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Effect of Long-Term Dietary Supplementation of High-Gamma-Linolenic Canola Oil versus Borage Oil on Growth, Hematology, Serum Biochemistry, and N-6 Fatty Acid Metabolism in Rats

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Dietary supplementation of a high-gamma-linolenic acid canola oil (HGCO) containing approximately 36% (w/w) of gamma-linolenic acid (GLA, 18:3n-6) from the seeds of a genetically transformed canola strain, was assessed for its long-term biological effects. Growing Sprague−Dawley rats ($n = 30$) were fed a purified AIN93G diet containing 5, 10, or 15% (w/w) of HGCO as the fat source. For comparison, a separate group of rats ($n = 10$) was given the diet containing 15% (w/w) of borage oil (BO), which contained 22% (w/w) of GLA. After 12 weeks of feeding, the growth, relative organ weights, hematology, and serum biochemistry were found to be similar among rats fed the 5, 10, and 15% HGCO diets. The GLA levels in plasma and liver phospholipids (PL) were also similar. However, the levels of GLA in peripheral tissues (muscle PL and adipose triacylglycerols) were significantly higher in rats fed the 10 and 15% HGCO diets than those fed the 5% HGCO diet. When the above biologic parameters were compared between the 15% HGCO and 15% BO dietary groups, there were no significant differences except for lower final body weights and higher tissue levels of GLA, dihomo-γ-linolenic acid (20:3n-6) and arachidonic acid (20:4n-6) in the 15% HGCO dietary group as compared with the 15% BO dietary group. This is due to a higher GLA content and possibly a more favorable stereospecific distribution of GLA in HGCO. Overall, long-term (12-week) feeding with diets containing up to 15% HGCO resulted in no adverse effects on growth, organ weight, hematology and serum biochemistry as compared to the diet containing 15% BO, suggesting that HGCO may be a safe alternative source of GLA.

KEYWORDS: Gamma-linolenic acid; stereospecific distribution of GLA; phospholipids

INTRODUCTION

In the biosynthetic pathway of polyunsaturated fatty acids (PUFA), linoleic acid (LA, 18:2n-6; all cis, 9,12-octadecadienoic acid) serves as the essential precursor for producing longer-chain n-6 PUFA. Linoleic acid (LA) is first converted to gamma-linolenic acid (GLA, 18:3n-6; all cis, 6,9,12-octadecatrienoic acid) by the action of Δ6-desaturase, and GLA is next converted to dihomo-gamma-linolenic acid (DGLA, 20:3n-6; all cis, 8,-11,14-eicosatrienoic acid) and arachidonic acid (AA, all cis, 20:4n-6; 5,8,11,14-eicosatetraenoic acid) by elongase and Δ5-desaturase, respectively (*1*). One of the important functions of DGLA and AA is their role in the production of hormone-like eicosanoids. DGLA is the precursor of antiinflammatory eicosanoids such as prostaglandin E_1 (PGE_1) and 15-hydroxy-eicosatrienoic acid (15-HETrE), while AA is the precursor of inflammatory eicosanoids such as PGE_2 and TXA_2 (*2*). Normally, the body maintains a delicate balance between the two series of eicosanoids. However, while AA is readily available from the diet, such as meats, eggs and dairy products, the formation of GLA and DGLA from LA can be significantly suppressed by numerous pathological, physiological, and nutritional factors (*3*). This would lead to a chronic imbalance between DGLA and AA, and promotion of inflammatory disorders due to low levels of antiinflammatory eicosanoids (*4*). Dietary supplementation of GLA to bypass the depressed Δ6-desaturation improves the balance between both DGLA and AA and their respective eicosanoids in the body (*5*) to alleviate the symptoms associated with inflammatory disorders.

GLA is available from the seed oils of borage, evening primrose, and blackcurrant. However, due to high cost and unreliable supply, there is a need to develop a more economic

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Table 1. Diet Composition

component	g/kg
oil	100
casein	200
L-cystine	3
corn starch	367.5
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 KH_2PO_4 (196.0), potassium citrate, monohydrate (70.78), sodium chloride NaCl (74.0), potassium sulfate K_2SO_4 (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 KIO_3 (0.01), sodium selenate Na_2SeO_4 (0.01025), ammonium paramolybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.00795), sodium metasilicate $Na_2SiO_3 \cdot 9H_2O$ (1.45), chromium potassium sulfate $CrK(SO_4)_2 \cdot 12H_2O$ (0.275), lithium chloride LiCl (0.0174), boric acid H_3BO_3 (0.0815), sodium fluoride NaF (0.0635), nickel carbonate, hydroxide, tetrahydrate (0.0318), ammonium vanadate NH_4VO_3 (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 B_{12} (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 D_3 (cholecalciferol, 500 000 IU/g) (0.2), vitamin K (phylloquinone) (0.075), sucrose, finely ground (974.705).

source of plant oil enriched with GLA. Through identifying and expressing a fungal Δ6-desaturase gene in the canola plant, we have recently developed seeds of genetically transformed canola, which produces high gamma-linolenic canola oil (HGCO) up to 43% GLA in the oil (*6*). The chemical and physical characteristics of HGCO have been evaluated (*7*). We have also compared the biological effects of feeding young male rats a diet containing 10% (w/w) of HGCO or borage oil at an equivalent level (23% of total fatty acids, w/w) of GLA (*8*) for 6 weeks. The results from the 6-week feeding study showed that HGCO and borage oil had a similar biological effect on the growth and hepatic metabolism of n-6 fatty acids in rats. The present study represents our continuous effort to assess the safety and biological effects of HGCO during long-term feeding. Here, we report the effect of feeding diets containing 5, 10, or 15% (w/w) of HGCO as compared with a diet containing 15% (w/w) of borage oil for 12 weeks on growth (body and organ weights), hematology, serum biochemistry, organ histology, and n-6 fatty acid composition in growing rats.

