Arch Pharm Res Vol 25, No 6, 747-758, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Biological Significance of Essential Fatty Acids/Prostanoids/ Lipoxygenase-Derived Monohydroxy Fatty Acids in the Skin

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(Received April 23, 2002)

The skin displays a highly active metabolism of polyunsaturated fatty acids (PUFA). Dietary deficiency of linoleic acid (LA), an 18-carbon (n-6) PUFA, results in characteristic scaly skin disorder and excessive epidermal water loss. Although arachidonic acid (AA), a 20-carbon (n-6) PUFA, is metabolized via cyclooxygenase pathway into predominantly prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$, the metabolism of AA via the 15-lipoxygenase (15-LOX) pathway, which is very active in skin epidermis and catalyzes the transformation of AA into predominantly 15S-hydroxyeicosatetraenoic acid (15S-HETE). Additionally, the 15-LOX also metabolizes the 18-carbon LA into 13S-hydroxyoctadecadienoic acid (13S-HODE), respectively. Interestingly, 15-LOX catalyzes the transformation of dihomo-γ-linolenic acid (DGLA), derived from dietary gamma-linolenic acid, to 15S-hydroxyeicosatrienoic acid (15S-HETrE). These monohydroxy fatty acids are incorporated into the membrane inositol phospholipids which undergo hydrolytic cleavage to yield substituted-diacylglycerols such as 13S-HODE-DAG from 13S-HODE and 15S-HETrE-DAG from 15S-HETrE. These substituted-monohydroxy fatty acids seemingly exert anti-inflammatory/antiproliferative effects via the modulation of selective protein kinase C as well as on the upstream/down-stream nuclear MAP-kinase/AP-1/apoptotic signaling events.

Key words: Fatty acids, Prostanoids, Lipoxygenase, Epidermal proliferation, 15S-HETE, 15S-HETrE, PUFA, Linoleic acid, 13S-HODE, Cell signal, PKC, Skin, Cox-2

ESSENTIAL FATTY ACIDS

Introduction/historical perspective

The first indication that dietary fat may be essential for healthy growing animals was presented in 1918 by Aron (Aron, 1919), who proposed that butter has a nutrient value that cannot be provided by other dietary components (Aron, 1919). This report suggested that there was a special nutritive value inherent in fat apart from its caloric contribution and that this possibility was related to the presence of certain lipids. In 1929, Burr and Burr (Burr and Burr, 1929) presented the first series of articles outlining a "new deficiency disease produced by the rigid exclusion of fat from the diet." In the series of conclusions put forth, they developed the hypothesis that warm-blooded animals in general, cannot synthesize appreciable quantities of certain fatty acids. In 1930, both investigators significantly added to their earlier work by presenting evidence that the dietary inclusion of linoleic acid alone could reverse all deficiency symptoms resulting from a fat-free diet and thus linoleic acid (LA or 18:2n-6)[1] was heralded as an *essential fatty acid* (EFA) (Burr and Burr, 1930). The recognition that some unsaturated fatty acids could not be synthesized from endogenous precursors by mammals and were essential dietary elements led to the designation of essential and nonessential fatty acids. It was originally thought that there are only two essential fatty acids, linoleic acid (9,12-octadecadienoic acid, LA, 18:2n-6) and α-linolenic acid (9,12,15-octadecatrienoic acid, ALA, 18:3n-3), but continued nutritional studies revealed positive essential growth responses not only for linoleic acid and α-linolenic acid, but also for arachidonic acid as well as the long-chain highly unsaturated fatty acids in fish oil (eicosapentaenoic acid, EPA, 20:5n-3) and (docosahex-

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[1] Fatty acids and acyl groups are denoted 18:2n-6, 18:3n-3 and so on, with the first number representing the number of carbons in a straight chain and the number following the colon indicating the number of methylene interrupted cis double bonds. The number after the "*n*" indicates the number of carbon atoms from the methyl end of the acyl chain to the nearest double bond.

aenoic acid, DHA, 22:6n-3) (Turpeinen, 1937; Burr *et al.*, 1940; Quackenbush *et al.*, 1942). More recent reports on the biological significance of the longer chain n-3 polyunsaturated fatty acids (PUFAs) do qualify these long-chain fatty acids as *essential* PUFAs.

BIOLOGICAL SIGNIFICANCE OF ESSENTIAL FATTY ACIDS

Structural form

The two major families of polyunsaturated fatty acids (PUFAs) characteristics of mammalian species are the n-6 and the n-3 PUFAs. The n-6 and n-3 PUFAs are defined by the position of the double bond closest to the terminal methyl group of the fatty acid molecule. In the n-6 family, the first double bond occurs between the sixth and the seventh carbons from the methyl group end of the molecule, whereas in the n-3 family, the first double bond occurs between the third and fourth carbons. PUFAs with these basic structures cannot be biosynthesized de novo in appreciable amounts by vertebrate animals nor are the n-3 and n-6 families of PUFAs interconvertible. Thus, these essential PUFAs must be supplied from dietary sources.

Dietary sources

The 18-carbon n-6 and n-3 polyunsaturated fatty acids (PUFAs) are synthesized on land by many plants and, therefore, are dietarily obtained from vegetable oils. The longer-chain members of each family are either biosynthesized in vivo after dietary ingestion of the shorter 18-carbon precursors or they are obtained directly from animal or marine sources. For example, the longer-chain n-3 PUFAs, especially eicosapentaenoic acid (5,8,11,14,17-eicosapentaenoic acid, EPA, 20:5n-3) and docosahexaenoic acid (4,7,10,13,16,19-docosahexaenoic acid, DHA, 22:6n-3) are found in fish and shellfish. They can be ingested directly from these sources. The longer-chain n-6 PUFA, arachidonic acid (AA, 20:4n-6), is found in the liver, brain, and meat, which are rich dietary sources of this PUFA.

