Comparison of Growth and Fatty Acid Metabolism in Rats Fed Diets Containing Equal Levels of γ-Linolenic Acid from High γ-Linolenic Acid Canola Oil or Borage Oil

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ABSTRACT: We have utilized transgenic technology to develop a new source of γ-linolenic acid (GLA) using the canola plant as a host. The aim of the present study was to compare the growth and fatty acid metabolism in rats fed equal amounts of GLA obtained from the transgenic canola plant relative to GLA from the borage plant. Young male Sprague-Dawley rats (n = 10/group) were randomized and fed a purified AIN93G diet (10% lipid by weight) containing either a mixture of high GLA canola oil (HGCO) and corn oil or a control diet containing borage oil (BO) for 6 wk. GLA accounted for 23% of the triglyceride fatty acids in both diets. Growth and diet consumption were monitored every 2–3 d throughout the study. At study termination, the fatty acid composition of the liver and plasma phospholipids was analyzed by gas chromatography. The growth and diet consumption of the HGCO group were similar to the BO group. There were no adverse effects of either diet on the general health or appearance of the rats, or on the morphology of the major organs. There was no significant difference between the diet groups for total percentage of n-6 polyunsaturated fatty acids present in either the total or individual phospholipid fractions of liver or plasma. The relative percentage of GLA and its main metabolite, arachidonic acid, in each phospholipid fraction of liver or plasma were also similar between groups. The percentage of 18:2n-6 in liver phosphatidylethanolamine and phosphatidylinositol/serine was higher ($P < 0.05$) and 22:5n-6 was lower in the HGCO group than the BO group. This finding could be attributed to the higher 18:3n-3 content in the HGCO diet than the BO diet. Results from this long-term feeding study of rats show for the first time that a diet containing transgenically modified canola oil was well-tolerated, and had similar biological effects, i.e., growth characteristics and hepatic metabolism of n-6 fatty acids, as a diet containing borage oil.

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Conversion of the essential fatty acid, linoleic acid (18:2n-6), to γ-linolenic acid (GLA, 18:3n-6) and dihomo-γ-linolenic acid (DHGLA, 20:3n-6) is rate-limited by Δ6 desaturase activity. GLA may become essential under certain pathological conditions that depress Δ6 desaturase activity and reduce production of DHGLA and its anti-inflammatory metabolites, prostaglandin E_1 (PGE_1) and 15-hydroxy-eicosatrienoic acid (15-HETrE) (1–3). A chronic imbalance between the fatty acids DHGLA and arachidonic acid and their respective derivatives (PGE_1 and 15-HETrE, vs. the 2-series of prostaglandins) has been proposed to be one contributing factor in the etiology of some inflammatory and cardiovascular disorders (4). In this regard, dietary studies have shown that provision of GLA to bypass Δ6 desaturase alleviated pathologic conditions associated with low levels of PGE_1 and 15-HETrE (5,6). More recently, experimental (7,8) and clinical studies (9) have revealed that supplementation of nutritional formulas with a combination of eicosapentaenoic (20:5n-3) and GLA can favorably reduce an inflammatory response while promoting vasodilation and oxygen delivery following acute lung injury.

These beneficial effects have increased the demand for GLA-enriched oils. At the present time, the predominant sources of GLA are oils from plants such as borage, evening primrose, and black currant. Since all GLA-containing oils on the market are relatively expensive due to large fluctuations in availability, and production and purification costs, there is a need for more economical sources of GLA. For this purpose, we have utilized transgenic technology to develop a new source of GLA using the canola plant as a host. The production of GLA in the canola plant starts with oleic acid (18:1n-9) and requires two desaturation steps at the Δ12 and Δ6 positions. Recently, cDNA clones encoding Δ12 and Δ6 desaturases from *Mortierella alpina* have been identified (10). By recombinant expression of both desaturases, the yield of GLA from the modified canola plant can range from 22 to 45% of the total fatty acids.

The aim of the present study was to compare, for the first time, the growth and fatty acid metabolism in rats fed diets containing equal levels of GLA from high GLA canola oil (HGCO) or borage oil (BO) for 6 wk. We also determined the fatty acid composition of the principal phospholipid fractions

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Abbreviations: ANOVA, analysis of variance; BO, borage oil; DHGLA, dihomo-γ-linolenic acid; GLA, γ-linolenic acid; GLC, gas–liquid chromatography; 15-HETrE, 15-hydroxy-eicosatrienoic acid; HGCO, high GLA canola oil; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PGE_1, prostaglandin E_1; PI/PS, phosphatidylinositol/serine; PUFA, polyunsaturated fatty acids; TLC, thin-layer chromatography.

of liver and plasma and triglycerides in epididymal adipose at study termination to compare the dietary effects on the metabolism of the principal n-6 fatty acids in these tissues.