MATERIALS AND METHODS

Animals. The study design was similar to that previously reported (*8*). The protocol was approved by the Institute Animal Care and Use Committee, and the care of the animals was in accordance with the National Institute of Health guidelines for the *Care and Use of Laboratory Animals*. Pathogen-free male (approximately 53−79 g) Sprague−Dawley rats ($n = 40$) (Taconic Farms, Germantown, NY) were housed in clear plastic cages (two rats per cage) with natural bedding materials and subjected to 12-h light−dark cycles in a temperature- and humidity-controlled room for the duration of the study.

Experimental Diets. After 3 days of acclimatization, the rats were weighed and randomly assigned (10 rats/diet) to receive one of four experimental diets for 12 weeks. The experimental diets were modified semisynthetic fatfree powdered diets fortified with vitamins (AIN-93) and minerals (AIN-93G; Harlan Teklad, Madison, WI) (**Table 1**) and supplemented with 5, 10, or 15% (w/w) of HGCO or 15% borage oil (BO). The HGCO and BO used in the present study were the same as those used previously (*8*). The levels of all essential nutrients were maintained at constant levels. However, the increase of fat was at the expense of carbohydrates in the diet. This resulted in a slightly higher energy density in the high fat diets. The fatty acid compositions of the four experimental diets are listed in **Table 2**. The levels of total nonsaponifiable fractions in HGCO and BO were 1.7 and 1.6 g/100 g oil, respectively, and the levels of total tocopherols were 922 and 763 mg/kg oil, respectively (*8*).

Table 2. Principle Fatty Acid Compositions (w/w) of Four Different Experimental Diets Containing 5, 10, or 15% (w/w) of High-Gamma-Linolenic Acid Canola Oil (HGCO) or 15%(w/w) of Borage Oil

	experimental diets			
fatty acid	5% HGCO	10%HGCO	15% HGCO	15% BO
16:0	5.9	5.4	5.3	11.3
18:0	2.9	2.7	2.6	4.3
18:1 n-9	19.4	19.6	19.4	17.1
18:1 n-7	3.1	2.7	3.1	0.7
18:2 n-6	26.9	27.3	27.4	35.1
18:3 n-6	35.5	36.3	36.5	21.8
18:3 n-3	1.5	1.5	1.4	0.2
22:1 n-9	0.1	0.04	0.1	2.5
others[a]	4.6	4.4	4.2	7.0

[a] Others include fatty acids such as 14:0, 16:1, 18:4 n-3, 20:0, 20:1, 20:2, 22:0, 22:1 n-7, and etc.

Each experimental diet was prepared, packed, and sealed in batches of 0.5 kg in zip-lock plastic freezer bags and stored at approximately 4 °C in the dark. During the study, the rats were allowed free access to the diets and water. Fresh foods were provided every other day.

Growth Measurements. The clinical appearances, body weights, and diet consumption were monitored weekly. At the end of 12 weeks, the rats were anesthetized with pentobarbital (50 mg/kg i.p. injection) and sacrificed by exsanguinization. Blood from each rat was collected into three different tubes; the two tubes containing the anti-coagulant (EDTA) were for hematological studies and analysis of cholesterol and fatty acid composition, respectively, and the third tube was allowed to coagulate, and serum was collected for biochemical analysis. Liver, heart, kidneys, and spleen were excised and weighed. The kidney, spleen, and the middle lobe of the liver were fixed in formalin and sent to Quest Diagnostics Incorporated (Boston, MA) for histologic assessment. Aliquots of liver, skeletal muscle, and epididymal adipose tissue from all the rats were taken for lipid analyses (total triacylglycerol content, total cholesterol content, and phospholipid fatty acid composition). Fecal materials were collected for analysis of total fat content.

Clinical Parameters. Evaluations of organ histology and common hematological and biochemical parameters were performed by Quest Diagnostics Incorporated. For organ histology, samples of liver, kidney, and spleen from subsets of rats ($n = 4$) from each group were placed in 10% formalin and evaluated for a level-1 assessment by a pathologist in blinded fashion. Hematological parameters included red blood cell count, white blood cell count, (monocytes, neutrophils, eosinphils, lymphocytes), platelet count, hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Biochemical tests on serum included alkaline phosphatase, lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, globulin, albumin/globulin ratio, total protein, blood urea nitrogen (BUN), creatinine, BUN/creatinine ratio, bilirubin (direct, indirect, and total), glucose, iron, sodium, potassium, chlorine, calcium, and phosphorus.

