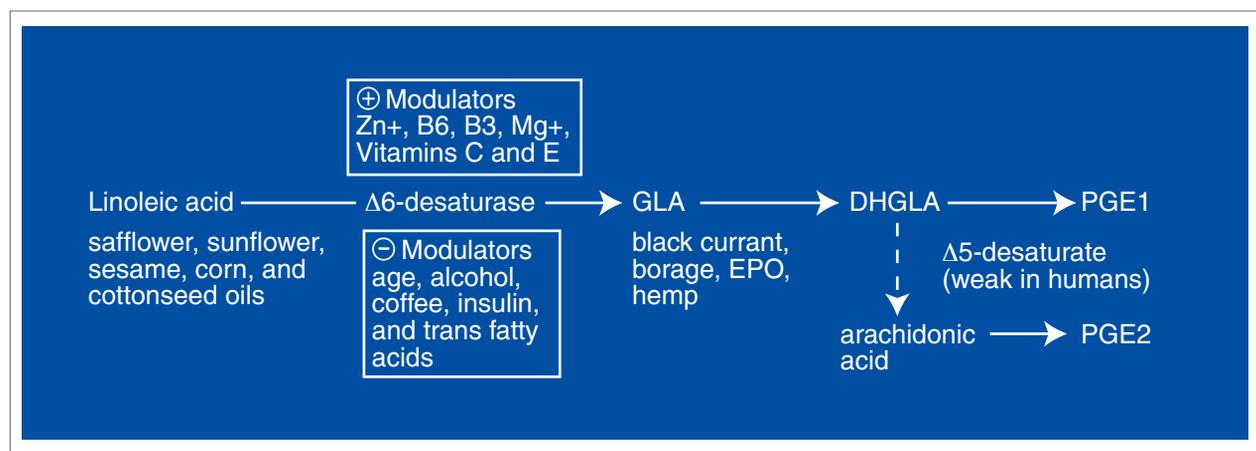


Gamma-Linolenic Acid (GLA)

Introduction

Gamma-linolenic acid (GLA) is an important conditionally essential fatty acid (EFA). 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 also known as 18:3n-6; 6,9,12-octadecatrienoic acid; cis-6, cis-9, cis-12-octadecatrienoic acid; and gamolenic acid.

GLA is found naturally in the fatty acid fractions of some plant seed oils. Most notably, sources of GLA include evening primrose oil (EPO), borage oil, black currant oil, and hemp seed oil. GLA is present in EPO at concentrations of 7-14 percent of total fatty acids; in borage seed oil at 20-27 percent; and in black currant seed oil at 15-20 percent. GLA is also found in some fungal sources, and a minimal amount is produced in the body as a downstream metabolite of the delta-6-desaturase induced conversion from the EFA linoleic acid. Under certain conditions, such as decreased enzymatic activity of delta-6-desaturase, GLA may become conditionally essential. GLA is also present naturally in the form of triglycerides.



Biochemistry

The stereospecificity 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.¹ 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.² The result will be more favorable, with an increase in anti-inflammatory and antithrombotic effects.

Pharmacokinetics

Although the ingestion of GLA-enriched oils results in the accumulation of DGLA in tissue phospholipids and triglycerides, the absolute level of GLA in the oil may not be the sole determinant of biological efficacy. Precise triglyceride stereospecific composition and cellular kinetics of phospholipases and acyltransferases may also influence GLA bioavailability.³ Although GLA concentration in borage oil is two-fold higher than in EPO, GLA-related effects, such as the formation of PGE1, are comparable for both dietary oils on a per gram basis.⁴ The intake of both fat and protein in the diet can affect an individual's essential fatty acid status. High intake of certain saturated fatty acids may increase the requirement for EFAs due to a decrease in essential fat production and decreased bioavailability.⁵

A randomized, double-blind trial on the pharmacokinetics and tolerability of essential fatty acids demonstrated that fasting plasma GLA levels plateaued within seven days of beginning treatment, regardless of dose.⁶ This suggests therapeutic levels of essential fatty acids can be achieved within a week.

