Published in final edited form as:

Food Chem Toxicol. 2012 June ; 50(6): 1911–1919. doi:10.1016/j.fct.2012.01.001.

Comparison of growth, serum biochemistries and *n*–6 fatty acid metabolism in rats fed diets supplemented with high-gamma-linolenic acid safflower oil or borage oil for 90 days

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Abstract

Recently, steps have been taken to further developments toward increasing gamma-linolenic acid (GLA) concentration and lowering costs in plant seed oils using transgenic technology. Through identification and expression of a fungal delta-6 desaturase gene in the high linoleic acid safflower plant, the seeds from this genetic transformation produce oil with >40% GLA (high GLA safflower oil (HGSO)). The aim of the study was to compare the effects of feeding HGSO to a generally recognized as safe source of GLA, borage oil, in a 90 day safety study in rats. Weanling male and female Sprague–Dawley rats were fed a semi-synthetic, fat free, pelleted diet (AIN93G) supplemented with a 10% (wt/wt) oil blend containing HGSO or borage oil, with equivalent GLA levels. Results demonstrated that feeding diets containing HGSO or borage oil for 90 days had similar biologic effects with regard to growth characteristics, body composition, behavior, organ weight and histology, and parameters of hematology and serum biochemistries in both sexes. Metabolism of the primary *n*–6 fatty acids in plasma and organ phospholipids was similar, despite minor changes in females. We conclude that HGSO is biologically equivalent to borage oil and provides a safe alternative source of GLA in the diet.

Keywords

Gamma-linolenic acid; Safflower; 90 Day toxicity; GLA safflower oil; Genetically modified safflower oil; Rats

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Conflict of Interest

The authors declare that there are no conflicts of interest.

1. Introduction

One of the fastest growing segments of nutritional products in the United States are those enriched with essential fatty acids, in particular the omega-3 fatty acids (eicosapentaenoic acid (EPA), docosahexaenoic acid) and gamma-linolenic acid (GLA; 18:3 n -6). The use of GLA in functional food, dietary supplements, personal care products, and animal health products has significantly increased recognition of its nutritional and health benefits.

Research on GLA has centered on its therapeutic role in alleviating chronic disease states such as inflammatory disease (Rheumatoid arthritis, critically ill patients and dermatitis), diabetic neuropathy, premenstrual syndrome, and cardiovascular disease risk factors (Fan and Chapkin, 1998). One of the proposed contributing factors in the etiology of these disease states is a chronic imbalance between GLA and its metabolites, dihomo-GLA (DGLA; 20:3 n -6) and arachidonic acid (20:4 n -6). Conversion of the essential fatty acid, linoleic acid (18:2 n -6) to GLA is rate limited by delta-6 desaturase activity (Fig. 1). GLA may become essential under certain pathological conditions (as noted above) that depress delta-6 desaturase activity and thus reduce the production of the elongase product of GLA, DGLA (Fan and Chapkin, 1998). This is important because DGLA can be converted by inflammatory cells to 15-hydroxy-eicosatrienoic acid and prostaglandin E_1 which possess anti-inflammatory and antiproliferative properties (Horrobin, 1992; Ziboh and Fletcher 1992). Numerous preclinical (Mancuso et al., 1997a; Mancuso et al., 1997b; Murray et al., 1995, 2000; Palombo et al., 1997, 1999) and clinical (Gadek et al., 1999; Pacht et al., 2003; Singer et al., 2006; Pontes-Arruda et al., 2006) studies have shown that nutritional formulas enriched with a combination of EPA (20:5 n -3) and GLA can favorably reduce an inflammatory response while promoting vasodilation and oxygen delivery in patients with acute lung injury. Recently Covar and colleagues (Covar et al., 2010) evaluated the safety and efficacy of a novel nutritional formula enriched in EPA and GLA and antioxidants in children 6–14 years of age with mild persistent asthma. Daily consumption of this nutritional formula for 12 weeks prevented the deterioration in bronchial hyper-responsiveness, while showing patterns of improvement in well established biomarkers of inflammation and pulmonary function.

These beneficial effects have increased the awareness and demand for GLA enriched oils. Presently, commercial sources of GLA are borage oil, evening primrose oil, and black current seed oil. The increased demand for GLA in functional foods will require a more concentrated, lower cost, and improved availability of oils containing GLA. Therefore, development of oil seed crops which produce substantial quantities of GLA using transgenic technology has been a major goal of plant biotechnology.

Through identification and expression of a fungal delta-6 desaturase gene in the canola plant, the developed seeds from this genetic transformation produced oil with >30% GLA (high GLA canola oil (HGCO) (Huang et al., 1999). The chemical and physical characteristics of this novel oil have been evaluated (Liu et al., 2001) as well as a series of animal studies conducted showing the bioequivalence and safety of HGCO to borage oil which is normally consumed by humans (Tso et al., 2002; Palombo et al., 2000; Liu et al., 2004; Wainwright et al., 2003). Recently, Arcadia Biosciences, Inc. (Davis, CA) has taken

the steps to further development toward increasing GLA concentration and lowered costs in plant seed oils by producing a high GLA safflower oil (HGSO) comprising >40% GLA with a significantly reduced level of linoleic acid. This level of GLA was achieved through genetic transformation of a high linoleic acid safflower variety with a delta-6 desaturase gene that codes for the enzyme which converts linoleic acid (18:2 n -6) to GLA (18:3 n -6). Arcadia Biosciences currently markets HGSO as a dietary supplement under the name SONOVA 400.

