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Perinatal supplementation of long-chain polyunsaturated fatty acids, immune response and adult diseases

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Summary

Both ω -6 and ω -3 long-chain polyunsaturated fatty acids (LCPUFAs) modulate T_H1 and T_H2 cell generation, their cytokine production, and cell proliferation and thus may serve as endogenous anti-inflammatory molecules. LCPUFAs suppress the production of tumor necrosis factor- α (TNF- α) (and so also of OX40, since it belongs to the family of TNFR) and the expression of *Bcl-2*, suggesting that these fatty acids have the ability to prevent/suppress autoimmune diseases. Human breast milk contains substantial amounts of both ω -3 and ω -6 fatty acids. This indicates that LCPUFAs present in human breast milk suppress the levels of OX40 and decrease the expression of *Bcl-xL* and *Bcl-2* on exposure to self-antigens and thus, protects against the development of autoimmune diseases in later life. In view of this, I propose that supplementation of appropriate amounts of LCPUFAs during perinatal period protects against atopy, asthma, auto-immune diseases, type 1 and type 2 diabetes mellitus, hypertension, coronary heart disease, metabolic syndrome X, lymphomas, leukemias and other cancers, schizophrenia, depression and other adult diseases in which low-grade systemic inflammation plays a significant role. It is also likely that perinatal supplementation of LCPUFAs in adequate amounts modulates the expression of genes concerned with immune response, angiogenesis, central osmo/sodium and glucose sensors etc. This renders various tissues and organs including T cells and macrophages, endothelial cells, hypothalamic neurons, and various cardiovascular tissues to be able to counteract the pathological mechanisms that tend to induce various adult diseases by blunting the inflammatory responses in those who received adequate amounts of LCPUFAs during the perinatal period compared to those who did not.

key words: long-chain polyunsaturated fatty acids • T cells • cytokines • atopy • asthma • adult diseases • perinatal programming • low-grade systemic inflammation • immune response

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BACKGROUND

Immune system plays a significant role both in cancer and autoimmune diseases. To prevent tumor cells from proliferating, immune system should recognize them as foreign and mount an attack. Conversely, immune system should not mount an attack on its own body tissues to prevent the occurrence of autoimmune diseases. Thus events that regulate self and non-self recognition by immune cells play a significant role both in cancer and autoimmune diseases. Peripheral T-cell tolerance is a mechanism to limit autoimmunity. The ability of immune system to develop self and non-self recognition and prevent the development of autoimmune diseases and cancer occurs in the early stages of fetal development itself. Hence, stimuli or insults during critical or sensitive periods in early life can have lifetime consequences and is called as 'programming'. One important environmental programming stimulus is that induced by early nutrition [1-3]. Since fetus depends on maternal ability to provide congenial atmosphere and supply adequate nutrition for its proper growth and development, any events or diseases of the mother are expected to adversely affect the fetus and may have lifetime consequences such that their impact is seen either immediately or at a later stage of life. Hence, development of a well-balanced immune system is not only essential for protecting the newborn from various infections but also to prevent some adult diseases.

In this context, it is important to note that inappropriate inflammation in the form of low-grade systemic inflammation plays a role in the pathobiology of adult diseases such as obesity, hypertension, type 2 diabetes mellitus, atherosclerosis, coronary heart disease (CHD), schizophrenia, depression, Alzheimer's disease, rheumatoid arthritis (RA), lupus, and cancer. On the other hand, adequate breast-feeding protects against the development of these conditions [4]. If so, what is the relationship between these two events?

LONG-CHAIN POLYUNSATURATED FATTY ACIDS AND T_H1 AND T_H2 CELL RESPONSES

Optimal T-cell activation requires multiple signals provided by recognition of peptide-major histocompatibility complex (MHC) proteins by the T-cell receptor (TCR), and by interaction of T-cell co-stimulatory receptors with their ligands on antigen-presenting cells (APCs). In the absence of co-stimulation, recognition of antigen leads to the induction of tolerance and prevention of autoimmune diseases. OX40 (CD134), a member of the TNF receptor (TNFR) family, is a primary co-stimulator of T cells that have encountered antigen rather than naive T cells. OX40 is not present on naive T cells, only peaks in expression 3-4 days after the initial activating signal, is rapidly and highly re-expressed on effector T cells and is induced by TCR signals. An agonistic antibody against OX40 could break an existing state of tolerance and promote T-cell expansion [5]. OX40 has the ability to positively regulate the anti-apoptotic molecules *Bcl-xL* and *Bcl-2*, and also strongly promote the activation of NF- κ B and thus breaks peripheral tolerance [5].