GLA is metabolized to the 20-carbon polyunsaturated fatty acid DGLA, which is cyclooxygenated to prostaglandin E1 (PGE1). PGE1 elicits biologic activities by binding to surface receptors on smooth muscle cells, increasing intracellular cAMP. GLA and DGLA are not normally found in the free state, but occur as components of phospholipids, triglycerides, neutral lipids, and cholesterol esters, mainly in cell membranes. PGE1 is me-

tabolized to smaller prostaglandin remnants – primarily dicarboxylic acids. The majority of metabolites are excreted in the urine.

Mechanism of Action

GLA, via conversion to PGE1, exhibits anti-inflammatory, antithrombotic, antiproliferative, and lipid-lowering potential. It also enhances smooth muscle relaxation and vasodilation. In addition, EFAs including GLA are important constituents of membrane phospholipids, including the mitochondrial membrane, where they enhance the integrity and the fluidity of the membrane.⁷

Deficiency States

Infants appear to lack sufficient delta-6-desaturase activity. Whereas breast milk is high in GLA and DGLA, infant formula is not. This can lead to a deficiency state in formula-fed infants, particularly skim milk-based formula.⁸ GLA-deficient infants present with dryness, desquamation and thickening of the skin, and growth failure.^{9,10}

Subclinical deficiency of essential fatty acids has been studied in pre-term and term infants. The fatty acid composition of structural membrane lipids can impact membrane function by modifying overall membrane fluidity, affecting membrane thickness, or by influencing the interaction of fatty acids with membrane proteins. Changes in neuronal membranes that affect membrane excitability have also been noted.¹¹

In a screening study on a Japanese population with high rates of atrophic gastritis, individuals with gastritis had significantly lower GLA levels than individuals without gastritis. Additionally, the same individuals also had elevated levels of DHA when compared to healthy subjects who had lower levels of DHA.¹² Studies also show disturbances in linoleic acid metabolism in obese individuals, particularly children.^{13,14}

Clinical Indications

Rheumatoid Arthritis

A series of clinical trials has measured the efficacy of various GLA dosages and sources on the progression of rheumatoid arthritis. Two placebo-controlled studies were conducted using borage seed oil. In a six-month study using daily doses of 1.4 g GLA from borage oil or cottonseed oil as placebo, the borage-treated group showed significant improvement (36.8%) compared to the placebo group (5.6%).¹⁵ Improvement was measured by joint tenderness counts and scores, joint swelling scores, physician global assessment, and pain. No patients went into remission. In the second study, 56 patients received 2.8 g GLA from borage seed or placebo for six months.¹⁶ A greater percentage of the borage-treated group showed improvement (64%) than did the placebo group (21%), defined as a 25-percent improvement in at least four measures, including tender joint counts, swollen joint counts, tender joint score, visual analogue score, and the Health Assessment Questionnaire Score.

Three trials have been conducted using EPO for rheumatoid arthritis. In the first trial, 40 patients took either 6 g EPO daily or placebo (olive oil) for six months.¹⁷ The EPO group revealed a significant improvement in morning stiffness, but not overall stiffness; whereas, the olive oil group experienced no change in morning stiffness, but an improvement in overall stiffness. A second study examined an EPO/fish oil combination, EPO, or placebo for a one-year period.¹⁸ Forty-nine patients were randomized to receive either EPO capsules providing 540 mg GLA or EPO/fish oil capsules containing 450 mg GLA and 240 mg EPA. A significant percentage of treated individuals reported subjective improvement compared to placebo (94% versus 30%). In addition, 73 percent of EPO patients and 80 percent of EPO/fish oil patients reduced or stopped non-steroidal anti-inflammatory drugs (NSAIDs) therapy compared to 33 percent of the placebo group. In the third trial, no significant changes occurred following three months of 20 mL/day of EPO.¹⁹

In a six-month trial, 34 patients took daily doses of 10.5 g black currant seed oil (containing 2.0 g GLA) or placebo (soybean oil).²⁰ Patients maintained NSAIDs and/or corticosteroid treatment throughout the study. Those in the treatment group who completed the study demonstrated significant improvement in joint tenderness scores compared to those on placebo who experienced no change.