MATERIALS AND METHODS

The study design was approved by the Institute Animal Care and Use Committee. The care of the animals was in accordance with the guidelines set forth by the National Institute of Health's, *Guide for the Care and Use of Laboratory Animals*. Pathogen-free male (78–100 g) Sprague Dawley rats (Taconic Farms, Germantown, NY) were housed in plastic cages (two rats per cage) and maintained on a 12-h light-dark cycle in a temperature- and humidity-controlled room.

After a 2-d acclimatization period, the rats were weighed and randomly assigned to receive a semisynthetic diet supplemented with 10% by weight of HGCO or BO (n = 10/group). Each diet was prepared at Ross Products Division (Columbus, OH) using a fat-free purified powder diet (AIN93G; Harlan Teklad, Madison, WI) fortified with vitamins and minerals according to recently established guidelines (11). The fatty acid compositions of the original HGCO oil and the two diets (HGCO and BO) are presented in Table 1. The HGCO was blended with corn oil (54:46, w/w) to approximate the relative percentages of GLA (23%) and LA (36%) in the BO. The percentage of α-linolenic acid (18:3n-3) was 1.1 and 0.3% in the HGCO and BO diets, respectively. Both HGCO and BO were extracted and processed in similar methods by the same processor (POS Pilot Plant Corp., Saskatoon, Canada). The mean total nonsaponifiable fractions in HGCO and BO were 1.7 and 1.6 g/100 g oil, respectively, while the mean total tocopherols were 922 and 763 mg/kg oil, respectively.

Gas chromatographic analysis of diet samples taken before and after the 6-wk feeding period indicated that no significant changes in the relative percentages of the fatty acids had occurred during the study period. Aliquots of each diet were stored under N_2 in sealed, plastic bags at –20°C. Every second or third day fresh aliquots of diet were weighed into clean, stainless steel feeders that hung vertically within the cages. Using this system, diet spillage within the cage was minimal. The rats were allowed free access to the diets and water during the study. Body weights and diet consumption were measured every 2–3 d throughout the study period. The rats' appearance and behavior were observed daily for signs of diet intolerance or toxicity.

TABLE 1
Principal Triglyceride Fatty Acids of Original HGCO and Study Diets[a]

Fatty acid	HGCO	HGCO diet	BO diet
16:0	4.2	8.3	10.8
18:0	3.7	2.8	4
18:1n-9	21.6	20.4	15.5
18:1n-7	3.4	2.1	0.7
18:2n-6	26.0	38.7	36.4
18:3n-6	37.0	23.3	22.6
18:3n-3	1.3	1.1	0.3
22:1n-9		0.4	2.9

[a]Expressed as the mole percentage of total fatty acids identified. HGCO, high γ-linolenic acid-canola oil; BO, borage oil.

After 6 wk of feeding, food was withheld from the animals overnight prior to study termination. Under pentobarbital anesthesia (50 mg/kg intraperitoneally), the abdomen was opened and blood drawn from the vena cava into heparinized syringes. The whole blood was centrifuged at 2500 × g at 4°C for 10 min to isolate the plasma. All rats were subjected to gross necropsy. The liver, kidneys, spleen, heart, and epididymal fat were excised, examined and weighed. Samples of plasma, liver, and epididymal fat were stored under N_2 at –20°C for compositional analysis of phospholipid or triglyceride fatty acids.

Fatty acid analysis of triglyceride and individual phospholipid fractions (10). Total tissue lipids were extracted immediately after samples had been thawed. Liver, epididymal tissue, plasma, and diet powder were extracted with chloroform/methanol, 2:1 (vol/vol). The extraction solvent mixture of each sample was allowed to stand in the refrigerator overnight. Saline was added to separate the chloroform and aqueous phase. Samples were centrifuged and the chloroform layer was transferred into Teflon-lined screw cap tubes. The chloroform was then evaporated at 40°C under N_2 and the lipid residues were redissolved in chloroform. The total lipid extracts were separated by thin-layer chromatography (TLC) into their neutral and phospholipid classes using LHPK Silica Gel on 10 × 20 cm plates with a thickness of 200 μm (Whatman, Fairfield, NJ). Neutral lipids were developed for 12 min with hexane/ethyl ether/glacial acetic acid, 70:30:1 (by vol). The solvent system for the phospholipids was chloroform/ethanol/de-ionized water/triethylamine, 4:5:1:5 (by vol). After visualization by spraying with 2,7-dichlorofluorescin in 2% ethanol, the areas corresponding to triglycerides and total phospholipids were scraped off the neutral TLC plate, and phosphatidylethanolamine (PE), phosphatidylinositol/serine (PI/PS), and phosphatidylcholine (PC) were obtained from the phospholipid plate. All lipid fractions were derivatized by methylation with 12% boron trifluoride in methanol. For fatty acid quantitation, a known amount of triheptadecanoin (17:0) was added as the internal standard to each lipid fraction. The fatty acid methyl esters obtained from each lipid fraction were extracted in hexane and analyzed by gas–liquid chromatography (GLC).