Though HGSO is considered a new plant seed oil containing GLA, it has similarities to borage oil and HGCO, possessing triglycerides with elevated levels of GLA as well as monounsaturated and n -6 fatty acids. Based upon the knowledge gained from the results of the absorption, growth and feeding studies in rats with diets containing HGCO, we chose to compare the effects of feeding HGSO to a generally recognized as safe source of GLA, borage oil, for a period of 90 days on growth characteristics, diet consumption, behavior, appearance, and organ morphology (relative organ size and histology) in male and female rats. In addition, the phospholipid fatty acid composition of plasma and various organs was determined to compare the metabolism of GLA given as either HGSO or borage oil.

2. Materials and methods

The in vivo portion of this study was conducted at the University of Cincinnati-Metabolic Diseases Institute (Cincinnati, Ohio), an Association for Assessment and Accreditation of Laboratory Animal Care, International (AAALAC) accredited facility, in accordance with the animal use protocol approved by the Institutional Animal Care and Use Committee (IACUC), using guidelines set forth by the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

2.1. Animals

Pathogen-free male (159–179 g; $n = 25$) and female (128–157 g; $n = 25$) Sprague–Dawley[®] rats were purchased from Harlan (Indianapolis, IN). The animals were housed in their own room, within a barrier animal facility, in conventional, plastic shoebox cages (Alternative Design, Siloam Springs, AK), one rat per cage, with corn cob bedding (Bed-o-cobs[®], The Andersons, Inc.) under light (12 h light–dark cycle), temperature (72 ± 1 °F) and humidity (30–70%) controlled conditions for the duration of the study. All animals had ad libitum access to food and water.

2.2. Test diets

Each diet was a semi-synthetic, fat free, pelleted diet fortified with vitamins and minerals (AIN93G; Harlan Teklad, Madison, WI) (Table 1) and supplemented with 10% (wt/wt) of an oil blend containing either HGSO or borage oil, with the resulting levels of GLA being equivalent between both diets. The fatty acid compositions of the original HGSO and borage oils, along with the two oil blends in the experimental diets, are presented (Table 2). All test diets were packed in batches of 0.5 kg in vacuum-sealed freezer bags and stored at approximately -20 °C. Fresh aliquots of diet were provided to the animals every other day.

2.3. Experimental design

After acclimation for 1 week, the male and female rats were placed in one of two weight matched groups for each sex, and assigned to receive one of the two experimental diets for the entire ninety day study period. Clinical appearance, body weight, and food intake were monitored weekly. At the end of twelve weeks, the rats were anesthetized with sodium pentobarbital (Fatal Plus, Vortech Pharmaceuticals, Ltd.) and euthanized by exsanguination. Blood from each rat was collected from the abdominal aorta and immediately placed into three different 4.0 ml sterile blood collection tubes (BD Vacutainer®); two tubes contained the anti-coagulant EDTA and the other tube contained Lithium Heparin. The blood in the EDTA tubes was stored in wet ice and covered with aluminum foil until centrifuged for fifteen minutes at 750 g and 4 °C. The plasma was carefully extracted and placed into an additive free tube. The plasma was stored at -20 °C until analyzed that evening for hematological studies and analysis of cholesterol and fatty acid composition. The Lithium Heparin tubes were placed on wet ice, covered with aluminum foil and allowed to coagulate. These tubes were stored at 37 °F until the serum was collected the same evening and used for biochemical analysis. Whole liver, heart, lung, kidney, spleen, and mesenteric adipose tissue were excised and weighed. A section of each of these tissues was fixed in 10% formalin and sent to Children's Hospital Medical Center, Pathology Research Core (Cincinnati, Ohio) for histological processing and hematoxylin and eosin staining. Another section of each tissue was flashfrozen in liquid nitrogen and stored at -80 °C until shipped to Abbott Nutrition (Columbus, Ohio) for phospholipid fatty acid composition analyses. A separate section of liver was snap-frozen in liquid nitrogen and stored at -80 °C until used for lipid analysis. Fecal material from each animal was collected for analysis of total fat content.

2.4. Body composition measurements

Body composition was determined using an EchoMRI (Echo Medical Systems, LLC, Houston, TX), an instrument that uses the differences in the nuclear magnetic resonance properties of hydrogen atoms in organic and non-organic environments to fractionate signals originating from adipose tissue, lean muscle tissue, and free water. This quantitative magnetic resonance (QMR) instrument measures body composition in live, un-anesthetized, small animals, and has become the method of preference for measuring their fat mass. The animals were placed in a clear plastic restraint tube of appropriate size. The tube was placed within the EchoMRI and each rat was scanned for one minute, while measurements were simultaneously recorded. The animal was then removed from the tube and returned to its cage. Echo-MRI was performed on all animals at baseline (week zero) and at week twelve to analyze for body composition.

2.5. Fatty acids analysis of triglycerides and tissue phospholipids

Total lipids in plasma, liver, kidney, spleen, mesenteric fat, and feces from male and female rats were extracted with chloroform/methanol (2/1, v/v) according to the procedure of Folch and colleagues (Folch et al., 1957). Aliquots of total phospholipid extracts from plasma and tissues were isolated from the total lipid fraction by thin-layer chromatography (TLC) using Whatman (Fairfield, NJ) LHPK silica gel plates (10 × 20-cm, 200 µm thickness) as

described previously (Huang et al., 1999). Fatty acid methyl esters were then separated and quantified by gas-liquid chromatography (GLC) using a Hewlett-Packard 6890 series II gas chromatograph, equipped with an Omegawax column (Supelco, Bellefonte, PA) and a flame ionization detector (FID) under the conditions as described previously (Huang et al., 1999). Oil samples were treated similarly beginning at the methylation step.