Atopic Eczema

Alterations in linoleic acid metabolism have been demonstrated in atopic conditions such as eczema.²¹ Conversion of linoleic acid to gamma-linolenic 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.²²⁻²⁵ These studies reveal subtle improvements, such as decreased inflammation and itching; however, overall numbers failed to reveal significant change.

In a multicenter trial, 179 patients with atopic dermatitis were treated with 4 g EPO daily. After 12 weeks, 62 percent of patients demonstrated a significant clinical response based on a standardized clinical assessment form.²⁶

Wright and Burton treated 60 adults and 39 children with atopic eczema with EPO or placebo for 12 weeks.²⁷ Adults received either 1.44 g EPO (180 mg GLA) or two times or four times that dose. Children received either 0.72 g EPO (90 mg GLA) or two times that dose. A moderate improvement in clinical signs, including itching, followed supplementation, particularly at the highest doses of EPO.

Most recently a double-blind, randomized, placebo-controlled trial was conducted on 118 formula-fed infants who were at high familial risk of developing atopic dermatitis.²⁸ Infants with a maternal history of atopic disease received a borage oil supplement (100 mg GLA) or placebo (sunflower oil) daily for the first six months of life. Outcome was based on incidence and severity of atopic dermatitis as well as total serum immunoglobulin E (IgE). Clinically, severity of atopic dermatitis was decreased favorably in the borage-oil group, although atopy was still present. Additionally, GLA had no effect on IgE levels during the first year. The authors

concluded the constant dose of GLA given over time was possibly insufficient with progressing age and body weight.

Acute Respiratory Distress Syndrome

The anti-inflammatory mechanism of GLA has successfully been evaluated in treating critically ill patients suffering with acute respiratory distress syndrome. Results of a randomized, double-blind, controlled, multicenter trial found that, compared to a control diet, enteral nutrition supplemented with EPA, GLA, and antioxidants for at least 4-7 days significantly reduced pulmonary neutrophil recruitment and inflammation in 146 patients.²⁹ The EFAs and antioxidants also benefited the amount of gas exchange, requirement for mechanical ventilation, length of intensive care unit stay, and reduction of new organ failure. Other clinical trials have demonstrated similar results.^{30,31}

Asthma

A randomized, double-blind, placebo-controlled trial in patients with mild-to-moderate asthma examined the effectiveness of supplementing an EPA/GLA combination to stimulate leukotriene biosynthesis.³² Forty-three patients were randomized to receive either 10 g (0.75 g GLA + 0.5 g EPA) or 15 g (1.13 g GLA + 0.75 g EPA) of an EPA/GLA emulsion or placebo (olive oil). After being followed for four weeks, patients' leukotriene biosynthesis was significantly reduced in the treatment group compared to placebo. Inhaler use also decreased. No other clinically significant changes were noted in four weeks, suggesting the need for longer patient follow-up.

Premenstrual Syndrome

A preparation containing nine-percent GLA (Efamol[®]) was used in the treatment of 68 women, ages 21-48, with severe cases of PMS.³³ Subjects were given two capsules of Efamol twice daily three days before the expected onset of symptoms until the start of menstruation. At the conclusion of the study, 61 percent experienced complete relief of symptoms, 23 percent had partial relief, and 15 percent reported no relief.

Cardiovascular Disease

Given the role of blood lipids in the progression of cardiovascular disease, recent research has focused on the effects of combined fatty acid supplementation. Dietary supplementation with GLA alone has yielded variable results on circulating lipid levels.³⁴⁻³⁶

A study of 32 women, ages 36-68, assessed the effects of different combinations of EFAs on serum lipids.³⁷ Subjects received either 4 g EPA + DHA; 4 g EPA + DHA plus 1 g GLA (4:1); 4 g EPA + DHA plus 2 g GLA (4:2); or 4 g EPA + DHA plus 4 g GLA (4:4) daily for 28 days. At the end of the 28-day period, the ratio of total-to-HDL cholesterol was significantly reduced in all four groups, by 11-, 9.6-, 14-, and 14.7 percent, respectively. Mean group reductions in LDL:HDL ratios from days 0-28 were statistically significant in the 4:1-, 4:2-, and most dramatically in the 4:4 group, with a 19.9-percent reduction. Triglyceride concentrations were most predominantly lowered in the 4:2 group. In general, the study demonstrated a combination of GLA and marine oils may be beneficial in improving lipid profiles.