A Hewlett-Packard 6890 series GLC (Palo Alto, CA) was set up with the following conditions: Omegawax 320™ capillary column (Supelco, Bellefonte, PA), 30 m × 0.32 mm i.d., 0.25 μm film; helium carrier gas; flame-ionization detector; pulsed splitless injection mode; injector 205°C; detector 235°C; column 120°C for 1 min, then raised 4°C/min to 205°C and held for 25 min. A sample volume of 2 μL was injected and analyzed with Hewlett-Packard ChemStation software version G2070AA. Peaks were identified based upon the relative responses of an external standard of pure fatty acid methyl esters.

Statistics. Descriptive and inferential statistics were computed on all continuous data for each parameter. The sample size was based primarily on the historical adequacy in similar studies using this model (12,13). Group differences were determined by *t* test and repeated measures of analysis of variance (ANOVA) across time points. Tukey's test was used for *post hoc* comparison of means of significant ANOVA. Estimates and 95% confidence intervals for group differences at each time point were also computed. Statistical significance was set at a P value less than 0.05.

RESULTS

The control (BO) and experimental (HGCO) diets were well tolerated throughout the study. There was no evidence of adverse effects of the diets with regard to the general appearance or behavior of the animals based on daily observations. The growth of the rats from each treatment group was similar. Initial and final body weights, mean body weight trends, and mean cumulative weight gain (288 vs. 286 g per rat for HGCO vs. BO groups, respectively) for each group were similar (Fig. 1). Diet consumption (Fig. 2) was also similar between treatment groups throughout the 6 wk study. The mean total consumption (±SD) of the HGCO and BO diets per two rats per cage over 6 wk was 2.13 (± 0.23) and 2.20 kg (± 0.14), respectively. Results from an earlier pilot study of Sprague-Dawley rats fed either the HGCO and BO diet for 3 wk revealed that the mean (±SD) outputs of fecal fat over three separate 48 h collections were similar for each diet group (0.07 ± 0.01 g/d for each group) (Palombo, J., unpublished observation).

Gross postmortem examination of the major organs did not reveal any adverse treatment effects. The mean liver weight (±SD) was not significantly different between diet groups (11.1 ± 1.1 vs. 11.5 ± 0.9 g for the HGCO and BO groups, respectively). In addition, mean weights (g) of heart (1.5 vs. 1.5), kidneys (2.7 vs. 2.7) or spleen (1.2 vs. 1.1) were similar for the HGCO vs. BO group, respectively.

Principal fatty acids in liver phospholipids. The effects of feeding either the HGCO or BO diets on the composition of n-6 fatty acids in the total and individual liver phospholipids are shown in Tables 2–5. There was no difference between diets for the total percentage of n-6 polyunsaturated fatty acids (PUFA) present in either the total (Table 2) or individual (Tables 3–5) phospholipid fractions. The relative percentages of 18:2n-6 and 18:3n-6 in the liver total phospholipids (Table 2) and PC fraction (Table 3) were also similar for both diets. The percentage of 18:2n-6 in liver PE (Table 4) and PI/PS (Table 5) was significantly higher ($P < 0.05$) in the HGCO diet group as compared with the BO group. The percentage of 20:3n-6 in the total phospholipids was slightly lower in the HGCO group than the BO group (Table 2); however, the percentages of 20:3n-6 in the individual phospholipid fractions (Tables 3–5) were similar between diets. There were no diet-induced differences in the percentages of 20:4n-6 and 22:4n-6 present in the total or individual phospholipids. The percentage of 22:5n-6, the end-product of the n-6 series of PUFA, in the HGCO group was significantly lower ($P < 0.05$) in the total phospholipids and each phospholipid fraction (Tables 2–5) in comparison with the BO group.