Analyses of Total Fat Content and Fatty Acid Composition. Total lipids in plasma and tissues were extracted with chloroform/methanol (2/1, v/v) according to the procedure of Folch et al (*9*). Aliquots of total lipid extracts were separated into different neutral lipid fractions (cholesteryl esters, triacylglycerols, diacylglycerols, monoacylglycerols, free fatty acids, cholesterol and total phospholipids) by thin-layer chromatography (TLC) using Whatman (Fairfield, NJ) LHPK silica gel plates (10- × 20-cm, 200-μm thickness). The TLC plates were developed with a solvent system of hexane/diethyl ether/glacial acetic

acid (70/30/1, v/v). After the TLC plates were developed, they were sprayed with 2,7-dichlorofluorescin (in 2% ethanol), and visualized under the UV light. The areas corresponding to triacylglycerols and total phospholipids on the plates were marked and scraped off for methylation. Each lipid fraction was spiked with a known amount of triheptadecanoin (internal standard), blanketed with nitrogen, and methylated with 14% boron trifluoride in methanol at 95 °C for approximately 20 min. Fatty acid methyl esters were then separated and quantified by using an Omegawax column (30-m × 0.32-mm i.d., 0.25-μm film thickness) (Supelco, Bellefonte, PA) and a flame ionization detector (FID) on a Hewlett-Packard 6890 II gas chromatograph (Palo Alto, CA) under the conditions as described previously (*8*).

Fecal fats were also extracted with chloroform/methanol (2/1, v/v) according to the procedure of Folch et al (*9*). The fecal total fat content was determined gravimetrically.

Cholesterol Analysis. Liver and plasma samples were analyzed for total cholesterol content. Aliquots of tissue lipid extracts were spiked with 5α-cholestane as the internal standard, and saponified with a solution of 50% (w/v) KOH at 80 °C for 20 min. The nonsaponified materials, including cholesterol, were extracted twice with hexane. The hexane extract of cholesterol was transferred into a screw-cap tube, dried under a stream of nitrogen, and derivatized with N, *O*-bis-(trimethylsilyl) trifluoracetamide (BSTFA). The derivatized cholesterol (TMS ether of cholesterol) was analyzed by gas chromatography using a 30-m × 0.25-mm i.d. SAC-5 capillary column (0.25 μm film thickness) (Supelco, Bellefonte, PA). The temperature of the oven and the FID was set at 285 °C and 300 °C, respectively.

Statistics. The hypotheses in this study were to assess the dose response to feedings of HGCO at 5, 10, and 15% levels, and to compare the effect of feedings of 15% HGCO versus 15% BO. The primary analysis for all variables except body weight was analysis of variance (ANOVA). When there were significant differences between the doses, multiple comparisons were made using Tukey's honestly significant difference (HSD) test. The 95% simultaneous confidence intervals for significant pairwise comparisons were calculated using Tukey's studentized range. Body weight over the days of collection was analyzed by repeated measures using SAS PROC MIXED. Two-sided statistical tests were made and were considered significant at the 0.05 level. The SAS software version 8.0 on the personal computer was used in the computation. All analyses were based on 10 rats per dietary group, except the analysis of eosinophils ($n = 7$).

RESULTS

Food Intake. Food consumption was 1.0, 1.1, 1.0, and 0.9 g/rat/day for rats fed the 5, 10, and 15% HGCO, and 15% BO diets, respectively.

Growth. The 12-week growth patterns for rats fed the 5, 10, and 15% HGCO diets and the 15% BO diet are shown in **Figure 1**. Among the rats fed the 5, 10, and 15% HGCO diets, the growth patterns were very similar, and their body weights were not significantly different from each other throughout the 12-week feeding period. At the time of sacrifice, the mean body weights were 479, 487, and 478 g, while the mean weight gains were 410, 417, and 405 g for the 5, 10, and 15% HGCO dietary groups, respectively. During the first 6 weeks, the three HGCO dietary groups collectively had similar body weights to the 15% BO dietary group. However, beyond week 6 and through the rest of study, rats fed the HGCO diets tended to have lower body weights and weight gains than those fed the 15% BO diet. At the end of study, rats fed the 15% BO diet had a mean body weight of 510 g, which was significantly higher than the 478 g of those fed the 15% HGCO diet. The total weight gain of 442 g in rats given 15% BO diet was approximately 8% higher than the 405 g in rats given 15% HGCO diet. Rats given 15% HGCO and 15% BO diets had similar fecal total fat contents of 5% (w/w), which was significantly higher than the 2.7 and 3.3% of rats given the 5 and 10% HGCO diets, respectively.