This suggests that if OX40 is suppressed, autoimmune diseases can be prevented.

Long-chain polyunsaturated fatty acids (LCPUFAs) suppress the production of TNF- α (and so also of OX40, since it belongs to the family of TNFR) and the expression of *Bcl-2* [reviewed in 6 and 7]. This suggests that LCPUFAs have the ability to prevent/suppress autoimmune diseases. The observation that LCPUFAs are of benefit in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [8,9] supports this view. In a recent study that assessed perinatal characteristics in relation to the risk of adult rheumatoid arthritis showed association with low frequency of breast-feeding [10]. This indicates that some components of human breast milk have a major role in the development of immune system in perinatal period. Human breast milk contains substantial amounts of both ω -3 and ω -6 fatty acids [4]. I suggest that LCPUFAs present in human breast milk suppress the levels of OX40 and decrease the expression of *Bcl-xL* and *Bcl-2* on exposure to self-antigens and thus, protects against the development of autoimmune diseases in later life.

LCPUFAS IN ATOPY AND ASTHMA

Normally a balance is maintained between T_H1 and T_H2 cells (T helper 1 and 2 lymphocytes respectively). T_H1 cells produce interferon- α (IFN- α), IL-2, and TNF- α , whereas T_H2 cells produce IL-4, IL-10, and IL-13 cytokines. IFN- α is essential for both innate and adaptive immunity and is produced by CD4 T_H1 cells, CD8 T cells, and NK cells. IFN- α binds to its receptor that is coupled to the Jak-STAT signaling pathway. Animals that lack IFN- α , the IFN- α receptor or STAT 1 have disrupted innate and adaptive immunity that results in death from infection by microbial pathogens and viruses. T-bet, a member of the T-box family of transcription factors, is rapidly induced in early developing T_H1 cells and is absent in developing T_H2 cells. Insertion of T-bet gene in to T cells results in the conversion of these cells into T_H1 cells that in turn produce IFN- α but not IL-4 and IL-5 [11]. Mice that lack T-bet do not have a functional T_H1 response in vivo and fail to control T_H1-dependent protozoan infection. But these animals are completely protected from developing inflammatory bowel disease (IBD) [11], and conversely, develop spontaneous airway hyper reactivity and asthma [12].

Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), ω -3 LCPUFAs, have a much stronger inhibitory action on the secretion of IL-1, IL-2, and TNF- α compared to γ -linolenic acid (GLA) and arachidonic acid (AA), which are ω -6 LCPUFAs. TNF- α synthesis and secretion is diminished by EPA and DHA and is unchanged or increased by AA and other ω -6 fatty acids. EPA and DHA supplementation enhances transforming growth factor- β (TGF- β) production and this delays autoimmune disease in experimental animals. TGF- β has a negative feed back control on the synthesis and release of TNF- α , whereas some of the actions of TGF- α depend on the presence of LCPUFAs [13]. Thus, depending on the type and amounts of LCPUFAs given,

T_H1 responses can be enhanced and T_H2 responses are blunted, which favor suppression of atopy and asthma. This favorable action on T_H1 and T_H2 responses may depend on the ratio between ω -3 and ω -6 fatty acids. A higher ratio of ω -6 to ω -3 LCPUFAs favors an enhancement in the T_H1 response, whereas a decrease in the ratio between ω -6 to ω -3 may favor the T_H2 response.

Galli et al. [14] demonstrated that newborns that subsequently developed atopic disease have lower levels of dihomog-GLA (DGLA, a ω -6 LCPUFA) and AA in cord blood compared to non-atopics. This suggests that low ω -6 LCPUFA levels may be predictive of atopic disease. Since T_H1 and T_H2 imbalance is present in asthma, it is likely that LCPUFAs enhance IFN- α production (i.e. activate T_H1 cells) and suppresses T_H2 cells. Further, EPA and DHA are reported to be of benefit in inflammatory bowel disease (IBD) [15], possibly by their action on PPARs, whereas in asthma it is due to their action on T_H1 subset of cells by suppressing IFN- α production. Thus, the mode of action of LCPUFAs may be different in different tissues and this in turn may depend on the concentrations of various LCPUFAs in the membranes of specific tissues. Such tissue specific actions of LCPUFAs, if demonstrated, may prove to be interesting and explains why LCPUFAs are useful in asthma, IBD, and other diseases.