Ulcerative Colitis

A randomized, double-blind, placebo-controlled study measured the efficacy of fatty acid supplementation in maintaining remission of ulcerative colitis.³⁸ Sixty-three patients were given EFAs (1.62 g GLA, 270 mg EPA, and 45 mg DHA) or placebo (500 mg sunflower oil) for 12 months. The study did not reveal a significant difference in relapse rates between active medication and placebo (placebo, 38%; EFAs, 55%). The study concluded that a different ratio of oils or larger doses may prove more beneficial.

Attention Deficit/Hyperactivity Disorder

In a small (n=18) double-blind, placebo-controlled, crossover trial, boys ages 6-12 with a clinical diagnosis of attention deficit/hyperactivity disorder (ADHD) were randomized to receive d-amphetamine or Efamol.³⁹ Zinc nutritional status was analyzed with regard to drug efficacy. In children with adequate zinc levels, d-amphetamine appeared to have the most profound effect based on the Conners Teacher Hyperactivity Index, while children with borderline-low zinc levels showed a profound improvement from Efamol. Results from this study indicate zinc nutrition may be an important factor in the treatment of ADHD. In children with borderline zinc status, EPO can improve or compensate for a mild deficiency, possibly because zinc is a cofactor in the conversion of linoleic acid to GLA.

Cancer

GLA has shown promise in the treatment of cancer, both as a cytotoxic agent and as an adjunct to chemotherapy. In the treatment of breast cancer, GLA, when used in combination with tamoxifen, was found to down-regulate estrogen receptor expression, both in an animal⁴⁰ and clinical⁴¹ trial. In the latter trial, 38 breast cancer patients were given 2.8 g GLA plus 20 mg tamoxifen daily and compared to matched controls who received only tamoxifen. GLA combined with tamoxifen was found to enhance the efficacy of tamoxifen and increase response rate – evident by the sixth week. There was a greater reduction in estrogen receptor expression in the combination group than the group taking only tamoxifen.

Additionally, animal studies have shown GLA, specifically in the form of borage oil, can inhibit a mammary tumorigenic response by increasing the activity of ornithine decarboxylase in mammary tumors.⁴² *In vitro* studies demonstrate various EFAs, particularly GLA, can enhance the effect of paclitaxel, a chemotherapy drug used for breast and ovarian cancers.⁴³

EFAs may also be effective in the treatment of certain cancers resistant to other

treatment.⁴⁴ In a small trial on human gliomas, a moderate improvement in patient survival was observed after 1 mg GLA was administered via cerebral reservoir for seven days.⁴⁵ GLA also demonstrated a synergistic effect with gemcitabine against pancreatic adenocarcinoma cell lines.⁴⁶ Intravesicular GLA has been shown to be cytotoxic in superficial bladder cancer, with a response rate of 43 percent, demonstrating a cytotoxic effect against transitional cell carcinoma.⁴⁷

Abnormal fatty acid concentrations have been detected in mononuclear blood cells from patients with lymphoblastic leukemia. In acute lymphoblastic leukemia, abnormal PUFA cells result from an increase in delta-6-desaturase, increasing production of tumor-promoting prostaglandin E2 (PGE2). In contrast, patients with myeloid leukemias do not show any significant differences.⁴⁸ An *in vitro* study demonstrated GLA induces apoptosis in isolated B-cells cultured from patients with chronic lymphocytic leukemia.⁴⁹

Dry Eye Syndrome

In a recent randomized, double-blind, clinical trial, 20 individuals with diagnosed keratoconjunctivitis sicca (dry eye syndrome) received either an oral dose of 28 mg linoleic acid plus 1 mg GLA twice daily in addition to a tear solution or a tear substitution and placebo for 45 days.⁵⁰ Statistically significant changes occurred in the treated group with a reduction in ocular surface inflammation and improvement in dry eye symptoms. These results are somewhat surprising given the small dosage size.

