

4

Gamma Linolenic Acid Oils

Rakesh Kapoor and Harikumar Nair

*Bioriginal Food & Science Corp.
Saskatoon, Saskatchewan, Canada*

1. INTRODUCTION

Essential fatty acids (EFAs) can be defined by classic definition, which defines EFAs as the fatty acids that are required for proper functioning of cells, but the body cannot synthesize them and, therefore, must be supplied by diet. According to this definition, there are only two EFAs: linolenic acid (LA, C18:2, n-6) and alpha-linolenic acid (ALA, C18:3, n-3). The functional definition of EFAs includes the fatty acids that can correct the symptoms produced by elimination of all EFAs from the diet. According to this definition, LA, gamma linolenic acid (GLA, C18:3, n-6), and arachidonic acid (AA, C20:4, n-6) are EFAs of n-6 family (1, 2).

Gamma linolenic acid (*cis*-6, *cis*-9, *cis*-12-octadecatrienoic acid) is an 18-carbon polyunsaturated fatty acid containing three double bonds. It is produced in the body from desaturation of LA by the reaction catalyzed by enzyme delta-6-desaturase (D-6-D) (Figure 1). GLA is rapidly elongated to DGLA by elongase enzyme. Cats do not have this enzyme; hence, they cannot synthesize GLA and subsequent metabolites from LA (3). Therefore, cats must eat a meat-based diet to obtain longer chain metabolites of LA (DGLA, AA). DGLA can be acetylated and incorporated into membrane phospholipids. A small amount can be converted into AA, and this

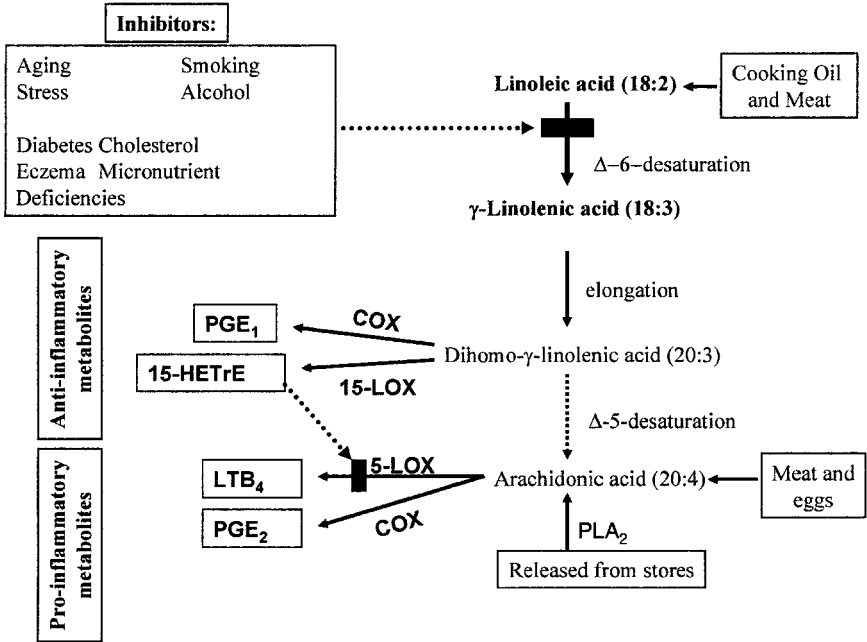


Figure 1. Metabolic pathway for linoleic acid. COX, Cyclooxygenase, LOX, Lipoxygenase, PGE₁, Prostaglandin E₁, PGE₂, Prostaglandin E₂, PGL₂, Prostaglandin I₂, TXA₂, Thromboxane A₂, 15-HETrE, 15-hydroxy eicosatrienoic acid, LTB₄, Leukotriene B₄.

reaction is catalyzed by delta-5-desaturase enzyme. Different animal species and different tissues differ in their capacity to convert DGLA to AA. Rat metabolizes DGLA to AA in significant amounts, whereas humans and other species have limited capacity to form AA from DGLA.

The reaction catalyzed by delta-6-desaturase enzyme is the slowest reaction in the metabolic pathway of LA and is considered as a rate-limiting step (4, 5). Activity of this enzyme further decreases with age and in people suffering from various diseases, including arthritis, diabetes, hypertension, eczema, psoriasis, and so on. Lifestyle factors like stress, smoking, excessive consumption of alcohol, linoleic acid (6), saturated and *trans*-fatty acids and nutritional deficiencies of Vitamin B6, zinc (7), and magnesium inhibit this desaturase. As a result of limitations in *in vivo* production of GLA, supplementation with preformed GLA is becoming important. This has led to interest in development and commercialization of the sources of GLA.

2. SOURCES OF GLA

GLA is present in small amounts in many plants belonging to the families Aceraceae, Boraginaceae, Cannabinaceae, Liliaceae, Onagraceae, Ranunculaceae,

Saxifragaceae, and Scrophulariaceae. Kleiman et al. (8) investigated 29 species of family Boraginaceae for the presence of GLA and tetraenoic (stearidonic acid, SDA) fatty acid. They observed 0–27% GLA, 0–56% ALA, and 0–17% SDA in seed oils from different plants in Boraginaceae. Janick et al. (9) identified 32 plants in which the content of GLA in seed oil can be more than 5% weight/weight (w/w) of total fatty acids (Table 1). The important crops that have been commercialized as sources of GLA-rich oils are discussed below.

TABLE 1. Selected Plant Species High in Gamma-Linolenic Acid.

Family Genus and Species	Oil Content of Seed (%)	GLA Content (%) of Oil	of Seed
Boraginaceae			
<i>Adelocaryum coelestinum</i>	22.0	12.0	2.7
<i>Alkanna orientalis</i>	23.0	12.0	2.8
<i>Anchusa azurea</i>	21.0	13.0	2.7
<i>Anchusa capensis</i>	29.0	10.0	2.9
<i>Anchusa hybrida</i>	20.0	13.0	2.6
<i>Borago officinalis</i>	28–38	17–25	5.0–8.4
<i>Brunnera orientalis</i>	27.0	15.0	4.2
<i>Cerintho minor</i>	10.0	10.0	1.0
<i>Cynoglossum amabile</i>	23.0	11.0	2.5
<i>Cynoglossum lanceolatum</i>	25.0	13.0	3.3
<i>Echium rubrum</i>	15.0	14.0	2.1
<i>Echium vulgare</i>	22.0	11.0	2.4
<i>Gastrocatyle hispida</i>	28.0	16.0	4.5
<i>Lithospermum arvense</i>	17.0	14.0	2.4
<i>Lithospermum purpureocaeruleum</i>	14.0	18.0	2.5
<i>Moltkia aurea</i>	10.0	10.0	1.0
<i>Moltkia coerulea</i>	10.0	11.0	1.1
<i>Nonea macrosperma</i>	39.0	13.0	5.1
<i>Onosma sericeum</i>	20.0	13.0	2.6
<i>Onosmodium molle</i>	17.0	20.0	3.4
<i>Onosmodium occidentale</i>	17.0	18.0	3.1
<i>Paracarum caelestinum</i>	21.0	12.0	2.5
<i>Pectocarpa platycarpa</i>	15.0	15.0	2.3
<i>Symphaticum officinale</i>	21.0	27.0	5.6
Cannabaceae			
<i>Cannabis sativa</i>	38.0	3–6	1.1–2.3
Onagraceae			
<i>Oenothera biennis</i>	17–25	7–10	1.2–2.5
<i>Oenothera grandifolia</i>	4.0	9.3	0.3
Saxifragaceae			
<i>Ribes alpinum</i>	19.0	9.0	1.7
<i>Ribes nigrum</i>	30.0	15–19	4.6–5.8
<i>Ribes rubrum</i>	25.0	4–6	1.0–1.5
<i>Ribes uva-crispa</i>	18.0	10–12	1.8–2.2
Scrophulariaceae			
<i>Scrophularia marilandica</i>	38.0	10.0	3.6

2.1. Borage (*Borago Officinalis* L.)

Borage is also known as star flower because of the shape of its flowers. Borage is the only member of the Boraginaceae family that is being grown commercially for its seeds at present. It is an annual herb native to Europe, Asia Minor, and North America. The morphology of this plant has been reviewed in many publications (9–11). It is an erect, hispid plant that can grow up to 100 cm in height. It has indeterminate vegetative growth habits that pose a significant challenge in commercial cultivation and harvesting of this plant. The plant has simple alternate leaves that are obovate, ovate, or oblong with an obtuse apex and create margin. The upper surface of leaves is dark-to-medium green, whereas the lower surface is light green. The stem is cylindrical, hollow, succulent, and occasionally susceptible to lodging. Stems, leaves, and calyx are covered with white, stiff, unicellular trichomes that can cause contact dermatitis to susceptible people. Flowers are bright blue, violet, pink, or white and are star shaped, hence, the other name for borage—star flower. Ovary is four lobed, and as the flower matures, it develops into 3–4 ovoid or oblong seeds, also known as nutlets. As they mature, they change color from green to brown to black and abscise rapidly. Flowering continues over a long period of time, and at any time, the plant bears the flowers and seeds in different stages of maturity. These factors pose a significant challenge in commercial production and harvesting.

Borage is mainly grown in the United Kingdom, Holland, Canada, New Zealand, and Poland, and it is estimated that about 95% of the world crop of borage is grown in these five countries. Borage is still not a major crop as its cultivation is very labor intensive. Most of the production of borage is under contract with oil producers with buy-back arrangement for seeds, and the production data regarding tonnage and acreage is not reported in any of the commercial publications. The acreage for borage grew consistently between 1980 and 1999 from a few hundred acres to about 7000 ha. There was an excellent harvest in 1999 in Canada, and the demand flattened leading to an over supply. This situation led to very limited production between 1999 and 2002 (unpublished information supplied by Bioriginal Food & Science Corp.). In 2002, the cultivation of borage increased again, but the severe drought in many of the borage-growing areas reduced the yield (unpublished information, Bioriginal Food & Science Corp.). Foliage is not used commercially any more, although in the folklore, it was used by early Europeans as a salad or vegetable and as a decoction for the treatment of various disease conditions.

As a result of seed drop and the continuous and long period of flowering and seed maturation, the seed yield is variable and a significant amount of potential crop is lost. The yields vary between 100 and 300 kg/ha in Canada and New Zealand and 300 and 500 kg/ha in the United Kingdom. The difference between Canada and Europe is probably caused by climatic conditions as borage prefers cool, moist conditions.

A limited amount of work has been done on optimization of cultivation, harvesting conditions, and plant variety development for borage and is reviewed by Clough (12) and Janik et al. (9) Because of the semiperennial nature of the crop and the seed drop, swathing and combining is the preferred harvest method. Simpson

(13) concluded from his studies that substantially higher yields could be obtained by swathng borage than by desiccating it with diquat or glyphosate. El Hafid et al. (14) studied the effect of seeding dates and nitrogen fertilization effects on borage in Alberta, Canada. In this case, early planting resulted in significantly higher seed yield and harvest index. Nitrogen fertility levels had no significant effect on the seed yield. The major objectives of borage variety development are to improve yield through development of varieties that are seed retentive on maturation and mature evenly. Other traits of interest include high oil content and high GLA content and morphological and yield stability. There is significant variability between the wild and cultivated borage accessions in terms of the amount of GLA and other fatty acid components as a percentage of oil indicating the possibility of developing lines with better quality parameters with additional breeding efforts (15). Hoffman La Roche has registered two varieties of borage (Tyreman and Spruce), but neither of these varieties are seed retentive. Bioriginal Food and Science Corp. has registered varieties of borage that have better seed retention compared with traditional borage.

From studies done on the *in vitro* propagation and somatic embryology (16–18) in borage, it appears that it is possible to produce GLA from cotyledonary somatic embryos. However, the authors observed that the system needs to be adapted to liquid media to facilitate large-scale production.

2.1.1. Chemistry The average weight of seed is between 16.1 and 24.5 mg (18). The oil content varies between 28% and 37% (w/w) and consists chiefly of linoleic (C18:2, 34–38%), GLA (C18:3, n-6; 16–27%), and oleic (C18:1, 14–18%) acids. The variation in GLA content is a result of geographical location, length of light period during growing season, average temperature, and diurnal temperature variations. There is no relationship between content of LA and GLA, although an inverse relationship is reported for content of 18:1 and GLA.

The average fatty acid composition of several seed lots over years, as provided by Bioriginal Food & Science Corp (unpublished data) and published information, is reported in Table 2. It has been observed that geographical location only affects the content of GLA and has no effect on the oil content of seeds, whereas plant density has no effect on the content of GLA (19). Oil also contains minor components that are characteristic of all vegetable oils and include sterols, tocopherols, and pigments. Borage oil contains about 650 ppm of γ - and 50 ppm of δ -tocopherols (20). The content of unsaponifiable fraction is dependent on the method of extraction of oil and the degree of refining and varies between 0.8% and 1.5%. The relative proportion of different sterols in borage oil is independent of the method of refining. The major proportion of sterols in borage oil belongs to 4-desmethylsterols, although small amounts of 4-monomethyl and 4,4-dimethylsterols are also present. Campesterol and sitosterol constitute more than 50% of 4-desmethylsterols (21). Major sterols belonging to 4-monomethylsterol family include gramisterol, obtusifoliol, and citrostadienol (21).

Like other members of the Boraginaceae family, the Borage plant also contains pyrrolizidine alkaloids. Seven pyrrolizidine alkaloids have been identified so far in

TABLE 2. Average Composition of Commercial Borage Crops.

Crop Year	2002	1999	1998	1997	1996
Sample size	17	87	71	53	43
Moisture (%)	10.39	10.54	8.90	9.80	8.80
Oil (%)	31.16	31.40	30.60	30.70	32.40
Free Fatty acid	1.23	1.34	1.70	2.40	1.50
Fatty Acid Profile					
C16:0	9.9	10.6	10.2	10.3	10.4
C 16:1	0.3	0.3	0.2	0.2	0.2
C18:0	4.1	4.3	3.3	3.5	3.6
C18:1	17.7	17.6	14.8	14.7	14.3
C18:2	36.7	35.7	37.9	37.6	37.3
C18:3 (n-3)	0.3	0.2	0.2	0.2	0.2
C18:3 (n-6)	22.4	21.6	24.6	24.1	24.9
C18:4	0.2	0.2	0.2	0.1	0.2
C20:0	0.3	0.3	0.2	0.1	0.2
C20:1	4.1	4.1	3.9	3.9	3.9
C20:2	0.2	0.2	0.2	0.2	0.2
C22:0	0.2	0.2	0.1	0.1	0.1
C22:1	2.6	2.7	2.6	2.5	2.5
C24:1	1.7	1.7	1.5	1.5	1.5

borage leaves, flowers, and seeds. Thesinine, a saturated pyrrolizidine alkaloid, is the major alkaloid, and the six unsaturated pyrrolizidine alkaloids identified so far include amabiline, supinine, lycopsamine, interemedine, acetyllycopsamine, and acetylinteremedine and are minor constituents. The total alkaloid content of the plant was reported to be less than 0.001%, whereas mature seeds yield about 0.03% crude alkaloids (22). Herrman et al. (23) reported the presence of thesinine as a glycoside (Thesinine-4'-O- β -D-glucoside) in seeds. As borage oil is an item of commerce for its content of GLA, there is a concern about the content of pyrrolizidine alkaloids in the oil. Published research to date could not detect the presence of pyrrolizidine alkaloids in borage oil. Dodson and Stermitz (22) used a method with a detection limit of 5 ppm, whereas Parvais and Stricht (24) employed a method with a detection limit of 0.1 ppm. These authors reported the absence of pyrrolizidine alkaloids in the oil samples tested. Mierendroff et al. (25) developed a method of detection of pyrrolizidine alkaloids at a limit of 4 ppb. Employing Mierendroff's method at an independent testing lab in Germany, Bioriginal tested several lots of borage oil over several years and could never detect any traces of pyrrolizidine alkaloids at such a low detection limit. The German Health Authority has limited the intake of unsaturated pyrrolizidine alkaloids to 1 μ g per day. Based on the above results, and assuming that borage oil contains PAs at a level of 4 ppb, one may have to consume more than 250 capsules (1000 mg each) every day to get a total of 1- μ g pyrrolizidine alkaloids. Based on this analysis, there is no likelihood of toxicity from pyrrolizidine alkaloids from borage oil ingestion.

2.2. Evening Primrose (*Oenothera biennis* L.)

Evening primrose of commerce consists of three species of family Onagraceae: *Oenothera biennis*, *O. lamarckiana*, and *O. parviflora*. It is native to North America and is now commonly found in many temperate zones around the world. Evening primrose thrives in open areas, especially in dunes and sandy soil. Evening primrose is grown commercially for its seed oil. China has become the major supplier of evening primrose seed and oil to world markets because it has the lowest cost of production and is estimated to supply about 90% of world supply of oil. Until 1985, it was mainly collected from wildy growing plants; however, increase in demand justified the commercial production. In the wild, seeds exhibit physiological dormancy, whereby they may cause problems in cultivation as the plant density depends on the conditions after seeds are sown into the ground, of which the farmer has no control. Seeds may lay dormant for a long period of time (up to several years in the wild). Seeds can be forced by cold stratification. Germination is equal in light and dark. Plant also flowers over a period of 2–3 months, with 2–4 flowers at any time, and seed maturation takes place in pods that split when ripe and shatters seeds on the ground. New varieties of evening primrose have been developed over the last two decades that germinate within 3 weeks under test conditions and retain pods intact on the plant. Seeds are small, angular, and dark brown, and 1000 seeds may weigh between 0.2 and 0.6 g and each pod may contain up to 180 seeds. Each plant can have up to 250 pods.

Some of the species of evening primrose plant, including *O. biennis* and *O. lamarckiana*, are very unique and are challenging for their unique genetics. They do not obey Mendelian genetics and have been a subject of study for over 100 years. These species differ from common plants in that, during meiosis, the chromosomes do not pair up but form a circle by joining end to end. This prevents reshuffling of genes through translocation. The plants are self-pollinating, but inbreeding does not occur because of the presence of a set of lethal genes.

2.2.1. Chemistry Evening primrose plant is cultivated for its seeds that contain about 16% protein and 23–28% fat. The oil is rich in linoleic acid (70–75%) and gamma-linolenic acid (8–14%). The average fatty acid content of commercial evening primrose oils is given in Table 3. The varieties containing up to 14% GLA have been developed (26) but not yet commercialized on a large scale, as the majority of evening primrose oil in trade contains between 9% and 10.5% GLA. The oil contains about 98% triacylglycerols, small amounts of other lipids (free fatty acids, diacylglycerols), and 1–2% unsaponifiable matter consisting chiefly of sterols. Sterols contain about 90% β -sitosterol, 5% citrastadienol, 1.5% gramisterol, and 1% obtusifoliol. It also contains traces of α -, γ -, and δ -tocopherols (27). Evening primrose also contains traces of phenolic compounds consisting of catechin, (-)-epicatechin, and gallic acid (28).

TABLE 3. Average Fatty Acid Profile of Oils of Evening Primrose, Black Currant, and Hemp.

Fatty Acid	Evening Primrose Oil	Black Currant Oil	Hemp Oil
C16:0	5.98	7.10	5.60
C16:1	0.05	0.10	0.10
C18:0	1.84	1.50	2.60
C18:1	7.20	10.90	11.50
C18:2	73.87	45.20	56.60
C18:3 (n-3)	0.28	13.20	18.50
C18:3 (n-6)	9.74	16.90	1.60
C18:4	0.07	3.30	0.50
C20:0	0.30	0.10	0.90
C20:1	0.19	0.80	0.60
C22:0	0.10	0.10	0.30
C22:1		0.20	
C24:0	0.04		0.10

2.3. Black Currant

Black currant (*Ribes nigrum*) belongs to the family Saxifragaceae. The seeds are byproducts of the fruit processing industry that mainly uses black currant juice for jam, jellies, and cordial. In the early 1900s, cultivation of any *Ribes* species in the United States was prohibited by a federal ban, as they are the alternative host of white pine blister rust, a problem for all five-needle pines and the lumber industry. The ban was rescinded in 1966, but several states continue to ban the cultivation of *Ribes* species. Limited commercial acreage, the short harvest season (from mid-June until mid-July), and limited access to fruit for commercial processing has prohibited widespread distribution in North America. For oil extraction, seeds and pomace left as residue from food processing industry is used to extract the oil. Black currant oil contains about 13–17% GLA, 10–14% ALA, and 2–4% SA. The average composition of black currant oil is given in Table 3.

