

IMPORTANCE OF γ -LINOLENIC ACID IN CLINICAL INDICATIONS

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ABSTRACT

Gamma linolenic acid (GLA, cis-6, cis-9, cis-12-octadecatrienoic acid) is a conditionally essential fatty acid of the n-6 family. GLA is an omega-6 polyunsaturated fatty acid (PUFA). The fatty acid molecule is comprised of 18 carbon atoms with three double bonds. It is produced in the mammalian body from enzymatic desaturation of linoleic acid by the enzyme delta-6-desaturase., an essential fatty acid that must be supplied by the diet. It is widely distributed in plant kingdom in trace amounts and is not present in major commercial vegetable seed oils. It is present in seeds of many species belonging to families *Aceraceae*, to the more favourable prostaglandins and leukotriene's, making it helpful for diseases that involve inflammation. GLA is widely used to treat diabetic neuropathy and eczema and is also used to treat cyclic mastalgia, a condition marked by breast pain associated with the menstrual cycle. GLA has also been proposed as a treatment for many other conditions *Boraginaceae*, *Cannabinaceae*, *Liliaceae*, *Onagraceae*, *Ranunculaceae*, *Saxifragaceae*, and *Scrophulariaceae*. The PUFA of omega 3 and omega 6 series play an significant role in health and disease by generating potent modulatory molecule for inflammatory responses, including eicosanoid (prostaglandin and leukotriene's), and cytokines (interleukins) and affecting the gene expression of various bioactive molecules. GLA is further metabolized to dihomogamma linoleic acid (DGLA) which undergoes oxidative metabolism by cyclooxygenase and lipoxygenase to produce anti-inflammatory eicosanoid. These substances influence inflammation and pain; some of them increase symptoms, while others decrease them. GLA may swing the balance over

Keywords:Gamma linolenic acid, Dihomogamma linoleic acid, Essential fatty acid, Poly unsaturated fatty acid, Eicosanoids.

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). 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). GLA is rapidly elongated to DGLA by elongase enzyme. DGLA can be acetylated and incorporated into membrane phospholipids. A small amount can be converted into AA, and this reaction is catalyzed by delta-5-desaturase enzyme. DGLA undergoes oxidative metabolism by cyclooxygenase and lipoxygenase to produce anti-inflammatory eicosanoid. These substances influence inflammation and pain; some of them increase symptoms, while others decrease them.

Acute inflammatory response is essential for survival of host as it plays an important role in host defence mechanism (in killing of invading microorganisms, damaged cells, wound healing, tissue repair etc.). Chronic/uncontrolled inflammatory response, on the other hand, leads to self-tissue damage, causing chronic disease like arthritis, psoriasis, etc. research in last five decade has confirmed involvement of inflammation in various chronic diseases like cancer, diabetes. Alzheimer disease, Parkinsonism, multiple sclerosis, and lupus nephritis, etc.

Different animal species and different tissues

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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 (2). 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, saturated and trans-fatty acids and nutritional deficiencies of Vitamin B6, zinc, and magnesium inhibit this desaturase. As a result of limitations 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.

HISTORY

Some of the plants with seeds that contain GLA have been used as folk remedies for hundreds, if not thousands, of years. However, the discovery of GLA and of these seed oils as a source of GLA is much more recent.

Research in the 1980s found that hormone-like substances called prostaglandins played a role in many of the body's processes. Prostaglandins play a role in making smooth muscles contract, controlling inflammation and body temperature, and in other body functions. Since GLA was known to be a building block for some prostaglandins, it was reasoned that GLA might help treat human disease. While GLA is widely touted for its health benefits, research on its effectiveness in human diseases is still at an early stage.

SOURCE

GLA dietary sources

GLA is found in human milk and in small amounts in a wide variety of common foods, notably organ meats (3). It is also obtained from vegetable oils such as: safflower oil (*Carthamus tinctorius*), evening primrose (*Oenothera biennis*) oil, blackcurrant seed oil, borage oil, and hemp seed oil. GLA is also found in considerable quantities in edible hemp seeds and from spirulina, a cyanobacterium. Each contains varying amounts of the fatty acid. Borage oil ranges from 15% to 20% GLA and evening primrose oil ranges from 8% to 10% GLA. (4-5). Ten grams of spirulina has been tested at 1% GLA. All are widely available in pharmacies, health food stores, and online shops. The triacylglycerol stereospecific structure of these oils is distinct, with GLA being