Dietary requirements

Attempts have been made to estimate the human EFA requirement, but these have been met with a plethora of problems regarding what criteria to use for physiological normality. For instance, human infants and children are generally thought to require 1-2% of total calories as LA in order to avoid EFA deficiency (Mohrhauer and Holman, 1963). It is generally believed that children require more LA as a percentage of total daily caloric intake than adults because growth increases the demand of this fatty acid for cell membrane components (Chapkin and Ziboh, 1984). Comparative studies on the metabolic equivalence of LA and AA demonstrate that AA has three times the potency of LA (Mohrhauer and Holman, 1963).

Assessment of essential fatty acid status

The classical biochemical method of establishing EFA deficiency (EFAD) is to calculate the ration of triene (5,8, 11-eicosatrienoic acid, 20:3n-9) to tetraene (AA, 20:4n-6) fatty acids (Chapkin *et al.*, 1986). A competitive interaction between the n-6 and n-3 acids results in the suppression of long-chain n-9 fatty acid biosynthesis. Furthermore, n-3 fatty acids have been shown to moderately suppress the metabolism of n-6 fatty acids. Thus, when the tissue level of 18:2n-6 is normal, relatively little oleic acid (OA, 18:1n-9) is desaturated and elongated into 20:3n-9, resulting in a low triene/tetraene ratio. On the other hand, when LA is very low or inadequate, as is the case in EFAD, OA (derived from *de novo* glucose metabolism) undergoes desaturation/elongation reactions resulting in the elevation of 20:3n-9 in tissue lipids. Correspondingly, the triene/tetraene ratio is elevated. The triene/tetraene ratios above 0.2 and 0.4 are considered the upper limits of normalcy in human plasma and tissue lipids.

Deficiency symptoms

The various deficiency symptoms apparent in the response to diets low or free from EFAs were first described by Burr and Burr (Burr and Burr, 1929; Burr and Burr, 1930). A salient feature of this deficiency syndrome is decreased growth rate, particularly in pair-fed male animals. Other symptoms largely compiled from EFA-deficient rat studies include scaly dermatoses, permeability of skin to water, hair loss, tail necrosis, fatty liver, kidney damage, impaired reproduction, fetal resorption in females, testicular degeneration in males and reduced ability to form and maintain cell membrane integrity.

DESATURATION/ELONGATION OF ESSENTIAL FATTY ACIDS

The shorter chain EFAs, linoleic acid (LA, 18:2n-6) and α-linolenic acid (ALA, 18:3n-3), serve as the initial unsaturated precursors for the in vivo biosynthesis of the longer chain PUFAs. Metabolism of the EFAs in most tissues involves an alternating sequence of Δ^6-desaturation, chain elongation and Δ^5-desaturation in which two hydrogen atoms are removed to create a new double bond followed by the addition of two carbon atoms from glucose metabolism to lengthen the fatty acid chain (Marcel *et al.*, 1968) (Fig. 1). The desaturations are catalyzed by two separate enzymes: the Δ^6-desaturase catalyzing the transformation of 18:2n-6 (LA) to 18:3n-6 (γ-linolenic acid, GLA) and the Δ^5-desaturase catalyzing the transformation of 20:3n-6 (DGLA) to 20:4n-6 (AA). The elongase enzyme

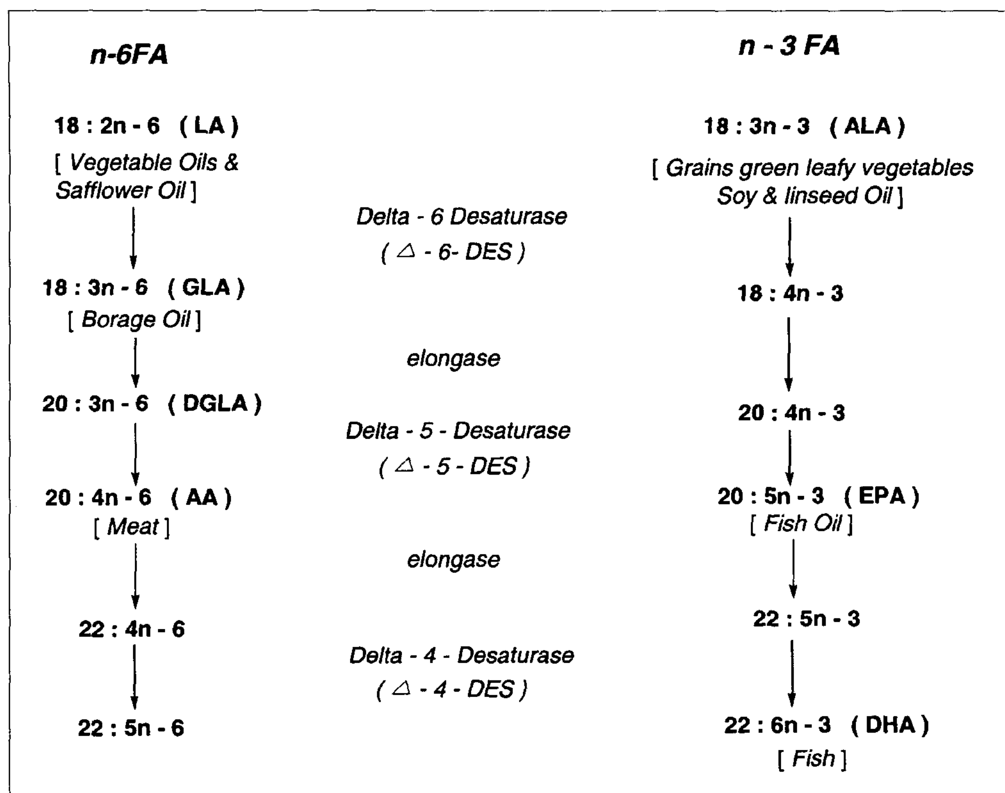

Fig. 1. Oxidative desaturation/elongation of n-3 and n-6 polyunsaturated fatty acids