2.4. Hemp

Hemp (*Cannabis sativa* L., family Cannabinaceae) is a fast-growing annual herbaceous plant that is well suited to the temperate climates. The largest supply of the world hemp production comes from China and Eastern Europe. Hemp is mainly used for fiber, although there is a long history of food use. As a source of GLA, it has recently become popular. The hemp contains psychoactive substances (cannabinoids), because of which its trade was restricted. Fiber hemp that is traditionally grown for fiber contains less than 0.3% delta-9 tetrahydrocannabinol (THC) and is classified as low THC hemp. However, no THC is present in the seed or seed oil. Seed contains 30–35% oil, of which 2.5% to 3.6% is gamma-linolenic acid and 15–20% alpha-linolenic acid (29). The average composition of the oil is given in Table 3.

When grown as a seed crop, a considerably lower seeding rate of 15–30 kg/ha is used as compared with a fiber crop. As hemp is a dioecious crop with male and female plants, for adequate seed set, approximately 3–5% of the plants should be male to ensure pollination (30). A few popular French monoecious varieties are also available in the market. Seed yields can vary greatly depending on the variety used and growing conditions but averages around 500 kg/ha and can be as high as 1200 kg/ha. In the northern climates, soil temperature should be above 10°C when planting. Although, the once growing vigorously hemp is a good competitor and will suppress most weeds, early establishment is a must to reduce weed pressure. Pesticides are generally considered not necessary to grow hemp. Crop length is between 3 and 4 months, depending on variety. Breeding and development work on this crop has been limited, and the movement of certified seed is highly regulated and expensive. Traditional breeding has concentrated on developing varieties with superior fiber yield. Thus, there is potential to improve seed yields domestically through breeding programs.

2.5. Echium

Echium has not been commercialized to any significant level because of regulatory requirements for registration. The genus *Echium* contains about 30 species distributed across Europe, the Mediterranean region, Madeira, the Canaries, and the Azores. The plants grow in the wild and are cultivated in home gardens as flowering plants. *Echium plantagineum* L. has been discovered to contain significant amounts of GLA, ALA, and stearidonic acid in seed lipids. *Echium plantagineum* is also known by the common names of Purple Vipers Bugloss, Paterson's Curse, and Salvation Jane. Agricultural production of this species is largely limited to Eastern and parts of Western Europe at present. Trials with *Echium* production in Canada have had reasonable success.

Echium plantagineum is an erect biennial 20–60 cm high, softly hairy, with one or many flowering stems. The basal leaves are ovate with prominent lateral veins and soft appressed setae. The cauline leaves are oblong to lanceolate, the uppermost being more or less cordate at the base. Inflorescence is usually branched. Calyx is 7–10 mm at anthesis, and up to 15 mm in fruit. Corolla is 18–30 mm long, infundibuliform is blue becoming pink through purple, and is hairy on veins and margins only. Two stamens are exerted from corolla tube, the remaining stamens are included or only slightly exerted, and the stigmas are distinctly bifid.

As a crop, echium is a spring-sown annual with crop duration of 3 to 3.5 months. It requires warm, sunny conditions for quick establishment, which helps in terms of early weed competition. The best oil quality is maintained when crop-growing temperatures are around 25°C. Well-worked medium-textured soils are preferable with adequate moisture. Seed bed should be prepared to a firm, fine, moist tilth, and the seed should be planted 1–2 cm deep at the seeding rate of 3–5 kg/ha. Weed control is an issue, and as a minor crop, no herbicides are currently registered for this crop. Pre-emergence weed control might be necessary in weedy fields to reduce the weed pressure during establishment. No significant problems have been reported on this

crop yet. Yields are highly variable, however, and usually average 300 kg/ha. Very little breeding work has been done to date on this crop, and no varietal information is available at this time.

Echium plantagineum occurs over significant areas of farmland in Australia (31). The young growth is eaten readily by livestock. The plant is considered a weed in good pastures, whereas on the poor land, it is considered a reserve fodder (32). The level of pyrrolizidine alkaloids is normally between 0.1% and 0.3% of the dry weight of the whole plant, but levels as high as 0.9% have been reported (33). Field evidence strongly indicates that horses, pigs, and, to a lesser extent, sheep are all affected. They are mainly cultivated as ornamental flowers. However, John K. Kings and Sons, Ltd. and Croda started a program on commercial cultivation of *E. platigenium* because of the presence of GLA and stearidonic acid (SA) in the seeds (unpublished information).

Echium oil is mainly composed of α -linolenic (30–33%), linoleic (14–18%), γ -linolenic (10–13%), stearidonic (13–15%), oleic (14–17%), and palmitic (6–7%) acids. Like other vegetable oils, echium oil contains between 0.6% and 1.8% unsaponifiable matter. The oil samples analyzed by Bioriginal Food & Science Corp. showed an average content of 0.91% total unsaponifiable matter, campesterol was 15.71%, beta-sitosterol was 12.53%, stigmasterol was 0.55%, and others were 33.52%. Tocopherols constituted 8.37% of total unsaponifiable matter and consisted of alpha- (0.53%), gamma- (6.92%), and delta-(0.92%) tocopherols. The fatty acid profile of echium oil is given in Table 3.

Echium seeds, like other members of family boraginaceae, contain pyrrolizidine alkaloids. The seeds contain echimidine as a major alkaloid and many minor alkaloids, including retronecine, lycopsamine, 7-acetyllycopsamine, and their derivatives.

3. EXTRACTION OF OIL

Of the above discussed sources of GLA, the commercial oils are mainly produced from borage and evening primrose. Black currant oil is limited as a result of availability of seeds for oil production, whereas hemp is still subject to trade restrictions in many countries because of the potential tetrahydrocannabinoid (THC) content. Echium is a new crop and has not been commercialized to any significant extent so far. All of these seeds are processed following general methods of oil extraction common in the vegetable oil industry as discussed here.

Borage, hemp, and black currant seeds have oil content in the range of 26–40% (on 8% moisture basis); they are best suited to mechanical pressing followed by solvent extraction. In North America, mechanically expressed borage oil is a major item of commerce, constituting about 80–90% of total sales of borage oil, whereas in Europe, solvent-extracted oil is the major item of commerce. Mechanically expressed oil is sold to a limited extent in the European markets, although it is gaining popularity. Evening primrose seeds are small and hard and contain 18–20% oil. Therefore, they are difficult to expeller press. Black currant seeds, being a byproduct

of the food industry, are usually associated with pomace and must be separated. The separation of pomace and seed can be done either by washing with alcohol or special cleaning processes. Washing with alcohol is not very common because of cost and environmental regulations.

3.1. Seed Cleaning

Seed cleaning is essential to protect the quality of the oil because seeds, as obtained from the farm, are contaminated with weed seeds, other grain seeds, and extraneous matter. These contaminating weed seeds may impart undesirable flavors and may negatively affect the stability of the oil. Seeds can be cleaned on the farm, but commercial seed-cleaning plants are used by most oil producers. The seed cleaning process involves aspiration of dust and lighter materials, followed by two-stage screening to remove larger and smaller sized particles. Cleaning is done to reduce the extraneous matter to less than 1.0%.

3.2. Expeller Pressing

The clean seed is stored in silos from where it is either conveyed to the screw-press or to a cooker, where they are conditioned by heating to about 50–90°C. The conditioning helps by improving the oil yield and inactivates the enzymes (lipase) that can affect the quality of the oil. Preheated seeds are conveyed to a continuous screw-press where they are crushed between a stationary cage (barrel) and rotating screw as they move forward. The pressure built up during their forward move causes oil to be released. The screw-press is similar to those used for other seeds, including canola and soybeans. The major manufacturers of these screw-presses include Anderson International, French Oil Mills, Krupp, and DeSmet. There are many smaller manufacturers of screw-presses whose equipment is better suited for small output plants common for GLA-containing oils. The oil is pumped to storage tanks and contains between 4% and 8% fines coming from the seed. The oil is clarified either by decantation or filtration. The press-cake so obtained contains between 12% and 18% oil and is either used as animal feed or subjected to solvent extraction to recover the remaining oil.

3.3. Solvent Extraction

The majority of evening primrose, black currant, and some borage seeds are extracted using this method. Prior to solvent extraction, the seed must be crushed or flaked to rupture the cell walls, enabling better extraction efficiency at a lower energy cost. The combination of expeller pressing and solvent extraction is a common practice for GLA-rich oils. The press-cake obtained from expeller press may be extruded or used as is. Flaking is commonly done for evening primrose seeds using a smooth roller mill. Food-grade hexane is the solvent of choice, although some work has been done replacing hexane with alcohol. Alcohol contains about 5% water. During the desolventization process, alcohol is removed first, leaving

water in the oil. Removal of water from the oil adds to the energy cost and adds additional steps in the processing, further complicating the process. The majority of the GLA oil producers are small and low-volume entities. They often use a batch process employing either percolation or immersion. Continuous process is also used but poses processing challenges in that the plant has to be optimized to produce different oils, as processing one type of seed will not be able to sustain the plant because of low-volume requirements. In either process, the cake or flaked seeds first come in contact with a solvent rich in oil (miscella) followed by an oil-poor solvent and the last stage, with a pure solvent.

3.4. Supercritical Extraction

Supercritical extraction using carbon dioxide under high pressure is also becoming popular for GLA-rich oils; however, it is not being used to any major extent because of the high cost of the plant and the oil obtained by this technology. Some work is being done on optimization with respect to operating conditions (34). Major emphasis is on the flow rate of carbon dioxide, pressure, and temperature to optimize the yield. The particle size of the seed pieces and the moisture content also play a role in extraction efficiency. It is reported that the lower the moisture content, the better the yield. The supercritical extraction usually results in an oil with similar fatty acid composition when compared with solvent extracted oil, but the oil is low in sterol content and may be more prone to oxidation. The biggest advantage of supercritical extraction is that it eliminates the need for further processing of oil such as distillation for desolventization, degumming, and so on.

3.5. Desolventization

The oil-solvent mixture and the meal is stripped of the solvent to recover solvent-free oil and meal. The solvent-enriched meal is conveyed to vertical desolventizer where heat and vacuum facilitate removal of solvent vapors. Desolventizer contains trays with sweeping arms to agitate the meal for improved efficiency. Some plants purge the cake with steam to remove the solvent, whereas others use hot air, although application of vacuum is most common. The solvent oil miscella are stripped of solvent in a three-stage evaporator. The hexane is reused for the extraction of oil.

3.6. Further Processing

The desolventized as well as expeller-pressed oil is further processed to reduce/remove the pigments and phosphatides (gums). Crude oils may contain 1–2% phosphatides, which are removed by the degumming process. The degumming process is similar to that employed in the vegetable oil industry and uses water, citric acid, phosphoric acid or a combination of acid, and water. After the oil is contacted with these agents, the phosphatides settle as sludge and are removed by either filtration or centrifugation.

The water degumming process involves addition of about 2% water to the oil, intensive mixing under vacuum at 80°C for 10–30 min, and filtration/centrifugation. Water degumming removes most of the hydratable phosphatides, leaving behind between 50 and 200 mg/kg of phosphorous depending on the extraction conditions employed. Acid degumming using a combination of citric acid or phosphoric acid with water also removes nonhydratable phosphatides. In this process, oil is heated to 60–80°C, and 0.1–0.4% citric acid or phosphoric acid is added with intense mixing for 1–5 min. To this mixture, 2% water is added and mixing is continued for 30–60 min. After contact with water, the oil is clarified of the precipitated gums by centrifugation or filtration using clays.

Degummed oil is further purified by physical or chemical refining. Alkali refining is rarely used in oil for the health food/dietary supplement industry, although it may be used for oil for cosmetic/pharmaceutical applications. In processing of oil for the health food/dietary supplement industry, water degumming and bleaching processes may be combined when the oil is heated with water and citric or phosphoric acid with activated bleaching clay to 80°C in a vacuum reactor. The mixture is intensely agitated under vacuum for 30–60 min. During this time, the gums are precipitated and are adsorbed onto the bleaching clay along with pigments and chloroplasts. The oil mixture is cooled and filtered to remove gums, and pigments. The resulting bleached oil has a lighter color and a phosphorous content of less than 50 ppm. As these oils also contain wax esters and other compounds that may settle with time at room temperature and are collectively called waxes, they are subjected to the winterization process where they are chilled to 4°C and filtered to remove the waxes. The oil for the dietary supplement industry is not winterized. Finally, the oil may be subjected to steam stripping (deodorization). In this process, the oil is steam distilled to remove free fatty acids and other volatile impurities. This is the last process in the refining of oils, and the oil is then packed in drums or totes under nitrogen atmosphere. The oil must be stored in a cool dry place, tightly packed in the container under nitrogen atmosphere to protect against oxidation.

3.7. Quality Control

The GLA-containing oils are used for nutritional and health-promoting or disease-preventive actions. They must be of high quality and free from contaminants. The quality of oil is dependent on many factors, including seed quality and purity, herbicide and pesticide residues in seed, processing and storage conditions for seed and oil, and so on. Improper storage and drying of seeds can raise the free fatty acid levels in seed that can result in off flavors in the oil. Being a polyunsaturated fatty acid, GLA is prone to oxidation. The oxidation process for GLA-rich oils involves addition of an oxygen atom at the double bond in unsaturated fatty acids leading to formation of hydroperoxides. These hydroperoxides are unstable and decompose to form aldehydes and ketones. These oxidation products not only impart off flavors to the oil, making it unacceptable organoleptically, but also may have adverse health effects. The quality of the oil is tested by checking for peroxide value, an indicator of primary oxidation product. Oil with a peroxide value of less than

TABLE 4. General Specifications of GLA-Rich Oils.

Parameter	Units	Value		
Peroxide Value	meq/kg	<5		
Anisidine Value		<15		
Acid Value (unrefined oils)	mg KOH/g	<4		
Acid Value (refined oils)	mg KOH/g	<0.7		
Unsaponifiable matter	%	<2		
Pesticides/Herbicides	mg/kg	<0.05		
Solvent residues	ppm	<1.0		
Color (Lovibond 1 inch)		<3 red		
Heavy metals	ppm	<10		
Lead	ppm	<0.1		
Mercury	ppm	<0.1		
Cadmium	ppm	<0.1		
Arsenic	ppm	<0.1		
	Evening			
Major Fatty Acid (% of total fatty acids)	Primrose Oil	Borage Oil	Black Currant Oil	
Oleic acid (C18:1)	6–9%	14–22%	9–15%	
Linoleic acid (C18:2)	70–77%	32–38%	40–50%	
Gamma Linolenic acid (C18:3, n-6)	8–12%	18–25%	15–19%	
Alpha Linolenic acid (C18:3, n-3)	0.1–1.0%	0.1–2.0%	12–15%	
Stearidonic acid (C18:4)	0.1–0.3%	0.1–0.3%	2–5%	

10 milliequivalent of KOH/kg oil is considered good for consumption. Peroxide value alone is not a good indicator of oxidative stability of oil as it measures the primary oxidation products, which degrade to secondary oxidation product, including aldehydes and ketones. These secondary oxidation products can be measured by several methods, including conjugated dienes, anisidine value, and so on. In addition to oxidative stability indices, the oils are tested for fatty acid profile to ensure the quality and purity of the oil. The presence of free fatty acids is tested by acid value. The free fatty acid content of the oil should be as low as possible. These oils are also tested for heavy metal contamination. The total content of heavy metal should be less than 10 ppm. The oils should be free of any herbicide or pesticide residues and the solvent used in extraction of oil. The quality parameters for these oils are listed in Table 4.

4. METABOLISM OF GLA

When GLA-rich oils are taken orally, GLA is rapidly absorbed. It first appears in serum phospholipids, and with continuous administration, it is distributed in other phospholipid fractions. Part of absorbed GLA is oxidized, and the rest is taken up by various tissues/cells and is rapidly elongated to dihomogammalinolenic acid (DGLA) (Figure 1). The oxidation rate of GLA was found to be 28% of that for

LA (35). DGLA can be acetylated and incorporated into membrane phospholipids, or it can be desaturated to AA by delta-5-desaturase. DGLA competes with AA for cyclooxygenase (COX) and lipoxygenase (LOX) enzymes. DGLA produced prostaglandins of series 1 (PGE₁) and thromboxane A₁ (TxA₁) by the action of COX. These products of COX action exert anti-inflammatory, vasodilatory, and anti-aggregatory actions. DGLA produces 15-hydroxyeicosatrienoic acid (15-HETrE) by the action of 15-lipoxygenase. 15-HETrE is a strong inhibitor of 5-lipoxygenase, whereby it inhibits production of leukotriene B₄ (LTB₄) from inflammatory cells, including neutrophils (36).

Hassam et al. (37) were the first to study the absorption and metabolism of GLA using ¹⁴C-labeled GLA in rats. They observed accumulation of labeled DGLA and AA in brain and liver after 22 hours of administration, suggesting that GLA is rapidly metabolized to DGLA and AA. Leyton et al. (35) reported that DGLA is preferentially incorporated in liver phosphoacylglycerols, mainly in choline and inositol phosphoacylglycerols. Feeding a GLA-rich diet to rats caused accumulation of DGLA in milk (38) and a rise in DGLA and AA in aorta and platelets (39) and in immune cells, including macrophages, kupfer cells, and endothelial cells (40, 41). Barre et al. (42) observed a rise in GLA and DGLA with no change in AA levels in different platelet phospholipid fractions in human volunteers following daily administration of 5.23-g GLA from borage oil for 42 days. There was a differential distribution of DGLA in various phospholipid fractions with phosphatidylcholine had maximal (67.6%) followed by phosphatidylethanolamine (16.7%), phosphatidylserine (12.9%), and phosphatidylinositol (2.6%). There was no change in sphingomyelin. In all phospholipid fractions, the ratio of DGLA/AA decreased significantly. In a later study, they observed a rise in GLA and DGLA levels in phosphatidylcholine fraction of plasma HDL and cholesteryl esters. AA levels increased only in phosphatidylcholine fraction of HDL (43). In these studies, the dose of GLA employed is much higher than used in any of the clinical trials. The difference in the observed rise in platelet AA levels after feeding of GLA sources in above studies is caused by species difference. Rat platelets have delta-5-desaturase enzyme required for conversion of DGLA to AA, whereas human platelets lack this enzyme and obtain preformed AA from the circulation.

In one study on six healthy volunteers, time of administration of GLA-rich oil was found to affect the peak serum levels of GLA (44). Administration of evening primrose oil (equivalent to 240-mg GLA) in the evening caused a rapid peak in serum levels (2.7 ± 1.2 hours) compared with administration in the morning (4.4 ± 1.9 hours). There was a small but insignificant increase in serum DGLA and AA levels. In this study, the second dose of evening primrose oil was given 12 hours after the morning dose. This might have contributed to the observed rapid rise in peak serum GLA levels, or this could reflect faster absorption of GLA in the evening. A rapid rise in plasma triacylglycerols level after the second meal has been observed. Manku et al. (45) studied the effect of feeding evening primrose oil (containing GLA) for a period of 10 days to 12 weeks on plasma fatty acid levels. In this study, they collected the blood samples of 392 individuals who were part of 20 different studies. In all of these studies, DGLA levels in plasma phospholipids were

increased significantly. In 17 of these studies, there was a small but significant rise in phospholipid AA levels, whereas in 3 studies, there was no rise, and in 2 of these studies, there was a fall in AA levels. In these 3 studies, EPO was administered for 10 days only. In all of the studies, the ratio of AA/DGLA fell, suggesting a greater rise in DGLA levels. These results indicate that, in humans, DGLA is slowly desaturated to AA. Feeding borage oil for 7 weeks to normotensive (WKY) and spontaneously hypertensive (SHR) rats resulted in an increase in GLA and DGLA levels in plasma, liver, aorta, and renal artery in both strains of rats, although AA was increased only in plasma and liver (46). These observations indicate that there is a tissue-specific rise in AA after administration of GLA.

