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Dietary gangliosides increase the content and molecular percentage of ether phospholipids containing 20:4n-6 and 22:6n-3 in weanling rat intestine

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Abstract

This study was conducted to determine whether dietary ganglioside (GG) increases the content of ether phospholipids (EPL) in intestinal mucosa. Weanling Sprague-Dawley rats were fed a semipurified diet consisting of 20% fat as a control diet. Two experimental diets were formulated by adding either 0.1% (w/w fat) GGs (GG diet) or 1.0% (w/w fat) sphingomyelin (SM diet) to the control diet. Fatty acid methyl esters from the alkenylacyl, alkylacyl and diacyl subclasses of phospholipids were measured to determine total and molecular percentage of EPL comprising the choline phosphoglyceride (CPG) and ethanolamine phosphoglyceride (EPG) fraction. Animals fed the GG diet significantly increased total EPL content both in CPG (by 36%) and in EPG (by 66%), and the molecular percentage of EPL in CPG (by 76%) and in EPG (by 59%) compared to animals fed the control diet. Dietary GG-induced increase in EPL resulted in a higher level of polyunsaturated fatty acids (PUFA) specifically in 20:4n-6 and 22:6n-3 compared to control animals, leading to a decrease in the ratio of saturated fatty acids (SFA) to PUFA both in CPG and in EPG. Feeding animals the SM diet showed a higher level of EPL than control animals with a concomitant increase in 22:6n-3 in EPL. The present data demonstrate that dietary GG increases the content and composition of EPL containing PUFA in the weanling rat intestine.

Keywords: Ganglioside; Ether phospholipid; Polyunsaturated fatty acid; Intestine; Diet

1. Introduction

Gangliosides (GG), sialic acid-containing glycosphingolipids, act as a receptor for *E. coli* and *Cholera* toxins [1] and a stimulator of the immune system in the intestine [2]. Rat intestine has 20–30% of the total lipids as glycosphingolipids, one third of which are GGs [3]. Change occurs in the composition and molecular structure of GGs during intestinal development [4]. Monosialoganglioside GM3 (GM3) is the major GG in rat intestine [5] and is localized at the brush border membrane while disialoganglioside GD3 (GD3) is present at the basolateral membrane [6]. These findings imply that intestinal function may be influenced by the presence and composition of constituent sphingolipids (SPL).

Exogenous GG and sphingomyelin (SM) are hydrolyzed by enterocyte membrane-bound enzymes such as sialidase, sphingomyelinase and/or ceramidase [7–10]. When rats were fed radiolabelled sphingosine, ceramide or SM, about 10–55% of radioactivity was found in the rat intestine, and

Abbreviations: CPG, choline phosphoglycerides; EPG, ethanolamine phosphoglycerides; EPL, ether phospholipids; GG, gangliosides; GLC, gas liquid chromatography; GD3, disialoganglioside GD3; GM1, monosialoganglioside GM1; GM3, monosialoganglioside GM3; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SM, sphingomyelin; SPL, sphingolipids; TLC, thin layer chromatography.

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30–60% was found in lymph lipids after 24 h [9]. Metabolites transported into enterocytes are reutilized in the synthesis of GGs or SM or both [7,8]. These studies indicate that dietary SPL are digested, metabolised and transported into other tissues.

Several studies suggest a possible interaction between SPL and phospholipids [7–9,11]. Sphingosine-1-phosphate, a metabolic derivative of SPL, is metabolized into phosphoethanolamine and hexadecanal, both prerequisite materials for phospholipid synthesis [7,8]. In animal studies, dietary [3-^{3}H] sphingosine–SM is absorbed, of which 70% is fatty acid and glyceride [9]. An appreciable amount of sphingosine is incorporated into hepatocyte phospholipids in the ether phospholipid (EPL) form when radiolabelled [^{3}H] sphingosine-GM1[2] was intraperitoneally injected into mice [11].