The percentage of total n-3 PUFA in each phospholipid fraction of liver was similar across the two diets. However, we observed small differences between the two diets for percentages of 22:5n-3 and 22:6n-3 within the phospholipid frac-

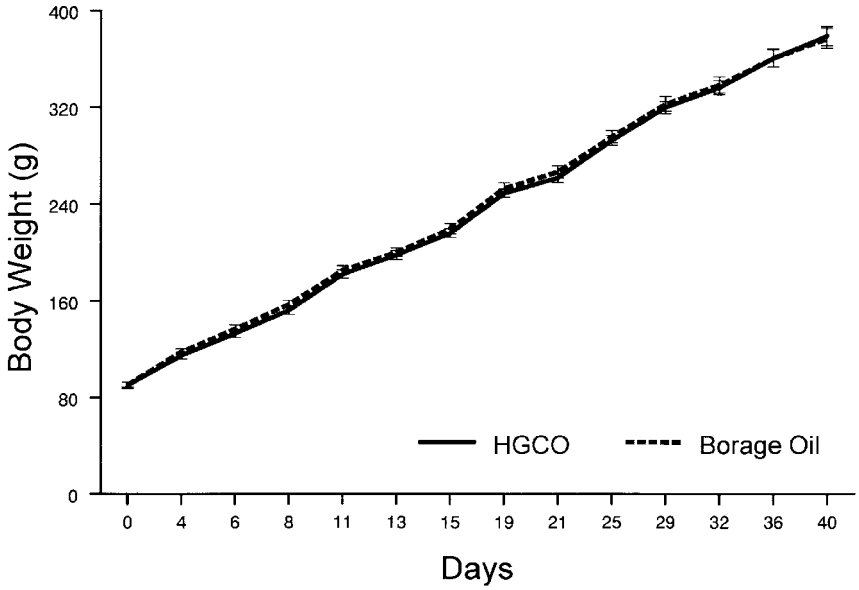

FIG. 1. Cumulative body weight gain (g) over 6 wk. Data points represent the mean ± SD for 10 male Sprague-Dawley rats fed an AIN93G diet containing 10% by weight high γ-linolenic acid (GLA) canola oil (HGCO) or borage oil for 6 wk.

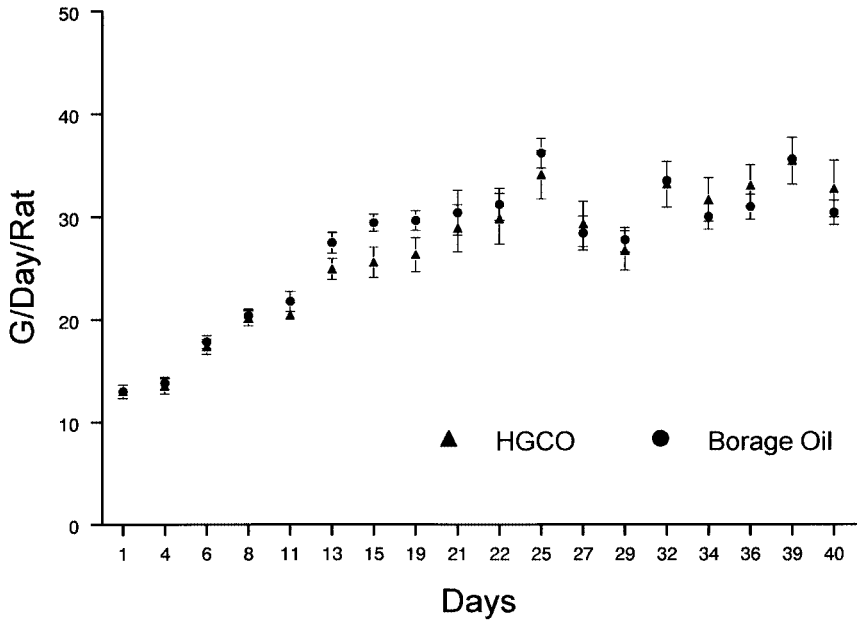

FIG. 2. Mean ± SD food consumption (g) per rat over 6 wk. Data points represent the calculated intake per rat of AIN93G diet containing 10% by weight HGCO or borage oil over a 2–3 d period (n = 10/group). See Figure 1 for abbreviations.

tions. Specifically, the relative percentage of 22:5n-3 was significantly higher, while the concurrent percentage of 22:6n-3 was lower in the total phospholipids (Table 2), and the PC (Table 3) and PE (Table 4) fractions from the HGCO group compared with the BO group. The mean percentage of 22:6n-3 in the PI/PS fraction of livers from the HGCO group was also lower as compared with the BO diet group (Table 5). Total monounsaturates (i.e., primarily the sum of 18:1n-9 and 18:1n-7) in the PC and PE fractions were higher in livers from rats fed the HGCO diet as compared with those from the BO diet group. The percentage of total saturates in each phospholipid fraction was similar between the treatment groups.