From the above discussion, it is clear that GLA is rapidly absorbed and elongated to DGLA. DGLA levels increase in most of the tissues after GLA administration, but the levels of AA rise to a smaller extent mainly in the liver. The capacity of other tissues to desaturate DGLA is limited and depends on the species. Chilton et al. (47) studied the effect of in-vivo administration of GLA and in vitro incubation of human neutrophils with GLA on metabolism of GLA. They observed that in vivo administration of GLA to humans caused an increase in DGLA in the neutrophils and no GLA was detected. Incubation of neutrophils with GLA resulted in a rise in the DGLA concentration of neutrophils. Stimulation of these neutrophils with ionophore A23187 caused a release of AA and DGLA from neutrophil phospholipids. DGLA was metabolized to 15-HETrE that inhibited LTB₄ production with an IC₅₀ of 5 μM.

4.1. Effect of Triacylglycerol Structure on Bioavailability of GLA

Major sources of GLA include borage oil, evening primrose oil, and fungal oils. GLA is mainly distributed at *sn*-2 position in triacylglycerols in borage oil, at *sn*-3 position in black currant and evening primrose oils, and at *sn*-1 and *sn*-3 positions in fungal oils (48). Evening primrose oil was reported to provide higher levels of GLA compared with borage oil in rats, although the latter oil contains a higher amount of GLA/g. This was a surprising finding and attributed to positional differences for GLA in the triacylglycerol structure and the inability of gastric and pancreatic lipases to hydrolyze fatty acids at *sn*-2 position. The fatty acid in the *sn*-2 position of triacylglycerols is preferentially absorbed as the 2-monoacylglycerol and serves as the template for re-esterification by intestinal cells to reform triacylglycerols. The *sn*-2 fatty acids are also preferentially preserved as components of chylomicrons and very-low-density lipoprotein particles for ultimate incorporation in tissue membranes. Subsequently, Raederstroff and Moser (49) repeated the studies in rats and failed to reproduce similar results. They observed that the levels of GLA and DGLA in liver, aortic, and erythrocyte phospholipids reflected the amount of GLA present in the source oil. This indicated that different oils were well absorbed and that the amount of GLA absorbed was dose dependent, and the source of GLA did not matter. To further resolve this matter, Chung et al. (50) studied the efficacy of borage oil, evening primrose oil, or a combination of borage oil with

safflower oil to match the GLA content to evening primrose oil in reversing the epidermal hyperproliferation induced by essential fatty acid deficient diets. In this study, they observed that GLA-rich diets reversed epidermal hyperproliferation caused by essential fatty acid deficiency and the potency order was borage oil greater than borage-safflower oil combination, greater than evening primrose oil. Finally, two diets had similar amounts of GLA, but they differed in the structural location of GLA on triacylglycerol molecule. There were higher levels of DGLA in epidermal phospholipids and ceramides on the borage oil or the borage oil and safflower oil diet than from the evening primrose oil diet. They proposed that borage oil, being richest in GLA at *sn*-2 position, is more bioavailable; hence, borage oil was more potent. Higher bioavailability of GLA at *sn*-2 position is also supported by data from other laboratories studying the effect of triacylglycerol structure on fat digestion and absorption. During digestion, gastric and pancreatic lipases hydrolyze fatty acids at *sn*-1 and *sn*-3 position forming free fatty acids and *sn*-2 monoacylglycerols. The absorption of free fatty acids is reduced in the presence of divalent ions (calcium and magnesium) because of soap formation, whereas *sn*-2 monoacylglycerols are favorably absorbed. The differences in the results obtained by these two groups [Chung et al. (50) and Raederstroff and Moser (49)] could be caused by species differences, or by a difference in study design, as Chung et al. (50) performed the studies in the essential-fatty-acid-deficient guinea pigs, or by tissue differences.

5. CARDIOVASCULAR EFFECTS

Cardiovascular disease is a major cause of mortality and morbidity in industrialized countries. Several risk factors have been linked to incidence of cardiovascular disease and include hypertension, lipid abnormalities (high plasma cholesterol and triacylglycerol levels), atherosclerosis, obesity, diabetes, smoking, stress, heredity, and diet. Dietary GLA affects many of these parameters and is discussed below.

5.1. Effect on Blood Pressure

Arterial blood pressure is regulated by the interaction of cardiac output and peripheral vascular resistance. Several factors can influence these interactions, and they can include renin-angiotensin system, local metabolic factors, stress hormones, and so on. Interventions that interfere with these modulators can affect the blood pressure regulation. In 1975, Rose et al. (51) observed a biphasic response of intravenously administered DGLA on systemic arterial pressure in dogs that was characterized by an initial fall in blood pressure followed by a sustained fall and an increase in myocardial contractility. Only the sustained fall in blood pressure was blocked by cyclooxygenase inhibition, whereas the early fall in blood pressure and positive inotropic effects were not affected, suggesting that DGLA causes a blood pressure-lowering effect directly and through PGE₁ pathways. In 1982, evening primrose oil was shown to inhibit the blood pressure-increasing activity of

intravenously administered renin and angiotensin II in rats given evening primrose oil for 3 months (52). This observation suggested that GLA-rich oils may reduce the blood pressure by interfering with the renin-angiotensin system in the body. GLA-inhibited isolation (psychological) stress-induced rise in blood pressure in rats when administered at a dose of 0.018 or 0.04 mg/hour via an osmotic pump (53). In the unstressed rats, there was no effect of GLA on blood pressure. No effect on heart rate, heart weight, or adrenal weight was observed in any animal. Mills et al. (54) repeated the experiments on humans to observe if GLA has similar actions on stress reactivity and performance. They selected 30 normotensive male university students for the study and divided into various groups. One group ($n = 10$) received olive oil capsules for 28 days, and another group received borage oil capsules ($n = 10$) providing 1.3-g GLA per day. These volunteers were given Stroop color word conflict test before commencement of supplement therapy and after 28 days of supplementation. Borage oil supplementation significantly reduced the stress-induced rise in systolic blood pressure and heart rate and did not affect diastolic blood pressure or plasma norepinephrine levels. Borage oil treatment increased the skin temperature and the performance as compared by number of correct responses. These data confirm the observations obtained earlier in rats and indicate increased tissue perfusion by borage oil treatment. Leng et al. (55) also observed a blood pressure-lowering effect in patients with peripheral arterial diseases. In their study, they used a combination of GLA with EPA, so the probable contribution of EPA to blood pressure-lowering effect cannot be entirely ruled out.

The exact mechanism of blood pressure-lowering effect is not very clear, and GLA-rich oils appear to act via several mechanisms. Borage (56) and evening primrose oils (57) were shown to reduce *in vivo* pressor responses to angiotensin-II and norepinephrine without affecting *in vitro* contractile response of aorta to potassium chloride and serotonin in rats. These observations suggest that GLA may be interfering with agonist-receptor interactions without affecting the contractility of vascular smooth muscles. Subsequent studies in spontaneously hypertensive rats demonstrated the blood pressure-lowering effect of borage oil (58) without affecting the pressor response to angiotensin and norepinephrine, suggesting the role of other mechanisms. These findings suggest that there may be a species difference in responsiveness to angiotensin II and norepinephrine, although the blood pressure-lowering effect was similar in magnitude. GLA was shown to prevent development of hypertension in SHR rats (59), which could have been mediated via the cyclooxygenase pathway as an increase in aortic levels of 6-keto $\text{PGF}_{1\alpha}$ was observed. In hypertensive rats, GLA was shown to significantly reduce the ratio of plasma aldosterone to renin that was caused by a insignificant decrease in plasma aldosterone levels and a small increase in plasma renin activity (60). There was no effect of borage oil treatment on plasma cortisol levels compared with rats fed control diet free of GLA. Borage oil treatments also reduced angiotensin receptor number and affinity in SHR rats, suggesting a reduction in the responsiveness of adrenal glomerulosa cells to angiotensin and interference with the renin-angiotensin-aldosterone axis might contribute to the hypotensive effects. These studies cannot alienate the exact mechanism by which borage oil interferes with angiotensin receptors.

Mills et al. (61) studied the effects of dietary borage oil on baroreflexes in normotensive, healthy males. These males were subjected to lower body negative pressure of -10 and -40 mm Hg. A negative pressure of -10 mm Hg unloads cardiopulmonary baroreceptors, whereas the negative pressure of -40 mm Hg unloads both cardiopulmonary and arterial baroreceptors. They observed that borage oil treatment augmented the baroreflex response to -40 mm Hg without affecting the response to -10 -mm Hg negative pressure, suggesting that GLA may be affecting only high-pressure arterial baroreflex responses. This could be mediated either by altering the sensitivity of baroreceptor stimulus-response relationship or by shifting the operating point of the reflex to a much steeper point on the baroreceptor stimulus-response relationship curve. In human hypertension, baroreceptor responses are decreased, which may be contributing to structural changes in hypertensive patients.

5.2. Platelet Function and Plasma Lipids

Increased levels of plasma triacylglycerols and cholesterol and platelet dysfunction (increased aggregation) are independent risk factors for cardiovascular disease. The effects of GLA on blood lipids and platelet function are controversial. Chaintreuil et al. observed a fall in serum triacylglycerols and cholesterol levels in insulin-dependent diabetic patients administered 2 g/day GLA, but not with 500 mg daily dose for 6 weeks (62, 63). In hypertriglyceridemic patients, GLA had no effect on plasma triacylglycerol levels or platelet function, although there was an increase in GLA and DGLA levels in plasma and platelet phospholipids (64). Viikari et al. (65) also failed to observe the lipid-lowering effect of evening primrose oil in hyperlipidemic subjects in an open study. They continued administration of evening primrose oil for 3 months but changed the dose every month from 2.4 ml (first month) to 7.2 ml (third month). They observed a rise in GLA levels in serum cholesteryl esters, phospholipids, and triacylglycerols. The differences in the results of above studies could be attributed to dose differences. Guivernau et al. (66) fed GLA at a dose of 240 mg/day for 12 weeks to 12 hypertriacylglycerolmic patients and 12 rats. They observed a significant decrease in plasma triacylglycerols, total cholesterol, and LDL cholesterol and an increase in HDL-cholesterol. Reactivity of platelets to low doses of adenosine diphosphate and epinephrine was significantly reduced. A reduction in plasma thromboxane B₂ levels was also observed in humans. In rats, a rise in plasma 6-keto-PGF_{1 α} levels was observed, suggesting an increase in PGE₁ production by GLA administration. Changes in eicosanoids may contribute to the observed effects of GLA on platelet aggregation as thromboxane B₂ is a potent platelet aggregator. GLA is rapidly metabolized to DGLA, and DGLA has been shown to inhibit platelet aggregation in *in vitro* (67) and *in vivo* studies (68, 69).

Ishikawa et al. (70), in a double-blind, cross-over trial in hypercholesterolemic patients, demonstrated that GLA lowered low-density lipoprotein cholesterol and apolipoprotein B in plasma and increased HDLC levels without affecting the levels of total cholesterol. Jantti et al. (71) observed a decrease in plasma

apolipoprotein B concentrations in rheumatoid arthritis patients given evening primrose oil at a dose of 20 ml (about 1.8 g GLA) per day for 12 weeks. In this trial, no effect on plasma triacylglycerols or total or high-density lipoprotein cholesterol was observed. Horrobin and Manku (72) found that evening primrose oil exerted cholesterol-lowering effects in people with plasma cholesterol levels above 5 mmol/l but had no effect in people having plasma cholesterol levels lower than 5 mmol/l. Fukushima et al. (73) fed conventional diets enriched with 10% borage oil, palm oil, perilla oil, evening primrose oil or mixed oils, and 0.5% cholesterol for 15-week to 8-week-old rats. GLA-rich diets lowered plasma total cholesterol and the sum of LDL, IDL, and VLDL cholesterol. Cholesterol-lowering effects of a GLA-rich diet could be mediated by changes in membrane lipid composition affecting absorption of cholesterol. This observation is confirmed by Koba et al. (74) in Cacao cells. When these cells were incubated with GLA, the absorption of cholesterol from the growth medium was attenuated and the cell membranes were enriched with GLA, DGLA, and AA.

5.3. Atherosclerosis

Atherosclerosis is the most common cause of morbidity and mortality in patients with cardiovascular diseases. The exact cause of atherosclerosis is not clear. Atherosclerosis is a culmination of several events, including vascular dysfunction, which may be caused by an injury to vasculature, recruitment of inflammatory cells including monocytes and neutrophils, activation of macrophages, vascular smooth muscle cell proliferation, deposition of lipids, and synthesis of extracellular matrix. Oxidized low-density lipoprotein cholesterol plays a role in initiation of atherogenesis. It stimulates monocytes with the resultant formation of foam cells. These cells release mediators that stimulate expression of adhesion molecules like cadherin, vcam, and so on. Macrophages, on stimulation, release eicosanoids and cytokines that may stimulate proliferation of vascular smooth muscle cells. Proliferation of vascular smooth muscle cells appears to be a central event in atherogenesis. Essential fatty acids are substrate for the production of eicosanoids, and the membrane composition of inflammatory cells reflects dietary intake of various fatty acids. It appears that dietary manipulation of the composition of cell membranes is the easiest target to control atherogenesis. Renaud et al. (75) demonstrated that dietary polyunsaturated fatty acids, including GLA, reduced severity of atherosclerotic lesion in rabbits compared with saturated-fatty-acid-rich diets. In Japanese quail, dietary primrose oil was shown to inhibit atherogenesis (76). Fan et al. (77) observed inhibitory action of dietary evening primrose oil either alone and in combination with fish oil on aortic smooth muscle cell proliferative action of peritoneal macrophages from mice. The inhibitory action appeared to be mediated through cyclooxygenase pathway as indomethacin (cyclooxygenase inhibitor)-inhibited PGE₁ release and antiproliferative actions. Addition of 5-lipoxygenase inhibitor to the culture medium had no effect on antiproliferative or DNA synthesis inhibitory actions of primrose oil. In vitro incubation of endothelial cells with PUFAs, including GLA, AA, ALA, EPA, or DHA, stimulated the oxidation of LDL and

metabolism of oxidized LDL by macrophages (78). These interventions also increased the release of superoxide anions by endothelial cells. These observations suggest pro-atherosclerotic actions of PUFAs in humans. In apolipoprotein E knockout mice, evening primrose oil inhibited aortic smooth muscle cell proliferation and reduced the aortic vessel wall medial layer thickness and the size of atherosclerotic lesion (79). This study confirms the beneficial effects of GLA in lowering cardiovascular risks by inhibiting atherosclerotic plaque development.

5.4. Cardiac Arrhythmia

Several studies have demonstrated that LA (present in vegetable oils) exert antiarrhythmic activity in several models, including ischemic-reperfusion injury and catechol-induced arrhythmias. Li et al. (80) observed that PGE₁ and PGI₂ exert antiarrhythmic activity in cultured, spontaneously beating neonatal rat cardiac myocytes, while PGD₂, PGE₂, PGF_{2α}, and TXA₂ exert proarrhythmic activity. Charnock et al. (81) studied the effects of evening primrose oil and black currant oil on ventricular fibrillation in rats induced by ischemia. They compared the effects of these two oils to sunflower oil (a source of LA) and sheep fat (saturated fat). They observed that, compared with the saturated fat group, all other dietary treatments significantly reduced the number of premature ventricular beats, however, there was no difference between the three PUFA groups. The effect on duration of ventricular fibrillation was dependent on diet with saturated fat showing the longest duration that was significantly reduced by the other three oils and the potency order of these three oils was sunflower oil < evening primrose oil < black currant oil. Evening primrose oil contains similar amounts of LA but additional amounts of GLA, suggesting GLA might have been playing an additional protective role. As black currant oil contains twice the amount of GLA and additional ALA, it is difficult to assess from this study if additional protection provided by black currant oil was caused by high amounts of GLA or synergistic action of GLA and ALA.

6. CANCER

Cancer is a collective term that defines a group of conditions caused by excessive growth of cells in any organ/tissue. It can occur in any part of the body. It is a complex phenomenon, the etiology of which is not very well understood. Risk of cancer increases with age, and about 77% of cancers are diagnosed in people after 55 years of age. Risk factors for cancer include lifestyle factors (diet, tobacco, excessive alcohol use, and physical inactivity), radiations, chemicals, infections, heredity (inherited mutations), immune conditions, obesity, and hormones. Heredity increases the predisposition to cancer but in itself is not responsible for initiation of cancer and requires interaction with other factors. About 5–10% of total cancers are hereditary because of inheritance of mutated gene. According to the American Cancer Society (<http://www.cancer.org/downloads/PRO/12>), about 1.37 million new cases of cancer are expected to be diagnosed in 2004. This estimate excludes

basal and squamous cell carcinoma of skin and carcinoma in situ of any site except urinary bladder. About 563,700 people are expected to die from cancer in the United States in 2004. Of these deaths, 170,000 deaths will be related to tobacco, and a similar number of deaths will be caused by nutrition, obesity, physical inactivity, and other lifestyle factors.

Basic treatment for cancer includes chemotherapy, radiations, and surgery. Strategies for prevention include modification of lifestyle factors and dietary interventions. The role of dietary fat in cancer is controversial. Many prospective studies found an increase in cancer risk (82–84), whereas others reported no association between fat intake and cancer (85–87).

GLA has been studied in several studies for its effects on various cancer cell lines in vitro. It has been observed to exert cytotoxic activities against several tumor cell lines in vitro and tumor implants in experimental animal models. There are limited studies on the effect of GLA on tumors in humans. In cell lines, the effect of GLA appears to depend on the cell line, dose, and incubation time. In a study by Dippenaar et al. (88), GLA caused significant (up to 70%) growth inhibitory effects on mouse BL6 melanoma cells in vitro at a dose of 20 $\mu\text{g/ml}$. At this dose, GLA did not affect the growth of normal bovine kidney epithelial MDBK cells, suggesting that GLA acts as an anticancer agent and inhibits the growth of cancer cells without affecting the normal cells. Human hepatoma cell lines differ in sensitivity to GLA as they require continuous presence of GLA in culture media for 4 days to observe growth inhibitory effects (88); withdrawal of GLA from the growth media after 5-day treatment suppressed the growth for 5 more days (89). This observation suggests that cancer cells may lack delta-6-desaturase and, hence, cannot make GLA and, subsequently, DGLA. Cancer cells incorporate GLA and DGLA in their cell membranes and DGLA may be acting via a cyclooxygenase pathway in inhibiting cancer cell growth as PGE_1 stimulates cyclic-AMP formation and induces cell death in cancer cell lines (90). In 1985, Begin et al. (91) confirmed that GLA has growth inhibitory actions against human prostate, breast, and lung cancer cells with no effect on normal cells.

Experiments were conducted to study the effects of GLA treatment on carcinogen-induced cancers in animals. Lee and Sugano (92) failed to observe any tumor inhibitory action of evening primrose oil in pathogen-free female Sprague Dawley rats in whom the tumor was induced by intragastric administration of 10 mg of 7,12-dimethylbenz(a)anthracene (DMBA) one week after animals were on experimental diets containing 5% evening primrose oil, sunflower oil, or palm oil. In another study, 50-day old female rats (Sprague Dawley) were given either 5 mg or 10 mg 7,12-dimethylbenz(a)anthracene (DMBA) intragastrically to induce mammary tumors (93). On 14-(5 mg DMBA rats) or 21-(10 mg DMBA rats) day post-DMBA administration, rats were divided into two groups and were fed a high-fat diet containing either 20% evening primrose oil or 20% corn oil (93). The group of rats on the evening primrose oil diet had significantly lower number of rats bearing tumors, and malignant tumors. Linoleic acid content of the primrose oil diet was higher than that of corn oil diet and linoleic acid has been linked to promote mammary tumorigenesis in rats and mice. The two diets differ in GLA only, which

suggests that GLA may be responsible for the tumor-inhibiting effects of the evening primrose oil diet. The different results of the above two studies (92, 93) can be due to differences in the dose of GLA given to rats. The other difference could be in the immune status of the rats, as Lee and Sugano conducted their studies on pathogen-free rats. Gonzalez et al. (94) performed a case controlled study in 4 regions of Spain investigating the association of dietary factors and risk of gastric cancer. Zaragoza is an area in Spain where people eat borage leaves and stem, usually cooked by boiling in water. After adjusting for intake of fruits and vegetables and caloric intake, a strong negative association was observed between risk of gastric cancer and borage intake. The negative association showed a strong dose-response effect, when the population was subdivided into quartiles. On analysis, they found that boiled borage leaves contained about 4.4% GLA, while boiled stems contained 14.6% GLA. This is the first study on association between dietary borage consumption and risk of gastric cancer. As very few populations are habitual borage eaters, it is difficult to repeat the studies and also this study cannot definitely link GLA as an anticancer agent in borage leaves and stems.