Ether phospholipid has an ester linkage at the sn-2 position, and an ether linkage, either to an alkyl or alkenyl group, at the sn-1 position [14]. Ether phospholipid tends to be enriched in mammalian intestinal cells [15]. One type of EPL known as plasmalogen, 1-*O*-alkenyl-2-acyl-glycero-phospholipids, accounts for about 6–12% of ethanolamine phosphoglyceride (EPG) in rat intestinal mucosa [15,16]. A high content of EPL may contribute to maintenance of cell integrity and function [17–23] such as permeability [18], fluidity [24] and endogenous antioxidant for membrane peroxidation [25,26]. Ether phospholipid also induces cell apoptosis [17], cytotoxicity [19,20] and antitumor activity [21–23], which could have potential in anticancer applications. These cellular functions of EPL seem to be dependent on membrane cholesterol content [27]. For example, cholesterol reduction in the membrane causes increased EPL uptake into the membrane [27] and increases activity of Δ-5 and Δ-6 desaturase enzymes [28].

Our previous work demonstrates that dietary GG increases the total content of GGs and decreases cholesterol content in developing rat intestine [6]. Thus it was logical to hypothesize that dietary GG will increase the content of polyunsaturated fatty acids (PUFA) in EPL in the intestine by increasing derivatives of SPL and by decreasing cholesterol content.

Suckling babies ingest about ~35–170 mg SPL from mothers' milk per day [29]. No clear information is available to explain the metabolic fate of dietary SPL to EPL in the developing intestine. Thus, the objective for this study was to determine whether dietary GG increases total membrane EPL content. This study also examined whether increased EPL content was accompanied with higher PUFA in EPL. Sphingomyelin and GG have a ceramide molecule anchored in the cell membrane, but attached to a different head group. Thus, SM was used as a second control to compare bioavailability with GGs. Using rats, the present study demonstrates that dietary GG increases total content and composition of EPL containing PUFA in the developing intestine.

[2] Nomenclature recommended by Svennerholm [12] and IUPAC-IUB [13].

2. Materials and methods

2.1. Animals and diets

The protocol for this study was approved by the Animal Care Committee at the University of Alberta, Canada. Male Sprague-Dawley rats (18-day-old, 40±4.5 g) were housed in polypropylene cages and maintained at a constant room temperature of 23°C and a 12-h light/dark cycle for 2 weeks. Animals had free access to water and were randomized to be fed one of three semipurified diets containing 20% (w/w) fat (Table 1). The control diet fat was a blend of triglyceride including corn oil, canola oil, coconut oil and olive oil, which reflected the fat composition of an existing infant formula. The composition of basal diet is reported elsewhere [30]. Two experimental diets were formulated by adding either SM (SM diet; 1.0% w/w fat; Sigma, MO) or GG (GG diet; 0.1% w/w fat; Fontera, Cambridge, New Zealand) to the control diet. The amount of SM and GGs added to diets was similar to that of human milk [31,32]. The GG fraction in the GG-enriched diet contained about 80% (w/w) GD3. Ganglioside GD1b, GM3 and other GGs were 9%, 5% and

Table 1
Composition of experimental diets

	Control	SM	GG
Basal diet[a] (g/100 g)	80.0	80.0	80.0
Casein	27.0	27.0	27.0
Starch	20.0	20.0	20.0
Glucose	20.765	20.765	20.765
Nonnutritive cellulose	5.0	5.0	5.0
Vitamin mix[b]	1.0	1.0	1.0
Mineral mix[c]	5.085	5.085	5.085
Choline	0.275	0.275	0.275
Inositol	0.625	0.625	0.625
L-Methionine	0.25	0.25	0.25
Oils	20.0	20.0	20.0
Triglyceride[d]	20.0 (100)[e]	19.8 (99.0)	19.9 (98.6)
Sphingomyelin	–	0.2 (1.0)	tr
Ganglioside	–	–	0.02 (0.1)
Phospholipid	–	–	0.05 (0.25)
Cholesterol	–	–	tr (0.002)

tr represents trace amount.

[a] The composition of the basal diet was described by Clandinin and Yamashiro [30].