2.6. Statistical analysis

Statistical analysis was performed on diet consumption, body and organ weights, body composition, hematologic and biochemistry parameters, plasma and tissue phospholipid fatty acids. Data are presented as means and standard errors with a statistical significance set at a p value ≤ 0.05 . Diet consumption and body weights were analyzed with a two-factor mixed-model ANOVA. Separate analyses were performed for male and female animals. Estimation was performed with the MIXED procedure in SAS version 9.2. The correlation among repeated measurements on the same animal was modeled with a first-order autoregressive structure (alternate structures, e.g., compound symmetry, were also tested but found to be vastly inferior according to the AIC criterion). The type I error rate for significance testing and confidence intervals was $\alpha = 0.05$, two sided. Organ weights, body composition, hematologic and biochemistry parameters, plasma and tissue phospholipid fatty acids were analyzed with an unpaired t -test with Welch's correction. Estimation was performed with the MIXED procedure in SAS version 9.2. Significance testing was $\alpha = 0.05$, two tailed.

3. Results

3.1. Food Intake and growth

All rats survived to the end of the 90-day feeding trial and scheduled sacrifice. There was no evidence of any adverse clinical, motor activity, or behavioral observations in any of the animals throughout the study period. Mean daily food consumption by the borage diet male controls (18.1 gm/rat/day) were similar to the HGSO males (18.5 gm/rat/day) ($P > 0.05$). Female rats had lower food consumption versus males, but daily food consumption was not different between the borage (13.1 gm/rat/day) and HGSO (12.7 gm/rat/day) ($P > 0.05$) groups. Based on the average daily food consumption data for males and females and analysis of diet samples to confirm GLA concentration, the mean overall daily intake of GLA for males was 888 mg/kg bw/day for the borage group and 913 mg/kg bw/day for rats given HGSO. Due to the lower food consumption, female rats had a lower mean overall daily intake of GLA with 1191 mg/kg bw/day for the borage group and 1161 mg/kg bw/day for the HGSO rats. Similar growth patterns were observed for both diets within the male and female rats with no differences in body weight changes between groups throughout the 12 week feeding period (Fig. 2). At the time of sacrifice, the mean body weights were 420 ± 11 gm for the male borage and 438 ± 10 gm for the male HGSO groups ($P > 0.05$). There were no significant differences in end of feeding period body weights for the females; 234 ± 4 and 236 ± 7 gm for the borage and HGSO groups, respectively. Female rats given borage or HGSO diets had similar fecal total fat contents of 0.24% and 0.21%, respectively. Male borage oil rats had higher fecal fat contents (1.1%) compared to HGSO animals (0.47%) ($P < 0.05$) (Fig. 3). Body composition analysis of male and female rats using nuclear magnetic

resonance is shown in Fig. 4. At the end of the 90 days of feeding, both male and female rats showed similar changes in fat and lean mass from baseline for the borage and HGSO diets.

3.2. Organ weights and histology

The absolute and relative mean weights of the liver, spleen, kidney, lung, and heart at the time of sacrifice were similar ($P > 0.05$; Table 3) among male and female rats fed the borage and HGSO. Organ weights were similar to previous research comparing 90-day feeding of diets containing either borage oil or HGCO (Liu et al., 2004). The evaluation of organ (kidney, spleen, and middle lobe of the liver) histology revealed slight variations from normal for kidney, spleen and liver for males and females given the borage or HGSO diets. There was very mild focal tubule dilation with protein and no histologic damage in the kidneys of some male and female rats given either borage oil or HGSO. Since these changes were not observed in all rats within the borage control and HGSO groups, the findings were not considered to be borage oil or HGSO related due to their random and rare occurrence for this age and strain of rats. The liver in one female rat given borage or HGSO had a mild diffuse hepatocellular vacuolization. This change is frequently observed in normal rodents and is not considered a significant alteration. The spleen had mild diffuse subcapsular edema in one male and female rat for both the borage and HGSO groups. Although edema is an abnormal finding, it is likely due to osmolarity alterations during fixation and is considered to be unrelated to the diets.

3.3. Hematology and serum biochemistries

Table 4 shows hematology values for male and female rats following 90 days of feeding diets containing either borage oil or HGSO. Overall, there were no adverse effects of HGSO or borage oil on any of the hematology parameters for both male and female rats. Male mean percent eosinophils, mean corpuscular hemoglobin concentration, and platelet counts were slightly higher ($P < 0.05$) in the HGSO group compared to rats given borage oil. Female mean red blood cell counts, percent basophils, hemoglobin, and hematocrit were slightly higher ($P < 0.05$) in the HGSO group compared to rats given borage oil. Mean platelet counts in females that were given HGSO were slightly lower ($P < 0.05$) than those fed the borage oil diet. All of the above differences were not considered adverse because they were minor and within the normal range of variation for each particular hematological parameter. Serum biochemistry profiles of male and female rats fed diets containing either borage oil or HGSO for 90 days are shown in Table 5. Mean potassium, chloride, calcium, glucose, and triglycerides were slightly lower ($P < 0.05$) in the male HGSO group compared to male rats given borage oil. Female mean sodium, total protein, albumin, and cholesterol were slightly lower ($P < 0.05$) in the HGSO group compared to rats given borage oil. Mean blood urea nitrogen and alanine aminotransferase in females that were given HGSO were slightly higher ($P < 0.05$) than those fed the borage oil diet. All of the above differences were not considered adverse because they were minor and within the normal range of variation for each serum biochemistry parameter.