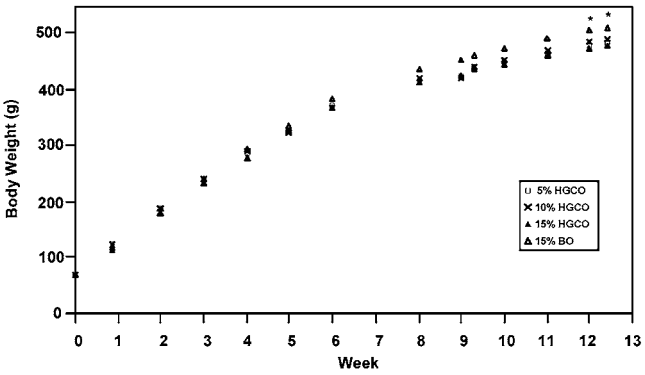

Figure 1. Cumulative body weights over 12 weeks. Data points represent the means of 10 male Sprague–Dawley rats fed an AIN93G diet containing 5, 10, or 15% (w/w) of high gamma-linolenic acid canola oil (HGCO) or 15% (w/w) of borage oil (BO) over a 1 to 2 week period. Data points noted with the symbol "*" at week 12 (day 84) and at the time of sacrifice (day 87) indicate that the 15% BO dietary group (△) had significant ($p < 0.05$) higher total body weights than did the 15% HGCO dietary group (▲).

Table 3. Absolute (abs) and Relative (rel) Weights of Liver, Heart, Spleen and Kidney from Male Sprague–Dawley Rats Fed an AIN93 Diet Containing 5, 10, or 15% (w/w) of High-Gamma-Linolenic Acid Canola Oil (HGCO) or 15% (w/w) of Borage Oil at the End of 12-Week Feeding[a]

	weight	5% HGCO	10% HGCO	15% HGCO	15% BO
liver	abs (g)	15.0 ± 0.6	15.7 ± 0.6	14.6 ± 0.5	15.8 ± 0.6
	rel (%)	3.1 ± 0.1	3.2 ± 0.1	3.1 ± 0.0	3.1 ± 0.1
heart	abs (g)	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	1.4 ± 0.0
	rel (%)	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
spleen	abs (g)	1.1 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.1
	rel (%)	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
kidney	abs (g)	3.0 ± 0.1	2.9 ± 0.1	2.8 ± 0.1	3.1 ± 0.1
	rel (%)	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
total body	abs (g)	479 ± 12.1	487 ± 17.3	478 ± 16.6	510 ± 10.3

[a] Data represent means ± standard error of the mean (SEM) ($n = 10$). Relative weights of organs are expressed as percentages of total body weights.

The absolute and relative weights of organs (heart, kidney, liver, and spleen) at the time of sacrifice were very similar among rats fed the 5, 10, and 15% HGCO and 15% BO diets (**Table 3**).

Organ Histology. The evaluation of organ histology revealed that the kidneys and spleens were comparable among the four dietary groups. There were localized fat infiltrations (focal fatty metamorphosis) in the peri-portal (triad) area of the livers in rats from all the dietary groups based on the visual examination of a subset ($n = 4$) of rats from each dietary group. On average, the 5 and 10% HGCO and 15% BO dietary groups had a slight fat infiltration in the peri-portal area of liver, while the 15% HGCO dietary group showed a moderate fat infiltration.

Hematology. Table 4 shows the effects of feeding the 5, 10, and 15% HGCO diets on RBC, total WBC, monocytes, neutrophils, eosinophils, lymphocytes, and platelet counts, and on hematocrit, hemoglobin, MCH, MCHC, and MCV in rats. There were no significant differences between the 5, 10, and 15% HGCO groups, and between the 15% HGCO and 15% BO dietary groups. The only exception was the MCV value, which was slightly but significantly lower in the 5% HGCO dietary group as compared with those in the 10 and 15% HGCO and 15% BO dietary groups. However, these differences were minor and within the normal range of variation.

Table 4. Hematological Parameters of Male Sprague–Dawley Rats Fed an AIN93G Diet Containing 5, 10, or 15% (w/w) of High-Gamma-Linolenic Acid Canola Oil (HGCO) or 15% (w/w) of Borage Oil (BO) at the End of 12-Week Feeding[a]

	5% HGCO	10% HGCO	15% HGCO	15% BO
RBC ($10^6/\mu$L)	8.2 ± 0.1	8.1± 0.1	7.8 ± 0.1	7.8 ± 0.1
total WBC ($10^3/\mu$L)	4.7 ± 0.4	4.6 ± 0.6	3.8 ± 0.4	4.8 ± 0.4
monocytes (% WBC)	3.8 ± 0.5	4.2 ± 0.5	5.2 ± 0.7	5.3 ± 1.2
neutrophils (% WBC)	8.5 ± 0.7	7.7 ± 1.1	11.0 ± 1.5	9.6 ± 1.3
eosinophils (% WBC)	2.2 ± 0.4	1.6 ± 0.2	1.9 ± 0.3	1.7 ± 0.3
lymphocytes (% WBC)	86.3 ± 1.1	86.9 ± 1.3	82.2 ± 1.5	83.7 ± 2.2
platelet count ($10^3/\mu$L)	802 ± 43.9	692 ± 33.5	762 ± 24.8	795 ± 26.8
hematocrit (%)	39.7 ± 0.5	40.8 ± 0.4	39.1 ± 0.5	40.4 ± 0.7
hemoglobin (g/dL)	14.5 ± 0.2	14.9 ± 0.1	14.4 ± 0.2	14.8 ± 0.3
MCH (pg)	17.9 ± 0.4	18.5 ± 0.3	18.7 ± 0.2	18.9 ± 0.3
MCHC (g/dL)	36.7 ± 0.3	36.7 ± 0.3	37.3 ± 0.4	36.9 ± 0.2
MCV (fL)	48.2 ± 0.8^x	50.5 ± 0.4^y	49.7 ± 0.6^{xy}	51.2 ± 0.4^y

[a] Mean ± SEM (n = 10 except n = 7 for eosinophils); RBC, red blood cell; WBC, white blood cell; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume. Data followed by the same letter are not significantly different (p < 0.05).