Principal fatty acids in plasma phospholipids. The effects of feeding either the HGCO or BO diets on the composition of n-6 fatty acids in the total and individual plasma phospholipids are shown in Tables 6–9. The total n-6 PUFA, n-3 PUFA, monounsaturates, and saturates in the total (Table 6) and individual (Tables 7–9) fractions of phospholipids were similar between diet groups. The relative percentages of individual n-6 PUFA, i.e., 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6,

TABLE 2
Fatty Acid Composition of Liver Total Phospholipids After 6 wk

Fatty acid	HGCO diet[a]	BO diet
16:0	24.5 ± 1.0	24.0 ± 1.1
18:0	24.7 ± 1.7	26.4 ± 2.4
18:1n-9	3.4 ± 0.3	3.0 ± 0.2*
18:1n-7	4.1 ± 0.5	2.9 ± 0.2*
18:2n-6	11.3 ± 0.9	10.3 ± 1.4
18:3n-6	0.6 ± 0.1	0.6 ± 0.1
20:2n-6	0.6 ± 0.2	0.4 ± 0.1*
20:3n-6	1.0 ± 0.1	1.4 ± 0.4*
20:4n-6	23.5 ± 1.7	23.4 ± 1.6
22:4n-6	1.3 ± 0.1	1.4 ± 0.3
22:5n-6	0.4 ± 0.2	1.4 ± 0.6*
22:5n-3	0.7 ± 0.1	0.4 ± 0.1*
22:6n-3	0.9 ± 0.2	1.2 ± 0.2*
Total saturates	50.5 ± 1.9	51.4 ± 2.6
Total monounsaturates	9.1 ± 0.8	8.1 ± 0.5
Total n-6 polyunsaturates	38.7 ± 2.0	38.9 ± 2.3
Total n-3 polyunsaturates	1.6 ± 0.3	1.6 ± 0.3

[a]Mean relative mole percentage ± SD, n = 10/group. *$P < 0.05$ by t test vs. HGCO group. See Table 1 for abbreviations.

TABLE 3
Fatty Acid Composition of Liver Phosphatidylcholine After 6 wk

Fatty acid	HGCO diet[a]	BO diet
16:0	27.7 ± 1.4	27.1 ± 2.0
18:0	17.2 ± 1.8	17.5 ± 4.4
18:1n-9	3.1 ± 0.4	2.7 ± 0.3*
18:1n-7	3.1 ± 0.5	2.0 ± 0.2*
18:2n-6	7.8 ± 0.8	7.7 ± 1.4
18:3n-6	0.7 ± 0.1	0.8 ± 0.2
20:3n-6	0.8 ± 0.1	0.9 ± 0.3
20:4n-6	34.0 ± 1.6	34.2 ± 3.9
22:4n-6	1.2 ± 0.1	1.1 ± 0.3
22:5n-6	0.5 ± 0.1	1.8 ± 0.9*
22:5n-3	0.8 ± 0.1	0.4 ± 0.1*
22:6n-3	1.2 ± 0.3	1.6 ± 0.3*
Total saturates	45.5 ± 1.9	45.2 ± 5.0
Total monounsaturates	7.2 ± 0.9	5.9 ± 0.5*
Total n-6 polyunsaturates	45.2 ± 1.7	46.8 ± 4.9
Total n-3 polyunsaturates	2.1 ± 0.4	2.1 ± 0.3

[a]Mean relative mole percentage ± SD, n = 10/group. *$P < 0.05$ by t test vs. HGCO group. See Table 1 for abbreviations.

TABLE 4
Fatty Acid Composition of Liver Phosphatidylethanolamine After 6 wk

Fatty acid	HGCO diet[a]	BO diet
16:0	19.9 ± 1.1	20.0 ± 1.6
18:0	26.2 ± 6.1	29.8 ± 6.9
18:1n-9	3.0 ± 0.4	2.5 ± 0.4*
18:1n-7	3.3 ± 0.6	1.9 ± 0.3*
18:2n-6	6.2 ± 1.1	4.6 ± 1.1*
18:3n-6	0.6 ± 0.1	0.6 ± 0.1
20:3n-6	0.9 ± 0.1	1.0 ± 0.4
20:4n-6	29.5 ± 4.4	26.8 ± 4.5
22:4n-6	2.5 ± 0.3	2.8 ± 0.6
22:5n-6	1.0 ± 0.2	3.4 ± 1.4*
22:5n-3	1.8 ± 0.3	1.1 ± 0.3*
22:6n-3	2.6 ± 0.6	3.2 ± 0.3*
Total saturates	46.8 ± 6.1	50.3 ± 5.9
Total monounsaturates	7.5 ± 0.9	5.7 ± 0.8*
Total n-6 polyunsaturates	41.2 ± 5.4	39.6 ± 5.5
Total n-3 polyunsaturates	4.4 ± 0.8	4.4 ± 0.5

[a]Mean relative mole percentage ± SD, $n = 10$/group. *$P < 0.05$ by t test vs. HGCO group. See Table 1 for abbreviations.