To confirm if the cytotoxic effects of GLA are, in fact, mediated by prostaglandin pathway, Botha et al. (95) cultured human breast carcinoma cell line NUB1 with 50 μ l GLA or DGLA and studied the effects on prostaglandin production and cell growth. They observed that GLA had inhibitory actions on NUB1 cell growth that were accompanied by an increase in production of prostaglandin E and F. On the other hand, DGLA caused a significantly higher increase in the level of these prostaglandins but had no effect on cell growth, indicating GLA exerts cancer-cell growth-inhibitory actions by some other mechanisms. Kenny et al. (96) co-administered 2.8 g GLA with 20 mg tamoxifen to 38 breast cancer patients. The control group consisted of 47 breast cancer patients on 20 mg tamoxifen only. They observed that GLA acted synergistically with tamoxifen in reducing the expression of estrogen receptors in tumor cells and enhanced the efficacy of tamoxifen. GLA + tamoxifen group of patients showed early response to therapy and had significantly better quality of life by 6 weeks on therapy. GLA treatment was well tolerated with 42% of patients reporting no side effect and a general feeling of well being, 34% of patients reported alterations in the bowel habits with a tendency towards loose stool (many elderly patients found this beneficial). In early responders, the GLA group had a much higher reduction in expression of estrogen receptors (ER) than tamoxifen alone. The GLA group also had downregulation of expression of bcl-2 gene at 6 weeks, compared with no effect or transient increase in bcl-2 protein in the tamoxifen group. As bcl-2 plays a role in prevention of apoptotic cell death, this observation suggests that, by reducing the expression of antiapoptotic protein, GLA stimulates apoptotic cell death in cancer cells, which may have contributed to faster response at 6 weeks.

GLA has been shown in experimental model of cancer to inhibit metastasis of cancer. Urokinase concentration is increased in malignant cancer cells, and it is reported to play a role in invasiveness and metastasis of cancer. du Toit et al. (97) studied the effect of GLA on urokinase activity. They observed that GLA is a competitive inhibitor of urokinase activity with a K_i value of 120 μ M. In a

subsequent study, they observed that GLA inhibited production of urokinase activity in human prostate tumor (DU-145) cells (98). These observations suggest that GLA, by inhibiting urokinase activity, may be playing a role in preventing metastasis of cancers. Jiang et al. (99) studied the effect of GLA on motility and invasiveness of three colon cancer cell lines (HT115, HT29, and HRT18) induced by hepatocyte growth factor. They observed GLA and its lithium salt reduced metastasis and invasiveness of all the cancer cell lines by upregulating expression of E-cadherin and inhibiting attachment of cancer cells to fibronectin without affecting fibronectin receptors. Dissociation of tumor cells from the main tumor is the first requirement for metastasis. By increasing the expression of E-cadherin, GLA increases the adhesiveness of tumor cells, so the incidence of metastasis is reduced. In subsequent studies, they further demonstrated reduced metastasis and increased adhesion of tumor cells that are E-cadherin negative (HT115 and MDA-MB 231) suggesting that other mechanisms play a role in reducing the invasiveness of cancer cells. They reported increased formation of desmosomes by increasing the expression of desmoglein. As desmosomes play a role in cell–cell adhesion, this observation indicates a role of GLA in preventing metastasis by increasing the adhesiveness of tumor cells so they fail to dissociate and, hence, metastasize. At the same time, GLA inhibits cell-matrix interaction and the exact mechanism is not clear. Integrins play a major role in cell-matrix interactions. GLA has been shown to inhibit this interaction at several stages by inhibiting focal adhesion kinase activation and paxilin activation. Both of these molecules are activated by tyrosine phosphorylation, which is inhibited by GLA in tumor cells. GLA also upregulates expression of metastasis suppressor nm-23 gene (100). A reduction in the level of nm-23 gene expression has been reported in various cancers, including colorectal, breast, liver, ovarian, and bladder cancers. These studies indicate that GLA may act on different targets at the gene level to reduce metastasis and invasiveness of cancers. Jiang et al. (101) demonstrated that GLA may be acting through activation of peroxisome proliferator activated receptor-gamma (PPAR- γ) through increased phosphorylation of these receptors. On phosphorylation, these receptors are translocated to the nuclear membranes and regulate the expression of various genes. They demonstrated that removal of PPAR- γ with antisense oligos abolished the effect of GLA on expression of adhesion molecules and tumor-suppressor genes.

6.1. Prostate Cancer

GLA has been shown to inhibit 5 α -reductase activity in androgen-sensitive (LNCaP) and androgen-insensitive (PC3) human-prostate cancer-cell lines (102). This observation may suggest that GLA could be acting as an anticancer agent against androgen-dependent prostate and skin cancers.

6.2. Glioma

Patient suffering from malignant cerebral glioma are treated aggressively with radiation, chemotherapy, and surgery, although surgery is the first option combined with

the other two treatments. The median survival time after aggressive treatment is about one year (103, 104). Naidu et al. (105) treated six patients suffering from histochemically confirmed malignant glioma with GLA. Of these patients, four patients received 1 mg GLA daily for 10 days, whereas the other two patients were treated only on alternate days. Treatment started 10 days after surgery; all these patients demonstrated marked necrosis of tumor immediately after the therapy. Of these six patients, three were alive after two years, whereas two were lost to follow-up and one died. No side effect of therapy was observed during or after treatment. During subsequent follow-up, authors did not observe any increase in size of residual tumor or recurrence of tumor. Based on the results of this study, authors extended the treatment to 15 more patients and found increased survival by one and one-half to two years. This study also confirmed necrosis of tumor cells and safety of GLA. They also injected GLA to normal dogs intracerebrally and found no cytotoxic effects (106). These studies demonstrated that GLA injected directly into tumor mass may potentially be useful treatment for malignant glioma.

6.3. Liver Cancer

Merve et al. (107) conducted a double-blind placebo-controlled trial of evening primrose oil in patients suffering from primary liver cancer, a fatal disease. The patients were randomly assigned to the GLA or placebo group. The GLA group patients received 36 capsules per day supplying 18 g evening primrose oil containing 1.44 g GLA. The control group received the same amount of olive oil. They observed a mean survival time of 58 days in the treatment group compared with 42 days in the placebo group, although the difference was not statistically significant. Gamma glutamyl transaminase enzyme activity was decreased in seven patients in the treatment group compared with two patients in the placebo group. This difference was statistically significant, suggesting that evening primrose oil may have some effect on tumor. In this study, patients had up to 3-kg tumor weight, suggesting an advanced stage of cancer. Probably, the dose of GLA was not sufficient to obtain a statistically significant effect on survival time. A major finding was that the quality of life was better for the evening primrose oil group as indicated by the patients self assessment. Falconer et al. (108) studied the effect of lithium salt of GLA on pancreatic cancer in 18 patients who had unresectable pancreatic cancer and had undergone either surgical bypass or had pancreas endoscopically stented. These patients were administered GLA intravenously for 10 days and then were switched to oral GLA therapy. During the infusion period, the dose of GLA was gradually increased for the first five days and then continued at maximal tolerated dose for a subsequent 5 days. Patients received a mean dose of 5.7g lithium GLA for the last 5 days and mean oral dose of 3 g afterwards. They observed a median survival of 8 months and 4 patients were still alive compared with normal life expectancy of 3–6 months for these patients. GLA treatment increased T-cell function and reduced TNF production. In this report, the study design was not well defined; therefore, it was difficult to assess if the protocol had any beneficial effect on patient survival, though the treatment was reported to be well tolerated.

6.4. Mechanism of Anticancer Effects of GLA

The exact mechanism of anticancer effects of GLA is not very clear and may depend on the cancer type. Many cancer cells have been shown to lack phospholipase A₂ (PLA₂) activity and delta-6-desaturase activity. PLA₂ is essential in releasing free fatty acids from the membrane phospholipids. Released free fatty acids, like DGLA, AA, EPA, etc, act as a substrate for cyclooxygenase and lipoxygenase enzyme to produce prostaglandins and leukotrienes. Delta-6-desaturase is essential for the conversion of dietary LA to GLA. Therefore, administration of GLA can bypass these metabolic steps and show anticancer effects. GLA, being a polyunsaturated fatty acid, can increase lipid peroxidation in the cancer cells. Free radicals have been implicated in cytotoxic actions of several anticancer drugs. It is possible that GLA may be showing its anticancer effects through oxidative mechanisms (109). Leaver et al. (110) analyzed the effect of GLA and AA on free radical production and cell death by necrosis and apoptosis in 30 human glioma types. The brain samples were obtained from patients undergoing surgery. Patients had grade 1 to grade 4 tumors. They observed that tumor cells in general produced less free radicals than normal cells and amongst the tumor cells, total free radical production was higher for advanced tumors (Grade 4). GLA and AA, both increased the production of free radicals in normal and tumor cells; however, tumor cells responded with a much higher increase in the production of free radicals and GLA was more potent than AA in increasing the free radical production in glioma cells. In this study, the necrotic cells produced less free radicals than nonnecrotic tumor cells and they showed a lower degree of rise in free radical production when incubated with GLA or AA. As necrotic cells are rich in phagocytic cells, this observation suggests that GLA or AA increase the production of free radicals in tumor cells mainly and the phagocytic cells are not the major source of free radical in gliomas incubated with GLA or AA. This observation also indicates that GLA is free from toxic effects on healthy cells in contrast to cancer therapeutics probably because it does not promote formation of free radicals from phagocytic cells that may release free radicals at several sites and damage healthy cells.

GLA could show its cancer-cell growth-inhibitory action by inhibiting cell proliferation or by increasing apoptotic cell death. de Kock et al. (111) demonstrated that GLA acts differently on human osteogenic sarcoma cells (MG-63 cells) and human epithelial cervix carcinoma cells (HeLa cells). In MG-63 cells, GLA-induced inhibition of mitosis was associated with abnormal metaphase cell spindle formation and inhibition of protein synthesis in G₁ and S-phase. HeLa cells respond differently, showing increased hypercondensation of chromosome, suggesting increased apoptotic cell death that was associated with increased protein synthesis for all of the G₁ proteins and selective S-phase proteins. In a subsequent study on HeLa cells, they further demonstrated that GLA inhibits MAP-kinase pathway and c-Jun expression. As c-jun is the transcription factor involved in cell proliferation and is activated by MAP-kinases, GLA is interfering with nuclear processes in inducing apoptosis in HeLa cells (112). Jiang et al. (113) observed a decrease in phosphorylation of p27^{kip1} and p57^{kip2} that are inhibitors of cyclin-dependent kinases

and play a role in progression of mitotic growth (progression from G1 to S phase). Decreased phosphorylation resulted in increased binding of these proteins to cyclin-dependent kinases including CDK4, cyclin E, and CDC2. Seegars et al. (114) studied the involvement of p53 protein in apoptotic cell death induction by GLA and AA in skin fibroblasts and lymphoblast cells containing wild type and mutant p53. They confirmed the earlier observations that normal cells are not affected by GLA to any appreciable extent. They also observed that AA was more toxic to normal cells than GLA, as GLA at much higher doses induced apoptosis in normal cells. Transformed cells were more susceptible to apoptotic cell death induction by GLA. The p53 does not appear to play a role in apoptosis induction by GLA as transformed cells containing wild type and mutant p53 responded to apoptosis induction by GLA.

7. IMMUNE FUNCTION AND AUTOIMMUNE DISEASES

Immune function is a very complex function that involves interplay of several cell types and humoral and cellular factors. Immune cells, including lymphocytes, polymorphonuclear leukocytes, monocytes, splenocytes, kuppfer cells, etc, have a high content of polyunsaturated fatty acids in their membrane phospholipids. The composition of PUFAs in membrane phospholipids can be altered by dietary interventions. GLA is taken up by inflammatory cells and is rapidly elongated to DGLA. In some species, it can be desaturated to AA but, in human immune cells, it is not desaturated probably because of, very limited to no delta-5-desaturase in immune cells. By the action of enzyme phospholipase A₂, free DGLA is released from the membrane phospholipids and competes with AA for cyclooxygenases and lipoxygenases. DGLA produces PGE₁ and thromboxane A₁ (TxA₁). The actions of PGE₁ have been reviewed in detail by Horrobin (115). It mainly exerts anti-inflammatory and vasodilatory properties. DGLA produces 15-hydroxyeicosatrienoic acid (15-HETrE) by the action of 15-lipoxygenase. This metabolite of DGLA is a strong inhibitor of 5-lipoxygenase whereby it inhibits production of leukotriene B₄ (LTB₄) from neutrophils (116). LTB₄ has a diverse array of inflammatory actions: It is a very potent chemotactic factor that attracts neutrophils at the site of inflammation, increases adherence of leukocytes to endothelial cells, enhances migration of T-lymphocytes in vitro stimulates release of interferon gamma and IL-2 production by T cells, and promotes the biosynthesis of IL-1 from monocytes. Thus, dietary administration of GLA-rich oils has a potential in modulating immune function. Several in vitro and in vivo studies have investigated the effect of GLA on immune functions.

Ziboh and Fletcher (117) observed a dose-dependent inhibition of calcium ionophore stimulated release of LTB₄ by human neutrophils obtained from healthy human volunteers fed either 0.48 or 1.5 g GLA per day for 6 weeks from borage oil. A linear relationship between rise in polymorphonuclear neutrophil (PMN) phospholipid DGLA and inhibition of LTB₄ production was not observed. Kaku et al. (118) observed inhibitory effects of dietary GLA on LTB₄ production by

rat peritoneal exudates cells. They also reported stimulation of immunoglobulin production from mesenteric lymph node leukocytes by GLA. This action may suggest that GLA may strengthen gut immune responses and may prevent allergic reactions.

Santoli and Zurier (119) studied the effect DGLA, AA, and EPA on mitogen-induced production of interleukin 2 (IL-2) by human peripheral blood mononuclear cells (PBMCs). They observed inhibition of IL-2 production by AA or DGLA in a dose-dependent manner. EPA showed inhibitory action in some donors only. Indomethacin, a cyclooxygenase inhibitor, caused an increase in IL-2 release and suppressed PGE release from PBMCs. It inhibited PGE release from fatty-acid-incubated PBMCs but did not attenuate IL-2 inhibitory action of fatty acids, suggesting that the suppressive effect of AA and DGLA on IL-2 release is not mediated through prostaglandin pathway. The inhibition of IL-2 release could be mediated by the effect of GLA and DGLA on early response genes, as both these fatty acids have been shown to reduce a rise in *c-fos* and a fall in *c-myc* oncogenes in T cells (120). DeMarco et al. (121) observed a reduction in IL-2-dependent proliferation of T-lymphocytes isolated from synovial tissue and synovial fluid from arthritic patients. Rotondo et al. (122) studied the effect of GLA, DGLA, AA, and EPA on IL-1-induced proliferation of thymic lymphocytes and observed that GLA was less potent than DGLA in inhibiting the IL-1-induced proliferation of lymphocytes. The actions of these fatty acids were not mediated through the prostaglandin pathway, as cyclooxygenase inhibitors had no effect on the actions of these fatty acids, which might exert a direct effect on lymphocytes. Rothman et al. (123) observed stimulation of production of IL-1 β in human peripheral mononuclear cells by DGLA. Incubation with LPS further stimulated the production of IL-1 β . Intracellular IL-1 β was entirely pro-IL-1 β . Incubation with DGLA also stimulated release of pro-IL-1 β and small amounts of mature IL-1 β . LPS failed to stimulate the further release of IL-1 β from PBMC. This could be due to the maturation of monocytes to macrophages during 16 hours of incubation. Mature macrophages are reported to release decreased amounts of IL-1 β in response to LPS stimulation. The observations of Rothman et al. (123) are contrary to expectations, as GLA and DGLA exert anti-inflammatory actions, one would expect a decrease in production and a release of IL-1 β . It could be caused by experimental design as they incubated the cells for 24 hours followed by incubation with LPS for 16 hours. DeLuca et al. (124) stimulated the PBMCs for 30 minutes followed by stimulation with LPS for 16 hours. They observed a dose-dependent decrease in LPS-induced release of IL-1 β and TNF α by GLA and DGLA. EPA also inhibited mediator release but required twice the amount. They observed a similar reduction in the release of these mediators when 2.4 g GLA was administered to human volunteers as a single dose.

A recent study by Furse et al. (125) demonstrated that LPS-stimulated IL-1 β release is further increased by IL-1, and this process is known as auto-induction. GLA inhibits IL-1 β release from LPS-stimulated monocytes mainly by inhibiting the auto-induction process. This information may suggest that GLA may be inhibiting excessive release of IL-1 β to prevent inflammation but may not interfere with basal release of IL-1 β , which plays a role in host defense.

GLA and DGLA inhibit protein kinase C (PKC) activity in PMA-stimulated T-lymphocytes. However, only GLA inhibited basal PKC activity. Both fatty acids stimulated translocation of PKC from cytosol to membrane (126). GLA and DGLA inhibited anti-CD3 monoclonal antibody induced early and late rise in intracellular calcium in T cells and also inhibited a rise in inositol-1,4,5-triphosphate (IP₃) production (127). Stimulation of T cells resulted in the formation of IP₃ and diacyl glycerol (DAG). IP₃ stimulates the early rise in intracellular calcium by releasing the calcium ions from intracellular stores (SR). DAG was found to stimulate the formation of PKC, which phosphorylates several proteins in the cells and plays a role in late rise in intracellular calcium. GLA and DGLA promote translocation of PKC to cellular membranes, whereby they may be inhibiting phosphatidylinositol turnover. These studies provide a strong support to the hypothesis that GLA and DGLA interfere with signal transduction pathways and exert antiproliferative actions on T cells, and these actions may mediate immune modulating and anti-inflammatory actions of these fatty acids.

Wu et al. (128) studied the effect of supplementation of black currant oil on immune function in healthy elderly volunteers. They isolated the mononuclear lymphocytes pre- and post-supplementation and studied the release of IL-1 β , IL-2, and PGE₂, and proliferation of lymphocytes in response to mitogens, including concanavalin A and phytohemagglutinin A (PHA). No effect of black currant oil administration was observed on lymphocyte proliferation in response to concanavalin A, but it increased in response to PHA. There was no effect on the release of IL-1 β and IL-2, while PGE₂ release was significantly decreased. Black currant oil supplementation also increased delayed type hypersensitivity (DTH) response as shown by the increase in the total diameter of induration at 24 hours and response to specific antigens (tetanus toxoid and *T. mentagrophides*). DTH response is depressed in aged populations and may contribute to increased mortality and morbidity. In this study, volunteers consumed 675-mg GLA and 653-mg ALA per day for 2 months. Therefore, it was not possible to ascribe the results to GLA only. Nerad et al. (129) demonstrated that administration of 2 g GLA for 12 weeks from borage oil to healthy volunteers caused an increase in total score of indurations, suggesting that the increase in induration observed by Wu et al. (128) may be contributed by GLA content of black currant oil. Immune enhancing activity observed in these studies could be contributed by a reduction in PGE₂, as it is well known inhibitor of lymphocyte proliferation and T-cell function. Zurier et al. (130) observed *in vitro* suppression of T-lymphocytes proliferation by GLA, DGLA, AA, and EPA. Of all fatty acids examined, GLA and DGLA were more potent than AA and EPA. In this study, preincubation of lymphocytes with fatty acids was required, but the continuous presence of GLA or DGLA was not needed for inhibition of proliferation suggesting that these fatty acids are incorporated in the membrane phospholipids of the cells and exert inhibitory actions on proliferation. Addition of these fatty acids along or after the addition of a stimulant has no effect on T-cell proliferation indicating that they interact at earlier stages of signal transduction, which leads to inhibition of proliferation. Thus, the fatty acids tested may reduce the stimulant-induced rise in cytosolic calcium that is required for proliferation.