catalyzes the elongation of GLA to DGLA (Fujiwara *et al.*, 1983). It is believed that the same enzymes catalyze equivalent steps in the n-3 and n-9 pathways (Brenner, 1974). The PUFA families interact in such a manner that the n-3 PUFAs competitively suppress the bioconversion of the n-6 PUFAs. Both the n-6 and the n-3 PUFAs respectively do suppress the formation of the nonessential long chain n-9 fatty acids, hence the negligible formation of the long chain n-9 PUFA (20:3n-9) in normally fed animals.

CUTANEOUS SIGNIFICANCE OF 18-CARBON LINOLEIC ACID

(1) Linoleic acid (18:2n-6)

(a) Role in skin water barrier system. The most abundant polyunsaturated fatty acid (PUFA) in human skin is the 18-carbon linoleic acid (Chapkin and Ziboh, 1984). There is good evidence to indicate that one functional significance of LA is its involvement in the maintenance of the epidermal water barrier (Hansen and Jensen, 1985), which is one of the major abnormalities of cutaneous EFA deficiency. The physical structure of the epidermal water barrier was ascribed to sheets of stacked lipid bilayers or lamellae which fill intercellular spaces of the uppermost layer of the epidermis (stratum corneum). These lipid bilayers contain large amounts of sphingolipids (Elias *et al.*, 1979) of which the linoleate-rich species have been characterized as acylglucosylceramide, acylceramide and a unique acylacid (Wertz et al, 1983; Wertz and Downing, 1983; Bower *et al.*, 1985). It has been suggested that the linoleyl moiety of the barrier acylsphingolipids is further metabolized by a lipoxygenase-like reaction to form first a hydroxyacylceramide and then a polyacylceramide before the barrier function is exhibited (Nugteren *et al.*, 1985).

(b) Role in epidermal hyperproliferation: generation of 13S-HODE by 15S-lipoxygenase. Although the feeding of LA to EFA-deficient animals is known to reverse the major cutaneous symptoms of EFA deficiency (which include hyperproliferation and increased transepidermal water loss), the mechanism for such a reversal has remained unknown. Thus, after the first recognition that skin enzyme preparations cannot biosynthesize arachidonic acid (Chapkin and Ziboh, 1984), we explored the possibility that accumulating cutaneous LA may undergo oxidative metabolism, presumably via the lipoxygenase pathway, generating bioactive products that in turn attenuate the skin hyperproliferative activity. To test this hypothesis, we incubated in vitro LA with soybean 15-lipoxygenase or 15-lipoxygenase prepared from skin epidermis. The major metabolite iden-

tified in these incubations was 13S-hydroxyoctadecadienoic acid (13S-HODE) and a minor metabolite was 9S-HODE. The skin epidermis is unique in that it preferentially metabolizes LA in vitro to 13S-HODE (presumably by 15-lipoxygenase-1, 15-LOX-1) with negligible amount being transformed to γ-linolenic acid (GLA), suggesting that 13S-HODE may serve an important metabolite in skin epidermal homeostasis. Similarly, the feeding of safflower oil to normal guinea pigs resulted in enhanced in vivo amounts of 13S-HODE in the epidermis. A schematic illustration of the metabolism of dietary LA in the epidermis is shown in Fig. 2.

In attempts to evolve a functional role for this monohydroxy fatty acid, subsequent studies revealed that 13S-HODE was preferentially incorporated into epidermal phosphatidylinositol 4,5-bisphosphate (PtdIns 4,5-P_2) (Cho and Ziboh, 1994). The 13S-HODE-substituted inositolphospholipid was subjected to epidermal phospholipase C-hydrolysis resulting in the release of 13S-HODE into a novel 13S-HODE-containing diacylglycerol (1-acyl-2-13S-HODE-glycerol) and inositol triphosphate. The formation of this novel 13S-HODE-containing diacylglycerol (13S-HODE-DAG) implies that it could function to modulate the activity of epidermal protein kinase C (PKC), which is associated with epidermal hyperproliferation or differentiation.

(c) Modulation of cellular cell signaling by 13S-HODE-substituted diacylglycerol. To determine whether or not 13S-HODE-substituted diacylglycerol can modulate cellular signaling, we synthesized 1-palmitoyl-2-13S-HODE-substituted diacylglycerol (13S-HODE-DAG) (Cho and Ziboh, 1994). The putative 13S-HODE-DAG was shown to exert selective inhibitory effect on the expression and activity of two epidermal membrane protein kinase C (PKC) isozymes: protein kinase-β (PKC-β) and PKC-α (Cho and Ziboh, 1994). Interestingly, the elevation of PKC-β was associated with the hyperproliferative scaly skin lesions of EFA-deficient guinea pigs. Reversal of the EFA-deficiency by dietary LA resulted in epidermal elevation of 13S-HODE/13S-HODE-DAG and the suppression of PKC-β expression and activity (Cho and Ziboh, 1995). Taken together, it appears that the signaling processes are associated with the attenuation of the EFA-deficient-induced hyperproliferation. A speculative scenario of dietary linoleic acid (as triglycerides) and the modulation of PKC activity is shown in Fig. 3.