TABLE 6
Fatty Acid Composition of Plasma Total Phospholipids After 6 wk

Fatty acid	HGCO diet[a]	BO diet
16:0	27.9 ± 1.2	27.1 ± 1.5
18:0	19.8 ± 1.2	21.5 ± 1.1
18:1n-9	2.8 ± 0.2	2.7 ± 0.2
18:1n-7	2.6 ± 0.4	1.8 ± 0.2*
18:2n-6	7.6 ± 0.6	7.6 ± 0.9
18:3n-6	0.6 ± 0.4	0.9 ± 0.8
20:3n-6	0.8 ± 0.1	1.0 ± 0.3
20:4n-6	29.1 ± 2.8	28.4 ± 1.6
22:4n-6	1.0 ± 0.2	1.2 ± 0.3
22:5n-6	0.1 ± 0.1	0.8 ± 0.8
22:5n-3	0.6 ± 0.2	0.3 ± 0.0*
22:6n-3	0.7 ± 0.2	0.9 ± 0.3
Total saturates	49.4 ± 2.0	50.0 ± 1.6
Total monounsaturates	9.6 ± 1.0	8.5 ± 0.9
Total n-6 polyunsaturates	40.1 ± 2.6	40.4 ± 2.1
Total n-3 polyunsaturates	0.9 ± 0.2	1.0 ± 0.3

[a]Mean relative mole percentage ± SD, $n = 10$/group. *$P < 0.05$ by t test vs. HGCO group. See Table 1 for abbreviations.

22:4n-6, and 22:5n-6, in the total phospholipids were similar between the HGCO and BO diet groups (Table 6). The percentage of 18:2n-6 in the PI/PS fraction of plasma was slightly higher for the HGCO group than the BO group (3.4 vs. 2.5%, respectively).

As observed in the liver, the percentages of 22:5n-3 in the total phospholipids (Table 6) and PC fraction (Table 7) of the plasma from the HGCO group were slightly higher ($P < 0.05$) than the corresponding levels of 22:5n-3 from the BO group. Unlike the liver, there was no significant difference in the relative percentage of 22:6n-3 in any phospholipid fraction between the groups.

Epididymal triglyceride fatty acids. The percentages of 18:2n-6, 20:3n-6, 20:4n-6, and 22:4n-6 in the epididymal triglycerides of the HGCO group were similar to the corresponding levels in the BO group (Table 10). The mean percentage of triglyceride 18:3n-6 was slightly higher (11.9 vs. 10.1%, $P < 0.05$) in the BO group than the HGCO group.

DISCUSSION

These data represent the first dietary studies undertaken to evaluate the biological effects of transgenic HGCO in mammals. The results revealed that, when feeding equal amounts of GLA from either HGCO or BO, no significant differences were observed in growth characteristics and diet consumption by the rats over 6 wk. In addition, there was no evidence of any adverse effect of feeding a diet containing HGCO on the rats' appearance, behavior, or organ morphology.

In both plasma and liver total phospholipids, we observed no significant changes in the levels of 18:3n-6, 20:4n-6, or 22:4n-6 in rats fed the HGCO diet as compared to those fed

TABLE 5
Fatty Acid Composition of Liver Phosphatidylinositol/Serine After 6 wk

Fatty acid	HGCO diet[a]	BO diet
16:0	8.7 ± 1.0	7.0 ± 0.9*
18:0	47.9 ± 7.6	53.4 ± 5.6
18:1n-9	1.7 ± 0.3	1.7 ± 0.2
18:1n-7	0.9 ± 0.2	0.7 ± 0.2
18:2n-6	2.5 ± 0.4	1.6 ± 0.3*
20:3n-6	1.3 ± 0.3	1.6 ± 0.7
20:4n-6	31.1 ± 6.2	27.1 ± 4.6
22:4n-6	1.3 ± 0.4	1.4 ± 0.3
22:5n-6	0.4 ± 0.2	1.1 ± 0.3*
22:5n-3	0.7 ± 0.2	0.6 ± 0.2
22:6n-3	0.7 ± 0.2	1.1 ± 0.4*
Total saturates	57.5 ± 7.0	61.3 ± 5.1
Total monounsaturates	4.0 ± 0.5	3.9 ± 0.4
Total n-6 polyunsaturates	37.1 ± 6.7	33.1 ± 5.3
Total n-3 polyunsaturates	1.4 ± 0.3	1.7 ± 0.5

[a]Mean relative mole percentage ± SD, $n = 10$/group. *$P < 0.05$ by t test vs. HGCO group. See Table 1 for abbreviations.

