do not integrate well into the fluid phospholipid bilayers, causing them to form microdomains. Upon T-cell activation, rafts compartmentalize the ligated T-cell receptor (TcR) (Leitenberg et al. 2001) and associated signal-transducing molecules, thus providing an environment conducive to signal transduction (Leitenberg et al. 2001). For example, the earliest mediators of T-cell proliferation [i.e., protein kinase C-θ (PKCθ), phospholipase C-γ (PLCγ), the linker for activation in T cells (LAT)], and T-cell apoptosis [i.e., Fas and Fas-ligand (Fas-L)], translocate to lipid rafts after stimulation (Ebinu et al. 2000; Bi and Altman 2001; Grassme et al. 2001). There is also an emerging paradigm that lipid rafts cluster at the T cell-antigen presenting cell interface, ultimately generating platforms specialized for progressive and sustained TcR signaling (Miceli et al. 2001). There is overwhelming evidence that lipid raft integrity is a prerequisite for optimized TcR signal transduction and immune response (Miceli et al. 2001; Janes et al. 1999; Burack et al. 2001; Hueber et al. 2000). PUFA added in culture are capable of modifying lipid rafts and suppressing signal transduction in vitro (Stulnig et al. 1998; Stulnig et al. 2001) and in vivo (Fan et al. 2003). Therefore, influence signaling complexes and modulate T-cell activation affecting the proliferation of these lymphocytes.

**Modulation of inflammatory diseases by ω-3 PUFA**

The effects of long-chain ω-3 PUFA supplementation on ex vivo lymphocyte proliferation, cytokine production by lymphocytes and monocytes in healthy subjects have been studied in twenty-seven, twenty-five and forty-six treatment cohorts respectively, at intake levels ranging from 0.2 g EPA+DHA/day to 7.0 g EPA+DHA/day. Most studies, particularly those with the highest quality study design, have found no effects on these immune markers. However, significant decrease of lymphocyte proliferation is observed in seven of eight cohorts, particularly in older subjects. The
direction of the significant changes in cytokine production by lymphocytes is inconsistent and only found at supplementation levels ± 2.0 g EPA+DHA/day. Inflammatory cytokine productions by monocytes are significantly decreased in all cohort studies. Overall, these studies fail to reveal a strong dose–response effects of EPA+DHA on the outcomes measured and suggest that healthy subjects are relatively insensitive to immunomodulation by LC ω-3 PUFA, even at intake levels that substantially raise their concentrations in phospholipids of immune cells. In patients with inflammatory conditions, cytokine concentrations or production are influenced by EPA+DHA supplementation in a relatively large number of studies. Some of these studies suggest that local effects at the site of inflammation might be more pronounced than systemic effects and disease-related markers are more sensitive to the immunomodulatory effects, indicating that the presence of inflamed tissue or ‘sensitized’ immune cells in inflammatory disorders might increase sensitivity to the immunomodulatory effects of long-chain ω-3 PUFA (Sijben and Calder 2007).

Various dietary components may modify chronic inflammatory processes at the stage of cytokine production, amplification of NFκB mediated inflammatory gene expression, release of anti-inflammatory cytokine and transforming growth factor-β (TGF β) (Figure-9). Dietary ω-3PUFA modulates murine Th1/Th2 balance towards the Th2 pole by suppression of Th1 development (Zhang et al. 2005). Fish oil induced shift away from a Th1-type response may explain the low incidence of inflammatory and autoimmune disorders among Greenland Eskimos (Kromann and Green 1980). Furthermore, it could explain some of the benefits that have been observed following the administration of fish oil to patients with rheumatoid arthritis (James and Cleland 1997; Geusens 1998), ulcerative colitis (Rodgers 1998), Crohn’s disease (Belluzzi and Miglio1998) and psoriasis (Ziboh 1998). These effects might be due to a shift away from a Th1-type response (Wallace et al. 2001). Differences in the composition of ω-3 and ω-6 PUFAs induce a shift in the Th1/Th2 balance in both mouse and human lymphocytes, even when ingested in normal dietary amounts of ALA (Mizota et al. 2009).