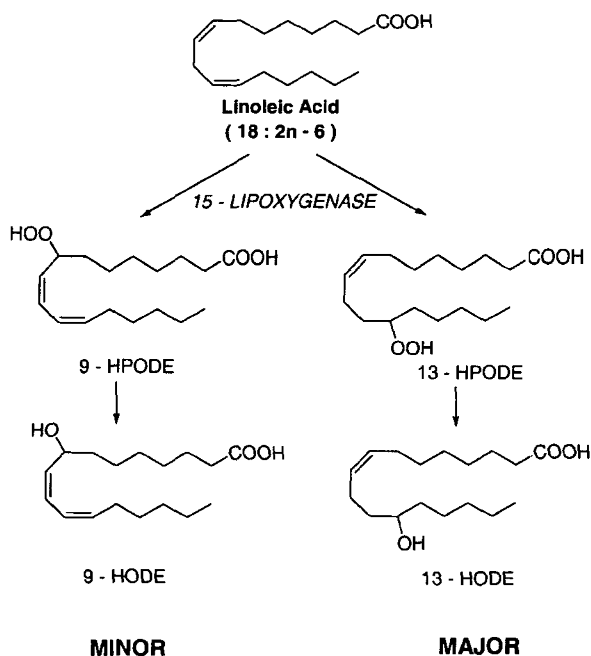

Fig. 2. Metabolism of linoleic acid (18:2n-6) by human epidermal preparation

CUTANEOUS SIGNIFICANCE OF γ-LINOLENIC ACID/DIHOMO-γ-LINOLENIC ACID

(1) γ-Linolenic acid (18:3n-6)

(a) Modulation of inflammatory process. Prompted by reports that γ-linolenic acid (GLA, 18:3n-6) does exert clinical efficacy in a variety of diseases such as the clinical improvement of patients with atopic eczema (Wright and Burton, 1982; Baker *et al.*, 1989) and the suppression of acute and chronic inflammation (Tate *et al.*, 1989; Chapkin and Ziboh, 1986), we embarked on a series of studies to discern the biological role of the monohydroxy fatty acid generated from dihomo-γ-linolenic acid (DGLA, 20:3n-6), an elongation product of GLA. A notable feature of dietary GLA in the epidermis is first, its in vivo elongation to DGLA (Chapkin and Ziboh, 1986), followed by its oxidative metabolism via the cyclooxygenase pathway to prostaglandin of the 1-series (PGE_1) and via its metabolism by 15-lipoxygenase enzyme into 15S-hydroxyeicosatrienoic acid (15S-HETrE) (Miller *et al.*, 1988). This metabolic transformation has been demonstrated after dietary supplementation of guinea pig diet with primrose oil and borage oil (both containing GLA). These include the in vivo elongation of GLA into DGLA and the latters transformation to 15S-HETrE in the epidermis (Miller and Ziboh, 1988). Metabolism of dietary γ-linolenic acid in the epidermis is shown in Fig. 4.

In a similar study in normal human volunteers, dietary supplementation with borage oil (containing GLA) resulted in elevated DGLA in the volunteers polymorphonuclear cell (PMN) phospholipids. This elevated increase of DGLA in the PMNs paralleled the inability of the isolated PMNs to ex vivo transform AA to leukotriene B_4 (LTB_4) (Ziboh and Fletcher, 1992), suggesting a possible inhibition of 5-lipoxygenase pathway. To determine whether the PMN inability was due to the inhibition of leukocyte 5-lipoxygen-

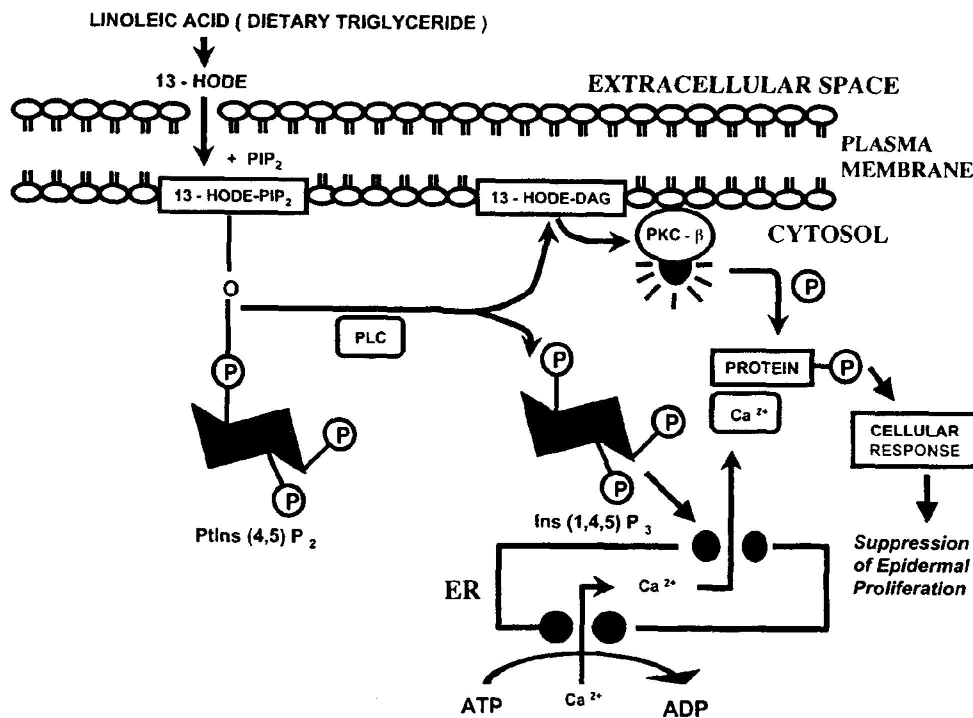

Fig. 3. A speculative scenario of dietary linoleic acid/13S-HODE modulation of epidermal proliferation via inositol phospholipid/PKC phosphorylation of intracellular proteins