BREAST-FEEDING, LCPUFAS AND LYMPHOMAS AND LEUKEMIAS

In a case-control study of 117 children diagnosed with acute lymphoblastic leukaemia (ALL), Hodgkin's disease and non-Hodgkin's lymphoma between 2 and 14 years of age, it was reported that there is a 70% reduction in risk for the babies who were breast fed for over 6 months [16]. In a review of 9 studies that tried to evaluate for an association between breast-feeding and risk of childhood leukaemia and lymphoma, it was concluded that there was evidence of a decreased risk for Hodgkin's lymphoma in those who were breast-fed [17]. These and other studies [18–20] concluded that there is an increased risk of all lymphoma or Hodgkin's lymphoma in those who were not breast-fed at all or who were breast-fed for less than 6 months in comparison to those who were breast-fed for a longer period of time. In a large scale case-control study of 1744 children with ALL and 456 with acute myeloid leukaemia (AML) in the USA, it was noted that the risk of leukaemia was 30% lower in those who were breast-fed for longer than 6 months [20]. It is interesting to note that the risk decreased progressively as duration of breast-feeding increased over the first year of life.

Although the mechanism(s) by which breast-feeding protects against leukaemia is not clear, it is tempting to suggest that substantial amounts of ω -6 and ω -3 LCPUFAs present in human breast milk may be responsible for this beneficial action. There is increasing evidence that in childhood leukaemia, the initiation occurs most probably *in utero*. A subsequent exposure to an oncogenic virus infection(s) may then lead to the development of acute leukaemia in children [21,22]. It is possible that breast

milk provides passive antibody protection and modulates the immune system. This later action of breast milk could modify the response to post-natal antigenic exposures and thereby reduce the susceptibility to potentially adverse actions of leukemogenic viruses.

Can the protective effect of breast milk against childhood leukaemia and lymphoma be explained in terms of its LCPUFAs content? Earlier, my colleagues and I showed that GLA prevents genetic damage induced by gamma-radiation, benzo(a)pyrene, and certain drugs both in vitro and in vivo [23,24]. GLA, AA, EPA, and DHA have selective tumoricidal action both in vitro and in vivo [25–27]. Limited clinical studies proved that GLA regresses human brain gliomas [28–30] and Hodgkin's lymphoma [31]. Fish oil, a rich source of EPA and DHA both prevents and regresses colon cancer [32–35]. This suggests that in the presence of adequate amounts of LCPUFAs damage to DNA is prevented and emerging tumor cells undergo apoptosis so that cancer development is prevented/eliminated. Thus, at least in part, the beneficial effect of breast-feeding in decreasing the incidence of leukemias and lymphomas can be ascribed to its content of LCPUFAs.

LOW-GRADE SYSTEMIC INFLAMMATION IN METABOLIC SYNDROME X AND LCPUFAS

Various features of metabolic syndrome X include: obesity (especially abdominal obesity), insulin resistance/hyperinsulinemia, dyslipidemia, impaired glucose tolerance (IGT), type 2 diabetes mellitus, hypertension, and CHD. It is believed that insulin resistance is the central feature of this syndrome. Although the exact cause for metabolic syndrome X is not known, there is mounting evidence to suggest that low-grade systemic inflammation induced by an increase in the levels of pro-inflammatory cytokines interleukin-6 (IL-6) and TNF- α and characterized by the presence of elevated amounts of C-reactive protein (CRP) plays a critical role in its pathogenesis [36–38]. This implies that the balance between pro- and anti-inflammatory cytokines (IL-6 and TNF- α vs. IL-4, IL-10) is tilted more towards the former. But why, how, and when this imbalance occurs is not clear. Recent studies suggest that this imbalance may be initiated early in life. This is supported by the observation that insulin resistance is seen South Asian children when they are 10–12 years old [39]. Metabolic syndrome X is common in South Asians and this implies that low-grade systemic inflammation is present since childhood. Exposure to a diabetic environment in utero is associated with increased occurrence of IGT and a defective insulin secretory response in adult offspring, independent of genetic predisposition to type 2 diabetes and this is related to low parasympathetic tone as observed by low levels of pancreatic polypeptide [40]. Radaelli et al. [41] reported that gestational diabetes mellitus leads to up regulation of ILs, leptin, and TNF- α receptors, and activation of stress-activated protein/c-Jun NH₂-terminal kinases, extracellular matrix components and angiogenic activators in the placenta. This suggests that adverse maternal environment activates inflammatory pathways in their offspring that have a critical role in the pathogenesis of