3.4. N–6 fatty acid metabolism in plasma phospholipids

The effects of 90 days of feeding diets with borage oil and HGSO on the *n*–6 fatty acid composition of plasma phospholipids of male and female rats are shown in Fig. 5. The level of 18:2*n*–6 in plasma phospholipids of both males and females were significantly higher ($P < 0.05$) in rats given HGSO compared to borage oil. This was expected due to the HGSO diets containing a higher percentage (39.1%) of 18:2*n*–6 as compared with the borage oil diet (34.1%). Compared with the HGSO dietary group, the male borage oil group had slightly higher ($P < 0.05$) levels of 18:3*n*–6, 20:3*n*–6, and 22:5*n*–6. Female rats had slightly higher ($P < 0.05$) levels of 22:4*n*–6 and lower levels of 22:5 *n*–6 after HGSO feeding compared to borage oil. These observed differences in *n*–6 fatty acid composition of plasma phospholipids for both males and female rats were minor in magnitude and not considered to reflect adverse or significant changes in fatty acid metabolism.

3.5. N–6 fatty acid metabolism in liver phospholipids

The effects of feeding diets with borage oil and HGSO on the *n*–6 fatty acid composition of liver phospholipids of male and female rats are shown in Figs. 6 and 7 (lower left panel). Similar to the observed changes in plasma phospholipid fraction, there was a significant increase ($P < 0.05$) in the level of 18:2*n*–6 in liver phospholipids of both male and female rats given HGSO compared to borage oil. In males, though statistically different, minor differences were observed in 22:4*n*–6 (HGSO > borage oil) and 22:5*n*–6 (borage oil > HGSO) between the two dietary groups. Female rats had slightly higher ($P < 0.05$) levels of 18:3*n*–6 and 22:5*n*–6 and lower levels of 22:4*n*–6 after borage oil feeding compared to HGSO. HGSO female rats showed significantly higher ($P < 0.05$) levels of 20:4*n*–6 versus the borage oil group. This difference in 20:4*n*–6 was not evident in the male animals.

3.6. N–6 fatty acid metabolism in kidney phospholipids

The effects of feeding diets with borage oil and HGSO on the *n*–6 fatty acid composition of kidney phospholipids of male and female rats are shown in Figs. 6 and 7 (upper left panel). Similar to the observed changes in plasma and liver phospholipid fractions, there was a significant increase ($P < 0.05$) in the level of 18:2*n*–6 in kidney phospholipids of both male and female rats given HGSO compared to borage oil. The observed differences in *n*–6 fatty acid composition of kidney phospholipids for both males and female rats were minor in magnitude and reflected the minor differences in fatty acid composition between the two dietary groups.

3.7. N–6 fatty acid metabolism in spleen phospholipids

Changes in the *n*–6 fatty acid composition of splenic phospholipids of male and female rats given either borage oil or HGSO for 90 days are shown in Figs. 6 and 7 (lower right panel). The level of 18:2*n*–6 was significantly higher ($P < 0.05$) for both male and female rats given HGSO compared to borage oil. In males, compared with the HGSO dietary group, borage oil rats had slightly higher ($P < 0.05$) levels of 20:3*n*–6, and lower levels of 18:3*n*–6. Female rats had slightly higher ($P < 0.05$) levels of 22:4*n*–6 and lower levels of 20:3*n*–6 and 22:5*n*–6 after HGSO feeding compared to borage oil.

3.8. N-6 fatty acid metabolism in mesenteric fat

The *n*-6 fatty acid composition of mesenteric fat of male and female rats after 90 days of feeding diets containing either borage oil or HGSO is shown in Figs. 6 and 7 (upper right panel). Following a similar pattern in the plasma, liver, kidney, and spleen, there was a significant increase ($P < 0.05$) in the level of 18:2*n*-6 after feeding HGSO as compared with borage oil for both male and female rats. There were no statistically significant differences in the other *n*-6 fatty acids among the diet groups in either male or female rats.

4. Discussion

These data represent the first dietary studies undertaken to evaluate the biological effects of HGSO in mammals. The levels of HGSO and GLA used in this study was chosen based upon findings from a previous long term feeding study with elevated levels of GLA (Liu et al., 2004). The one dosage level of GLA (10% w/w of an oil blend containing 22% GLA) for this study represents a high level of GLA intake; 913 mg/kg bw/day and 1161 mg/kg/bw/day for male and female HGSO rats respectively. The results showed that when feeding diets containing equal amounts of GLA from either HGSO or borage oil, no significant differences were observed in food intake, growth characteristics, or body composition in either male or female rats. In addition, there was no evidence of any adverse effect of feeding a diet containing HGSO on the appearance, behavior, organ weight and histology of growing Sprague–Dawley rats. The administration of approximately 913 mg/kg bw/day (males) and 1161 mg/kg bw/day (females) of GLA (18:3*n*-6) for 90 days via a diet containing HGSO did not show adverse changes in hematology, clinical chemistries, or coagulation parameters in male and female rats. The observed statistical differences between the borage and HGSO groups for either male or female rats were minor in magnitude and well within the normal range of variation for each particular hematological or biochemical parameter. The variation in hematological and biochemical parameters were similar in magnitude as those observed in a previous 90 day feeding study using high levels of GLA (Liu et al., 2004). Thus these findings were considered clinically insignificant.