Two polarized CD4 T cell subsets have been identified by their signature cytokines and mutually exclusive helper functions. Th1 effector cells produce IL-2, IFN-γ and
lymphotoxin, Th2 cells produce IL-4, IL-5, IL-10, and IL-13 (Mosmann and Sad 1996). The pathogenic role of Th1 and the protective role of Th2 cells have been described for certain autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS), and insulin-dependent diabetes mellitus (Liblau et al. 1995). In some cases, the Th1/Th2 balance was an important indicator of the disease state (Kleemann et al. 1998). A shift from Th1 to Th2 cytokine profiles was observed in many clinical interventions that resulted in improvement of these diseases (Tisch and McDevitt 1996; Delovitch and Singh 1997). Modulation of the Th1/Th2 balance has provided a new paradigm for immunomodulatory therapy in some autoimmune diseases (Liblau et al. 1995).

Chronic inflammatory diseases are characterized by a dysregulated, overactive Th1-type response and often by an inappropriate production of AA derived eicosanoids, especially PGE₂ and LTB₄. The effects of fish oil outlined in Figure-9 suggest that it might have a role in prevention and therapy of these diseases.

Dietary fish oil has shown to have beneficial clinical, immunological and biochemical effects in various animal models of human chronic inflammatory diseases, including decreased incidence and severity of inflammation in mice with type II collagen-induced arthritis (Leslie et al. 1985) and less inflammation in rat models of colitis (Wallace et al. 1989; Vilaseca et al. 1990). It was recently reported (Volker et al. 2000) that both EPA and DHA suppress streptococcal cell wall-induced arthritis in rats. EPA was more effective among the two and this fits with the more potent effects of EPA than DHA on inflammation and immunity. There have been a number of clinical trials assessing the benefits of dietary supplementation of fish oil in several inflammatory diseases in humans including rheumatoid arthritis, Crohn’s disease, ulcerative colitis, and psoriasis (Table 2). Many of the placebo controlled, double-blind trials of fish oil in chronic inflammatory diseases reveal significant benefits including decreased disease activity and a lowered use of anti-inflammatory drugs. The evidence for the beneficial effect of FO is strongest in rheumatoid arthritis (Table-2).
Figure-9: Potential sites of action of ω-3 PUFA in ameliorating chronic inflammatory diseases. Chronic inflammatory diseases are characterized by a dysregulated Th1 response resulting from inappropriate recognition of self-antigen in genetically predisposed individuals. ω-3 PUFA can exert anti-inflammatory effects at several points (∅) to induce clinical benefit. (Adopted from Calder et al. 2002)
Fats in Indian diet

Dietary fat is the principle source of essential fatty acids and fat soluble vitamins. The recommended daily intake of fats for Indians range between 20-35% of the total calorie intake as per the ICMR expert group of 2009. The amount of fat contributed by foods other than visible fat called invisible fat must not exceed 10% of total energy and fat from visible fat or vegetable oils can contribute up to 25% of total calorie intake (Narasinga...
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Rao 2010). Cereals and millets being staple of Indians, contribute greatly to the invisible fat content whereas vegetables, fruits, seeds and nuts contribute the rest. Though legumes, green leafy vegetables and nuts are good source of ALA (Ghafoorunissa and Pangrekar 1993) it is cereals, millets and animal foods containing higher amount of $\omega$-6 PUFA (LA/AA) which increase the $\omega$-6 PUFA content of total invisible fats being consumed by Indians (Ghafoorunissa 1998).