Table 5. Serum Biochemistry of Male Sprague–Dawley Rats Fed an AIN93G Diet Containing 5, 10, or 15% (w/w) of High-Gamma-Linolenic Acid Canola Oil (HGCO) or 15% (w/w) of Borage Oil at the End of 12-Week Feeding[a]

	5% HGCO	10% HGCO	15% HGCO	15% BO
ALT(U/L)	34.8 ± 5.7	39.1 ± 6.9	33.0 ± 2.3	36.2 ± 4.0
AST (UL)	112 ± 14.8	149 ± 29.8	169 ± 42.2	178 ± 46.4
LDH (U/L)	882 ± 167	1001 ± 295	1014 ± 224	1377 ± 341
alkaline phosphatase (U/L)	91.9 ± 3.0	105 ± 5.4	104 ± 5.0	95.1 ± 7.1
direct bilirubin (mg/dL)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
indirect bilirubin (mg/dL)	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
total bilirubin (mg/dL)	0.1 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	0.2 ± 0.0
albumin (g/dL)	2.9 ± 0.1	3.0 ± 0.0	3.0 ± 0.0	3.1 ± 0.1
globulin (g/dL)	3.7 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	3.5 ± 0.1
albumin/globulin ratio	0.8 ± 0.0	0.9 ± 0.0	0.8 ± 0.0	0.9 ± 0.1
total protein (g/dL)	6.7 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.6 ± 0.1
BUN (mg/dL)	28.9 ± 1.6	30.0 ± 1.0	28.1 ± 2.1	29.3 ± 2.0
creatinine (mg/dL)	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	1.1 ± 0.4
BUN/Creatinine Ratio	44.7 ± 2.1	46.0 ± 1.6	42.6 ± 3.3	45.6 ± 4.7
glucose (mg/L)	178 ± 11.0	173 ± 10.6	173 ± 7.7	190 ± 8.6
carbon dioxide (mEq/L)	25.8 ± 0.6	27.3 ± 0.3	27.4 ± 0.8	25.9 ± 0.8
anion gap	23.4 ± 1.1	22.7 ± 0.7	21.4 ± 1.1	22.7 ± 1.0
iron (μg/dL)	260 ± 25.2	281 ± 16.1	293 ± 18.4	313 ± 10.3
chloride (mEq/L)	98.9 ± 0.4	99.2 ± 0.5	99.6 ± 0.3	99.1 ± 0.5
potassium (mEq/L)	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.5 ± 0.2
sodium (mEq/L)	148 ± 0.3	149 ± 0.5	148 ± 0.7	148 ± 0.5
phosphorus (mg/dL)	7.0 ± 0.2	7.3 ± 0.2	6.5 ± 0.2	6.7 ± 0.2
calcium (mg/dL)	10.7 ± 0.1^{xy}	10.7 ± 0.1^{xy}	10.5 ± 0.1^x	11.0 ± 0.1^y

[a] Data represent means ± SEM (n=10). ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; BUN, blood urea nitrogen. Data followed by the same letter are not significantly different (p < 0.05).

Serum Biochemistry. Table 5 shows that the serum biochemistry results were not significantly different between the 5, 10, and 15% HGCO dietary groups, or between the 15% HGCO and 15% BO dietary groups. The only exception was that the calcium level of the 15% HGCO dietary group was only slightly but significantly lower than that of the 15% BO dietary group. These changes were minor and within the normal range of variation.

Cholesterol and Triacylglycerol Contents in Plasma and Liver. The plasma cholesterol content (671 mg/L) of the 15% HGCO dietary group was similar to the 701 and 723 mg/L of the 5% HGCO and 10% HGCO dietary groups, respectively, but was significantly (p < 0.05) lower than that (862 mg/L) of the 15% BO dietary group (**Figure 2**). The liver cholesterol content of rats fed the 15% HGCO diet was 0.557 g/100 g, which was not different from the 0.415 g/100 g of rats fed the 10% HGCO diet, but was significantly higher than the 0.354 and 0.393 g/100 g of those fed the 5% HGCO and 15% BO diets, respectively. There was a dose-dependent effect of feeding different levels of HGCO on liver total cholesterol content.