7.1. Rheumatoid Arthritis

Rheumatoid arthritis is an autoimmune disease characterized by inflammation of the joints and cartilage destruction. Several studies discussed above have demonstrated anti-inflammatory potential of GLA-containing oils. These studies suggest that GLA-rich oils can be used to treat inflammatory conditions.

Tate et al. (131) demonstrated in rats that a GLA-rich diet can reduce the inflammation induced by injection of monosodium urate. GLA inhibited polymorphonuclear leukocyte recruitment, crystal phagocytosis, and lysosomal enzyme release. In a subsequent study, the authors demonstrated anti-inflammatory effects of GLA in Freund's adjuvant induced arthritis in rats (132). The anti-inflammatory effect of GLA was associated with inhibition of proliferation of pouch-lining cells and maintenance of architecture of these cells. Hansen et al. (133) administered 4 g of evening primrose oil supplying 360-mg GLA per day along with zinc, ascorbic acid, niacin, and pyridoxine to a group of 20 arthritis patients for 12 weeks. They did not observe any effect of treatment on several parameters of arthritis (number of tender and swollen joints, pain, erythrocyte sedimentation rate, and duration of morning stiffness). The failure of GLA to exert any beneficial effect could be caused by low dosage short duration of treatment. Belch et al. (134) studied the effect of 540-mg GLA or 450-mg GLA and 240-mg EPA per day on symptoms of arthritis and NSAID requirement. They continued the treatment for 12 months and observed that a significant number of patients had reduced requirements for NSAIDs at the end of 12 months. After 12 months, the treatment with GLA was stopped and three months after stopping the treatment, all the patients needed a full dose of NSAIDs, indicating that GLA or EPA had NSAID sparing effects and were not disease-modifying agents. In an open label clinical study, 1.1-g GLA given for 12 weeks reduced inflammation in arthritic patients and also reduced release of PGE₂, LTB₄, and LTC₄ (135). Laventhal et al. (136), in a randomized, placebo-controlled trial, observed that 1.4-g GLA given as borage oil for 6 months resulted in significant reduction in swollen joint count and score, tender joint count and score, and platelet counts. They also observed a 33% reduction in duration of morning stiffness. The only side effects of GLA treatment were belching, flatulence, and soft stools. Zurier et al. (137) repeated the trial by increasing the dose to 2.8 g of GLA. In this trial, the patients were randomized to receive either 2.8-g GLA/day from borage oil or placebo for 6 months and, after 6 months, all the patients were switched to GLA arm. Patients on GLA group at the end of 6 months showed reductions in swollen joint count and score, morning stiffness, and tender joint count and score. At the end of 12 months, the patients who started with GLA from the beginning continued to show improvement in their symptoms. Patients who started GLA after 6 months on placebo also started to improve. None of the patients in the GLA group experienced deterioration of condition in first 6 months, but, at the end of 12 months, two patients (out of 21) reported deterioration in condition. Seven of these 21 patients required a reduction in the dosage of nonsteroidal anti-inflammatory drugs or prednisone. Three months after stopping the treatment with borage oil, most of the patients showed exacerbation of disease condition suggesting that borage oil must be continued for relief of symptoms.

Overall, research with GLA-containing oils has shown that GLA provides benefit in the reduction of morning stiffness by about 73 minutes and exerts a NSAID-sparing effect. However, the dosage of GLA required for the treatment of arthritis is not well established as, in various studies, from 340-mg to 2.8-g GLA per day has been used.

7.2. Acute Respiratory Distress Syndrome

Acute respiratory distress syndrome (ARDS) is an acute, severe injury to the lungs. Patients with ARDS suffer from severe shortness of breath, requiring mechanical ventilation. It is associated with increased pulmonary capillary permeability, pulmonary edema, increased pulmonary vascular resistance, and progressive hypoxemia. ARDS can also lead to damage and failure of other organs. The exact cause of ARDS is not known but several factors can contribute, including chest trauma, sepsis, bacterial infections, and so on. At the cellular level, oxygen-free radicals, cytokines, and prostaglandins can play a role. Recent research is focused on dietary manipulations that help in reducing the inflammation and generation of pro-inflammatory mediators. Oxidative metabolism of AA results in the formation of pro-inflammatory mediators, including PGE₂, TXB₂, LTB₄, etc. LTB₄ is a potent chemotactic factor and attracts neutrophils and exacerbates the damage to tissues. GLA and its metabolic product DGLA counter the effects of AA by forming anti-inflammatory mediators, such as PGE₁ and 15-HETrE, and reduce the formation of AA-derived inflammatory mediators. Kumar et al. (138) observed a significant reduction in plasma phospholipid levels of GLA, DGLA, ALA, and EPA in patients at risk of developing ARDS while patients with established ARDS additionally had lower amounts of AA. This study suggests that treatment with GLA and EPA is warranted in these patients. Gadek et al. (139) conducted a multicenter double-blind, placebo-controlled clinical trial on patients with ARDS. The treatment group (51 patients) was administered a mixture of borage oil, fish oil (providing 5.8 ± 0.3-g GLA, 6.9 ± 0.3-g EPA, and 2.9 ± 0.1-g DHA per day), and antioxidants via gastric or jejunal tube. They observed a significant reduction in the number of total cells and neutrophils in bronchoalveolar fluid by day 4 compared with the fluid obtained from the control group. This was associated with improved arterial oxygenation. Patients in the treatment group had a lesser requirement for ventilator support, supplemental oxygen, and lower number of days of stay in ICU compared with patients in the control group. Significantly fewer patients in the treatment group developed new organ failure, and there was about a 17% reduction in the total number of infections in the treatment group. As this study used a combination of EPA and GLA with antioxidants, it is difficult to differentiate the effects of GLA alone, although this study provides a strong support for using a combination of EPA and GLA. Murray et al. (140) studied the effect of fish oil alone or in combination with borage oil on cardiac function in pigs during acute lung injury induced by infusion of *E. coli* endotoxin. They observed that fish oil or a fish oil and borage oil combination attenuated lung injury induced depression of cardiac function. A combination of fish oil and borage oil acted synergistically compared with fish

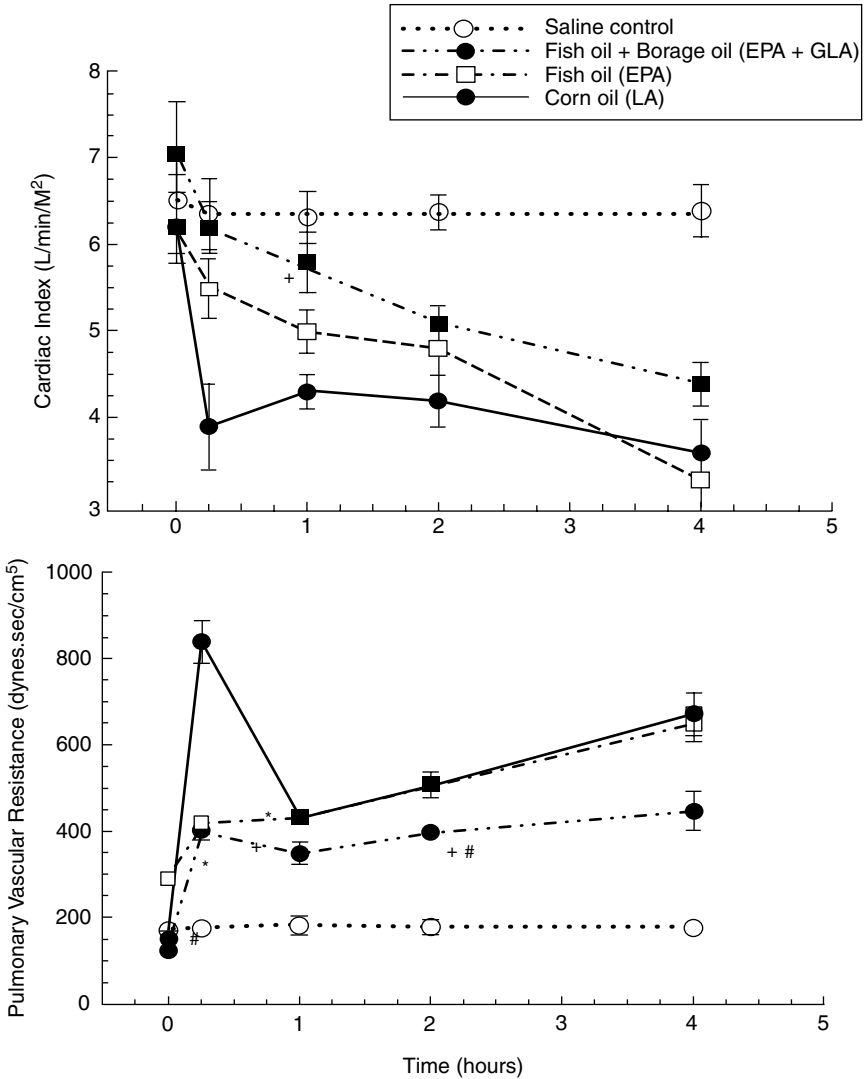


Figure 2. Effect of various treatments on cardiac index (upper panel) and pulmonary vascular resistance (lower panel). Data adapted from (140).

oil alone in attenuating the cardiac depression (Figure 2, Upper panel). A fish oil and borage oil combination had lower pulmonary vascular resistance during the 4-hour experiment duration than either the control group or the group given fish oil alone (Figure 2, Lower panel). The other interesting finding of this study was that GLA in combination with EPA prevented the loss of platelets from circulation, whereas EPA alone did not exert this effect. This observation indicates that GLA decreased the aggregatory and adhesive properties of platelets in vivo. A significant

reduction in the amount of TXB₂ (EPA, and EPA + GLA groups) and 6-keto prostaglandin F₁ α in the alveolar fluid in EPA + GLA group was also observed, suggesting that the beneficial effects of the treatment may be mediated by a reduction in the formation of pro-inflammatory and vasoconstrictor metabolites of AA. In a subsequent study, the administration of EPA or EPA+GLA to pigs altered the composition of pulmonary surfactant by reducing the concentration of oleic acid and increasing the concentration of DGLA, EPA, and DHA. However, there was no effect on the pulmonary compliance or surfactant function. Mancuso et al. (141) observed that a combination of fish oil and borage oil attenuated endotoxin induced rise in pulmonary microvascular protein permeability in rats, which was associated with a decrease in LTB₄, TxA₂, and PGE₂ production by pulmonary alveolar macrophages. Additionally, this treatment also attenuated endotoxin induced early and late hypotension.

8. SKIN CONDITIONS

Skin is the largest organ of the body and provides a barrier function, whereby it protects the inner organs from environmental toxins and bacteria. It also plays an important role in temperature regulation, sensory perception, and excretion. It undergoes constant renewal. Burr and Burr (142) observed that rats on a fat-free diet developed dried, scaly skin suggesting the role of fats in normal physiology of skin. Subsequent studies have demonstrated that skin contains essential fatty acids and is metabolically an active organ. It has the capability to elongate the fatty acids but lacks the capacity to desaturate. This information suggests that dermal cells take up preformed long-chain metabolites of LA (GLA, DGLA, and AA) and ALA (EPA, DPA, and DHA).

During EFA deficiency, the levels of LA, DGLA, and AA are reduced in the skin and may contribute to dry and scaly appearance of the skin, with the increase in epidermal water loss. In studies with EFA-deficient rats, mice, and guinea pigs, it has been demonstrated that skin undergoes hyperproliferation (acanthosis, hypergranulosis, and hyperkeratosis) with increased DNA synthesis. LA levels were significantly decreased with an increase in mead acid (20:3 n-9, abnormal fatty acid characteristic of EFA deficiency). Supplementing diets with a large dose of safflower oil (rich in LA) or much smaller dose of evening primrose oil (rich in LA and GLA) reversed the signs of EFA deficiency on skin, whereas fish oil failed to reverse these symptoms (143). In this study, a rise in EPA, DPA, and DHA levels in skin phospholipids was observed, but the levels of LA did not increase. This study also confirmed, by labeled fatty acid incubation, that skin lacks delta-6 and delta-5 desaturase activities, indicating that skin cannot metabolize LA or ALA.

8.1. Atopic Dermatitis

Atopic dermatitis, or eczema, is a skin disorder characterized by dry, itchy, and hypersensitive skin. It is common in children but can occur at any time and age.

The exact cause of eczema is not well understood and it can be hereditary. Soon after the discovery of essential fatty acids, it was observed that infant patients suffering from atopic eczema had low levels of LA and AA (144) and responded well to supplemental lard containing LA and AA (144). Very high doses of LA (20–50 g) provided partial relief from symptoms of eczema but failed to raise the levels of metabolites of LA in the blood. Later on, it was confirmed that the plasma phospholipids of adults suffering from atopic dermatitis had higher concentrations of LA and a lower concentration of GLA, DGLA, and AA (145), suggesting that atopic patients may suffer from defective delta-6-desaturation. These patients also failed to show flushing response to topically applied niacin, suggesting that they have defects in prostaglandin pathways and fail to produce vasodilatory prostaglandins. Subsequent studies have shown lower levels of DGLA in breast milk of atopic mothers than in the normal mothers (146, 147). As the breast-fed infants get their nutrient requirements from breast milk, they do not receive sufficient quantities of DGLA and may be prone to dermatitis.

Based on these observations, it seems logical that dietary GLA or DGLA should help prevent/treat atopic dermatitis. Wright and Burton (148) conducted a double-blind, placebo-controlled clinical trial of evening primrose oil. They recruited 60 adults and 39 children suffering from moderate to severe atopic dermatitis in the study. Adult patient groups received 4, 8, or 12 capsules daily, whereas children were given 2 or 4 capsules daily providing 45 mg GLA per capsule, and the placebo was liquid paraffin. Treatment was continued for 12 weeks. All of the patients had moderate to severe eczema. They observed that the lower dose of GLA only provided relief from itch, while the other two groups of adult patients on higher doses of GLA showed better improvements in itch, scaling, and the general impression of severity as assessed by the physician and the patient. Children in this study did not perform as well as the adults, possibly caused by either insufficient dose of GLA or high placebo effects in children. Manku et al. (149) analyzed the blood samples of adult patients from the above study for plasma phospholipid fatty acids. They observed that LA levels were higher in the atopic patients, and the scatter of values for LA was also very high. Levels of DGLA and AA were lower in these patients. Treatment with 4 capsules per day did not affect blood GLA or DGLA or plasma PGE₁ levels, whereas 8 and 12 capsules per day caused a significant elevation in the levels of DGLA and PGE₁. Schafer and Kragballe (150) observed that neutrophils and epidermis of atopic dermatitis patients have high levels of monounsaturated fatty acids (MUFAs), which correlated positively with the severity of disease, and lower ratios of n-6 PUFAs/MUFA. Feeding 6 g of evening primrose oil for 10 weeks increased the ratio of n-6 PUFA/MUFA and increased the levels of DGLA in neutrophils and epidermal phospholipids. They did not evaluate the effect of the treatment on dermatitis as the patients were allowed to use emollients; this study cannot shed any light on the efficacy of GLA in dermatitis. In another large multicenter study, 179 patients with eczema were treated with 4.0 g of evening primrose oil per day, and they demonstrated clinical improvements as evaluated by a dermatologist. Scarff and Lloyd (151) studied the effect of treatment with evening primrose oil in dogs suffering from dermatitis. In this study, the dogs

were on olive oil placebo for 3 weeks followed by either olive oil or evening primrose oil for 9 weeks. At the end of 9 weeks, the treatments were switched over without any wash out period. They observed a deterioration in condition during first 3 weeks on olive oil in all of the dogs; however, during the first treatment period, all dogs showed improvement that could be ascribed to placebo effect in the olive oil group. In the second treatment period, dogs on olive oil worsened, whereas those on evening primrose oil improved. They observed an interaction in the order of treatment with the evening primrose oil that could be caused by a change in treatment between active and placebo without any washout period. Fiocchi et al. (152) evaluated safety and efficacy in children suffering from atopic eczema. They treated these children (average age 11.4 months) with 3.0 g/day of GLA for 28 days. None of the children showed complete recovery, although gradual improvement in erythema, excoriations, and lichenification was reported. They also reported a significant reduction in itching and the use of antihistamines without observing any side effect because of the treatment. Borrek et al. (153) compared the effect of borage oil with corn oil in 24 subjects suffering from atopic eczema in a double-blind cross-over trial. The subjects were between 3 and 17 years old and received 360-mg GLA daily for 10–14 weeks. They did not observe any difference between the two groups, and the placebo treatment also showed improvements. In this study, 10 patients on borage oil treatment showed improvements but they did not differ from nonresponders in any of the characteristics (age, sex, symptom severity, etc.). As there was a large placebo effect, the effectiveness of borage oil may have been masked due to a small number of subjects in this study.

Eczematous skin also has high transepidermal water loss compared with normal skin. Hartop and Protty (154) observed that application of pure GLA triacylglycerol to rats with dry skin due to essential fatty acid deficiency reduced the transepidermal water loss. In essential fatty acid deficiency, there was a loss of LA, ALA, and their long-chain metabolites in plasma and other organs. However, the eczema differed from essential fatty acid deficiency in which there is usually an excess of LA with imbalance of longer chain metabolites. Tolleson and Frithz (155) studied the effects of topically applied borage oil on the transepidermal water loss from the skin of infants suffering from seborrheic dermatitis. They observed that topically applied borage oil relieved the symptoms of dermatitis within 3–4 weeks and also normalized the elevated transepidermal water loss. Topically applied borage oil also caused a rise in serum LA content, suggesting transdermal absorption of LA from borage oil. The site of application of borage oil was not important as borage oil in the napkin area of the infants also relieved the symptoms at other sites.

Henz et al. (156) evaluated the efficacy of borage oil in the treatment of atopic dermatitis in a double-blind, placebo-controlled, multicenter clinical trial. In this study, 160 patients with moderate eczema (Costa score between 20 and 36 points) were divided into two groups. The active group received 3.0 g borage oil (690 mg GLA) daily for 24 weeks and the placebo group received migliol, an oil containing no GLA. Patients were allowed to use a steroid cream during the trial. Some patients did not follow the guidelines and violated the conditions of protocol and included poor compliance (less than 70% of dose consumed; 7 patients on placebo,

6 on borage oil), excessive use of steroid cream (three times above median dose; 1 patient on placebo and 4 on borage oil), and less than 11 weeks of treatment (6 patients on each treatment), and patients with unstable disease (Costa score of less than 18 at week 2; 32 patients on placebo and 21 on borage). When all the patients, including those who did not follow the protocol, were included in the data analysis, no significant differences in Costa scores between the two groups was observed, although borage oil treatment improved erythema, vesiculation, crusting, excoriation, lichenification, and insomnia scores over placebo group. A marked reduction in serum IgE levels was observed, but the difference was statistically insignificant due to large intersubject variations. Borage oil treatment also increased plasma and erythrocyte levels of GLA and DGLA in the majority of patients. When the subgroup of patients who did not follow the protocol was excluded from the analysis, the borage oil treatment showed significant improvement on the reduction of steroid cream use. Borage oil was well tolerated with minor side effects (headache, nausea, vomiting, and diarrhea). The frequency of side effects was not different from that observed with the placebo treatment.