Vegetable oils used for cooking and frying constitute up to 80% of visible fat. Vanaspathi and ghee are other sources of fat especially in India. Fat intake is income dependent and there are regional preferences in both quality and quantity of fat consumed among Indians. In northern parts of India mustard oil is used in many households (Rasthogi et al 2004). Along with mustard oil, soybean, sunflower, safflower, and sesame seed oils are consumed readily in northeastern states. Coconut oil, ground nut oil, gingili oil, sunflower oil is preferred by people in southern India. Corn oil and sunflower oil are the major choice of richer community (Nigam 2000) and RBD palm oil is the single largest edible oil consumed in India because of low cost and affordability. Further, consumption of trans fats from hydrogenated vegetable oils called vanaspathi and clarified butter or ghee is higher in comparison to its consumption Western countries (Singh et al 1996). Table 3 represents type and quantity of vegetable oils consumed by Indians from 2000 to 2006. India consumes around 12.5-13.0 million tons of vegetable oil per annum. India's per capita consumption of edible oil was 12.78 kg/annum in the 2008-09, up by 1.38 kg as compared to 11.40 kg/annum previous year due to cheaper availability of edible oil and increasing per capita income. The present transition in dietary fat consumption pattern has led to lower intake of MUFA, $\omega$-3 PUFA and high intake of $\omega$-6 PUFA, SFA, and trans fatty acids which are associated with myriad health complications. The consumption pattern clearly indicate that, Indians are consuming fats which are rich in one type of EFA (LA, 18:2, $\omega$-6) and lacking in the other (ALA, 18:3, $\omega$-3) (Ghafoorunisaa 1996) and this dietary habit has increased the $\omega$-6/ $\omega$-3 PUFA ratio among rural Indians up to 6.1 and 38-50 among the urban dwellers (Pella et al. 2003). Further, LC $\omega$-3 PUFA levels of plasma phospholipids of Indian men were found to be lower than their counterparts in America, Belgium, and Japan indicating the need to improve $\omega$-3 status in Indians (Ghafoorunissa 1998).
Several sources of information suggest that human beings evolved on a diet with a ratio of ω-6 to ω-3 PUFAs of ~1. Excessive amounts of ω-6 PUFA and a very high ω-6/ω-3 ratio, is found in Indian diets, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, inflammatory and autoimmune diseases (Simopoulos 2004). In India, the epidemics of non-communicable diseases (NCD) are presently emerging (Murray and Lopez 1996). The rates of cardiovascular diseases have increased by 300% in India over the last three decades (Enas 2000) and cause 3 million deaths every year accounting for 25% of all mortality (Rastogi 2004). India has become the capital of diabetes. Consumption of ω-3 PUFAs has shown beneficial effects by reducing risk factors, inflammatory mediators, and immune cells implicated in progression of CVD and a 70% decrease in total mortality due to CVD. Further, a positive correlation between ω-6 / ω-3 PUFA ratio and prevalence of type 2 diabetes in India was reported (Raheja et al. 1993) (Figure-10). Other less known diseases among Indians such as ulcerative colitis (UC) and Crohn’s disease (CD) together called as inflammatory bowel disease (IBD) is raising and the incidence is as high as in other developed countries (Sood et al. 2003; Binder 1998; Desai and Gupte 2005). Dietary fat was recognized as an important etiological factor for IBD and higher amount of ω-6 PUFA have been

<table>
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<tr>
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<th>2000</th>
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<td>3,681</td>
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<td>4,259</td>
<td>3,500</td>
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<td>2,210</td>
<td>2,355</td>
<td>2,032</td>
<td>2,049</td>
<td>2,767</td>
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<td>RFO</td>
<td>1,813</td>
<td>1,654</td>
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<td>1,323</td>
<td>2,021</td>
<td>1,793</td>
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<td>1,345</td>
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<td>494</td>
<td>462</td>
<td>597</td>
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<td>752</td>
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<tr>
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<td>620</td>
<td>287</td>
<td>455</td>
<td>447</td>
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<td>444</td>
<td>455</td>
<td>424</td>
<td>421</td>
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<td>Butter/Ghee</td>
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<td>494</td>
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<td><strong>Total</strong></td>
<td><strong>11,735</strong></td>
<td><strong>12,142</strong></td>
<td><strong>12,394</strong></td>
<td><strong>12,099</strong></td>
<td><strong>12,487</strong></td>
<td><strong>13,082</strong></td>
<td><strong>13,616</strong></td>
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</table>

Table-3: Consumption of vegetable oils 2000-2006 (x1000 tones) (Adopted from Global oils and fats 2008)
implicated in the origin of IBD, given that they affect the AA metabolism by increasing
the production of LTB4, with pro-inflammatory action. Whereas early epidemiological
studies have demonstrated a clear link between low incidences of IBD among population
eating fish which is a rich source of ω-3 LC PUFA (Kromann and Green 1980; Bang and
Dyerberg 1980). It is evident that increased levels of ω-3 PUFA (a lower ω-6/ω-3 ratio),
exert suppressive effects. Moreover a ratio of 2.5/1 reduced rectal cell proliferation in
patients with colorectal cancer. The lower ω-6/ω-3 ratio in women with breast cancer was
associated with decreased risk. A ratio of 2-3/1 suppressed inflammation in patients with
rheumatoid arthritis, and a ratio of 5/1 had a beneficial effect on patients with asthma,
whereas a ratio of 10/1 had adverse consequences. These studies indicate that the optimal
ratio may vary with the disease under consideration. This is consistent with the fact that
chronic diseases are multigenic and multifactorial. Therefore, it is quite possible that the
therapeutic dose of ω-3 PUFA will depend on the degree of severity of disease resulting
from the genetic predisposition. A lower ratio of ω-6/ω-3 PUFA is more desirable in
reducing the risk of many of the chronic diseases of high prevalence in Western societies,
as well as in the developing countries (Simopoulos 2004).