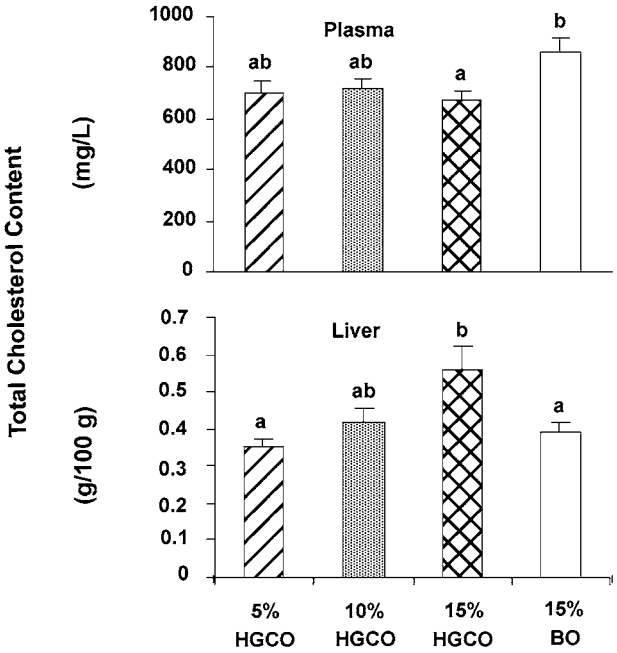

Figure 2. Total cholesterol contents of plasma and liver in male Sprague–Dawley rats fed an AIN93G diet containing 5 (diagonal lined bar), 10 (shaded bar), or 15% (cross-hatched bar) (w/w) of high gamma-linolenic acid canola oil (HGCO) or 15% (w/w) of borage oil (BO) (white bar) at the end of 12-week feeding. n = 10 rats per group. Bars with the same letter are not significantly different (p < 0.05). Error bars represent standard errors of the means (SEM).

Rats fed the 15% HGCO diet had a plasma triacylglycerol content of 251 mg/mL, which was significantly lower than the 368, 386, and 398 mg/mL in those fed the 5 and 10% HGCO and 15% BO diets, respectively (**Figure 3**). The 15% HGCO dietary group had a liver total triacylglycerol content of 16 mg/g, which was not significantly different from the 13.2 and 14.1 mg/g of the 15% BO and the 10% HGCO dietary groups, respectively; however, it was significantly higher than the 9.8 mg/g of the 5% HGCO dietary group.

N-6 Fatty Acid Composition in Plasma Phospholipids. The effects of feeding 5, 10, 15% HGCO and 15% BO diets on the n-6 fatty acid composition of plasma phospholipids are shown in **Figure 4** (top panel). There were no significant differences among the 5, 10, and 15% HGCO dietary groups for all the n-6 fatty acids except 18:2 n-6. The level of 18:2 n-6 was significantly higher in the 10 and 15% HGCO dietary groups than in the 5% HGCO dietary group. Compared with the 15% HGCO dietary group, the 15% BO dietary group had significantly higher levels of 18:2 n-6 and 22:5n-6 but a significantly lower level of 18:3n-6 and 20:3n-6.

N-6 Fatty Acid Composition in Liver Phospholipids. The effects of feeding the 5, 10, 15% HGCO and 15% BO diets on the n-6 fatty acid composition of liver phospholipids (PL) are shown in **Figure 4** (middle panel). With an increasing HGCO content in the diet, there was an increase in the proportion of 18:2n-6 and 18:3n-6 and a decrease in the percentage of 22:5n-6. However, these differences, although statistically significant, were mostly small. With respect to the levels of 20:3n-6,

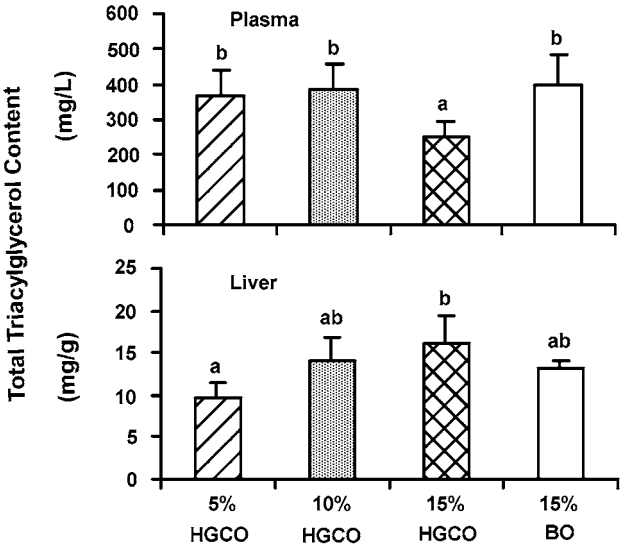

Figure 3. Total triacylglycerol contents of plasma and liver in male Sprague–Dawley rats fed an AIN93G diet containing 5 (diagonal lined bar), 10 (shaded bar), or 15% (cross-hatched bar) (w/w) of high gamma-linolenic acid canola oil (HGCO) or 15% (w/w) of borage oil (BO) (white bar) at the end of 12-week feeding. $n = 10$ rats per group. Bars with the same letter are not significantly different ($p < 0.05$). Error bars represent SEM.