Osteoporosis

A placebo-controlled trial examined the effect of either a combination of GLA/EPA or placebo (coconut oil) for 18 months on 65 elderly women with senile osteoporosis.⁵¹ In addition, all women were given 600 mg calcium carbonate daily. During the first eighteen months, lumbar spine density remained the same in the treatment group, but decreased 3.2 percent in the placebo group. Femoral bone density in the treated group increased 4.7 percent, but did not change in the placebo group.

Diabetic Neuropathy

GLA has shown promising results in the treatment of diabetic complications in several human and animal studies.⁵²⁻⁵⁵ In a double-blind, placebo-controlled, parallel trial of 111 patients with mild diabetic neuropathy, patients were given either 480 mg GLA or placebo daily.⁵⁶ After one year, GLA-treated patients showed favorable improvement in all parameters, including hot and cold threshold, sensation, tendon reflexes, and muscle strength compared to placebo.

In a smaller, double-blind, placebo-controlled trial of 22 patients with distal diabetic neuropathy, similar results were achieved at a dose of 360 mg GLA daily for six months.⁵⁷

Other Indications

A large cross-sectional Japanese study demonstrated a positive association between improvement in seasonal allergic rhinoconjunctivitis and GLA supplementation.⁵⁸ GLA supplementation has also been shown to provide benefit for insomnia,⁵⁹ tardive dyskinesia,⁶⁰ and uremic skin symptoms in hemodialysis patients.⁶¹ A recent clinical trial found GLA (1.5 g borage oil) and EPA (1.5 g fish oil) daily benefited adults with periodontitis.⁶²

Nutrient-Nutrient Interactions

Zinc, ascorbic acid, and vitamin B6 regulate delta-6-desaturase activity and aid in the conversion of GLA to PGE1.⁶³ 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.⁶⁴

Drug-Nutrient Interactions

GLA has shown promising results when combined with the anticancer drugs tamoxifen^{40,41} and paclitaxol.⁴³

Research demonstrates that some individuals on NSAIDs, as well as patients on corticosteroids, have been able to decrease dosages or even discontinue medications completely after supplementation with GLA.¹⁸

Side Effects and Toxicity

Dietary and supplemental sources of GLA have been reliably investigated and have been found to be nontoxic. Limited cases of soft stool, belching, and abdominal bloating have been reported.⁶⁵ Several long-term studies have demonstrated up to 2.8 g GLA daily is well tolerated.^{15,16}

Dosage

Dose is dependent on age and the condition being treated. Children with eczema are often given 2-4 g GLA-containing oil daily (90-100 mg GLA). Rheumatoid arthritis in an adult is often treated with 5-10 g GLA-containing oil daily (2 g GLA), although some studies reveal higher dosages. Supplementation should be given with food to avoid gastrointestinal distress.

References

1. Lawson LD, Hill EG, Holman RT. Dietary fats containing concentrates of cis or trans octadecenoates and the patterns of polyunsaturated fatty acids of liver phosphatidylcholine and phosphatidylethanolamine. *Lipids* 1985;20:262-267.
2. Cleland LG, James MJ, Proudman SM. The role of fish oils in the treatment of rheumatoid arthritis. *Drugs* 2003;63:845-853.
3. Fan YY, Chapkin RS, Ramos KS. Dietary lipid source alters murine macrophage/vascular smooth muscle cells interaction *in vitro*. *J Nutr* 1996;126:2083-2088.
4. Fan YY, Chapkin RS. Mouse peritoneal macrophage prostaglandin E1 synthesis is altered by dietary gamma-linolenic acid. *J Nutr* 1992;122:1600-1606.
5. Alfin-Slater RB, Morris RS, Hansen H, Proctor JF. Effects of non-essential fatty acids on essential fatty acid deficiency. *J Nutr* 1965;87:168-172.