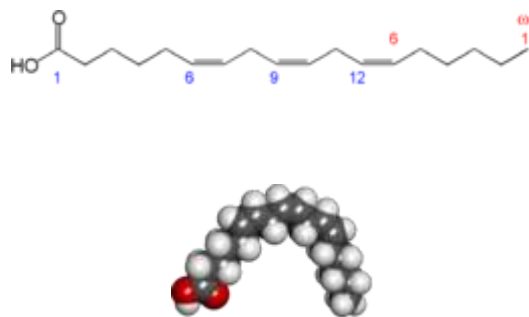
concentrated in the sn-3 position of evening primrose oil and blackcurrant seed oil, the sn-2 position of borage oil and the sn-2 and sn-3 positions of fungal oil (6). The development of oilseed crops designed to produce substantial quantities of GLA is a major goal of plant biotechnology, and the cyanobacterial $\Delta 6$ desaturase gene has recently been successfully expressed in transgenic tobacco, resulting in GLA accumulation (7). In addition, the efficient production of GLA by mutants of *Mortierella ramanniana* has been investigated (8). Although the ingestion of GLA-enriched oils results in the accumulation of dihomo- γ -linolenic acid [DGLA, 20:3(n-6)] in tissue phospholipids and triacylglycerol's, the absolute level of GLA in the oil may not be the sole determinant of biological efficacy. The precise triacylglycerol stereospecific composition and the cellular kinetics of phospholipases and acyltransferases may also influence GLA bioavailability. For example, although the GLA concentration in borage oil is twofold higher than in primrose oil, GLA-related effects, such as formation of prostaglandin E1 (PGE1), is comparable for both dietary oils on a per gram basis (9). LA is consumed sufficiently in most diets, from such abundant sources as cooking oils and meats. However, a lack of GLA can occur when there is a reduction of the efficiency of the D6D conversion (for instance, as people grow older or when there are specific dietary deficiencies) or in disease states wherein there is excessive consumption of GLA metabolites. (10)



Evening primrose flower

BIOCHEMISTRY

Molecular formula: $C_{18}H_{30}O_2$



IUPAC: all-cis-6,9,12-octadecatrienoic acid

Molar mass: 278.43 g/mol.

The stereo specificity of GLA varies from source to source. In EPO and black currant oil GLA is concentrated in the n-3 position, while in borage oil it is concentrated in the n-2 position. GLA is concentrated evenly in both the n-2 and n-3 positions of fungal oils. (11) Conversion of linoleic acid to GLA is induced by the enzyme delta-6-desaturase. Linoleic acid is converted first to GLA then to arachidonic acid by an alternating sequence of delta-6-desaturation, chain elongation, and delta-5-desaturation, in which hydrogen atoms are selectively removed to create new double bonds. Dietary GLA supplementation bypasses the rate-limiting step of delta-6-desaturation and is quickly elongated to dihomo-gamma-linolenic acid (DGLA). GLA formation is dependent on the activity of the delta-6-desaturase enzyme, which is hindered by numerous factors, including aging, nutrient deficiency, trans-fatty acids, hydrogenated oils, smoking, and excessive alcohol consumption. Unopposed omega-6 supplementation may cause an increase in arachidonic acid and the undesirable, pro-inflammatory, 2-series prostaglandins. A combination of alpha-linolenic acid (or eicosapentaenoic (EPA) and docosahexaenoic (DHA) with GLA may antagonize conversion to arachidonic acid. (12) The result will be more favourable, with an increase in anti-inflammatory and antithrombotic effects.

MECHANISM OF ACTION

GLA (cis 6, cis 9, cis 12-Octadecatrienoic acid) is produced in the body as an intermediate in the metabolism of linoleic acid (LA), an essential fatty acid of omega-6 series by the action of enzyme delta-6-desaturase. This reaction is very slow and is further restricted during nutritional deficiency of vitamins, minerals (zinc, cobalt etc.) and also during inflammatory condition like arthritis, psoriasis, hypertension, diabetes, and several other disease also impair the activity of this enzyme, leading to insufficient production of GLA

in the body. Life style factors like stress, smoking and alcohol, saturated and Trans fat and preformed arachidonic acid and eicosapentaenoic acid also inhibit the enzyme. Once formed or administered, GLA is rapidly elongated to dihomo-gamma linolenic acid (DGLA) which is incorporated into the cell membrane phospholipid following acylation reaction catalysed by acyl transferase. DGLA is the active form produced from GLA that mediates most of the physiological action of GLA. A small amount of DGLA can also be converted to arachidonic acid (AA) by the enzyme delta-5-desaturase. However, this reaction is slow and the extent of formation of AA from DGLA is dependent on the dietary and environmental factors. Preformed AA (from meat and dairy) (13) Eicosapentaenoic acid (EPA, from fish) (14), and sesamin (from sesame seeds) inhibit the enzyme and prevent formation of AA.