TABLE 7
Fatty Acid Composition of Plasma Phosphatidylcholine After 6 wk

Fatty acid	HGCO diet[a]	BO diet
16:0	29.0 ± 1.3	28.1 ± 1.5
18:0	18.2 ± 1.0	19.9 ± 1.0*
18:1n-9	2.9 ± 0.3	2.7 ± 0.2
18:1n-7	2.9 ± 0.4	2.0 ± 0.2*
18:2n-6	8.6 ± 0.9	8.5 ± 1.0
20:1n-9	0.5 ± 0.1	0.7 ± 0.1*
20:3n-6	0.9 ± 0.1	1.1 ± 0.3*
20:4n-6	32.5 ± 1.9	31.3 ± 1.4
22:4n-6	1.2 ± 0.1	1.3 ± 0.3
22:5n-6	0.3 ± 0.1	1.5 ± 0.9
22:5n-3	0.6 ± 0.1	0.3 ± 0.0*
22:6n-3	0.9 ± 0.2	1.3 ± 0.2
Total saturates	47.7 ± 1.5	48.7 ± 1.5
Total monounsaturates	6.8 ± 0.7	6.0 ± 0.5
Total n-6 polyunsaturates	43.9 ± 1.6	43.8 ± 1.5
Total n-3 polyunsaturates	1.6 ± 0.3	1.5 ± 0.3

[a]Mean relative mole percentage ± SD, $n = 10$/group. *$P < 0.05$ by t test vs. HGCO group. See Table 1 for abbreviations.

TABLE 8
Fatty Acid Composition of Plasma Phosphatidylethanolamine After 6 wk[a]

Fatty acid	HGCO diet[a]	BO diet
14:0	2.5 ± 0.7	2.2 ± 0.9
16:0	21.1 ± 4.0	20.4 ± 2.9
18:0	29.5 ± 4.4	30.9 ± 1.7
18:1n-9	5.3 ± 1.4	4.9 ± 0.6
18:1n-7	2.1 ± 0.9	1.8 ± 0.4
18:2n-6	6.5 ± 1.6	5.8 ± 0.6
18:3n-6	2.3 ± 1.3	2.0 ± 0.5
18:3n-3	1.1 ± 0.3	1.0 ± 0.3
20:1n-9	2.7 ± 1.6	2.2 ± 0.5
20:3n-6	0.7 ± 0.4	0.8 ± 0.3
20:4n-6	18.3 ± 6.5	22.7 ± 3.0
22:4n-6	4.3 ± 1.6	4.6 ± 1.9
Total saturates	55.1 ± 4.8	54.2 ± 4.2
Total monounsaturates	10.2 ± 1.4	8.9 ± 1.0
Total n-6 polyunsaturates	32.9 ± 9.3	36.6 ± 4.8
Total n-3 polyunsaturates	1.1 ± 0.3	1.0 ± 0.3

[a]Mean relative mole percentage ± SD, n = 10/group. See Table 1 for abbreviations.

TABLE 10
Fatty Acid Composition of Epididymal Triglycerides After 6 wk

Fatty acid	HGCO diet[a]	BO diet
16:0	19.9 ± 2.2	19.9 ± 1.2
18:0	2.1 ± 0.2	2.3 ± 0.3
18:1n-9	20.7 ± 1.6	19.3 ± 1.5
18:1n-7	2.4 ± 0.3	1.5 ± 0.1*
18:2n-6	29.2 ± 2.7	29.0 ± 1.7
18:3n-6	10.1 ± 1.4	11.9 ± 1.4*
18:3n-3	1.0 ± 0.2	0.5 ± 0.1*
20:3n-6	1.5 ± 0.2	1.7 ± 0.2
20:4n-6	2.7 ± 0.5	3.1 ± 0.5
22:4n-6	0.4 ± 0.1	0.5 ± 0.1
Total saturates	23.8 ± 2.2	24.7 ± 0.8
Total monounsaturates	29.9 ± 2.2	28.9 ± 1.5
Total n-6 polyunsaturates	45.0 ± 4.0	45.7 ± 2.1
Total n-3 polyunsaturates	1.3 ± 0.2	0.7 ± 0.1

[a]Mean relative mole percentage ± SD, n = 10/group. *$P < 0.05$ by t test vs. HGCO group. See Table 1 for abbreviations.

the BO diet. Similar findings were reported by Raederstorff and Moser in a GLA feeding study comparing BO with primrose oil (14). They found no changes in liver GLA and DHGLA levels within groups given equal amounts of dietary GLA from either oil source, indicating that the relative percentages of GLA and DHGLA in tissue phospholipids are dependent upon the GLA content in the diet, but not on the oil source of GLA. The only exception in the present study was that we found that the percentage of 20:3n-6 in liver total phospholipids from the rats given the BO diet was slightly higher than that in rats fed the HGCO diet (1.4 vs 1.0, $P < 0.05$) (Table 2). However, there were no dietary differences in the relative percentage of 20:3n-6 present in the liver PC, PE, or PI/PS fractions (Tables 3–5), suggesting that this may have been a statistical anomaly.