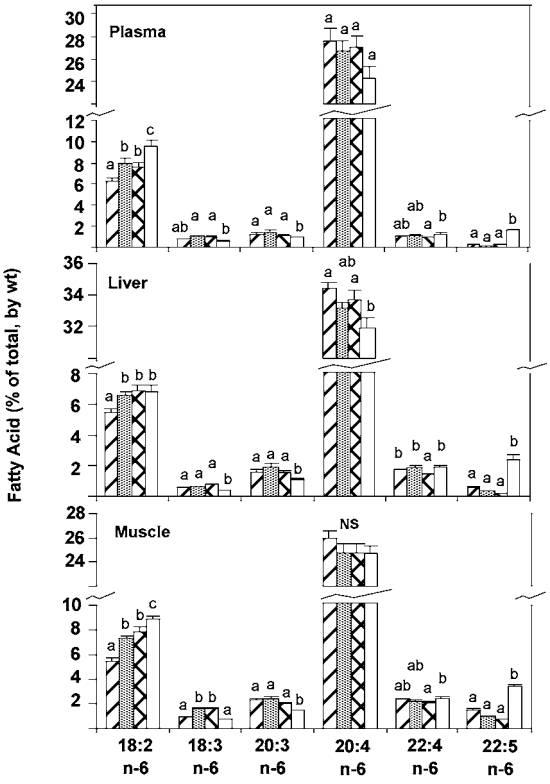

Figure 4. n-6 fatty acid profiles of plasma, liver, and muscle phospholipids in male Sprague–Dawley rats fed an AIN93G diet containing 5 (diagonal lined bar), 10 (shaded bar), or 15% (cross-hatched bar) (w/w) of high gamma-linolenic acid canola oil (HGCO) or 15% of borage oil (BO) (white bar) at the end of 12-week feeding. $n = 10$ rats per group. Bars with the same letter are not significantly different ($p < 0.05$). Error bars represent SEM. The error bar denoted with NS indicates that there was no significant difference among the three HGCO dietary groups and between the 15% HGCO and 15% BO dietary groups.

20:4n-6, and 22:4n-6 in liver PL, there were generally very little differences among the three HGCO dietary groups.

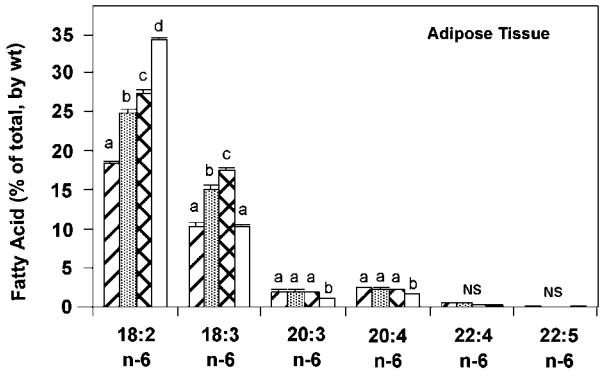

Figure 5. n-6 fatty acid profile of triacylglycerols from adipose tissue in male Sprague–Dawley rats fed an AIN93G diet containing 5 (diagonal lined bar), 10 (shaded bar), or 15% (cross-hatched bar) (w/w) of high gamma-linolenic acid canola oil (HGCO) or 15% (w/w) of borage oil (BO) (white bar) at the end of 12-week feeding. $n = 10$ rats per group. Bars with the same letter are not significantly different ($p < 0.05$). Error bars represent SEM. Bars denoted with NS indicate that there was no significant difference among the three HGCO dietary groups and between the 15% HGCO and 15% BO dietary groups.

In comparison with rats fed the 15% BO diet, rats fed the 15% HGCO diet had a slightly but significantly higher percentage of 18:3n-6 and 20:4n-6, whereas they had a lower percentages of 22:4n-6 and 22:5n-6 in liver PL. However, there were no differences in other n-6 fatty acids between the two dietary groups.

N-6 Fatty Acid Composition in Muscle Phospholipids. The effects of 5, 10, and 15% HGCO and 15% BO diets on the n-6 fatty acid composition of muscle phospholipids are shown in **Figure 4** (bottom panel). The relative percentages of 18:2n-6 and 18:3n-6 were generally higher, whereas that of 22:5n-6 was lower in the 10 and 15% HGCO dietary groups than those in the 5% HGCO dietary group. The proportions of 18:3n-6 and 20:3n-6 were significantly higher, whereas those of 18:2n-6 and 22:5n-6 were significantly lower in the 15% HGCO dietary group, as compared to the 15% BO dietary group.

N-6 Fatty Acid Composition in Adipose Triacylglycerols. The effects of the four different diets on n-6 fatty acid composition in adipose TG are shown in **Figure 5**. There was a significant dose-dependent effect of HGCO on the levels of 18:2n-6 and 18:3n-6, which increased with increasing contents of HGCO in the diet. There were no differences in other n-6 fatty acids among the three HGCO dietary groups. When compared with the 15% HGCO dietary group, the level of 18:2n-6 was significantly higher, whereas the levels of 18:3n-6, 20:3 n-6, and 20:4 n-6 were significantly lower in the 15% BO dietary group.