Takwale et al. (157) conducted a single-center, double-blind clinical trial to study the efficacy and tolerability of borage oil in the treatment of atopic eczema in children and adults. Adult patients were given 8 capsules of borage oil (supplying 920-mg GLA) and children were given 4 capsules (supplying 460 mg of GLA) daily for 12 weeks. Patients were allowed to continue using a steroid ointment for symptomatic relief. The efficacy of the treatment was evaluated from the change in total symptom score measured with the six-area, six-sign, atopic dermatitis (SASSAD) score as the primary endpoint. Secondary end points included symptom score assessment on visual analogue scales, topical corticosteroid requirement, and global assessment of response by participants. This study failed to observe any effect of borage oil treatment on eczema, although the treatment was safe, well tolerated, and was free from major side effects. This study suffers from several major limitations. They recruited 151 patients, of which 11 were lost at week two of the 12-week study. An additional 16 participants withdrew during the trial, leaving only 124 subjects who completed the trial. However, they analyzed the data for 140 patients, including those who did not complete the protocol. Good clinical trial demands inclusion of data only from those patients that follow the protocol. Noncompliance with the treatment protocol is the single most important reason for failure of treatment in dermatological practice and was evident in the study by Henz et al. (158). They used two different placebo treatments: Liquid paraffin for adults and olive oil for children. Liquid paraffin is an inert material for its effect on atopic dermatitis, whereas olive oil is not as inert because it can modify the cellular fatty acid profile. It has been reported to increase tissue levels of DGLA (159, 160). By increasing tissue levels of DGLA, olive oil may increase the dermal levels of lipooxygenase and cyclooxygenase metabolites of DGLA that are reported to exert anti-inflammatory actions (161, 162). Therefore, olive oil may show some beneficial effects because of the above-mentioned biochemical pathways, and hence, may not be a true placebo and dampen the effect of treatment. Therefore, separate analysis in adults and children was highly desirable to avoid the potential variations in

outcome induced by different placebo. The scoring system (SASSAD) used in this study as a primary outcome parameter is reported to have a very high interobserver variation (7–30, median 15.5, out of a possible score of 108) (163).

8.2. Other Skin Conditions

Radiation-induced damage: Skin is sensitive to radiations. Hopewell et al. (164) studied the effect of GLA or GLA and EPA combinations on radiation-induced skin damage in pigs. They treated female pigs for 4 weeks prior to and 10–16 weeks after irradiation of skin. Control pigs were treated with a placebo oil devoid of GLA and EPA. The pigs were irradiated with a single or fractionated (20 F/28 days) dose of β -rays. They observed that prior administration of GLA or GLA + EPA had no protective effect, while given before and after irradiation, both of these interventions reduced the development of early (bright red erythema or moist desquamation) and late (dusky/mauve erythema and dermal necrosis) reactions of radiations. This observation suggests that GLA alone or in combination with EPA may help improve the efficacy of radiation treatment by reducing the side effects.

9. DIABETES

Diabetes is a metabolic syndrome resulting in disturbed glucose homeostasis. This can be mediated because of decreased production of insulin (because of damage to insulin-producing pancreatic β -cells), as in type 1 diabetes, or increase in tissue resistance to the action of insulin (type 2 diabetes). According to the American Diabetes Association, approximately 17 million people in the United States, or 6.2% of the population, have diabetes. Although an estimated 11.1 million have been diagnosed, unfortunately, 5.9 million people (or one-third) are unaware that they have the disease. It affects people of all ages and races, although it is more common in African Americans, Latinos, Native Americans, Asian Americans, and Pacific Islanders. Incidence of diabetes increases with age in all populations. Diabetics are also at high risk of other complications, including cardiovascular (atherosclerosis, heart attack, stroke, peripheral vascular disease), neurological (neuropathies), renal failure, skin diseases (dry, itchy skin, skin infections, dermopathy), slow wound healing, retinopathy, and impotence. A combination of neuropathy and vascular disease in diabetics leads to more amputations. The mechanism of complications of diabetes is not well understood. High blood-glucose levels may be contributing to various complications, although several pathways, including increased oxidative stress, modification of proteins by glycosylation, reduction in production of vasodilator mediators including nitric oxide, prostacyclin, altered cytokine production, and so on, may be involved. Reduced tissue perfusion and resulting cellular damage contribute to several pathophysiological situations. The management of diabetes involves controlling the blood-glucose levels by diet, exercise, or drugs.

Several studies have confirmed that diabetes inhibits the activity of delta-6-desaturase (165–167), which is the first enzyme in the metabolism of LA and ALA. As a result of inhibition of this enzyme, diabetics have a lower content of DGLA and AA in various tissues (168). This may lead to imbalance in different eicosanoids in diabetics and may contribute to various complications common in diabetics. Based on these observations, it was hypothesized that GLA may help correct many of the complications of diabetes. To test this hypothesis, many studies were conducted in animals and humans. Some of these studies are discussed here.

Diabetic patients and animals show reduced nerve conduction velocity. Julu (169) from the University College of London performed a detailed study investigating the effect of GLA alone or in combination with EPA on nerve conduction velocity. During diabetes, nerve conduction velocity was reduced. Rats made diabetic by injection of streptozotocin (a pancreatic toxin) suffered about a 20% decline in motor nerve-fiber conduction velocity. Supplementation with GLA alone significantly attenuated the diabetes-induced deficit in nerve conduction velocity. Combined treatment with GLA and EPA completely prevented diabetes-induced reduction in motor nerve conduction velocity. In this study, they observed no effect of GLA or EPA on diabetes-induced weight loss, increase in blood-glucose, and glycosylated hemoglobin levels. In the subsequent study, Julu and Mutamba (170) studied the comparative effect of GLA and insulin for 3 or 5 days of treatment after induction of diabetes with streptozotocin in rats. They again observed a reduction in conduction velocity in myelinated sensory and motor nerve fibers. Unmyelinated sensory fiber also showed a trend for reduction in conduction velocity, but because of high intersubject variability, the fall could not reach statistical significance. Treatment with insulin for 3 days partially corrected the deficit in sensory-nerve conduction velocity, whereas motor-nerve conduction velocity was brought back to normal levels. Treatment for 5 days with insulin returned the motor-nerve conduction velocity to normal levels. GLA treatment for 3 days overcorrected the sensory-nerve conduction velocity, whereas the motor-nerve conduction velocity was brought back to normal levels. Treatment with GLA for 5 days brought sensory-nerve conduction velocity to normal levels. The overcorrection in sensory-nerve conduction velocity by three-day treatment with GLA cannot be explained. GLA treatment had no effect on blood-glucose levels or diabetes-induced weight loss, whereas insulin corrected both of these parameters. Cameron et al. (171) studied the effect of GLA alone or in combination with fish oil on diabetes-induced reduction in nerve conduction velocity and resistance to conduction block and found that diabetes increased the resistance of nerves to hypoxic conduction block and reduced the nerve conduction velocity. Treatment with GLA prevented these changes, whereas a combination with fish oil was less effective. Their results on conduction velocity are different from those reported by Julu and Mutamba (170) who observed better efficacy of GLA and fish oil combination. This difference is difficult to explain except for the sex differences in the rats in the two studies. Julu and Mutamba (170) used female rats, whereas Cameron's (171) group used male rats. The role of female sex hormones in differential observation of two groups cannot be discounted as the polyol pathway is differentially affected in

males and females. This pathway may be mediating a greater role in conduction velocity reduction in males than in females because of reduction of perfusion of vasa nervosum. In addition, Cameron et al. (171) observed that GLA treatment reduced the resistance to ischemic conduction block that was increased by diabetes. This may be caused by improved perfusion of vasa nervosum. During diabetes, the perfusion to vasa nervosum is reduced, which may cause ischemic preconditioning of nerves leading to increased resistance to subsequent ischemic conduction block. They also demonstrated increased capillary density in the sciatic nerve of GLA-treated diabetic rats leading to improved perfusion. The fatigue index of skeletal muscle was increased in diabetes, which increase was attenuated significantly by GLA treatment. This was also associated with increased capillary vascularization of muscle. In the subsequent study, Cameron and Cotter (172) observed that streptozotocin-induced diabetes reduced endoneural blood flow and oxygen tension that may be causing the hypoxic injury to the nerve cells leading to reduced nerve conduction and neuropathy. Treatment with evening primrose oil prevented the decrease in endoneural blood flow that was primarily caused by increased perfusion through capillaries leading to normal endoneural oxygen tension. However, in non-diabetic rats, evening primrose oil treatment increased the bulk flow (flow through major arteries, arteriols, veins, and arterio-venous shunts) without affecting capillary blood flow. The chronic increased blood flow through major vessels may be a stimulus to increased angiogenesis observed in an earlier study. Treatment with vasodilator drugs similarly caused an increase in blood flow and stimulated angiogenesis. After confirming the beneficial effects of GLA treatment in amelioration of diabetic nerve-conduction deficit, Cameron and Cotter (173) studied the effect of GLA and antioxidant treatment on nerve conduction velocity in diabetes. They selected a dose of GLA that would only correct the nerve conduction velocity reduction by about 20%. When the diabetic rats were given 20 mg/kg/day of GLA alone or in combination with an antioxidant (BM15.0639), the combination had a synergistic effect in improving the velocity. This synergistic effect on nerve conduction velocity was mediated by synergistic improvement in sciatic nerve capillary endoneural blood flow by the combined treatment. Several later studies confirmed the beneficial effects of antioxidants and GLA given in combination or as conjugates. In these studies, investigators studied the effects of GLA conjugates with ascorbic acid or α -lipoic acid or in combination with various antioxidants, including Vitamin E, ascorbic acid, α -lipoic acid, n-acetylcystein, butylated hydroxytoluene, and so on. These observations confirm previously reported observations that diabetic complications may be mediated by a combination of increased oxidative stress and abnormalities in essential fatty acid metabolism.

Jamal and Carmichael (174) conducted a double-blind, placebo-controlled trial of evening primrose oil in patients with type 1 and 2 diabetes with established neuropathy for at least 6 months. The patients were given 380 mg of GLA per day for 6 months and were evaluated before and after the treatment for neurological symptoms (pain, parasthesia, numbness, weakness, and abnormal sensation to heat and cold) as well as nerve conduction. At the end of the 6-month treatment, a significant improvement in 9 out of 12 variables was observed on GLA treatment. Treatment

with GLA also increased plasma phospholipid content of GLA, DGLA, and AA that are reduced in diabetes. The GLA treatment had no effect on glycosylated hemoglobin levels. These observations indicate that GLA-induced improvements may be mediated by improved perfusion of nerves rather than correction in metabolic derangements. By improving the tissue perfusion, treatment may have prevented hypoxic insult and related injury to the nerves, whereby improving the nerve conduction and reducing the associated pain and numbness. Keen et al. (175) conducted a double-blind, placebo-controlled, multicenter clinical trial of GLA in 111 diabetic patients with mild neuropathy. They studied the effect of treatment over 1 year on 13 symptoms, including motor-nerve conduction velocity, muscle strength, hot and cold thresholds, sensation, and tendon reflexes. GLA treatment at a dose of 480 mg per day was demonstrated to render benefits on functions, and the effects were more pronounced in well-controlled diabetics than in poorly controlled subjects. Keen et al. (175) used a higher dose of GLA than Jamal (174), and the study was of longer duration.

Diabetes is also known to impair immune response in humans (176) and animals, which may contribute to slow wound healing in diabetics. In streptozocin-induced diabetes in rats, a number of circulating T and B lymphocytes decreased with no effect on the number of circulating monocytes and neutrophils. In a study by Oon et al. (177), it was demonstrated that feeding evening primrose oil prevents a diabetes-induced fall in lymphocyte number that may have been mediated by increased production of PGE₁ in this group.

10. PREMENSTRUAL SYNDROME (PMS)

Premenstrual syndrome (PMS) is a recurrent cyclic disorder associated with the cyclic hormonal rhythms of the menstrual cycle. A large number of symptoms have been associated with PMS that are divided into physical, behavioral, and emotional symptoms. PMS may be associated with dysmenorrhea and other menstrual irregularities. Physical symptoms include bloating, abdominal and back cramps and discomfort, change in appetite, weight gain, breast tenderness and pain, and headache. Behavioral changes include anxiety, depression, lethargy, hypersomnia or insomnia, moodiness, irritability, anger, and social withdrawal. These symptoms vary in intensity from mild to severe and affect up to 90% of women some time in their child-bearing age. About 40% of women in industrialized countries suffer from mild to moderate symptoms of PMS, whereas about 10% of North American women suffer from moderate to severe symptoms affecting their daily life activities (178).

The objective of treatment is to prevent the symptoms of PMS. Several therapeutic agents are available to control the symptoms of PMS, but none of the agents are effective in controlling more than a few symptoms. In a study on 42 women suffering from PMS, a deficiency of long-chain metabolites of LA was reported with above-normal LA levels in plasma phospholipids, suggesting inhibition of delta-6-desaturase enzyme (179). As prostaglandins play an important role in regulation

of reproductive function, it was suggested that an imbalance between different prostaglandins may contribute to the symptoms of PMS. GLA, being the only natural source of DGLA that can be supplemented, may benefit these women by improving the ratio of prostaglandins of series 1 and 2. Puolakka et al. (180) performed a placebo-controlled trial of evening primrose oil in 30 women suffering from severe PMS. They observed that evening primrose oil treatment reduced the symptoms of PMS, although it was more effective in reducing depression. Treatment with evening primrose oil also reduced the production of thromboxane B2 by the platelets during clotting. The effectiveness of GLA oils in treatment of PMS is not equivocally proven. Some studies reported no effect of evening primrose oil treatment over placebo (181).

11. INFANT NUTRITION AND DEVELOPMENT

The role of GLA in infant nutrition and development as such is not very clear. The body weight of infants at birth was positively associated with the proportions of AA and DGLA in plasma triacylglycerols and choline phosphoacylglycerols in premature infants (182) and infants born at full term (182, 183). The positive association of DGLA with birth weight is more consistent than that observed for AA or DHA. This information suggests that DGLA is playing an important role in fetal development. The exact role, however, is not clear. Careful analysis of breast milk composition from women of different geographical areas revealed that women who had a higher amount of DHA in their breast milk lipids also had a higher amounts of DGLA (Figure 3) (184). The role of DHA is well established in infant development,

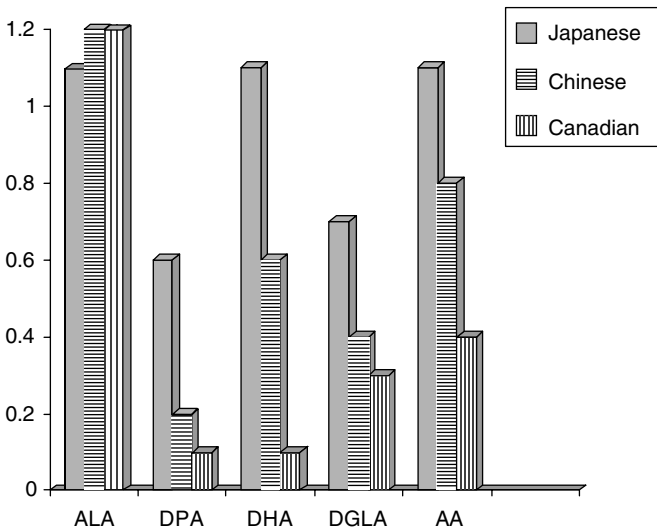


Figure 3. Breast milk fatty acid composition from Japanese, Chinese, and Canadian women. Data adapted from (184).

while the role of GLA and DGLA is not clear. This observation suggests that DGLA may play an important role in infant development. Earlier discussion in this chapter revealed that GLA and DGLA may be playing a role in atopy in infants as the breast milk of mothers of atopic children had lower GLA and DGLA levels in breast milk. Recently popularized, the Barker hypothesis (185) (the fetal origins hypothesis) states that, during fetal development, the environmental exposures set the limit to metabolic capacity. When this capacity is exceeded later in life, it sets as an overt disease. According to this theory, the effect of nutrient deficiency may be more marked than that during later life and may cause predisposition to many chronic diseases, such as heart disease, diabetes, metabolic syndrome, etc. (186, 187). In support of this hypothesis, it was observed that children who had higher levels of GLA in umbilical cord plasma phospholipids at birth did not develop insulin resistance at the age of 7 years, while those with lower levels of DGLA had increased insulin resistance, body fat, insulin, proinsulin, and leptin concentrations (188). The exact mechanism of how DGLA in infancy and fetal development can affect the health conditions in later life is not clear. It may be possible that GLA, as a ligand for peroxisome proliferator activated receptors (PPARs), affects the transcriptional regulation of glucose and lipid homeostasis.

12. DRUG/NUTRIENT INTERACTIONS

GLA modulates the second messengers at the cellular levels. Many drugs act by interfering with these second messengers. This suggests that GLA may interact with drugs to affect their actions. These actions could include alterations in the therapeutic potential or side effect profiles. Cyclosporin is a potent immunosuppressant drug that is commonly used in the prevention of graft rejection in transplant recipients and in the treatment of severe psoriasis. The major side effects of cyclosporin include hypertension and renal toxicity (189, 190). In borderline hypertensive rats, GLA was shown to inhibit hypertensive and glomerular filtration-rate-reducing actions of cyclosporin (191). In Wistar rats, GLA was shown to attenuate nephrotoxic effects of cyclosporin (192). These actions could be mediated by increased production of PGE₁, as demonstrated by the increased ratio of 6-keto-PGF_{1 α} /TXB₂. GLA has been shown to enhance the sensitivity of 36B10 astrocytoma cells to radiation (193). The enhanced sensitivity could be mediated by increased free radical production as it was blocked by Vitamin E. GLA has been shown in vitro to enhance the cytotoxicity of paclitaxel to various breast cancer cell lines, including MDA-MB-231, MCF-7, SK Br3, and T47D (194). In this study, the authors observed the synergistic action of GLA when the cells were co-incubated with paclitaxel, whereas preincubation of cancer cell lines with GLA, followed by treatment with paclitaxel, resulted in only additive effects. These actions of GLA were only partly inhibited by Vitamin E, suggesting that increased oxidative stress may partly be contributing to the cytotoxic actions of GLA against breast cancer cell lines. Thus, GLA was found to be most potent in enhancing cytotoxic actions of paclitaxel followed by ALA, EPA, DHA, and OA, whereas LA had no effect.

Similar results were obtained for vinorelbine and GLA in breast cancer cells (MDA-MB-231, T47D, and SK-Br3) (195).

Recently, it was shown that GLA acts synergistically with tamoxifen in enhancing the antitumor activity (196). This study was conducted in nude mice implanted with estrogen receptor positive breast cancer cells (MCF-7 B1M). GLA acted synergistically with tamoxifen in inhibiting tumor growth and expression of estrogen receptors.

Ikushima et al. (197) studied the interaction of GLA on cytotoxicity of various anticancer drugs in human neuroblastoma cell lines in culture. They observed that GLA enhanced absorption and cytotoxicity of vinca alkaloids (vincristine, vinblastin, and vindesine) 2–2.5-fold. This was associated with increased lipid peroxidation of cancer cells. In the same cell lines, GLA inhibited the cytotoxic action of platinating agents like cisplatin and carboplatin. This study suggests that GLA may react differently with various anticancer drugs. Liu and Tan (198) observed that GLA and DHA increase the absorption of doxorubicin into doxorubicin-sensitive and-resistant lymphoma cancer cells. The resistant cells became sensitive to doxorubicin toxicity, which was associated with increased superoxide dismutase activity with no effect on catalase activity and p-glycoprotein levels. This observation suggests that GLA has no effect on p-glycoprotein, which plays a role in multidrug resistance development. However, by increasing the levels of only superoxide dismutase and not catalase activity, GLA may stimulate the formation of hydrogen peroxide in the cancer cells, which may contribute to oxidative toxicity of doxorubicin. Hydrogen peroxide can form hydroxyl radicals, which are highly toxic to adjacent molecules.

Kaku et al. (199) studied the interaction of GLA with soy protein and casein in mediating immune response and LTB₄ production by rat peritoneal exudates cells. They observed that dietary borage oil reduced production of LTB₄ from the peritoneal exudates cells and the effect was stimulated by soy protein but not by casein. This study suggests that soy protein, but not casein, may stimulate anti-inflammatory action of GLA-rich oils.