many diseases. Hence, suppressing these pro-inflammatory pathways early in life could be beneficial. This is supported by the observation that use of cod liver oil (a rich source of EPA and DHA) during pregnancy, and first year of life was associated with a significantly lower risk of type 1 diabetes mellitus [42,43]. Previously, my colleagues and I showed that both ω -6 and ω -3 fatty acids prevent alloxan-induced type 1 diabetes in experimental animals [44–46]. It was observed that plasma and RBC membrane concentrations of EPA, DHA, AA, GLA and other LCPUFAs are low in patients with obesity, hypertension, diabetes mellitus, CHD, RA, lupus, depression, and schizophrenia [47–52]. In addition, low serum concentrations of DHA predispose to the development of Alzheimer's disease [52], whereas EPA/DHA are of benefit not only in all these conditions but also in hyperlipidemias [7], and in decreasing the severity of atopy and asthma [53].

SCHIZOPHRENIA, BIPOLAR DISORDERS, INFLAMMATION, AND LCPUFAS

Plasma concentrations of IL-6 and TNF- α are increased in depression and schizophrenia suggesting that inflammation plays a role in these conditions [54]. IL-2, IL-6, IL-8 and TNF- α levels are elevated in patients with schizophrenia [55] and relapse-prone patients had higher levels of CSF IL-2 than patients who did not relapse. Haloperidol and perazine decreased IL-1 α and TNF- α in schizophrenic patients whereas IL-2-induced behavioral changes are blocked by dopamine D1 receptor antagonist or by a high dose of a D2 antagonist [56]. Mice born to mothers who had respiratory tract infection at mid-gestation show increased levels of IL-2 and IL-6 and features of schizophrenia suggesting that cytokines have a role in schizophrenia [57].

Membrane DHA, EPA, and AA levels are reduced in schizophrenics [58] and their levels were higher in chronic medicated patients than drug-naïve first-episode patients. EPA suppresses IL-2, IL-6 and TNF- α production and oral EPA is useful in schizophrenia [59]. Hence, it is likely that increased maternal IL-2, IL-6 and TNF- α that occurs due to infections during pregnancy damage fetal brain, alter the balance between dopaminergic and serotonergic neurons and predispose them to develop schizophrenia in adult life. Antipsychotics possibly, increase the levels of EPA and DHA and thus, are beneficial.

LCPUFAS ARE ENDOGENOUS NEGATIVE REGULATORS OF INFLAMMATION

LCPUFAs especially ω -3 EPA and DHA suppress the synthesis and release of IL-6 and TNF- α and thus may serve as endogenous negative regulators of inflammation [8]. Low concentrations of LCPUFAs seen in diabetes mellitus, hypertension, CHD, schizophrenia and depression may explain why CRP, IL-6, and TNF- α levels are elevated in these conditions. Infants accumulate LCPUFAs from maternal/placental transfer, consumption of human milk and synthesis from essential fatty acids: linoleic acid and α -linolenic acid [4]. Concen-

trations of LCPUFAs in plasma, red blood cell membrane, and cerebral cortex are lower in formula-fed infants than they are in infants receiving human milk [4]. Newborn infants have limited capacity to form LCPUFAs from their precursors. Hence, in the event maternal stores and/or mother's breast milk does not contain adequate amounts of various LCPUFAs as may happen when they have diabetes mellitus, hypertension, and other diseases their offspring are likely to receive sub-optimal amounts of LCPUFAs. A marginal or sub-clinical deficiency of various LCPUFAs may predispose them to develop low-grade systemic inflammation due to the absence of the negative regulatory influence of these fatty acids on IL-6 and TNF- α that ultimately leads to the development of various diseases.

Macaubas et al. [60] showed that antenatal attenuated T_H1 and T_H2 cytokine production can be related to the development of atopy and asthma outcomes at age 6 years suggesting that perinatal factors are important in determining susceptibility to diseases in later life. Recent report that fish oil (a rich source of EPA and DHA) supplementation in pregnancy modifies neonatal allergen-specific immune responses by decreasing neonatal IL-5, IL-13, IL-10 and IFN- α responses compared to control and that infants in the fish oil group had significantly less severe atopic dermatitis supports this view [61]. Joshi et al. [62] showed that maternal supplementation of fish oil to a diet containing marginal protein maintained circulating glucose, insulin, cholesterol and homocysteine levels near normal levels compared to control (marginal protein deficient diet fed rats) in the offspring as adults. This clearly suggests that fish oil given during perinatal period ameliorates the negative effects of maternal malnutrition and prevent the development of features of metabolic syndrome X possibly by restoring the glucose sensors to normal both in the CNS (central nervous system) and peripheral tissues. Similarly, perinatal supplementation of appropriate amounts of ω -6 and ω -3 fatty acids in the right proportion could prevent other adult diseases such as type 2 diabetes mellitus, hypertension, and CHD lending support to the concept presented here [63–65].