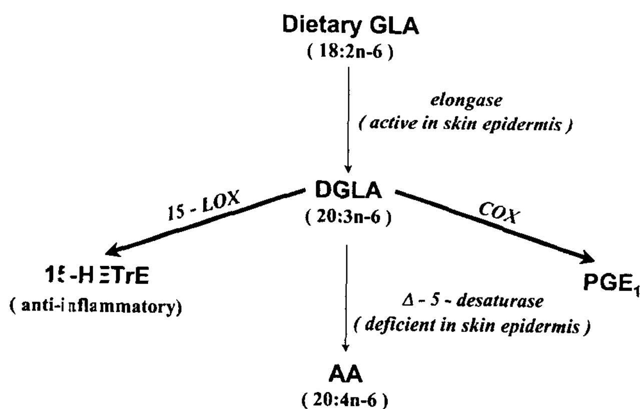

Fig. 4 Metabolism of γ-linolenic acid (18:3n-6) by human epidermal preparations

ase activity, we incubated rat basophilic leukemia (RBL-1) cells with varying concentrations of 15S-HETrE in vitro. Generation of LTB_4 by the RBL-1 cells was markedly suppressed (Miller *et al.*, 1991). The in vitro effects of 15S-HETrE on the inhibition of pro-inflammatory LTB_4 by PMNs are consistent with the reported beneficial effects of dietary oil containing GLA on inflammatory conditions (Lovell *et al.*, 1981; Tate *et al.*, 1989).

In another dietary study, we tested the effects of dietary supplementation of GLA-containing primrose oil on bleomycin-induced lung fibrosis in hamsters (Ziboh *et al.*, 1997). Bleomycin is a glycopeptide known to cause pulmonary injury and thought to induce generation of reactive oxygen species and release various inflammatory mediators (Wang *et al.*, 1992). Our data revealed marked suppression of lung leukotriene B_4 (LTB_4), a pro-inflammatory mediator generated from arachidonic acid, and suppression of lung hydroxyproline (a marker of lung fibrosis). The decrease in LTB_4 was accompanied by marked elevation of lung 15-HETrE and PGE_1 levels seemingly generated from dietary GLA. Taken together, the results from these studies suggest that dietary GLA-containing oils may contribute to tissue elevation of PGE_1 and 15-HETrE which in vivo may attenuate lung inflammation and fibrosis.

(b) Possible role of 15-HETrE in cutaneous cell signaling. Since a previous report from our laboratory had revealed that 13S-HODE (15-lipoxygenase metabolite of LA) was incorporated into phosphatidylinositol 4,5-bisphosphate (PtdIns 4,5-P_2) resulting in PLC-catalyzed release of 13S-HODE containing diacylglycerol (13S-HODE-DAG) (Cho and Ziboh, 1994), we investigated whether a similar occurrence could take place in skin epidermis when 15S-HETrE is elevated. The findings from subsequent experiments revealed that 15S-HETrE was similarly incorporated into PtdIns 4,5-P_2 resulting in PLC-catalyzed release of novel putative 15S-HETrE-substituted diacylglycerol (15S-HETrE-DAG) (Cho and Ziboh, 1997). Next we tested whether or not this putative substituted-DAG does exert any effect on the cutaneous PKC-isoforms. Our results re-

vealed that 15-HETrE-DAG competitively inhibited diolein-activated membrane associated PKC-β expression and activity (Cho and Ziboh, 1995).

MODULATION OF DOWN-STREAM SIGNALING EVENTS BY 15-HETrE

(1) Modulation of nuclear PKC/mitogen-activated protein-kinase

Because cell growth and proliferation are regulated by biochemical events in the nuclear compartment of the cell, we established an in vivo model of epidermal hyperproliferation in guinea pig (Xi *et al.*, 2000) in order to delineate the cellular biochemical events that take place during the hyperproliferative process. To evaluate 15-HETrE in the skin hyperproliferative model, we demonstrated that PKC-α and the atypical PKC-ζ are the two major PKC isozymes in the epidermal nuclear membrane (Mani *et al.*, 1999) which is in contrast to PKC-α and PKC-β previously observed in the epidermal plasma membrane (Cho and Ziboh, 1994) and that epidermal hyperproliferation paralleled the enhanced nuclear PKC-α and the atypical PKC-ζ isozymes. The increase in the nuclear PKC isozymes paralleled the marked increase in the expression of nuclear MAP-kinase. Interestingly, our findings are consistent with other reports which have indicated that PKC is extremely important in mitogenic signaling, starting with its dramatic activation of the downstream extracellular signal regulated kinase (ERK) family which includes MAP-kinase kinase (MEK) and MAP-kinase (MAPK) (Nishida and Goter, 1993; Berra *et al.*, 1995). Treatment of the epidermal hyperproliferative model suppressed the PKC-isozymes and MAP-kinase. Since PKC/MAPK constitute the upstream events of the cell signal transduction cascade, the alteration of PKC/MAPK could be expected to affect the regulation of the downstream nuclear events such as those involving activator protein-1 (AP-1). The activator protein is altered during cell proliferation (Saez *et al.*, 1995; Schuh *et al.*, 1990) and apoptosis (Smeyne *at al.*, 1993; Xia *et al.*, 1995), a process of programmed cell death. Both 13-HODE and 15-HETrE have recently been reported to modulate these nuclear events (Xi and Ziboh, 2000). Since 15S-HETrE is a metabolite derived from dietary GLA, it is possible that increased dietary intake of PUFA may result in the in vivo generation of biologically active metabolites which could alleviate hyperproliferative skin conditions via the modulation of the transcription factor AP-1 and apoptosis.