13. SAFETY OF GLA-CONTAINING OILS

GLA-containing oils have been studied in several clinical trials on humans, in addition to laboratory animal studies. All of these studies have revealed that these oils are safe and are devoid of serious side effects. The more commonly observed side effects with these oils include gastric upsets (nausea, vomiting, diarrhea, belching), and headache. Evening primrose oil was associated with reducing sensitivity to seizure threshold in patients suffering from temporal lobe epilepsy (200). Although similar effects with borage or black currant oils have not been reported, it is advisable to observe caution when giving these oils to epileptic patients. Potential of hepatotoxicity from pyrrolizidine alkaloids in borage is nonexistent (as discussed in section 2.1.1) as the content of pyrrolizidine alkaloids in borage oil is less

than 4 ppb. At this level, one may have to consume more than 250 g of oil per day to expose to toxic levels of alkaloids.

14. CURRENT RESEARCH FOCUS

Current focus of research is on increasing the concentration of GLA in oils and to find new sources of GLA for commercial use. The strategies include genetic manipulations, variety development, and concentrations of existing GLA-rich oils like borage and evening primrose. GLA-containing oils can be concentrated to higher GLA levels by employing common techniques such as hydrolysis of oil to form free fatty acids followed by urea complexation to remove saturated and monounsaturated fatty acids (201). Employing this technique, the oil can be concentrated to 40–80% GLA (202, 203). The resultant oil contains GLA as a free fatty acid or can be converted to ethyl ester or triacylglycerol form by chemical/enzymatic esterification. The triacylglycerol form produced in this way contains about 50–70% triacylglycerols, 10–25% diacylglycerols, and 5–10% monoacylglycerols (Bioriginal Food and Science Corp). The enzymatic process involves the use of microbial lipases (from *Pseudomonas* sp.). Other areas of research include increasing the content of GLA in foods and alternative crops by genetic engineering. Cook et al. (204) inserted the delta-6-desaturase gene from borage into tomato. This strategy resulted in an increase in the content of GLA in tomato fruit along with a reduction in LA content. Although this variety has not been commercialized, there is a potential in optimizing the variety. Similar efforts have been made on other plants, including tobacco (205, 206) and canola (207). GLA levels in tobacco plants could be increased to about 14% of total fatty acids. At this level, it is not an economical source for production of GLA. Recently, Ross Labs has been successful in producing a transgenic variety of canola plant that contains up to 40% GLA in its seed oil (207). The transgenic canola oil containing GLA was compared with borage oil for its pharmacological actions and was found to be similar to borage oil, demonstrating that the transgenic canola oil could become an economically viable source of GLA.

Another area of current research is development of structured lipids where GLA is combined with a fatty acid of omega-3 family, preferably EPA or DHA, into one triacylglycerol molecule. Structured lipids can be produced by interesterifying a mixture of conventional fats and oils of interest using chemical or enzymatic methods. Chemical methods provide random distribution of different fatty acids on the glycerol backbone, whereas enzymatic reactions could be position specific, affording controlled production of triacylglycerols with desired configuration (208). Interesterification using the chemical method usually involves a reaction between two oils using metal alkoxide (sodium methoxide) as a catalyst. The unreacted fatty acids are removed by vacuum distillation. The alternative and more researched process involves acidolysis using lipases. In this process, either pure fatty acid is reacted with a triacylglycerol molecule or relatively rich fraction of fatty acid of interest is taken/prepared before acidolysis reaction.

The structured lipids have unique chemical, physical, or physiologic properties that are not observed by simply blending mixtures of the starting fats and oils (209). At present, the enzymatic process is under development but has not been widely commercialized so far due to the economy of the process. Laboratory research is in progress with the objective to develop a process that can be economically scaled up. The current emphasis is on optimization of lipases, reaction conditions including water activity of the reaction mixture, mole ratios of fatty acids to triacylglycerol, amount of enzyme, reaction temperature, and duration. The nonspecific lipases can be obtained from *Candida rugosa*, *Pseudomonas* sp, while 1, 3-position specific lipase are obtained from *Aspergillus niger*, *Mucor javanicus*, *Rhizomucor miehei*, *Rhizopus* sp., *Geotrichum candidum*, *Candida cylindracea*, *Candida parapolitytica*, *Rhizopus delemar*, etc. One can utilize either pure EPA or DHA as free acid or ethyl esters for incorporation into borage or evening primrose oil or GLA can be added to fish oils containing EPA and DHA. In these approaches, a structured lipid containing EPA, DHA, and GLA in one triacylglycerol molecule is produced. Spurvey et al. (210) studied the effect of reaction conditions on the incorporation of GLA into menhaden and seal blubber oils. They observed that the best conditions include a mole ratio of 3:1 for GLA to triacylglycerol, enzyme concentration of 500 units/g of oil, reaction temperature of 40°C, and time of 24 hours for incorporation of GLA into fish oils. They utilized concentrated GLA from borage oil for acidolytic reaction. The GLA of 91% concentration was prepared by chemical hydrolysis followed by urea complexation (201). This concentrate was reacted with fish oils using lipase from *Pseudomonas* sp (non-specific enzyme) and *Mucor miehei* (sn 1,3-specific enzyme). Lipase from *Pseudomonas* sp. gave higher incorporation of GLA into fish oil. Using a similar approach, production of EPA-rich borage oil and evening primrose oil has been produced (208).

REFERENCES

1. A. G. Hassam, J. P. Rivers, and M. A. Crawford, *J. Nutr.*, **107**(4), 519–524 (1977).
2. A. G. Hassam, J. P. Rivers, and M. A. Crawford, *Nutr. Metab.*, **21**(Suppl 1): 190–192 (1977).
3. J. P. Rivers, A. J. Sinclair, and M. A. Crawford, *Nature*, **258**(5531), 171–173 (1975).
4. H. Sprecher, *Prog. Lipid Res.*, **20**, 13–22 (1981).
5. R. R. Brenner, *Adv. Exp. Med. Biol.*, **83**, 85–101 (1977).
6. G. D. de, I. M. J. de Alaniz, and R. R. Brenner, *Lipids*, **19**(2), 91–95 (1984).
7. S. Ayala and R. R. Brenner, *Acta Physiol. Lat. Am.*, **33**(3), 193–204 (1983).
8. R. Kleiman, F. R. Earle, I. A. Wolff, and Q. Jones, *J. Am. Oil Chem. Soc.*, **41**(7), 459–460 (1964).
9. J. Janick, J. Simon, J. Quinn, and N. Beaubaire, *Herbs, Spices and Med. Plants*, **4**, 147–167 (1989).
10. K. R. Fell and J. M. Peck, *Planta Medica*, **16**, 29–42 (1968).

11. M. L. Fernald, *Grey's Manual of Botany*, 8th ed., D. Van Nostrand Co., New York, NY, 1970, 1198.
12. P. Clough, in *Structured and Modified Lipids*, F. D. Gunstone, ed., Marcell Dekker, Inc., New York, NY, 2000, pp. 75–117.
13. M. J. A. Simpson, *Ann. Appl. Biol.*, **123**, 105–108 (1993).
14. R. El Hafid, S. F. Blade, and Y. Hoyano, *Industrial Crops and Products*, **16**, 193–199 (2002).
15. A. De Haro, V. Dominguez, and M. del Rio, *J. Herbs, Spices and Med. Plants*, **9**(4), 297–304 (2002).
16. J. Janick, J. E. Simon, and A. Whipkey, *Hort. Sci.*, **22**, 493–495 (1987).
17. J. Quinn, J. E. Simon, and J. Janick, *J. Am. Soc. Hort. Sci.*, **114**(3), 511–515 (1989).
18. A. Whipkey, J. Simon, and J. Janick, *J. Am. Oil Chem. Soc.*, **65**(6), 979–984 (1988).
19. N. A. Beabaire, Master's Thesis, Purdue University, West Lafayette, Indiana, 1987.
20. A. Sensidoni, G. Bortolussi, C. Orlando, G. Lognay, P. Fantozzi, and M. Paquot, *Lebensm. Wiss. Technol.*, **28**(3), 343–346 (1995).
21. I. Wretnesjo and B. Karlberg, *J. Amer. Oil Chem. Soc.*, **79**(11), 1069–1074 (2002).
22. C. Dodson and F. R. Stermitz, *J. Nat. Prod.*, **49**(4), 727–728 (1986).
23. M. Herrmann, H. Joppe, and G. Schmaus, *Phytochemistry*, **60**(4), 399–402 (2002).
24. O. Parvais and V. Stricht, *J. Planar Chromat.*, **7**(1/2), 80–82 (1994).
25. H. J. Mierendorff, *Fett. Wissenschaft Technologie.*, **97**(1), 33–37 (1995).
26. H. W. Hulan, I. Hall, and D. M. Nash, *Crop. Res.*, **27**, 1–9 (1987).
27. W. W. Christie, *Industrial Crops and Products*, **10**, 73–83 (1999).
28. M. Wettasinghe, F. Shahidi, and R. Amarowicz, *J. Agric. Food Chem.*, **50**(5), 1267–1271 (2002).
29. B. D. Oomah, M. Busson, D. V. Godfrey, and J. C. G. Drover, *Food Chem.*, **76**(1), 33–43 (2002).
30. Hemp Oil Canada Inc. (2004) *Nutritional Composition*. Available: <http://hempoilcanada.com/compo.html>.
31. C. C. J. Culvenor, *Trends Pharmacol. Sci.*, **6**(1), 18–22 (1985).
32. C. C. J. Culvenor, *Aust. J. Chem.*, **9**, 512 (1956).
33. A. A. Seawright, M. P. Hegarty, L. F. James, and R. F. Keeler, in *Plant Toxicology—Proceedings of the Australia / USA Poisonous Plants Symposium Yeerongpilly, Brisbane, Australia*, 1985.
34. A. Molero Gomez and E. Martinez de la Ossa, *Chem. Eng. J.*, **88**(1–3), 103–109 (2002).
35. J. Leyton, P. J. Drury, and M. A. Crawford, *Lipids*, **22**(8), 553–558 (1987).
36. L. Chilton, M. E. Surette, D. D. Swan, A. N. Fonteh, M. M. Johnson, and F. H. Chilton, *J. Immunol.*, **156**(8), 2941–2947 (1996).
37. A. G. Hassam, A. J. Sinclair, and M. A. Crawford, *Lipids*, **10**(7), 417–420 (1975).
38. A. G. Hassam and M. A. Crawford, *Nutr. Metab.*, **20**(2), 112–116 (1976).
39. M. M. Engler, J. W. Karaian, and N. Salem, Jr., *Nutr. Res.*, **11**, 753–763 (1991).
40. J. D. Palombo, S. J. DeMichele, E. E. Lydon, T. J. Gregory, P. L. Banks, R. A. Forse, and B. R. Bistriani, *Am. J. Clin. Nutr.*, **63**(2), 208–219 (1996).

41. J. D. Palombo, S. J. DeMichele, E. Lydon, and B. R. Bistrrian, *J. Parenter. Enteral Nutr.*, **21**(3), 123–132 (1997).
42. D. E. Barre and B. J. Holub, *Lipids*, **27**(5), 315–320 (1992).
43. D. E. Barre and B. Holub, *J. Nutr. Res.*, **12**(10), 1181–1194 (1992).
44. J. Martens-Lobenhoffer and F. P. Meyer, *Int. J. Clin. Pharmacol. Ther.*, **36**(7), 363–366 (1998).
45. M. S. Manku, N. Morse-Fisher, and D. F. Horrobin, *Eur. J. Clin. Nutr.*, **42**(1), 55–60 (1988).
46. M. M. Engler and M. B. Engler, *Prostaglandins Leukot. Essent. Fatty Acids*, **59**(1), 11–15 (1998).
47. L. Chilton, M. E. Surette, D. D. Swan, A. N. Fonteh, M. M. Johnson, and F. H. Chilton, *J. Immunol.*, **156**(8), 2941–2947 (1996).
48. L. D. Lawson and B. Hughes, *Lipids*, **23**(4), 313–317 (1988).
49. D. Raederstorff and U. Moser, *Lipids*, **27**(12), 1018–1023 (1992).
50. S. Chung, S. Kong, K. Seong, and Y. Cho, *J. Nutr.*, **132**(10), 3090–3097 (2002).
51. J. C. Rose, M. Johnson, P. W. Ramwell, and P. A. Kot, *Proc. Soc. Exp. Biol. Med.*, **148**(4), 1252–1256 (1975).
52. B. A. Scholkens, D. Gehring, V. Schlotte, and U. Weithmann, *Prostaglandins Leukot. Med.*, **8**(3), 273–285 (1982).
53. D. E. Mills and R. Ward, *Proc. Soc. Exp. Biol. Med.*, **176**(1), 32–37 (1984).
54. D. E. Mills, K. M. Prkachin, K. Harvey, and R. Ward, *J. Hypertens.*, **3**(2), 111–116 (1989).
55. G. C. Leng, A. J. Lee, F. G. R. Fowkes, R. G. Jepson, and G. D. O. Low, *Clin. Nutr.*, **17**(6), 265–271 (1998).
56. M. M. Engler, M. B. Engler, S. Erickson, and S. M. Paul, *J. Hypertens.*, **10**(10), 1197–1204 (1992).
57. B. A. Scholkens, D. Gehring, V. Schlotte, and U. Weithmann, *Prostaglandins Leukot. Med.*, **8**(3), 273–285 (1982).
58. M. M. Engler, M. B. Engler, S. K. Erickson, and S. M. Paul, *J. Hypertens.*, **10**(10), 1197–1204 (1992).
59. P. Hoffmann, H. U. Block, J. Beitz, C. Taube, W. Forster, P. Wortha, P. Singer, E. Naumann, and H. Heine, *Lipids*, **21**(12), 733–737 (1986).
60. M. M. Engler, M. Schambelan, M. B. Engler, D. L. Ball, and T. L. Goodfriend, *Proc. Soc. Exp. Biol. Med.*, **218**(3), 234–237 (1998).
61. D. E. Mills, M. Mah, R. P. Ward, B. L. Morris, and J. S. Floras, *Am. J. Physiol.*, **259**(6 Pt 2), R1164–R1171 (1990).
62. J. Chaintreuil, L. Monnier, C. Colette, P. Crastes de Paulet, A. Orsetti, D. Spielmann, F. Mendy, and A. Crastes de Paulet, *Hum. Nutr. Clin. Nutr.*, **38**(2), 121–130 (1984).
63. P. Singer, P. Hoffmann, J. Beitz, W. Forster, M. Wirth, and W. Godicke, *Prostaglandins Leukot. Med.*, **22**(2), 173–177 (1986).
64. M. Boberg, B. Vessby, and I. Selinus, *Acta. Med. Scand.*, **220**(2), 153–160 (1986).
65. J. Viikari and A. Lehtonen, *Int. J. Clin. Pharmacol. Ther. Toxicol.*, **24**(12), 668–670 (1986).
66. M. Guivernau, N. Meza, P. Barja, and O. Roman, *Prostaglandins Leukot. Essent. Fatty Acids*, **51**(5), 311–316 (1994).

67. M. Lagarde, M. Burtin, M. Dechavanne, B. Sicard, and B. Coiffier, *Prostaglandins Med.*, **4**(3), 177–183 (1980).
68. P. B. A. Kernoff, A. L. Willis, K. J. Stone, J. A. Davies, and G. P. McNicol, *Br. Med. J.*, **2**(6100), 1441–1444 (1977).
69. J. C. Rose, M. Johnson, P. W. Ramwell, and P. A. Kot, *Proc. Soc. Exp. Biol. Med.*, **148**(4), 1252–1256 (1975).
70. T. Ishikawa, Y. Fujiyama, O. Igarashi, M. Morino, N. Tada, A. Kagami, T. Sakamoto, M. Nagano, and H. Nakamura, *Atherosclerosis*, **75**(2–3), 95–104 (1989).
71. J. Jantti, T. Nikkari, T. Solakivi, H. Vapaatalo, and H. Isomaki, *Ann. Rheum. Dis.*, **48**(2), 124–127 (1989).
72. D. F. Horrobin and M. S. Manku, *Lipids*, **18**(8), 558–562 (1983).
73. M. Fukushima, K. Shimada, E. Ohashi, H. Saitoh, K. Sonoyama, M. Sekikawa, and M. Nakano, *J. Nutr. Sci. Vitaminol. (Tokyo)*, **47**(3), 228–235 (2001).
74. K. Koba, J. W. Liu, L. T. Chuang, S. N. Anderson, T. Bowman, E. Bobik, Jr., M. Sugano, and Y. S. Huang, *Biosci. Biotechnol. Biochem.*, **64**(12), 2538–2542 (2000).
75. S. Renaud, L. Mcgregor, R. Morazain, C. Thevenon, C. Benoit, E. Dumont, and F. Mendy, *Atherosclerosis*, **45**(1), 43–51 (1982).
76. A. M. Sadi, T. Toda, H. Oku, and S. Hokama, *Exp. Anim.*, **45**(1), 55–62 (1996).
77. Y. Y. Fan, K. S. Ramos, and R. S. Chapkin, *Arterioscler. Thromb. Vasc. Biol.*, **15**(9), 1397–1403 (1995).
78. C. Maziere, F. Dantin, M. A. Conte, J. Degonville, D. Ali, F. Dubois, and J. C. Maziere, *Biochem. J.*, **336**(Pt 1) 57–62 (1998).
79. Y. Y. Fan, K. S. Ramos, and R. S. Chapkin, *J. Nutr.*, **131**(6), 1675–1681 (2001).
80. Y. Li, J. X. Kang, and A. Leaf, *Prostaglandins*, **54**(2), 511–530 (1997).
81. J. S. Charnock, P. L. McLennan, M. Y. Abeywardena, and W. F. Dryden, *Ann. Nutr. Metab.*, **29**(5), 306–318 (1985).
82. A. R. Kristal, J. H. Cohen, P. Qu, and J. L. Stanford, *Cancer Epidemiol. Biomarkers Prev.*, **11**(8), 719–725 (2002).
83. F. Levi, C. Pasche, F. Lucchini, and C. La Vecchia, *Ann. Oncol.*, **13**(3), 369–373 (2002).
84. A. J. Littman, S. A. Beresford, and E. White, *Cancer Causes Control*, **12**(8), 691–702 (2001).
85. C. Byrne, H. Rockett, and M. D. Holmes, *Cancer Epidemiol. Biomarkers Prev.*, **11**(3), 261–265 (2002).
86. R. Jarvinen, P. Knekt, T. Hakulinen, H. Rissanen, and M. Heliövaara, *Br. J. Cancer*, **85**(3), 357–361 (2001).
87. S. A. Smith-Warner, D. Spiegelman, H. O. Adami, W. L. Beeson, P. A. van den Brandt, A. R. Folsom, G. E. Fraser, J. L. Freudenheim, R. A. Goldbohm, S. Graham, L. H. Kushi, A. B. Miller, T. E. Rohan, F. E. Speizer, P. Toniolo, W. C. Willett, A. Wolk, A. Zeleniuch-Jacquotte, and D. J. Hunter, *Int. J. Cancer*, **92**(5), 767–774 (2001).
88. N. Dippenaar, J. Booyens, D. Fabbri, P. Engelbrecht, and I. E. Katzeff, *S. Afr. Med. J.*, **62**(19), 683–685 (1982).
89. J. Booyens, N. Dippenaar, D. Fabbri, P. Engelbrecht, C. C. Louwrens, and I. E. Katzeff, *S. Afr. Med. J.*, **65**(15), 607–612 (1984).
90. P. Coffino, H. R. Bourne, and G. M. Tomkins, *Am. J. Pathol.*, **81**(1), 199–204 (1975).