Figure-10: Relation between the ratio of ω-6 to ω-3 PUFAs in dietary lipids in
Indian diet and prevalence of type-2 diabetes. (Adopted from Raheja et al. 1993)

Among the ω-3 PUFA the precursor ALA must be distinguished from long chain
derivatives like EPA and DHA. Indeed only ALA is strictly essential since it cannot be
synthesized and must be provided in the diet whereas the longer chain counterparts are formed in the body after elongation and desaturation of ALA. International agencies recommend ALA as the main ω-3 PUFA to meet the dietary requirement of ω-3 PUFA (Gebauer et al. 2006). The ω-3 PUFA recommendation to achieve nutritional adequacy defined as the amount necessary to prevent deficiency symptoms is 0.6–1.2% of energy for ALA; up to 10% of this can be provided by EPA or DHA (Gebauer et al. 2006). The international society for study of fatty acids and lipids (ISSFAL) recommends 1.6g/ day of ALA and 500 mg/day of EPA and DHA as adequate intake (AI) for ω-3 PUFA for humans (ISSFAL 2004). The AI for ALA ranges between 1.4 to 2 g/day as per recommendations of various agencies across the world (Gebauer et al. 2006). Much higher amounts of ALA used in various epidemiological studies and randomized clinical trials have demonstrated the beneficial and protective effects against coronary diseases (Davis and Kris-Etherton 2003). Inflammatory events are central to pathogenesis of atherosclerosis and other related coronary diseases (Hansson 2005; Libby 2006). Cardiovascular benefits conferred by high ALA in diet are mediated by its anti-inflammatory effects at least in part. Consumption of higher amount of ALA by humans decreased the inflammatory mediators like IL-1β, IL-6, and TNF-α by immunocompant cells in ex vivo (Caughey et al. 1996; Zhao et al. 2007). Further, production of a number of pro inflammatory mediators was blunted by ALA in in vitro as well as in animal studies (Zhao et al. 2005). These studies have substantiated the anti-inflammatory efficacy of ALA in animal models and humans. Estimates of the amount of ALA converted to EPA range from 0.2% to 8%, with young women showing a conversion rate as high as 21% (Burdge and Calder 2005; Burdge and Wootton 2002) whereas conversion of ALA to DHA appears limited in humans, with most studies showing a conversion rate of about 0.05%, although one study reported a figure of 4%, and a conversion rate of 9% was reported in young women (Burdge and Wootton 2002). The variation in the extent of conversion of ALA to its long chain counterparts reported could be due to differences in study methodologies and LA content used. Since diets rich in LA can affect the conversion of ALA to EPA and DHA (Liou et al. 2007) many investigators have indicated a need to reduce the levels of LA in diet and increase levels of ALA for improving the ω-6 to ω-3 balance in experimental animals (Morise et al. 2004). Although
the magnitude of lipid lowering and anti inflammatory effects of ALA is modest as compared to fish oils (Indu and Ghafroonissa 1992), long term intakes of ALA may provide health benefits (Nettleton 1991). Moreover ALA has an important role in reducing chronic disease through conversion to EPA and DHA, as well as through its own unique metabolic activities. Additionally, what many fail to understand is the critical need for both plant and fish based ω-3 PUFAs, especially considering the increasing predominance of ω-6 PUFAs in the diet.

Despite the evidence on beneficial effects of fish and fish oils, its consumption is relatively among Indians. Some populations do not eat fish because of concerns about environmental toxins like mercury and polychlorinated biphenyls (PCBs). There is evidence suggesting that the levels of mercury along the Indian cost line range between 0-2100 ng/l in seawater which is higher than the global ocean averages (Ramaiah and De2003). In aquatic environments, inorganic mercury is microbiologically transformed into lipophilic organic compound ‘methylmercury’. This transformation makes mercury more prone to bio-magnification in food chains. Recent epidemiological studies have shown that fish-eating human populations may be exposed to Hg sufficient to cause significant developmental effects (Chan et al. 2003). Further, many people might not like the taste of fish and might avoid its consumption. Most importantly an analysis of consumption data originating from National Sample Survey (NSS) shows that 42 percent of households are vegetarian who never eat fish meat or eggs (Mehta et al 2003). Therefore ALA is the main, if not only, ω-3 source in the diet of at least one billion vegetarians worldwide.