To assess whether the absorption and metabolism of 18:3*n*-6 from HGSO was similar to 18:3*n*-6 provided as borage oil, total fecal fat and the fatty acid composition of the phospholipids in plasma, liver, kidney and spleen were measured after 90 days of feeding. Total fecal fat content were similar among the diet groups for females with the exception of male rats given borage oil had higher fecal fat levels compared to males given HGSO. Though the percentage of fecal fat was higher in borage fed males, the percentages of fecal fat for all four dietary groups in this study were lower when compared to similar studies assessing the metabolic effects of 30-day or 90-day feeding of diets containing either borage oil or HGCO (Liu et al., 2004; Palombo et al., 2001). Thus with a percentage of fecal fat below 1.5% for all groups, this would suggest that there were no abnormalities in the digestion and absorption of either HGSO or borage oil. These data are also consistent with a study by Hoy and Christensen (Hoy and Christensen, 1996) which found no significant differences in the absorption and metabolism of 18:3*n*-6 from current commercial sources of 18:3*n*-6 (borage, evening primrose, black currant seed oils).

In plasma and all tissue phospholipids, a significantly higher level of 18:2 n -6 was observed in both male and female rats given HGSO compared to borage oil. This observation is not unexpected since the HGSO diets contain a higher percentage (39.1%) of 18:2 n -6 as compared with the borage oil diet (34.1%). Another possibility could be due to an elevation in 18:2 n -6 which may indicate a decrease in the delta-6 desaturation of 18:2 n -6 in rats given HGSO. The decrease in desaturation could be due to the competition for binding of delta-6 desaturase by linolenic acid (18:3 n -3), which was present in higher amounts in the HGSO diet compared with the borage oil diet (2.0% vs 1.0%, Table 2).

Though our main goal was to maintain equivalent levels of GLA between the two diets, there was a higher ratio of n -3/ n -6 fatty acids within the HGSO diet compared to borage oil. This may have contributed to a reduction in 18:2 n -6 desaturation as n -3 fatty acids are the preferred substrates for the delta-6 desaturase enzyme (Holman, 1964; Stubbs and Smith, 1984). When the phospholipid fatty acid profiles were examined closely for all n -6 fatty acid intermediates and metabolites, only minor changes in the relative percentage of 18:3 n -6, 20:3 n -6, 20:4 n -6, and 22:4 n -6 were evident between the two dietary groups. There was no consistent pattern of differences between the two diets or between male and female rats. Most of the statistical differences were minor in magnitude and similar to n -6 fatty acid metabolism changes observed in previous borage oil and HGCO studies (Palombo et al., 2000; Liu et al., 2004). We also observed a decrease in the levels of 22:5 n -6 in the phospholipids of plasma, liver, kidney, and spleen of female rats only fed the HGSO diet as compared with rats given the borage oil diet. This finding, in the absence of observing meaningful differences in the levels of 18:3 n -6, 20:3 n -6, 20:4 n -6 and 22:4 n -6, suggests that any inhibition on the desaturation of 18:2 n -6 after feeding the HGSO diet may affect the levels of its end metabolite, 22:5 n -6. In the biosynthesis of 22:5 n -6, (Sprecher and colleagues, 1996) proposed a biosynthetic pathway which requires the elongation of 22:4 n -6 to 24:4 n -6 followed by a delta-6 desaturation to 24:5 n -6, which in turn undergoes partial beta-oxidation to form 22:5 n -6. As noted above, the n -3/ n -6 fatty acid ratio in the HGSO diet was higher and thus the n -3 fatty acids could have suppressed the delta-6 desaturase activity thereby decreasing the production of 22:5 n -6. Similar changes in 22:5 n -6 have been reported by others who also fed equal amounts of 18:3 n -6 but with higher n -3 fatty acids (Palombo et al., 2000; Raederstorff and Moser, 1992). The amount of 22:5 n -6 however, represents only a minor fraction of the overall fatty acid composition of tissue phospholipids. Of note, only HGSO female rats showed significantly higher levels of 20:4 n -6 in liver phospholipids versus the borage oil group. Though these results may be an isolated occurrence, it is important to note that the level of 20:4 n -6 in the liver phospholipids in all groups in this study were within the normal ranges of liver 20:4 n -6 observed in previous studies which assessed feeding 18:3 n -6 or non-18:3 n -6 containing oils in rats (Palombo et al., 2000; Palombo et al., 2001; Liu et al., 2004).

The findings in this study were similar to those from a previous set of metabolic studies which were designed to evaluate the bioequivalence and safety of HGCO, a genetically modified plant seed oil. In vivo studies using a lymph fistula rat model (Tso, 1985) showed that the intestinal absorption and lymphatic transport as well as the normal physiologic changes associated with fat absorption (lymph flow and the increase in lymphatic

endogenous lipid outputs, cholesterol and phospholipids) of HGCO were similar to those of borage oil (Tso et al., 2002). The biological effects of short (6 weeks) (Palombo et al., 2000) and long-term (12 weeks) (Liu et al., 2004) feeding of varying levels of HGCO versus borage oil in normal rats have been reported. Results from the 6-week feeding study showed that HGCO and borage oil were well tolerated and had a similar biological effect on growth characteristics and hepatic metabolism of *n*-6 fatty acids (Palombo et al., 2000). Long-term feeding of diets containing HGCO at three different levels (5, 10, and 15% w/w) had similar long-term biological effects as a diet containing 15% w/w borage oil on the growth, relative organ size, organ histology, parameters of hematology and serum biochemistries, and *n*-6 fatty acid metabolism in plasma and various organs (Liu et al., 2004). Further evaluation of HGCO showed no adverse effects on the reproduction, brain and behavioral development in mice when compared to borage oil (Wainwright et al., 2003).