91. M. E. Begin, U. N. Das, G. Ells, and D. F. Horrobin, *Prostaglandins Leukot. Essent. Fatty Acids*, **19**(2), 177–186 (1985).
92. J. H. Lee and M. Sugano, *Nutr. Rep. Int.*, **34**(6), 1041–1049 (1986).
93. S. Abou El-Ela, K. W. Prasse, R. Carroll, and O. R. Bunce, *Lipids*, **22**(12), 1041–1044 (1987).
94. C. A. Gonzalez, J. M. Sanz, G. Marcos, S. Pita, E. Brullet, E. Saigi, A. Badia, A. Agudo, and E. Riboli, *Cancer Epidemiol. Biomarkers Prev.*, **2**(2), 157–158 (1993).
95. J. H. Botha, K. M. Robinson, N. Ramchurren, and R. J. Norman, *Prostaglandins Leukot. Essent. Fatty Acids*, **35**(2), 119–123 (1989).
96. F. S. Kenny, S. E. Pinder, I. O. Ellis, J. M. Gee, R. I. Nicholson, R. P. Bryce, and J. F. Robertson, *Int. J. Cancer*, **85**(5), 643–648 (2000).
97. P. J. du Toit, C. H. van Aswegen, and D. J. du Plessis, *Prostaglandins Leukot. Essent. Fatty Acids*, **51**(2), 121–124 (1994).
98. P. J. du Toit, C. H. van Aswegen, and D. J. du Plessis, *Prostaglandins Leukot. Essent. Fatty Acids*, **55**(3), 173–177 (1996).
99. W. G. Jiang, S. Hiscox, M. B. Hallett, C. Scott, D. F. Horrobin, and M. C. Puntis, *Br. J. Cancer*, **71**(4), 744–752 (1995).
100. W. G. Jiang, S. Hiscox, R. P. Bryce, D. F. Horrobin, and R. E. Mansel, *Br. J. Cancer*, **77**(5), 731–738 (1998).
101. W. G. Jiang, A. Redfern, R. P. Bryce, and R. E. Mansel, *Prostaglandins Leukot. Essent. Fatty Acids*, **62**(2), 119–127 (2000).
102. T. Liang and S. Liao, *Biochem. J.*, **285**(Pt 2), 557–562 (1992).
103. L. Kleinberg, S. A. Grossman, K. Carson, G. Lesser, A. O'Neill, J. Pearlman, P. Phillips, T. Herman, and M. Gerber, *J. Clin. Oncol.*, **20**(14), 3149–3155 (2002).
104. A. A. Brandes, U. Basso, and L. M. Pasetto, *Expert. Rev. Anticancer Ther.*, **1**(3), 357–370 (2001).
105. M. R. C. Naidu, U. N. Das, and A. Kishan, *Prostaglandins Leukot. Essent. Fatty Acids*, **45**, 181–184 (1992).
106. U. N. Das, W. S. K. Prasad, and D. R. Reddy, *Cancer Let.*, **94**(2), 147–155 (1995).
107. C. F. van der Merwe, J. Booyens, H. F. Joubert, and C. A. van der Merwe, *Prostaglandins Leukot. Essent. Fatty Acids*, **40**(3), 199–202 (1990).
108. J. S. Falconer, K. C. Fearon, J. A. Ross, and D. C. Carter, *World Rev. Nutr. Diet.*, **76**, 74–76 (1994).
109. P. Sangeetha, U. N. Das, R. Koratkar, G. Ramesh, M. Padma, and G. Sravan Kumar, *Cancer Let.*, **63**, 189–198 (1992).
110. H. A. Leaver, J. R. Williams, J. W. Ironside, E. P. Miller, A. Gregor, B. H. Su, R. J. Prescott, and I. R. Whittle, *Eur. J. Clin. Invest.*, **29**(3), 220–231 (1999).
111. M. de Kock, M. L. Lottering, and J. C. Seegers, *Prostaglandins Leukot. Essent. Fatty Acids*, **51**(2), 109–120 (1994).
112. M. de Kock, M. L. Lottering, C. J. Grobler, T. C. Viljoen, M. le Roux, J. C. Seegers, *Prostaglandins Leukot. Essent. Fatty Acids*, **55**(6), 403–411 (1996).
113. W. G. Jiang, R. P. Bryce, D. F. Horrobin, and R. E. Mansel, *Int. J. Oncol.*, **13**(3), 611–617 (1998).

114. J. C. Seegers, M. de Kock, M. L. Lottering, C. J. Grobler, D. H. van Papendorp, Y. Shou, R. Habberset, and B. E. Lehnert, *Prostaglandins Leukot. Essent. Fatty Acids*, **56**(4), 271–280 (1997).
115. D. F. Horrobin, *Wien. Klin. Wochenschr.*, **100**(14), 471–477 (1988).
116. L. Chilton, M. E. Surette, D. D. Swan, A. N. Fonteh, M. M. Johnson, and F. H. Chilton, *J. Immunol.*, **156**(8), 2941–2947 (1996).
117. V. A. Ziboh and M. P. Fletcher, *Am. J. Clin. Nutr.*, **55**(1), 39–45 (1992).
118. S. Kaku, K. Ohkura, S. Yunoki, M. Nonaka, H. Tachibana, M. Sugano, and K. Yamada, *Prostaglandins Leukot. Essent. Fatty Acids*, **65**(4), 205–210 (2001).
119. D. Santoli and R. B. Zurier, *J. Immunol.*, **143**(4), 1303–1309 (1989).
120. W. V. Williams, H. Rosenbaum, and R. B. Zurier, *Pathobiology*, **64**(1), 27–31 (1996).
121. D. M. DeMarco, D. Santoli, and R. B. Zurier, *J. Leukocyte Biol.*, **56**(5), 612–615 (1994).
122. D. Rotondo, C. R. Earl, K. J. Laing, and D. Kaimakamis, *Biochim. Biophys. Acta*, **1223**(2), 185–194 (1994).
123. D. Rothman, H. Allen, L. Herzog, A. Pilapil, C. M. Seiler, and R. B. Zurier, *Cytokine*, **9**(12), 1008–1012 (1997).
124. P. DeLuca, R. G. Rossetti, C. Alavian, P. Karim, and R. B. Zurier, *J. Investig. Med.*, **47**(5), 246–250 (1999).
125. R. K. Furse, R. G. Rossetti, and R. B. Zurier, *J. Immunol.*, **167**(1), 490–496 (2001).
126. R. G. Rossetti, C. M. Seiler, M. Laposata, and R. B. Zurier, *Clin. Immunol. Immunopathol.*, **76**(3), 220–224 (1995).
127. D. Vassilopoulos, R. B. Zurier, R. G. Rossetti, and G. C. Tsokos, *Clin. Immunol. Immunopathol.*, **83**(3), 237–244 (1997).
128. D. Wu, M. Meydani, L. Leka, Z. Nightingale, G. Handelman, and J. Blumberg, *Am. J. Clin. Nutr.*, **70**(4), 536–543 (1999).
129. J. L. Nerad, S. N. Meydani, and C. A. Dinarello, *Cytokine*, **3**, 513 (1991).
130. R. B. Zurier, R. G. Rossetti, C. M. Seiler, and M. Laposata, *Prostaglandins Leukot. Essent. Fatty Acids*, **60**(5–6), 371–375 (1999).
131. G. A. Tate, B. F. Mandell, R. A. Karmali, M. Laposata, D. G. Baker, H. R. Schumacher, Jr., and R. B. Zurier, *Arthritis Rheum.*, **31**(12), 1543–1551 (1988).
132. G. A. Tate and R. B. Zurier, *Agents Actions*, **43**(1–2), 35–38 (1994).
133. T. M. Hansen, A. Lerche, V. Kassis, I. Lorenzen, and J. Sondergaard, *Scand. J. Rheumatol.*, **12**(2), 85–88 (1983).
134. J. J. Belch, D. Ansell, R. Madhok, A. O'Dowd, and R. D. Sturrock, *Ann. Rheum. Dis.*, **47**(2), 96–104 (1988).
135. S. Pullman-Mooar, M. Laposata, D. Lem, R. T. Holman, L. J. Leventhal, D. DeMarco, and R. B. Zurier, *Arthritis Rheum.*, **33**(10), 1526–1533 (1990).
136. L. J. Leventhal, E. G. Boyce, and R. B. Zurier, *Ann. Intern. Med.*, **119**(9), 867–873 (1993).
137. R. B. Zurier, R. G. Rossetti, E. W. Jacobson, D. M. DeMarco, N. Y. Liu, J. E. Temming, B. M. White, and M. Laposata, *Arthritis Rheum.*, **39**(11), 1808–1817 (1996).
138. K. V. Kumar, S. M. Rao, R. Gayani, I. K. Mohan, and M. U. Naidu, *Clin. Chim. Acta*, **298**(1–2), 111–120 (2000).

139. J. E. Gadek, S. J. DeMichele, M. D. Karlstad, E. R. Pacht, M. Donahoe, T. E. Albertson, C. Van Hoozen, A. K. Wennberg, J. L. Nelson, and M. Noursalehi, *Crit. Care Med.*, **27**(8), 1409–1420 (1999).
140. M. M. Murray, M. Kumar, T. Greogory, P. Banks, H. D. Tazelaar, and S. J. DeMichele, *Am. J. Physiol.*, **269**(6 pt 2), H2090–H2099 (1995).
141. P. Mancuso, J. Whelan, S. J. DeMichele, C. C. Snider, J. A. Guszczka, K. J. Claycombe, G. T. Smith, T. J. Gregory, and M. D. Karlstad, *Crit. Care Med.*, **25**(3), 523–532 (1997).
142. G. O. Burr and M. M. Burr, *J. Biol. Chem.*, **82**(2), 345–367 (1929).
143. R. S. Chapkin, V. A. Ziboh, and J. L. McCullough, *J. Nutr.*, **117**(8), 1360–1370 (1987).
144. A. E. Hansen, *Proc. Soc. Exp. Biol. Med.*, **31**, 160–161 (1933).
145. M. S. Manku, D. F. Horrobin, N. Morse, V. Kyte, K. Jenkins, S. Wright, and J. L. Burton, *Prostaglandins Leukot. Med.*, **9**(6), 615–628 (1982).
146. S. Wright and C. Bolton, *Br. J. Nutr.*, **62**(3), 693–697 (1989).
147. L. Businco, M. Ioppi, N. L. Morse, R. Nisini, and S. Wright, *J. Allergy Clin. Immunol.*, **91**(6), 1134–1139 (1993).
148. S. Wright and J. L. Burton, *The Lancet*, **2**(8308), 1120–1122 (1982).
149. M. S. Manku, D. F. Horrobin, N. L. Morse, S. Wright, and J. L. Burton, *Br. J. Dermatol.*, **110**(6), 643–648 (1984).
150. L. Schafer and K. Kragballe, *Lipids*, **26**(7), 557–560 (1991).
151. D. H. Scarff and D. H. Lloyd, *Vet. Record*, **131**(5), 97–99 (1992).
152. A. Fiocchi, M. Sala, P. Signoroni, G. Banderali, C. Agostoni, and E. Riva, *J. Int. Med. Res.*, **22**(1), 24–32 (1994).
153. S. Borrek, A. Hildebrandt, and J. Forster, *Klin. Padiatr.*, **209**(3), 100–104 (1997).
154. P. J. Hartop and C. Prottey, *Br. J. Dermatol.*, **95**(3), 255–264 (1976).
155. A. Tolleson and A. Frithz, *Acta Derm. Venereol.*, **73**(1), 18–20 (1993).
156. B. M. Henz, S. Jablonska, P. C. van de Kerkhof, G. Stingl, M. Blaszczyk, P. G. Vandervalk, R. Veenhuizen, R. Muggli, and D. Raederstorff, *Br. J. Dermatol.*, **140**(4), 685–688 (1999).
157. A. Takwale, E. Tan, S. Agarwal, G. Barclay, I. Ahmed, K. Hotchkiss, J. R. Thompson, T. Chapman, and J. Berth-Jones, *Br. Med. J.*, **327**(7428), 1385 (2003).
158. B. M. Henz, S. Jablonska, P. C. van de Kerkhof, G. Stingl, M. Blaszczyk, P. G. Vandervalk, R. Veenhuizen, R. Muggli, and D. Raederstorff, *Br. J. Dermatol.*, **140**(4), 685–688 (1999).
159. M. D. Giron, F. J. Mataix, and M. D. Suarez, *Biochim. Biophys. Acta.*, **1045**, 69–73 (1990).
160. K. L. Campbell and G. P. Dorn, *Res. Vet. Sci.*, **53**, 172–178 (1992).
161. C. C. Miller and V. A. Ziboh, *Biochem. Biophys. Res. Commun.*, **154**(3), 967–974 (1988).
162. V. A. Ziboh, C. C. Miller, and Y. Cho, *Am. J. Clin. Nutr.*, **71**(1 Suppl), 361S–366S (2000).
163. C. R. Charman, A. J. Venn, and H. C. Williams, *Br. J. Dermatol.*, **146**(6), 1057–1060 (2002).
164. J. W. Hopewell, G. J. van den Aardweg, G. M. Morris, M. Rezvani, M. E. Robbins, G. A. Ross, E. M. Whitehouse, C. A. Scott, and D. F. Horrobin, *Int. J. Radiat. Oncol. Biol. Phys.*, **30**(5), 1119–1125 (1994).
165. S. Ayala and R. R. Brenner, *Acta Physiol. Lat. Am.*, **25**(5), 371–378 (1975).

166. J. P. Poisson, *Enzyme*, **34**(1), 1–14 (1985).
167. O. Mercuri, R. O. Peluffo, and R. R. Brenner, *Biochim. Biophys. Acta*, **116**(2), 409–411 (1966).
168. M. Arisaka, O. Arisaka, and Y. Yamashiro, *Prostaglandins Leukot. Essent. Fatty Acids*, **43**(3), 197–201 (1991).
169. P. O. Julu, *J. Diabet. Complications*, **2**(4), 185–188 (1988).
170. P. O. Julu and A. Mutamba, *J. Neurol. Sci.*, **106**(1), 56–59 (1991).
171. N. E. Cameron, M. A. Cotter, and S. Robertson, *Diabetes*, **40**(5), 532–539 (1991).
172. N. E. Cameron and M. A. Cotter, *Acta Diabetologica*, **31**(4), 220–225 (1994).
173. N. E. Cameron and M. A. Cotter, *Am. J. Physiol.*, **271**(3 Pt 1), E471–E476 (1996).
174. G. A. Jamal and H. Carmichael, *Diabetic Medicine*, **7**(4), 319–323 (1990).
175. H. Keen, J. Payan, J. Allawi, J. Walker, G. A. Jamal, A. I. Weir, L. M. Henderson, E. A. Bissessar, P. J. Watkins and M. Sampson, *Diabetes Care*, **16**(1), 8–15 (1993).
176. B. Singh, J. Lauzon, J. Venkatraman, A. B. Thomson, R. V. Rajotte, and M. T. Clandinin, *Diabetes Res.*, **8**(3), 129–134 (1988).
177. B. B. Oon, D. Muggleston, and A. Warley, *Exp. Physiol.*, **77**(1), 185–190 (1992).
178. D. N. Ugarriza, S. Klingner, and S. O'Brien, *Nurse Pract.*, **23**(9), 40–52 (1998).
179. M. G. Brush, S. J. Watson, D. F. Horrobin, and M. S. Manku, *Am. J. Obstet. Gynecol.*, **150**(4), 363–366 (1984).
180. J. Puolakka, L. Makarainen, L. Viinikka, and O. Ylikorkala, *J. Reprod. Med.*, **30**(3), 149–153 (1985).
181. S. K. Khoo, C. Munro, and D. Battistutta, *Med. J. Aust.*, **153**(4), 189–192 (1990).
182. A. A. Leaf, M. J. Leighfield, K. L. Costeloe, and M. A. Crawford, *Early Hum. Dev.*, **30**(3), 183–191 (1992).
183. P. Rump, R. P. Mensink, A. D. M. Kester, and G. Hornstra, *Am. J. Clin. Nutr.*, **73**(4), 797–806 (2001).
184. L. Wang, Y. Shimizu, S. Kaneko, S. Hanaka, T. Abe, H. Shimasaki, H. Hisaki, and H. Nakajima, *Pediatrics. Int.*, **42**(1), 14–20 (2000).
185. D. J. Barker, P. D. Winter, C. Osmond, B. Margetts, and S. J. Simmonds, *Lancet*, **2**(8663), 577–580 (1989).
186. D. W. Lamont, L. Parker, M. A. Cohen, M. White, S. M. Bennett, N. C. Unwin, A. W. Craft, and K. G. Alberti, *Public Health*, **112**(2), 85–93 (1998).
187. A. A. Jackson, *Adv. Exp. Med. Biol.*, **478**, 41–55 (2000).
188. P. Rump, C. Popp-Snijders, R. J. Heine, and G. Hornstra, *Diabetologia*, **45**(3), 349–355 (2002).
189. J. M. Rabkin, C. L. Corless, H. R. Rosen, and A. J. Olyaei, *Am. J. Surg.*, **183**(5), 595–599 (2002).
190. T. Markham, A. Watson, and S. Rogers, *Clin. Exp. Dermatol.*, **27**(2), 111–114 (2002).
191. D. E. Mills, M. Mah, R. P. Ward, B. Morris, and J. Floras, *Am. J. Physiol.*, **259**(6 Pt 2), R1164–R1171 (1990).
192. P. Morphake, J. Bariety, I. Darlametsos, and G. Tsipas, *Prostaglandins Leukot. Essent. Fatty Acids*, **50**(1), 29–35 (1993).
193. S. Vartak, M. E. Robbins, and A. A. Spector, *Lipids*, **32**(3), 283–292 (1997).

194. J. A. Menendez, M. D. Barbacid, S. Montero, E. Sevilla, E. Escrich, M. Solanas, H. Cortes-Funes, and R. Colomer, *Eur. J. Cancer*, **37**(3), 402–413 (2001).
195. J. A. Menendez, S. Ropero, M. M. del Barbacid, S. Montero, M. Solanas, E. Escrich, H. Cortes-Funes, and R. Colomer, *Breast Cancer Res. Treat.*, **72**(3), 203–219 (2002).
196. F. S. Kenny, J. M. Gee, R. I. Nicholson, I. O. Ellis, T. M. Morris, S. A. Watson, R. P. Bryce, and J. F. Robertson, *Int. J. Cancer*, **92**(3), 342–347 (2001).
197. S. Ikushima, F. Fujiwara, S. Todo, and S. Imashuku, *Anticancer Res.*, **10**(4), 1055–1059 (1990).
198. Q. Y. Liu and B. K. Tan, *Life Sci.*, **67**(10), 1207–1218 (2000).
199. S. Kaku, S. Yunoki, K. Ohkura, M. Sugano, M. Nonaka, H. Tachibana, and K. Yamada, *Biosci. Biotechnol. Biochem.*, **65**(2), 315–321 (2001).
200. K. S. Vaddadi, *Prostaglandins Med.*, **6**(4), 375–379 (1981).
201. S. P. Senanayake and Shahidi F, *J. Food Lipids*, **7**, 51–61 (2000).
202. J. C. Philips and Y. S. Huang, in *Gamma-linolenic acid: Metabolism and its roles in Nutrition and Medicine*, Y. S. Huang and D. E. Mills, eds. AOCS Press, Champaign, IL, 1996, pp. 1–13.
203. F. C. Huang, Y. H. Ju, and J. C. Chiang, *J. Am. Oil Chem. Soc.*, **76**(7), 833–837 (1999).
204. D. Cook, D. Grierson, C. Jones, A. Wallace, G. West, and G. Tucker, *Mol. Biotechnol.*, **21**(2), 123–128 (2002).
205. A. S. Reddy and T. L. Thomas, *Nat. Biotechnol.*, **14**(5), 639–642 (1996).
206. O. Sayanova, M. Smith, P. Lapinskas, and A. K. Stobart, *Proc. Natl. Acad. Sci. USA*, **94**(8), 4211–4216 (1997).
207. P. E. Wainwright, Y. S. Huang, S. J. DeMichele, H. Xing, J. W. Liu, L. T. Chuang, and J. Biederman, *Lipids*, **38**(2), 171–178 (2003).
208. S. P. Senanayake and F. Shahidi, *J. Agric. Food Chem.*, **50**(3), 477–483 (2002).
209. K. T. Mok, A. Maiz, K. Yamazaki, J. Sobrado, V. K. Babayan, L. L. Moldawer, B. R. Bistrrian, and G. L. Blackburn, *Metabolism*, **33**(10), 910–915 (1984).
210. S. A. Spurvey, S. P. Senanayake, and F. Shahidi, *J. Am. Oil Chem. Soc.*, **78**(11), 1105–1112 (2001).