Apart from invisible fat sources (green leafy vegetables, legumes, nuts and seeds), vegetable oils like linseed oil (58%), perilla oil (55%), camelina seed oil (38%), hemp seed oil (20%) are main sources of ALA. In vegetarian diets their effectiveness as a source of ω-3 PUFA, bioavailability, lipid lowering properties and anti inflammatory properties have been studied. Due high content of ω-6 PUFA in the Indian diet, there is an immediate requirement of newer visible fat sources of ALA which contribute substantially to total fat consumed by Indian population (5-20kg/person/year). Since, there is regional preference of vegetable oils consumed by Indians, there is a need to
identify and develop locally available sources of ALA rich vegetable oils and study their
efficacy.

**Garden cress seed oil- a novel source of ALA**

Garden Cress (GC) is an annual, erect, herbaceous plant, belongs to the family
Brassicaceae. The plant is native to Persia and a well known culinary herb throughout the
world. It is cultivated in India (Nadkarni and Nadkarni 1954), North America and parts
of Europe (Nuez and Bermejo 1994). It is used in the form of vegetable, most commonly
its succulent hypocotyls are used in salads in Europe and America. In India the seeds are
harvested for food and medicinal purposes. GC seeds are claimed to possess varied
medicinal properties like galactogogue, aperient, diuretic, alterative, tonic, demulcent,
aprodisiac, carminative and emmenagogue. Mucilage of the seeds allays the irritation of
the mucous coat of intestines. Seeds are also useful in hicc up, dysentery, diarrhea and
skin diseases caused by impurities and toxins in blood and chronic enlargements of
spleen (Nadkarni and Nadkarni 1954). A preliminary pharmacological study on seeds of
L. sativum has suggested the presence of cardioactive substance and is shown to have
probable action through adrenergic mechanisms (Vohora and Khan 1977). Further the
seeds have shown to be effective in alleviating the symptoms of asthma in patients
(Paranjape and Mehta 2006)

**Taxonomy**

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Table-4: Trivial names of Garden cress (*Lepidium sativum* L.)

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<tr>
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</table>

Figure-11: picture of garden cress plant in field
The GC grows as a wild plant in regions of Madhya Pradesh, Chhattisgarh, Uttar Pradesh, Maharashtra and Northern Karnataka. There is no data or statistics available from India about its cultivation or yield. It is domesticated by population in the above said regions mainly for seeds to be used in diet or medicinal purposes. The morphology of GC seeds resemble that of an oil seed with 80–85% of the seed matter is dicotyledonous endosperm whereas the seed coat and the embryo account for 12–17% and 2–3% respectively (Sumangala et al. 2004). Recent studies have identified GC as an underexploited, alternative low input oil seed crop for profitability of farming systems owing to nutritionally and industrially desirable fatty acid composition in the oil extracted from these seeds (Angelini et al. 1997). There are reports indicating the use of oil obtained from GC seeds by Ethiopians and ancient Greeks (Uphof 1959; Lotfy et al. 1957). The particular interest in GC seeds as source of vegetable oil is higher amount of ω-3 PUFA, ALA in its oil. In the wake of burgeoning need to improve the ω-3 status of Indian diet, GC seed oil emerges as a potential, locally available and affordable source of ALA.
Scope and objectives of the study

Garden cress seeds contains 18-22% oil and ALA content of 30-35% of total fatty acids. Garden cress seed oil (GCO) contains good amount of lignans (29.4%) and other antioxidants such as tocopherols and carotenoids which can stabilize the ω-3PUFAs in its seed oil. GCO has considerable potential for altering the balance of ω-6/ω-3 ratio when supplemented as a dietary fat or health oil by blending with other oils. In spite of its good oil content comparable to that of any other oil seeds, it is not been explored as a source of oil rich in LA and has remained an underutilized seed crop. A number of studies have demonstrated that ω-3 PUFA in dietary fats can modulate immune function and inflammatory disorders. The main aim of the proposed study is to evaluate the oil from the Garden cress seeds as a novel source of ALA and assess its immunomodulatory effects in animal models.

Objectives

1. Studies on physicochemical characterization of Garden cress oil (GCO) and nutritional evaluation in experimental animals.

2. To study the effect of Garden cress oil on indices of immune status in albino rats.

3. To assess the modulatory effect of Garden cress oil in experimentally induced ulcerative colitis in animal model.