CUTANEOUS SIGNIFICANCE OF 20-CARBON ARACHIDONIC ACID

(1) Arachidonic acid (20:4n-6)

The skin performs critical homeostatic functions including thermoregulation, sensory perception, protection against microbial invasion and protection against absorption of potentially toxic environmental agents. To perform these functions, the skin must maintain a structural and functional integrity that derives from an array of complex cellular and biochemical interactions, some of which are biosynthesized from polyunsaturated fatty acids. The 20-carbon arachidonic acid (AA) is the second prominent PUFA in the skin. It is approximately 6-10% of the total fatty acids in the epidermal phospholipids of guinea pigs and approximately 9% in the epidermal phospholipids of human skin (Chapkin *et al.*, 1986). Its functional role in the epidermis depends largely on its generation of biologically potent metabolites such as the prostaglandins and the hydroxy fatty acids.

(a) Cyclooxygenase (prostanoids). It is now recognized that metabolism of AA via the cyclooxygenase pathway is catalyzed by two isoforms of cyclooxygenase, commonly referred to as COX-1 and COX-2 (Smith and Dewitt, 1996; Herschman *et al.*, 1997). The genes for these two isozymes exist on different chromosomes in humans as well as in mice (Crofford, 1997). Structurally, the two isozymes share about 60% homology in overall amino acid sequences and about 90% in the active site region where arachidonic acid as well as COX inhibitors bind (Kurumbail *et al.*, 1996). In the binding site region, the COX-2 gene encodes for a valine rather than an isoleucine as found in COX-1 (Gierse *et al.*, 1996). This single amino acid substitution alters the inhibitor binding domain in such a way that inhibitor selectivity becomes possible.

The occurrence of COX-1 and COX-2 has been reported in mouse epidermis (Sholtz *et al.*, 1995). For instance, when mouse skin is treated with phorbol ester tumor promoter (an initiator of epidermal hyperplasia), COX-2 is expressed similar to the inducible patterns reported in the inflammatory cells. Expression of COX-2 protein were reported in the basal layer of mouse epidermis. Human epidermis also has been demonstrated to express both COX-1 and COX-2 isozymes using immunohistochemical techniques as well as by Western blotting of protein extracts from cultured human keratinocytes (Leong et al, 1996). However, unlike mouse skin, COX-2 protein in human skin is constitutively expressed in the more differentiated layers of the epidermis. Consistent with this view, increased expression of COX-2 was present in biopsies of squamous cell carcinomas that derive from more differentiated keratinocytes; in contrast, little if any expression of COX-2 was observed in basal cell carcinomas that derive from the least differentiated basal cells (Leong *et al.*, 1996; Buckman *et al.*, 1998; Muller-Decker *et al.*, 1999). It is important to point out that there are types of cells in which differentiation appears to involve COX-1, rather than COX-

2, induction (Smith *et al.*, 1993). Consequently, the physiological roles of the COX isozymes are most likely cell and tissue specific, and to extrapolate the findings from one type of cell or tissue study to untested cells and tissues need to be undertaken with caution. In a pathophysiological context, increased COX-2 expression in keratinocytes occurs following exposure to UV light and chronic exposure to UV light is associated with the generation of both actinic keratoses and squamous cell carcinomas (reviewed in Salasche, 2000). Overall, COX-2 inhibitors may play a role in the attenuation of epidermal neoplasms such as squamous cell carcinomas and actinic keratoses that exhibit COX-2 expression.

(b) Lipoxygenases (hydroxy fatty acids)

(i) 12-Lipoxygenase

12S-Hydroxyeicosatetraenoic acid

The lipoxygenase pathway is very active in the epidermis. For instance, the 12-lipoxygenase product of AA, 12-hydroxyeicosatetraenoic acid (12S-HETE) was first reported to accumulate in the lesion of psoriatic patients (Hammerstrom *et al.*, 1975). This observation provoked the first interest in the possible role of this monohydroxy fatty acid in cutaneous homeostasis. Although 12S-HETE can exert moderate inflammatory effects in a variety of cell types, its full significance in epidermal inflammatory/proliferative processes is not fully understood. Advances in chiral phase chromatography now reveal that 12S-HETE which originally was reported to accumulate in the lesion of psoriatic patients is primarily the 12(R) epimer and not the 12(S) epimer that is the typical 12-lipoxygenase product form in platelets (Woollard, 1986). The 12(R)-HETE has been shown to be more potent than the 12(S)-HETE as a chemo-attractant. It has been reported that 12(R)-HETE and 12(S)-HETE are products of an epidermal membrane-bound monoxygenase (Holtzman *et al.*, 1989).

(ii) 15-Lipoxygenase

15S-Hydroxyeicosatetraenoic Acid

The 15-lipoxygenase enzyme, on the other hand, is very active in the epidermis. Products of a variety of polyunsaturated fatty acids (PUFAs) which are generated via this pathway yield biologically active metabolites in this tissue. For example, the 15S-hydroxyeicsatetraenoic acid (15S-HETE), a product of AA, has been demonstrated in human epidermis (Kragballe *et al.*, 1986), in cultured murine and human epidermal cells (keratinocytes), in isolated human neonata foreskin (Burrall *et al.*, 1985) and in guinea pig epidermis (Camp *et al.*, 1983). This monohydroxy fatty acid was detected in biologically active concentrations in human patients with psoriatic lesions (Camp *et al.*, 1983; Cunningham *et al.*, 1985). Although attempts to stimulate keratinocytes in culture with calcium ionophore to release 15S-HETE into the medium have been met with limited success, probably due to the use of inappropriate agonists, 15S-HETE has been extracted and demonstrated in vivo in the epidermis (Duell *et al.*, 1988) where it is esterified to the phospholipids.