Membrane bound DGLA is released by the action of enzyme phospholipase A2 (PL A2). Once released, it competes with AA for enzymes cyclooxygenase (COX) and lipoxygenase (LOX) to produce short lived secondary messengers that are responsible for cell-cell communications and mediation of physiological effects of these fatty acids. The cyclooxygenase products of DGLA include prostaglandin of series 1 (PGE1) and thromboxane A1 (TX A1). These products of COX exert anti-inflammatory, vasodilatory and anti-aggregatory actions. DGLA produces 15-hydroxyeicosatrienoic acid (15-HETrE) by the action of 15-LOX. 15-HETrE is a strong inhibitor of 5-lipoxygenase, whereby inhibits production of leukotriene B4 (LTB4) from inflammatory cells including neutrophils.

GLA has gained importance in last four decades for its anti-inflammatory and anti-cancer actions. It also improves nerve conduction velocity in diabetic patients, leading to improved blood flow and reduced tingling of extremities. Due to these actions of GLA, many sources of GLA, many sources of GLA have been commercialized.

CLINICAL INDICATIONS (USES)

Diabetic neuropathy

Diabetic neuropathy is a gradual degeneration of nerves caused by diabetes. There is some evidence that GLA can be helpful, if you give it long enough to work. In one double-blind placebo-controlled study, 111 people with mild diabetic neuropathy received either 480 mg daily of GLA or placebo. After 12 months, the group taking GLA was doing significantly better than the placebo group. Good results were seen in a smaller study

as well. In addition, numerous studies in animals have found that evening primrose oil can protect nerves from diabetes-induced nerve injury. There is some preliminary evidence that GLA may be more effective for this condition when it is combined with lipoic acid.

Rheumatoid arthritis

According to many studies, fish oil, a source of omega-3 essential fatty acids, definitely improves symptoms of rheumatoid arthritis. A few studies suggest that GLA may also work. One double-blind study followed 56 people with rheumatoid arthritis for 6 months. Participants received either 2.8 g daily of GLA or placebo. The group taking GLA experienced significantly fewer symptoms than the placebo group, and the improvements grew over time. Other small studies have found similar results. The overall conclusion appears to be that purified GLA may offer some benefit for rheumatoid arthritis, especially when used along with standard treatment for rheumatoid arthritis.

Raynaud's phenomenon

High dosages of evening primrose oil may be useful for Raynaud's phenomenon, a condition in which a person's hands and feet show abnormal sensitivity to cold temperature. A small double-blind study found that GLA produced significantly better results than placebo. Similar results have been obtained with the omega-3 fatty acids found in fish oil.

Attention deficit/hyperactivity disorder (ADHD)

Clinical studies suggest that children with ADHD have lower levels of essential fatty acids (EFAs), both omega-6s and omega-3s. EFAs are important to normal brain and behavioural function. Some studies suggest that taking fish oil (containing omega-3 fatty acids) may help reduce ADHD symptoms, though the studies have not been well designed. Studies that used evening primrose oil have found it was no better than placebo at reducing symptoms.

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.

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 tumour cell lines in vitro and tumour implants in experimental animal models. There are limited studies on the effect of GLA on tumours in humans. In a study by Dippenaar et al., GLA caused significant (up to 70%) growth inhibitory effects on mouse BL6 melanoma cells in vitro at a dose of 20 mg/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 (15); withdrawal of GLA from the growth media after 5-day treatment suppressed the growth for 5 more days (16). 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 PGE1 stimulates cyclic-AMP formation and induces cell death in cancer cell lines. In 1985, Begin et al. (17) confirmed that GLA has growth inhibitory actions against human prostate, breast, and lung cancer cells with no effect on normal cells.

Eczema

Alterations in linoleic acid metabolism have been demonstrated in atopic conditions such as eczema. Conversion of linoleic acid to gammalinolenic acid is inhibited in individuals with atopic dermatitis. During the past two decades several studies have reported mixed results on the use of GLA-containing oils, particularly EPO, for atopy. (18-21) these studies reveal subtle improvements, such as decreased inflammation and itching; however, overall numbers failed to reveal significant change. In a multicentre trial, 179 patients with atopic dermatitis were treated with 4 g EPO daily. After 12 weeks, 62 % of patients demonstrated a significant clinical response based on a standardized clinical assessment form (22). Conflicting data are found for GLA in the treatment of eczema. The UK's

Medicines and Healthcare products Regulatory Agency has withdrawn GLA's product licence for atopic eczema. (23) Still, the US National Institute of Health's Medline Plus states that there is 'B' grade evidence ('good scientific evidence') for the efficacy of evening primrose oil in the treatment of eczema and skin irritation. But it cautions that large well-designed studies are still needed. A controlled study of borage oil for eczema found no benefit to GLA; it underperformed placebo. (24)

High blood pressure (Hypertension)

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. (25) 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

Mastalgia

Cyclic mastalgia, also known as fibrocystic breast disease, cyclic mastitis, and mastodynia, is a condition in which a woman's breasts become painful during the week or two before her menstrual period. The discomfort is accompanied by swelling, inflammation, and sometimes actual cysts that form in the breasts. It is often associated with other symptoms of premenstrual syndrome (PMS).