LCPUFAS AND GENE/ONCOGENE(S) EXPRESSION

One important question that needs to be addressed is how perinatal supplementation of LCPUFAs prevents/arrests the development of adult diseases. In this context, the interaction(s) between environment and the fetus assumes significance. During the early critical periods of life and development (first and second trimesters of pregnancy when organogenesis is occurring) the organism responds to environmental stimuli by adaptations at the gene, biochemical, and cellular levels which may permanently change an organism's metabolism and physiology such that it may lead to the development of various adult diseases. Such fetal adaptation to an environmental insult/stimuli called 'programming' [reviewed in 4] can occur in several tissues including hypothalamus, cardiovascular system, pancreas, etc. Early nutrition is an important environmental signal that induces lifetime effects on metabolism, growth, and neurodevelopment that will

have a major impact on the development or susceptibility to develop hypertension, diabetes mellitus, CHD, and obesity in adult life. A low protein diet or caloric restriction during gestation and lactation is known to induce lifetime effects on metabolism, growth, and neuroendocrine development and predispose them to develop obesity, diabetes mellitus, hypertension, atherosclerosis and CHD in adult life [4,66]. Studies by Patel and Srinivasan [67] revealed that four-day old rats reared on a high carbohydrate milk formula developed chronic hyperinsulinemia and obesity. Female rats fed high carbohydrate milk during their suckling period spontaneously transmitted the metabolic characteristics of hyperinsulinemia and obesity to their progeny. Furthermore, the growth pattern of high carbohydrate rats in the second generation paralleled that of first generation that were fed a high carbohydrate diet. Crossbreeding experiments revealed that only high carbohydrate females transmit these traits to the progeny suggesting that intrauterine programming is essential for the transmission of these traits and that early adaptations are programmed and accompanied by additional changes probably triggered by adult-onset factors. It is likely that a similar programming occurs with the ingestion of the present day high fat or energy dense foods by the pregnant and lactating mothers that leads to metabolic maladaptations in their progeny such that it ultimately renders them susceptible to develop adult diseases. Both high carbohydrate and high fat diets are known to inhibit Δ^6 and Δ^5 desaturases leading to low tissue concentrations of LCPUFAs that might predispose to the development of adult diseases as discussed above [4,68].

Support to the concept that perinatal supplementation of LCPUFAs prevents adult diseases is derived from the observation that they modulate the expression of Toll-like receptors [69], protect endothelial cells by activating Akt gene [70], influence the expression of various genes related to cell proliferation, growth and adhesion, as well as for many transcription factors including sterol regulatory element binding proteins (SREBP) [71], stimulate the expression of CD36 receptor that plays an important role in atherosclerosis [72], and alter the expression of brain genes that control synaptic plasticity, cytoskeleton and membrane association, signal transduction, ion channel formation, energy metabolism, and regulatory proteins [73], enhance the expression of anti-oncogene p53. This suggests that when adequate amounts of LCPUFAs are provided during the perinatal period optimal expression and function of various beneficial genes and suppression of noxious genes occurs such that various tissues are able to counteract the pathological processes in an efficient manner to prevent the development of various diseases in adult life [74]. This is further illustrated by the studies of Weisinger et al. [75] who showed that DHA deficiency in the perinatal period results in raised blood pressure later in life, even when animals were subsequently replete with this fatty acid. It was observed that animals raised on LCPUFAs-deficiency diet under drank water and over ingested sodium, suggesting an aberration in central osmo/sodium sensors and/or angiotensinergic mechanisms. This suggests that LCPUFAs should be available

in optimal amounts during the critical periods of growth and development of various tissues and organs to prevent diseases in adult life.

CONCLUSIONS

In summary, I propose that sub-clinical/marginal deficiency of LCPUFAs during perinatal period initiates low-grade systemic inflammation that leads to the development of several adult diseases. Hence, supplementation of adequate amounts of these fatty acids during the perinatal period may prevent atopy/bronchial asthma, IBD, RA, SLE, Alzheimer's disease, depression, metabolic syndrome X, type 1 and type 2 diabetes mellitus, hypertension, CHD, and certain forms of cancer. Although the exact amount and proportion in which various LCPUFAs need to be provided during perinatal period is not known, giving them in the proportion in which they are present in human breast milk may prove to be optimum.

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