In conclusion, these data show that feeding diets containing HGSO or borage oil for 90 days had similar biologic effects in vivo with regard to growth characteristics, body composition, behavior, organ weight and histology, and common parameters of hematology and serum biochemistries in either male or female rats. When feeding equal amounts of 18:3*n*-6, the metabolism of primary *n*-6 fatty acids in plasma and organ phospholipids were similar despite some minor changes observed in female rats. No adverse effects were observed for HGSO when fed at levels of 1980 mg/kg bw/day and 2530 mg/kg bw/day, for male and female rats, respectively. Based upon the body surface area conversion (EPA, 1992), the human equivalent of these doses is approximately 330–422 mg of HGSO/kg bw/day, an equivalent of 19.8–25.3 gm/day HGSO for a 60 kg adult. Thus a high safety margin for HGSO would be provided up to 25 or 10.7 gm GLA a day. It is concluded that HGSO is biologically equivalent to borage oil and provides a safe alternative source of GLA in the diet.

Acknowledgments

The authors gratefully acknowledge Normanella Dewille, PhD, Judy Atkinson, Kelley Lowe, and Suzette Pereira, PhD for their assistance in the preparation of the oil blends and animal diets and Jeff Welge, PhD for performing statistical analyses. We also acknowledge Christine Hastilow for her expert fatty acid analyses and Heather Ferguson, PhD for her advice and review of the manuscript.

Abbreviations

AAALAC	Association for assessment and accreditation of laboratory animal care
DGLA	dihomo-gamma-linolenic acid
EPA	eicosapentaenoic acid
FID	flame ionization detector
GLA	gamma-linolenic acid
GLC	gas-liquid chromatography
HGCO	high gamma-linolenic acid canola oil
HGSO	high gamma-linolenic acid safflower oil

QMR	quantitative magnetic resonance
TLC	thin-layer chromatography

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The Metabolic Pathway for n-6 Fatty Acids

Fig. 1.
The metabolic pathway for *N*-6 fatty acids.

Fig. 2.
Weekly body weight measurements of male and female animals fed either HGSO or borage oil diet for 12 weeks.

Fig. 3. Fecal fat content in male and female rats given either the HGSO or borage oil diet. * $P < 0.05$ versus male HGSO.

Fig. 4. Body composition measurements showing fat and lean body masses represented as percentages of the total body mass. Measurements were taken using QMR at baseline (week zero) and at week twelve.

Fig. 5.

N-6 fatty acid composition of plasma phospholipids in male and female rats after being fed 12 weeks with HGSO or borage oil diet. (18:2*n*-6, linoleic acid; 18:3*n*-6, gamma-linolenic acid; 20:3*n*-6, dihomogamma-linolenic acid; 20:4*n*-6, arachidonic acid; 22:4*n*-6, adrenic acid; 22:5*n*-6, docosapentaenoic acid). **P* < 0.05.

Fig. 6.

N-6 fatty acid composition of kidney, mesenteric fat, liver, and spleen phospholipids in male rats after being fed 12 weeks with HGSO or borage oil diet. (18:2*n*-6, linoleic acid; 18:3*n*-6, gamma-linolenic acid; 20:3*n*-6, dihomo-gamma-linolenic acid; 20:4*n*-6, arachidonic acid; 22:4*n*-6, adrenic acid; 22:5*n*-6, docosapentaenoic acid). **P* < 0.05.

Fig. 7.

N-6 fatty acid composition of kidney, mesenteric fat, liver, and spleen phospholipids in female rats after being fed 12 weeks with HGSO or borage oil diet. (18:2*n*-6, linoleic acid; 18:3*n*-6, gamma-linolenic acid; 20:3*n*-6, dihomo-gamma-linolenic acid; 20:4*n*-6, arachidonic acid; 22:4*n*-6, adrenic acid; 22:5*n*-6, docosapentaenoic acid). * $P < 0.05$.

Table 1**Diet Composition.**

Component	g/kg
Oil	100
Casein	200
L-cystine	3
Corn starch	397.5
Maltodextrin	132
Sucrose	100
Cellulose	50
Mineral mix, ^a AIN-93G-MX	35
Vitamin mix, ^b AIN-93-VX	10
Choline bitartrate	2.5
TBHQ (antioxidant)	0.02

^a Mineral mix (g/kg): calcium carbonate (357.0), potassium phosphate, monobasic KH₂PO₄ (196.0), potassium citrate, monohydrate (70.78), sodium chloride NaCl (74.0), potassium sulfate KH₂PO₄ (46.6), magnesium oxide MgO (24.3), ferric citrate (6.06), zinc carbonate (1.65), manganous carbonate (0.63), cupric carbonate (0.31), potassium iodate KIO₃ (0.01), sodium selenate Na₂SeO₄ (0.01025), ammonium paramolybdate (NH₄)₆ Mo₇ O₂₄·4H₂O (0.00795), sodium metasilicate Na₂SiO₃·9H₂O (1.45), chromium potassium sulfate CrK(SO₄)₂·12H₂O (0.275), lithium chloride LiCl (0.0174), boric acid H₃BO₃ (0.0815), sodium fluoride NaF (0.0635), nickel carbonate, hydroxide, tetrahydrate (0.0318), ammonium vanadate NH₄VO₃ (0.0066), and sucrose, finely ground (220.716).

^b Vitamin mix (g/kg): nicotinic acid (3.0), calcium pantothenate (1.6), pyridoxine HCl (0.7), thiamin HCl (0.6), riboflavin (0.6), folic acid (0.2), D-biotin (0.02), vitamin B₁₂ (0.1% in mannitol) (2.5), DL- α -tocopheryl acetate (500 IU/g) (15.0), vitamin A palmitate (500,000 IU/g) (0.8), vitamin D₃ (cholecalciferol, 500,000 IU/g) (0.2), vitamin K (phyloquinone) (0.075), sucrose, finely ground (974.705).