DISCUSSION

The present study assessed the long-term effect of feeding purified diets containing different levels (5, 10, and 15%, w/w) of HGCO, as compared to a diet containing 15% (w/w) BO on the growth and metabolism of growing rats. The four groups of rats had a similar growth pattern for the first 6 weeks of the study. This is consistent with our previous findings in a 6-week feeding study with 10% HGCO/corn oil and 10% BO diets (*8*). However, beyond week 6, the rats fed the three HGCO diets tended to gain less weight than those fed the BO diet (**Figure 1**). At the end of study, the mean body weight of the rats fed the 15% HGCO diet was significantly lower than that of rats fed the 15% BO diet. On the average, rats fed the 15% HGCO

diet weighed 32 g less than those fed the 15% BO, despite the fact that the two groups had similar levels of food intake and fecal fat excretion. It has been reported that GLA reduces body fat content but not lean body mass and facilitates fatty acid β-oxidation in the liver (*10*, *11*). In the present study, the lower weight gain observed in rats fed the 15% HGCO diets as compared with those fed 15% BO diet may be partly attributed to a higher GLA intake and possibly a greater β-oxidation in the liver. To verify this possibility in future studies, it would be necessary to measure the total body fat content of rats from these two dietary groups.

Results from the organ histology revealed that the kidneys and spleens for rats in the four dietary groups were comparable to the chow-fed rats used as a reference. Localized fat infiltration (focal fatty metamorphosis) was found in the peri-portal (triad) area of liver in rats from all four groups, although only four animals per group were examined. The 5 and 10% HGCO and 15% BO dietary groups had a slight localized fat infiltration, while the 15% HGCO dietary group had a moderate localized fat infiltration. Hayes et al. (*12*) reported that rats fed a powdered diet containing 10% (w/w) corn oil had a higher incidence of fat vacuoles in the liver than those fed a diet containing 10% (w/w) of a saturated fat. They suspected that these fat vacuoles resulted from higher absorption of long-chain unsaturated fatty acids in the corn oil group. Hempenius et al (*13*) have also reported the presence of fat vacuoles in the liver in rats fed a diet containing 13% of a highly unsaturated fat. Similar results were observed in the present study, presumably due to the high levels of long-chain unsaturated fatty acids in both HGCO and BO (**Table 2**). Nevertheless, the four dietary groups were similar in their relative liver sizes (**Table 3**) and comparable in their levels of liver enzymes (ALT, AST, LDH, and alkaline phosphatase) and bilirubins (direct, indirect, and total) (**Table 5**), indicating that liver function was similar among the four dietary groups.

We found that the rats fed the 15% HGCO diet had a higher level of liver total cholesterol than those fed the other diets. Previously, n-6 PUFA (mainly 18:2n-6) have been shown to increase the activity of liver LDL receptor, and consequently, the removal of low-density lipoproteins (LDL) from plasma through enhanced activities of LDL receptor and acyl coenzyme A/cholesterol acyltransferase (ACAT) in the liver (*14*). GLA (18:3 n-6), as compared to 18:2n-6, is even more effective in lowering plasma cholesterol in human and experimental animals (*15*, *16*, *17*). It has been reported that the effect of GLA intake on plasma total cholesterol and LDL-cholesterol is dose-dependent in healthy humans (*18*). Huang et al (*16*) suggested that the hypocholesterolemic effect of GLA was exerted through enhancing the deposition of plasma cholesterol into liver. Accordingly, the higher GLA content (5.5 g GLA per 100 g of diet) in the 15% HGCO diet, as compared to that (3.3 g GLA per 100 g of diet) in the 15% BO diet, would have exerted a greater effect of removing cholesterol from plasma and depositing it in liver (**Figure 2**).

However, the hypocholesterolemic effect could not be attributed solely to the amount of GLA in the diet. Results in **Figure 2** show that the 5% HGCO dietary group had a similar level of plasma cholesterol as the 15% BO dietary group, even though the former was fed a much lower amount of GLA than the latter (1.8 g vs 3.3 g/100 g of diet). One possible explanation is that the bioavailability of GLA may differ between HGCO and BO. Wainwright et al (*19*) have noted that feeding HGCO and BO decreased the level of docosahexaenoic acid (DHA) in brain phospholipids by competition between the n-6 fatty acid (GLA) and the n-3 fatty acid (DHA), but this effect was significantly greater from HGCO than from BO at an equivalent level of GLA. Wainwright et al (*19*) attributed the greater biochemical effects of HGCO to the higher bioavailability of GLA from HGCO. Comparison of the stereospecific distribution of GLA between the two oils showed that the majority (75%) of 18:3n-6 in HGCO was located at the *sn*-1, 3 positions and only 25% at *sn*-2 position of the triacylglycerol (TG) molecules (*7*), whereas less than half (45.8%) of 18:3n-6 in BO was located at *sn*-1 and *sn*-3 positions and 54.2% at *sn*-2 position (*20*). Because lipoprotein lipase more readily hydrolyzes the fatty acids at the *sn*-1 and *sn*-3 positions of TG molecules, the greater distribution of GLA at these two positions in HGCO would allow the GLA molecules of HGCO to be more readily available to the tissues when compared with BO. Indeed, in the present study, the levels of GLA and its two immediate metabolites (DGLA and AA) in the phospholipids of plasma, liver, and muscle, and in the adipose TG were higher and in most cases were significantly higher in all the HGCO dietary groups than in the 15% BO dietary group.

In summary, this study demonstrates that 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, common parameters of hematology and serum biochemistry, and n-6 fatty acid metabolism in rats. Moreover, a smaller dosage (5 or 10%) of HGCO is sufficient to produce similar levels of biological effects as 15% BO. We conclude that HGCO may be used as a safe and cost-effective alternate source of GLA.

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