Osteoporosis

Some studies suggest that people who don't get enough of some essential fatty acids (particularly EPA and GLA) are more likely to have bone loss than those with normal levels of these fatty acids. In a study of women over 65 with osteoporosis, those who took EPA and GLA supplements had less bone loss over 3 years than those who took placebo. Many of these women also experienced an increase in bone density.

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, behavioural, 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. Behavioural 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 although most studies have found no effect, some women report relief of PMS symptoms when using GLA. The symptoms that seem to be helped the most are breast tenderness and feelings of depression, as well as irritability and swelling and bloating from fluid retention.

Other Indications

A large cross-sectional Japanese study demonstrated a positive association between improvement in seasonal allergic rhino conjunctivitis and GLA supplementation (26). GLA supplementation has also been shown to provide benefit for insomnia, (27) tardive dyskinesia (28), and uremic skin symptoms in haemodialysis patients (29). A recent clinical trial found GLA (1.5 g borage oil) and EPA (1.5 g fish oil) daily benefited adults with periodontitis (30).

INTERACTIONS

Ceftazidime

The activity of ceftazidime against *Pseudomonas aeruginosa* appears to be potentiated by fatty acids, including GLA (31). In vitro, GLA exerts bacteriostatic effects on *Escherichia coli* and, in combination with arachidonic acid, is bactericidal to *P. aeruginosa*.

Cisplatin; Doxorubicin

In ovarian cancer cells, pre-incorporation of either gamma-linolenic acid (GLA) or eicosapentaenoic acid (EPA) increased sensitivity to doxorubicin and cisplatin; however, it was difficult to distinguish the cytotoxicity of the drug from the fatty acid (32). The cytotoxicity of the fatty acids was additive with that of the drugs. Another study investigating the effectiveness of

this combination therapy on human neuroblastoma cell lines found that addition of GLA to the growth medium reduced the cytotoxic effects of cisplatin and carboplatin (33).

Idarubicin; Mitoxantrone

Preclinical data suggest a possible enhancement of cellular uptake of idarubicin when cells are enriched with GLA (34). In multidrug resistant human breast cancer and bladder cancer cells, pre-incorporation of GLA at non-cytotoxic levels increased the cellular uptake of idarubicin. In addition, GLA changed the intracellular distribution of mitoxantrone.

Tamoxifen

GLA may interact with the estrogen receptor antagonist tamoxifen. In one short-term phase II clinical trial, high-dose GLA therapy (8 capsules/day supplying 2.8 g GLA) potentiated the effects of tamoxifen (20 mg OD) in 38 stage I-II breast cancer patients, 90% of whom were estrogen receptor-positive (35). Patients given GLA exhibited a faster treatment response that was evident by 6 weeks.

Vincristine; Vinblastine

In human neuroblastoma cell lines, addition of GLA to the growth medium enhanced the cytotoxic effects of the vinca alkaloids, vincristine, vinblastine, and vindesine (49). In both vincristine-sensitive and -resistant human cervical cancer cells, GLA was cytotoxic (36).

Drug-Nutrient Interactions

GLA has shown promising results when combined with the anticancer drugs tamoxifen and paclitaxel.

- 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.
- 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. In this study, the authors observed the synergistic action of GLA when the cells were co-incubated with paclitaxel, whereas pre-incubation 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) (37).
- Recently, it was shown that GLA acts synergistically with tamoxifen in enhancing the antitumor activity. 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 tumour growth and expression of estrogen receptors.
- Ikushima et al. (33) 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, vinblastine, 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 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. 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.

Nutrient-Nutrient Interactions

Zinc, ascorbic acid, and vitamin B6 regulate delta-6-desaturase activity and aid in the conversion of GLA to PGE1. Deficiencies of these vitamins and minerals may contribute to low levels of EFAs. Optimal absorption and activity of vitamin D and calcium is dependent on sufficient fatty acids in the diet. GLA and EPA enhance calcium absorption and activity with a corresponding decrease or reversal of bone loss.

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. Employing this technique, the oil can be concentrated to 40–80% GLA (38). 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% triacylglycerol's, 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. 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 (39) and canola. 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.

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 triacylglycerol's with desired configuration (40).

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. 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. (41) 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 40C, and time of 24 hours for incorporation of GLA into fish oils.

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