Table 2

Fatty acid composition of dietary oils and experimental diets.

Fatty Acid	Borage oil	HGSO	10% Borage oil ^a blend	10% HGSO ^b blend
Myristic acid (14:0)	0.07	0.12	0.14	0.17
Palmitic acid (16:0)	9.80	6.50	10.00	7.70
Stearic acid (18:0)	4.30	2.00	4.50	2.80
Oleic acid (18:1n-9)	15.20	28.20	20.00	24.80
Linoleic acid (18:2n-6)	35.60	14.30	34.10	39.10
Gamma-linolenic acid (18:3n-6)	23.50	42.80	21.60	21.60
Alpha-linolenic acid (18:3n-3)	1.50	0.12	1.00	2.00
Stearidonic acid (18:4n-3)	0.18	0.07	0.14	0.12
Arachidic acid (20:0)	0.30	0.41	0.34	0.37
Gadoleic acid (20:1n-9)	4.10	0.20	3.70	0.23
Erucic acid (22:1n-9)	2.50	0.02	2.40	0.07
Nervonic acid (24:1n-9)	1.50	0.15	1.40	0.13
Others	1.45	5.11	0.68	0.91

Values are presented as percents of total fatty acids.

^a Borage blend = 90% borage oil, 10% canola oil.^b HGSO blend = 47% High-GLA Safflower oil (HGSO), 27% soybean oil, 26% safflower oil.

Table 3

Absolute (abs) and relative (rel) weights of major organs at the end of 12 week feeding for male and female Sprague–Dawley rats fed on AIN93G diet containing either a borage oil blend or a High-GLA safflower oil (HGSO) blend.

Organ	Weight	Male		Female	
		Borage oil	HGSO	Borage oil	HGSO
Liver	Abs (g)	10.30 ± 0.30	10.20 ± 0.30	5.80 ± 0.10	5.60 ± 0.20
	Rel (%)	2.40 ± 0.04	2.30 ± 0.03	2.50 ± 0.03	2.40 ± 0.03
Spleen	Abs (g)	1.20 ± 0.04	1.10 ± 0.04	0.90 ± 0.02	0.90 ± 0.03
	Rel (%)	0.27 ± 0.00	0.25 ± 0.00	0.37 ± 0.00	0.36 ± 0.00
Kidney	Abs (g)	1.50 ± 0.03	1.50 ± 0.03	0.90 ± 0.01	0.90 ± 0.01
	Rel (%)	0.35 ± 0.00	0.34 ± 0.00	0.40 ± 0.00	0.40 ± 0.00
Mesenteric Fat	Abs (g)	4.00 ± 0.30	4.00 ± 0.20	1.70 ± 0.10	1.90 ± 0.20
	Rel (%)	0.95 ± 0.08	0.92 ± 0.04	0.72 ± 0.05	0.78 ± 0.07
Lung	Abs (g)	1.90 ± 0.04	1.80 ± 0.05	1.60 ± 0.04	1.50 ± 0.02
	Rel (%)	0.44 ± 0.00	0.42 ± 0.00	0.67 ± 0.02	0.65 ± 0.02
Heart	Abs (g)	1.70 ± 0.03	1.70 ± 0.04	1.20 ± 0.01	1.20 ± 0.03
	Rel (%)	0.40 ± 0.00	0.39 ± 0.00	0.50 ± 0.01	0.50 ± 0.01
Total Body Weight	(G)	420 ± 11	438 ± 10	234 ± 4	236 ± 7

Data represents means ± standard error of the mean (N = 12/group). Relative weights of organs are expressed as percentages of total body weights.

Table 4

Hematological parameters of male and female Sprague–Dawley rats fed on AIN93G diet containing either the borage oil blend or the High-GLA Safflower oil (HGSO) blend at the end of 12-week feeding. ^{*}.

Parameter [normal range]	Male		Female	
	Borage Oil	HGSO	Borage Oil	HGSO
RBC ($\times 10^6/\mu\text{L}$) [5–10]	8.3 $\pm$ 0.1	8.6 $\pm$ 0.1	7.4 $\pm$ 0.1	8.1 $\pm$ 0.2 ^a
TotalWBC ($\times 10^3/\mu\text{L}$) [3–17]	4.1 $\pm$ 0.4	4.6 $\pm$ 0.6	1.8 $\pm$ 0.2	2.2 $\pm$ 0.2
Neutrophils (% WBC) [13–26]	17.7 $\pm$ 2.5	21.9 $\pm$ 1.8	14.6 $\pm$ 1.3	13.5 $\pm$ 1.7
Lymphocytes(%WBC) [65–83]	76.8 $\pm$ 2.6	70.7 $\pm$ 2.3	79.9 $\pm$ 1.9	81.3 $\pm$ 2.0
Monocytes (% WBC) [0–4]	4.5 $\pm$ 0.8	3.0 $\pm$ 0.6	1.6 $\pm$ 0.8	1.0 $\pm$ 0.5
Eosinophils (% WBC) [0–4]	0.8 $\pm$ 0.2	2.5 $\pm$ 0.4 ^a	4.1 $\pm$ 1.0	2.5 $\pm$ 0.6
Basophils (% WBC) [0–1]	0.0 $\pm$ 0.0	0.0 $\pm$ 0.0	0.1 $\pm$ 0.1	1.5 $\pm$ 0.6 ^a
Hemoglobin (gm/dl) [11–19]	15.7 $\pm$ 0.2	14.4 $\pm$ 1.3	14.3 $\pm$ 0.1	15.1 $\pm$ 0.2 ^a
Hematocrit (%) [35–57]	53.1 $\pm$ 4.0	44.8 $\pm$ 0.4	38.8 $\pm$ 2.0	44.5 $\pm$ 1.1 ^a
MCH (pg) [18–23]	18.5 $\pm$ 0.2	18.3 $\pm$ 0.1	19.2 $\pm$ 3.5	19.3 $\pm$ 0.2
MCHC (gm/dl) [31–40]	33.8 $\pm$ 0.1	34.5 $\pm$ 0.2 ^a	28.7 $\pm$ 3.0	34.7 $\pm$ 0.1
MCV (fl) [46–65]	54.7 $\pm$ 0.6	52.9 $\pm$ 0.3	55.2 $\pm$ 0.4	55.7 $\pm$ 0.4
Platelets ($\times 10^3/\mu\text{L}$) [200–1500]	761 $\pm$ 14	832 $\pm$ 14 ^a	858 $\pm$ 26	782 $\pm$ 15 ^a
PT (sec) [19.3–32.6]	20.5 $\pm$ 0.5	18.7 $\pm$ 0.2	18.5 $\pm$ 0.1	18.5 $\pm$ 0.2