When fatty acid profiles within individual liver phospholipids were examined more closely, we observed minor changes in the relative percentage of several fatty acids in rats fed diets containing HGCO as compared to BO. For example, the levels of 18:2n-6 in PE (Table 4) and PI/PS (Table 5) were increased in the HGCO group. The elevation of 18:2n-6 may indicate a decrease in the hepatic Δ6 desaturation of 18:2n-6 in rats fed the HGCO diet. This could be due to competition for binding of Δ6 desaturase by 18:3n-3, which was present in higher amounts in the HGCO diet as compared with the BO diet (1.1 vs. 0.3, Table 1). A greater ratio of n-3/n-6 is known to suppress the metabolism of n-6 fatty acids given that n-3 PUFA are the preferred substrates for the Δ6-desaturase binding (15,16) and acyltransferase (17). The above finding was not unexpected as our main objective was to maintain the ratio of comparable levels of n-6 PUFA (18:2n-6 and 18:3n-6) in both diets. As a consequence the n-3/n-6 fatty acids were not balanced. In addition to an increase in 18:2n-6, we have also observed a decrease in the levels of 22:5n-6 in rats fed the HGCO diet as compared with rats fed the BO diet (Tables 2–5). This finding, in the absence of differences in levels of 18:3n-6, 20:3n-6, 20:4n-6, and 22:4n-6, suggests that any inhibition on desaturation of 18:2n-6 after feeding the HGCO diet may exclusively affect the levels of its end metabolite, 22:5n-6. In this regard, Sprecher *et al.* (18) have proposed a revised pathway for the biosynthesis of 22:5n-6, which requires elongation of 22:4n-6 to 24:4n-6 followed by Δ6 desaturation to 24:5n-6, which in turn undergoes partial β-oxidation to 22:5n-6. Thus, preferential binding of n-3 vs. n-6 fatty acids at this later Δ6 desaturation step would result in decreased production of 22:5n-6 as observed in the liver phospholipids for the HGCO group. Similar changes in 22:5n-6 have been reported by others who also fed diets with equal amounts of GLA, but unbalanced n-3/n-6 fatty acids (14). It should be noted, however, that the level of 22:5n-6 represents only a minor fraction of the overall PUFA content in liver lipids.

TABLE 9
Fatty Acid Composition of Plasma Phosphatidylinositol/Serine After 6 wk

Fatty acid	HGCO diet[a]	BO diet
16:0	11.7 ± 1.6	10.1 ± 1.6*
18:0	40.4 ± 3.4	41.2 ± 5.2
18:1n-9	2.0 ± 0.3	1.8 ± 0.4
18:1n-7	1.1 ± 0.2	0.8 ± 0.2*
18:2n-6	3.4 ± 0.8	2.5 ± 0.4*
18:3n-6	0.6 ± 0.2	0.6 ± 0.1
20:1n-9	0.7 ± 0.2	0.7 ± 0.1
20:3n-6	1.0 ± 0.2	1.3 ± 0.5
20:4n-6	34.6 ± 2.8	34.4 ± 3.2
22:4n-6	0.8 ± 0.2	0.8 ± 0.4
Total saturates	53.0 ± 3.6	52.3 ± 5.2
Total monounsaturates	4.4 ± 1.8	3.4 ± 0.6
Total n-6 polyunsaturates	42.4 ± 3.4	43.8 ± 4.6
Total n-3 polyunsaturates	0.4 ± 0.8	0.7 ± 1.0

[a]Mean relative mole percentage ± SD, n = 10/group. *$P < 0.05$ by t test vs. HGCO group. See Table 1 for abbreviations.

In summary, the growth and hepatic metabolism of the principal n-6 fatty acids was similar between rats fed diets containing the same amounts of fat (10%, by weight) and GLA (23% of total fats) whether the source of GLA was HGCO or BO. Additional bioequivalency studies are now warranted to determine the effects of long-term feeding (i.e., >12 wk) of diets containing HGCO on clinically relevant biochemical and hematologic parameters.

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