Functionally, 15S-HETE in vitro was reported to inhibit 5-lipoxygenase activity and generation of LTB_4 in neutrophils and basophils (Vanderhoek, 1980). Similarly, 15S-HETE generated from epidermis from AA has also been shown to inhibit the generation of 12S-HETE from AA (Miller *et al.*, 1988). These in vitro effects suggest, at least in part, the anti-inflammatory potential of 15S-HETE. Consistent with this anti-inflammatory potential, 15S-HETE was reported to improve the symptoms of psoriasis vulgaris after intralesional injections (Fogh *et al.*, 1988). Although the mechanism of this antilesional effect is unclear, one possibility is that 15S-HETE in pharmacological active doses may function in vivo to inhibit the formation of products of 5- and 12-lipoxygenase (LTB_4 and 12S-HETE), both of which are markedly elevated in psoriatic lesion. It is therefore, reasonable to speculate that an elevation of this monohydroxy fatty acid in the epidermis may provide a local in vivo anti-inflammatory molecule which will inhibit AA-derived inflammatory metabolites.

(iii) 5-Lipoxygenase

(a) Leukotrienes

Both leukotrienes and hydroxyeicosatetraenoic acids are generated from arachidonic acid by the action of 5-lipoxygenase. For instance, the 5-lipoxygenase pathway generates mainly leukotrienes: LTB_4, LTC_4 and LTD_4 from AA by leukocytes. These metabolites are known to accumulate in lesions of psoriasis (Brian *et al.*, 1984; Ruzicka *et al.*, 1988), prompting controversy as to their in vivo source. Because the psoriatic lesion is infiltrated with leukocytes (particularly the PMNs), one possibility is that these leukocytes contribute largely to the leukotrienes found in the psoriatic lesion. However, there is now new information indicating that some of the leukotrienes in the psoriatic lesion are synthesized locally by the epidermis. For example, the incubation of human epidermal preparations with PMN-derived LTA_4, or the co-incubation of human PMNs with keratinocytes (transcellular metabolism) in culture, results in the generation of LTB_4 (Solar *et al.*, 1992; Iversen *et al.*, 1993). These studies underscore the possibility that keratinocytes does amplify local LTB_4 formation by utilizing PMN-derived LTA_4 (from infiltrating PMNs) during cutaneous inflammation when PMNs migrate into the epidermis as psoriasis. Transcellular leukotriene synthesis as demonstrated by PMN/keratinocytes may be an important mechanism by which human epidermis can contribute significantly to LTB_4 formation inflammatory skin diseases.

The leukotrienes are known to exert a variety of effects on a variety of cells. For instance, when LTB_4 is tested on cells in vitro, it exerts a potent chemo-attractant effect for neutrophils (Ternowitz *et al.*, 1987), eosinophils and neutrophils (Czarnetski and Rosenback, 1986) and fibroblasts (Mensing and Czarnetski, 1989). Notably, LTB_4 induces chemotaxis and chemokinesis at very low concentrations. Intradermal injection of LTB_4 into skin induces transient weal, flare and erythematous induration (Julin and Hammerstrom, 1983; Soter *et al.*, 1983). Topical application of LTB_4 to skin induces intraepidermal microabcesses (Camp *et al.*, 1984) and epidermal proliferation in guinea pig (Chan *et al.*, 1985) and human skin (Bauer *et al.*, 1986). These effects implicate LTB_4 as an important mediator in cutaneous inflammatory and proliferative disorders.

(b) 5S-Hydroxyeicosatetraenoic acid

Although the 5-lipoxygenase (5-LOX) is typically studied because of its ability to catalyze the biosynthesis of inflammatory leukotrienes, there is increasing evidence that it can also in several tissues catalyze the generation of 5-hydroxyeicosatetraenoic acid (15S-HETE). For instance, 5-lipoxygenase reportedly is expressed in the central nervous system neurons (Manev *et al.*, 2000), prompting the suggestion that 5-lipoxygenase is needed for proliferation of cerebellar granule neurons. On the other hand, selected inhibitors of 5-LOX have been reported to inhibit proliferation and induce apoptotic cell death in cultures of prostate PC-3 cells (Anderson *et al.*, 1998). Addition of 5-LOX metabolites: leukotrienes and 5S-HETE to cultures revealed that 5S-HETE and not leukotrienes stimulated growth of prostate cancer cells. Furthermore, 5-LOX inhibitors have been reported to reduce proliferation of pancreatic cancer cells. Interestingly, only cancerous and not normal pancreatic cells exhibited high 5-LOX expression (Ding *et al.*, 1999). Although the leukotrienes (5-LOX metabolites) have been reported to play a role in the skin inflammatory/ proliferative aspects of psoriasis (Iversen *et al.*, 1993; Iversen *et al.*, 1994), the role of 5S-HETE in the physiology and/or pathophysiology of cutaneous biology has not been rigidly explored.