6. Surette ME, Koumenis IL, Edens MB, et al. Inhibition of leukotriene synthesis, pharmacokinetics, and tolerability of a novel dietary fatty acid formulation in healthy adult subjects. *Clin Ther* 2003;25:948-971.
7. Mayes P. Metabolism of unsaturated fatty acids and eicosanoids. In: Murray R, Granner D, Mayes P, Rodwell V, eds. *Harper's Biochemistry*. 24th ed. Appleton & Lange: Stamford, CT; 236-244.
8. Horrobin DF. Nutritional and medical importance of gamma-linolenic acid. *Prog Lipid Res* 1992;31:163-194.
9. Caldwell MD, Jonsson HT, Othersen HB Jr. Essential fatty acid deficiency in an infant receiving prolonged parenteral alimentation. *J Pediatr* 1972;81:894-898.
10. Hansen AE. Essential fatty acids and infant nutrition; Borden award address. *Pediatrics* 1958;21:494-501.
11. Salem N Jr, Shingu T, Kim HY, et al. Specialization in membrane structure and metabolism with respect to polyunsaturated lipids. In: Karnovsky ML, Leaf A, Bollis LC, eds. *Biological Membranes: Aberrations in Membrane Structure and Function*. New York, NY: Alan R. Liss; 1988:319-333.
12. Ito Y, Suzuki K, Imai H, et al. Effects of polyunsaturated fatty acids on atrophic gastritis in a Japanese population. *Cancer Letters* 2001;163:171-178.
13. Decsi T, Csabi G, Torok K, et al. Polyunsaturated fatty acids in plasma lipids of obese children with and without metabolic cardiovascular syndrome. *Lipids* 2000;35:1179-1184.
14. Desci T, Molnar D, Koletzko B. Long-chain polyunsaturated fatty acids in plasma lipids of obese children. *Lipids* 1996;31:305-311.
15. Leventhal LJ, Boyce EG, Zurier RB. Treatment of rheumatoid arthritis with gammalinolenic acid. *Ann Intern Med* 1993;119:867-873.
16. Zurier RB, Rossetti RG, Jacobson EW, et al. Gamma-linolenic acid treatment of rheumatoid arthritis. A randomized, placebo-controlled trial. *Arthritis Rheum* 1996;39:1808-1817.
17. Brzeski M, Madhok R, Capell HA. Evening primrose oil in patients with rheumatoid arthritis and side effects of non-steroidal anti-inflammatory drugs. *Br J Rheumatol* 1991;30:370-372.
18. Belch JJ, Ansell D, Madhok R, et al. Effects of altering dietary essential fatty acids on requirements for non-steroidal anti-inflammatory drugs in patients with rheumatoid arthritis: a double-blind placebo-controlled study. *Ann Rheum Dis* 1988;47:96-104.
19. Jantti J, Seppala E, Vapaatalo H, Isomaki H. Evening primrose oil and olive oil in the treatment of rheumatoid arthritis. *Clin Rheumatol* 1989;8:238-244.
20. Leventhal LJ, Boyce EG, Zurier RB. Treatment of rheumatoid arthritis with blackcurrant seed oil. *Br J Rheumatol* 1994;33:847-852.
21. Melnik BC, Plewig G. Is the origin of atopy linked to deficient conversion of omega-6-fatty acids to prostaglandin E1? *J Am Acad Dermatol* 1989;21:557-563.
22. Andreassi M, Forleo P, Di Lorio A, et al. Efficacy of gamma-linolenic acid in the treatment of patients with atopic dermatitis. *J Int Med Res* 1997;25:266-274.
23. Schalin-Karrila M, Mattila L, Jansen CT, Uotila P. Evening primrose oil in the treatment of atopic eczema: effect on clinical status, plasma phospholipid fatty acids and circulating blood prostaglandins. *Br J Dermatol* 1987;117:11-19.
24. Landi G. Oral administration of borage oil in atopic dermatitis. *J Appl Cosmetol* 1993;11:115-120.
25. Henz BM, Jablonska S, van de Kerkhof PC, et al. Double-blind, multicentre analysis of the efficacy of borage oil in patients with atopic eczema. *Br J Dermatol* 1999;140:685-688.
26. Steward JC, Morse PF, Moss M, et al. Treatment of severe and moderately severe atopic dermatitis with evening primrose oil (Epogam): a multi-center study. *J Nutr Med* 1991;2:9-15.
27. Wright S, Burton JL. Oral evening-primrose-seed oil improves atopic eczema. *Lancet* 1982;2:1120-1122.
28. van Gool CJ, Thijs C, Henquet CJ, et al. Gamma-linolenic acid supplementation for prophylaxis of atopic dermatitis – a randomized controlled trial in infants at high familial risk. *Am J Clin Nutr* 2003;77:943-951.
29. Gadek JE, DeMichele SJ, Karlstad MD, et al. Effect of enteral feeding with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants in patients with acute respiratory distress syndrome. Enteral Nutrition in ARDS Study Group. *Crit Care Med* 1999;27:1409-1420.