Values in brackets indicate the normal range for the laboratory rat (Johnson-Delaney and Harrison 2000; Sharp and La Regina 1998).

^{*} Data represent means $\pm$ SEM ($n = 6$ male borage, $n = 12$ male HGSO, $n = 11$ female borage, $n = 12$ female HGSO).

RBC, Red Blood Cell; WBC, White Blood Cell; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; MCV, Mean Corpuscular Volume; PT, Prothrombin time.

^a indicates statistically significant difference from borage oil blend diet ($P < 0.05$).

Table 5

Serum Biochemistry profiles of male and female Sprague–Dawley rats fed on AIN93G diet containing either the borage oil blend or the High-GLA Safflower oil (HGSO) blend at the end of 12-week feeding. *.

Parameter [normal range]	Male		Female	
	Borage Oil	HGSO	Borage Oil	HGSO
Sodium (mEq/l) [142–154]	143 ± 0.8	143 ± 0.4	144 ± 0.3	142 ± 0.4 ^a
Potassium (mEq/l) [3.6–9.2]	5.4 ± 0.2	4.5 ± 0.2 ^a	4.1 ± 0.2	4.7 ± 0.3
Chloride (mEq/l) [84–110]	108 ± 0.6	106 ± 0.5 ^a	107 ± 0.5	106 ± 0.3
Phosphorus (mg/dl) [4.7–16]	6.8 ± 0.2	6.2 ± 0.2	5.6 ± 0.2	5.8 ± 0.3
Calcium (mg/dl) [9.1–15.1]	10.2 ± 0.1	9.8 ± 0.1 ^a	9.4 ± 0.1	9.3 ± 0.1
Bicarbonate (mEq/l) [12.6–32]	22.4 ± 0.8	22.8 ± 0.6	19.1 ± 0.7	22.1 ± 0.7
BUN (mg/dl) [11–23]	16.8 ± 0.5	16.8 ± 0.4	14.9 ± 0.5	18.0 ± 0.7 ^a
Total Protein (gm/dl) [4.5–8.4]	5.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.7 ± 0.0 ^a
Albumin (gm/dl) [2.9–5.9]	3.2 ± 0.1	3.2 ± 0.0	3.5 ± 0.0	3.3 ± 0.0 ^a
Glucose (mg/dl) [50–135]	153 ± 4	125 ± 5 ^a	74.8 ± 1.4	86.2 ± 5.3
Globulin (gm/dl) [1.8–3.0]	2.6 ± 0.0	2.7 ± 0.0	2.5 ± 0.0	2.5 ± 0.0
Alb/Glob Ratio [0.72:1.21]	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.0
Uric Acid (mg/dl) [1.2–7.5]	0.8 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1
Cholesterol (mg/dl) [40–130]	92.6 ± 1.8	85.8 ± 3.6	103.4 ± 3.1	80.1 ± 3.4 ^a
Triglycerides(mg/dl) [26–145]	34.4 ± 2.2	26.7 ± 1.2 ^a	25.6 ± 1.8	26.7 ± 1.2
<i>Liver Function Test</i>				
AST (IU/l) [46–81]	159 ± 16	159 ± 43	98 ± 5	92 ± 5
ALT (IU/l) [17.5–30.2]	45.7 ± 3.7	46.1 ± 10.2	19.0 ± 1.1	27.1 ± 1.3 ^a
LDH (IU/l) [61–121]	1350 ± 168	1426 ± 128	769 ± 39	800 ± 62
AP (IU/l) [57–128]	63.6 ± 3.1	72.8 ± 3.1	52.3 ± 1.8	53.9 ± 1.3
Total Bilirubin (mg/dl) [0–0.64]	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0

ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen; AP, Alkaline Phosphatase, Alb/Glob, Albumin/Globulin Ratio.

Values in brackets indicate the normal range for the laboratory rat (Johnson-Delaney and Harrison 2000; Sharp and La Regina 1998).

* Data represent means ± SEM. (*n* = 10 male borage, *n* = 11 male HGSO, *n* = 12 female borage, *n* = 12 female HGSO).

^a indicates statistically significant difference from borage oil blend diet (*P* < 0.05).