(iv) 8-Lipoxygenase

8S-Hydroxyeicosatetraenoic acid

Although the stereospecific oxygenation of arachidonic acid at the 8-carbon resulting in activation of 8-lipoxygenase and generation of 8(R)-hydroxyeicosatetraenoic acid (8-HETE) has been detected in a variety of diverse natural sources such as sea whip and sea fan corals (Bundy *et al.*, 1986; Brash *et al.*, 1987) of the caribbean, it is relatively recent that 8S-HETE was reported to occur in higher animals. Treatment of the dorsal skin of NMRI mice with a single application of phorbol ester (12-O-tetradecanoylphorbol-13-acetate, TPA) induced an enzymatic activity that converts arachidonic acid to 8S-HETE (Gshwendt *et al.*, 1986). An investigation of the chirality of the mouse skin product revealed that it is a novel 8S-lipoxygenase. The dose-dependent activation of 8-lipoxygenase by TPA also prompted the suggestion that the 8-lipoxygenase gene may belong to the group of TPA-responsive genes which are thought to be regulated via a protein kinase C-dependent pathway. The TPA-induced 8-lipoxygenase from mouse skin has been cloned (Jisaki *et al.*, 1997). The immunohistochemical analysis revealed the strongest reaction in differentiated epidermal layer, the stratum granulosum. The significance of this observation is presently unclear. Furthermore, comparison of the cloned 8S-lipoxygenase cDNA with human 15S-lipoxygenase (15-LOX-2) revealed that the two lipoxygenases have 78% amino acid identity prompting the suggestion that 8S-lipoxygenase is the mouse homologue of the human 15-LOX-2 (Jisaki *et al.*, 1997). An interesting reported property of 8S-HETE is its high binding capacity for peroxisome proliferator-activated receptor-α (PPAR-α) (Kliewer *et al.*, 1997). The extent to which the mouse skin 8-LOX and the human 15-LOX-2 are functionally similar deserve further exploration.

CONCLUSION AND PERSPECTIVE FOR FUTURE RESEARCH

Taken together, PUFAs are uniquely metabolized by skin epidermal enzymes, implying that if present in adequate amounts in the skin epidermis because of dietary supplementation, the tissue cyclooxygenase and 15-lipoxygenase enzymes can generate local in vivo inflammatory and anti-inflammatory metabolites. However, the lipoxygenase-catalyzed metabolites of PUFAs derived from both the n-6 and the n-3 families generate potent mediators which exert dose-dependent in vitro inhibitory effects on the generation of PMN-derived LTB_4 (Miller *et al.*, 1991). Interestingly, 15S-HETrE, the metabolite derived from DGLA, the elongase metabolite from GLA, exerts the most potent in vitro inhibitory effect when compared with the other monohydroxy acids derived from AA, EPA and DHA. In contrast, the 18-carbon LA and its 15-lipoxygenase metabolite, 13-HODE, while exerting moderate anti-inflammatory effects, does exert a potent antiproliferative effect on skin hyperproliferation. A speculative scenario of GLA/ DGLA/15-HETrE/upstream and down-stream signaling cascade is illustrated in Fig. 5.

Although the mechanism involved in the antiproliferative effects of 15S-HETrE is not completely understood, the finding of our previous study that 15S-HETrE inhibits the expression of PKC/MAPK after its incorporation into DAG to form 15S-HETrE-DAG is of interest (Cho and Ziboh, 1997). Thus, we speculate that, since DAG is a natural

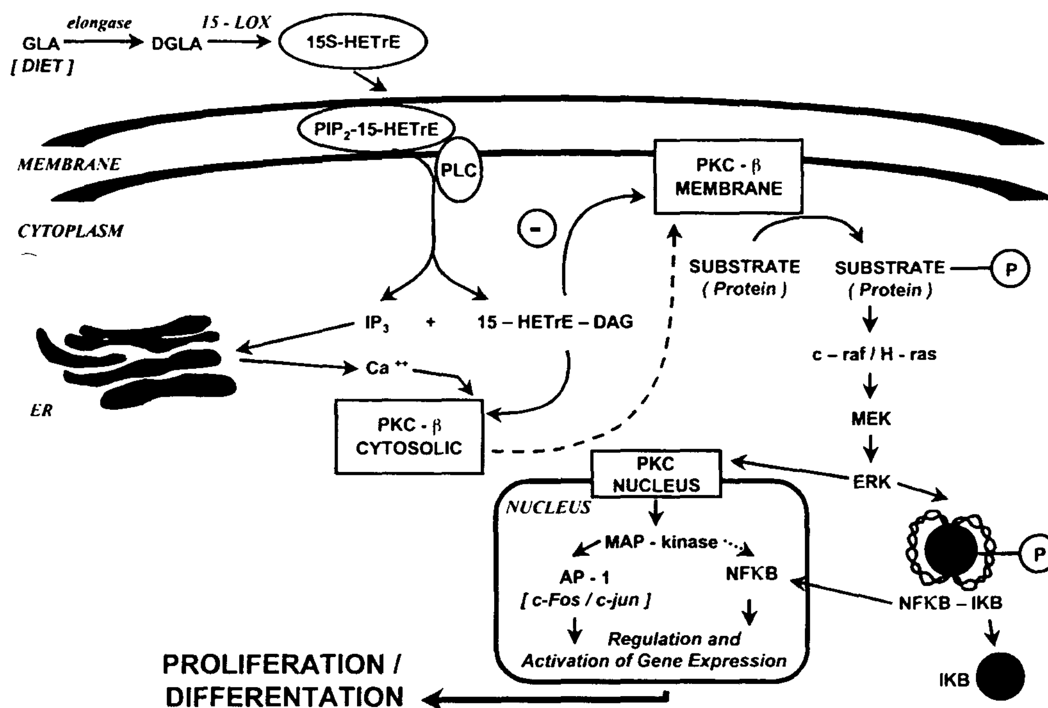

Fig. 5 A speculative scenario of GLA/DGLA/15S-HETrE-induced modulation of epidermal proliferation via nuclear PKC/MAP-kinase/transcription factors

activator of PKC and activation of PKC was associated with epidermal hyperproliferation (Cho and Ziboh, 1995), inhibition of PKC expression and modulations of subsequent events including nuclear transcription and apoptosis by GLA/DGLA/15S-HETrE may be a plausible explanation for the reversal of epidermal hyperproliferation. The present results add to our understanding of the mechanisms of how dietary nutrients can be involved in the suppression of skin hyperproliferation. Although additional studies remain to be done, these results provide information for exploring future novel antiproliferative signaling pathways via nuclear transcription factors and apoptosis.

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