30. Pacht ER, DeMichele SJ, Nelson JL, et al. Enteral nutrition with eicosapentaenoic acid, gamma-linolenic acid, and antioxidants reduces alveolar inflammatory mediators and protein influx in patients with acute respiratory distress syndrome. *Crit Care Med* 2003;31:491-500.
31. Kumar KV, Rao SM, Gayani R, et al. Oxidant stress and essential fatty acids in patients with risk and established ARDS. *Clin Chim Acta* 2000;298:111-120.
32. Surette ME, Koumenis IL, Edens MB, et al. Inhibition of leukotriene biosynthesis by a novel dietary fatty acid formulation in patients with atopic asthma: a randomized, placebo-controlled, parallel-group, prospective trial. *Clin Ther* 2003;25:972-979.
33. Brush MG, Watson SJ, Horrobin DF, Manku MS. Abnormal essential fatty acid levels in plasma of women with premenstrual syndrome. *Am J Obstet Gynecol* 1984;150:363-366.
34. Graham J, Franks S, Bonney RC. *In vivo* and *in vitro* effects of gamma-linolenic acid and eicosapentaenoic acid on prostaglandin production and arachidonic acid uptake by human endometrium. *Prostaglandins Leukot Essent Fatty Acids* 1994;50:321-329.
35. Guivernau M, Meza N, Barja P, Roman O. Clinical and experimental study on the long-term effect of dietary gamma-linolenic acid on plasma lipids, platelet aggregation, thromboxane formation, and prostacyclin production. *Prostaglandins Leukot Essent Fatty Acids* 1994;51:311-316.
36. von Schacky C. n-3 fatty acids and the prevention of coronary atherosclerosis. *Am J Clin Nutr* 2000;71:224S-227S.
37. Laidlaw M, Holub BJ. Effects of supplementation with fish oil-derived n-3 fatty acids and gamma-linolenic acid on circulating plasma lipids and fatty acid profiles in women. *Am J Clin Nutr* 2003;77:37-42.
38. Middleton SJ, Naylor S, Woolner J, Hunter JO. A double-blind, randomized, placebo-controlled trial of essential fatty acid supplementation in the maintenance of remission of ulcerative colitis. *Aliment Pharmacol Ther* 2002;16:1131-1135.
39. Arnold LE, Pinkham SM, Votolato N. Does zinc moderate essential fatty acid and amphetamine treatment of attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol* 2000;10:111-117.
40. Kenny FS, Gee JM, Nicholson RI, et al. Effect of dietary GLA +/-tamoxifen on the growth, ER expression and fatty acid profile of ER positive human breast cancer xenografts. *Int J Cancer* 2001;92:342-347.
41. Kenny FS, Pinder SE, Ellis IO, et al. Gamma linolenic acid with tamoxifen as primary therapy in breast cancer. *Int J Cancer* 2000;85:643-648.
42. Bunce OR, Abou-El-Ela SH. Eicosanoid synthesis and ornithine decarboxylase activity in mammary tumors of rats fed varying levels and types of n-3 and/or n-6 fatty acids. *Prostaglandins Leukot Essent Fatty Acids* 1990;41:105-113.
43. Menendez JA, del Mar Barbacid M, Montero S, et al. Effects of gamma-linolenic acid and oleic acid on paclitaxel cytotoxicity in human breast cancer cells. *Eur J Cancer* 2001;37:402-413.
44. Plumb JA, Luo W, Kerr DJ. Effect of polyunsaturated fatty acids on the drug sensitivity of human tumour cells lines resistant to either cisplatin or doxorubicin. *Br J Cancer* 1993;67:728-733.
45. Bakshi A, Mukherjee D, Bakshi A, et al. Gamma-linolenic acid therapy of human gliomas. *Nutrition* 2003;19:305-309.
46. Whitehouse PA, Cooper AJ, Johnson CD. Synergistic activity of gamma-linolenic acid and cytotoxic drugs against pancreatic adenocarcinoma cell lines. *Pancreatol* 2003;3:367-373.
47. Harris NM, Crook TJ, Dyer JP, et al. Intravesical meglumine gamma-linolenic acid in superficial bladder cancer: an efficacy study. *Eur Urol* 2002;42:39-42.
48. Agatha G, Hafer R, Zintl F. Fatty acid composition of lymphocyte membrane phospholipids in children with acute leukemia. *Cancer Lett* 2001;173:139-144.
49. Mainou-Fowler T, Proctor SJ, Dickinson AM. Gamma-linolenic acid induces apoptosis in B-chronic lymphocytic leukemia cells *in vitro*. *Leuk Lymphoma* 2001;40:393-403.
50. Barabino S, Rolando M, Camicione P, et al. Systemic linoleic and gamma-linolenic acid therapy in dry eye syndrome with an inflammatory component. *Cornea* 2003;22:97-101.
51. Kruger MC, Coetzer H, de Winter R, et al. Calcium, gamma-linolenic acid and eicosapentaenoic acid supplementation in senile osteoporosis. *Aging (Milano)* 1998;10:385-394.

52. Jack AM, Keegan A, Cotter MA, Cameron NE. Effects of diabetes and evening primrose oil treatment on responses of aorta, corpus cavernosum and mesenteric vasculature in rats. *Life Sci* 2002;71:1863-1877.
53. Simoncikova P, Wein S, Gasperikova D, et al. Comparison of the extrapancreatic action of gamma-linolenic acid and n-3 PUFAs in the high fat diet-induced insulin resistance. *Endocr Regul* 2002;36:143-149.
54. Suresh Y, Das UN. Long-chain polyunsaturated fatty acids and chemically induced diabetes mellitus. Effect of omega-6 fatty acids. *Nutrition* 2003;19:93-114.
55. Horrobin DF. The use of gamma-linolenic acid in diabetic neuropathy. *Agents Actions Suppl* 1992;37:120-144.
56. Keen H, Payan J, Allawi J, et al. Treatment of diabetic neuropathy with gamma-linolenic acid. The gamma-Linolenic Acid Multicenter Trial Group. *Diabetes Care* 1993;16:8-15.
57. Jamal GA, Carmichael H. The effect of gamma-linolenic acid on human diabetic peripheral neuropathy: a double-blind placebo-controlled trial. *Diabet Med* 1990;7:319-323.
58. Wakai K, Okamoto K, Tamakoshi A, et al. Seasonal allergic rhinoconjunctivitis and fatty acid intake: a cross-sectional study in Japan. *Ann Epidemiol* 2001;11:59-64.
59. Yehuda S, Rabinovitz S, Mostofsky DI. Essential fatty acids and sleep: mini-review and hypothesis. *Med Hypotheses* 1998;50:139-145.
60. Soares-Weiser KV, Joy C. Miscellaneous treatments for neuroleptic-induced tardive dyskinesia. *Cochrane Database Syst Rev* 2003;2:CD000208.
61. Yoshimoto-Furuie K, Yoshimoto K, Tanaka T, et al. Effects of oral supplementation with evening primrose oil for six weeks on plasma essential fatty acids and uremic skin symptoms in hemodialysis patients. *Nephron* 1999;81:151-159.
62. Rosenstein ED, Kushner LJ, Kramer N, Kazandjian G. Pilot study of dietary fatty acid supplementation in the treatment of adult periodontitis. *Prostaglandins Leukot Essent Fatty Acids* 2003;68:213-218.
63. Horrobin DF. Loss of delta-6-desaturase activity as a key factor in aging. *Med Hypotheses* 1981;7:1211-1220.
64. Schlemmer CK, Coetzer H, Claassen N, Kruger MC. Oestrogen and essential fatty acid supplementation corrects bone loss due to ovariectomy in the female Sprague Dawley rat. *Prostaglandins Leukot Essent Fatty Acids* 1999;61:381-390.
65. DeLuca P, Rothman D, Zurier RB. Marine and botanical lipids as immunomodulatory and therapeutic agents in the treatment of rheumatoid arthritis. *Rheum Dis Clin North Am* 1995;21:759-777.