[b] AOAC vitamin mix (Teklad Test Diets, Madison, WI): 20,000 IU vitamin A; 2000 IU vitamin D; 100 mg vitamin E; 5 mg menadione; 5 mg thiamine-HCl; 8 mg riboflavin; 40 mg pyridoxine-HCl; 40 mg niacin; 40 mg pantothenic acid; 0.4 mg biotin; 2 mg folic acid; 30 mg vitamin B_{12} per kilogram of complete diet.

[c] Bernhart-Tomarelli mineral mix (General Biochemicals, Chagrin Falls, OH): 77.5 mg Mn^{2+}; 0.06 mg Se^{2+} per kilogram of complete diet.

[d] The fatty acid composition of the triglyceride fed was similar to that of an infant formula (Table 2).

[e] Values in parenthesis represent the percentage of total fat.

6% w/w, respectively. The fatty acid composition of experimental diets was quantitatively analyzed by gas liquid chromatography (GLC, Varian Vista 3400CX, Varian Instruments, ON, Canada). The fatty acid composition was consistent among the three diets: 18:1n-9 (50%), 18:2n-6 (20%), 16:0 (16%), 18:0 (8%), 18:3n-3 (2.8%) and other fatty acids (3.2%). The control diet and experimental diets provided an n-6 to n-3 ratio of 7:1. Cholesterol content was negligible (<0.002% w/w of total fat). Body weight and food intake were measured every other day throughout the experiment.

2.2. Collection of intestinal mucosa

After anesthetizing the animals with halothane, the small intestine (jejunum to ileum) was excised. The intestine was washed with 0.9% cold saline solution to remove visible mucus and debris, opened longitudinally, and moisture was carefully removed. Intestinal mucosa was scraped off with a glass slide on an ice-cold glass plate. All samples were weighed and kept in a −70°C freezer until used.

2.3. Lipid extraction and phospholipid separation

Total lipid was extracted by using the Folch method [33]. For extracting total lipid, the intestinal mucosa was washed twice with Folch lower phase solution (chloroform/methanol/water, 86:14:1, by volume). The lower phase lipid was pooled, dried and dissolved in chloroform/methanol (2:1, v/v). Extracted lipid was applied onto precoated silica gel "H" thin layer chromatography (TLC) plates (Analtech, Newark, DE) and developed in the solvent (chloroform/methanol/2-propanol/0.25% KCl/triethylamine, 45:13.5:37.5:9:27, by vol) to separate individual phospholipid classes. After spraying with 0.1% ANSA (anilino naphthalene sulfonic acid) and identifying CPG and EPG bands under UV light, the two bands were scraped into test tubes.

2.4. Fatty acid composition and quantification

Separation and purification of three subclasses of CPG and EPG were prepared [34,35]. Solid phases containing CPG and EPG were eluted with 5 ml of chloroform/methanol (2:1, v/v) and dried under N_2. Phospholipids were dephosphorylated with phospholipase C (*B. cereus*, Sigma, St. Louis, MO) by the method of Bernett et al. [36] to provide for alkenylacyl, alkylacyl and diacyl fractions from each phospholipid. After extraction of diradylglycerols with diethyl ether, lipids were acetylated in 0.1 ml of pyridine and 0.5 ml of acetic anhydride at 80°C for 1 h. The acetylated derivatives of three subclasses of CPG and EPG were applied onto a silica gel high-performance TLC plate (HPTLC, Whatman, Clifton, NJ) and developed in petroleum ether/diethyl ether/acetic acid (90:10:1, by vol) to a migration distance of 10 cm from the solvent line, followed by a second development in toluene [37]. Three subclasses of the CPG and EPG fractions scraped from the plate were then methylated in 3N-methanolic-HCl (Supelco, Bellefonte, PA) for 16 h at 70°C with a known amount of heptadecanoic acid as an internal standard. The fatty acid composition of each of the six fractions was analyzed by GLC equipped with a flame ionization detector and BP-20 fused capillary column (SGE, Australia). The flow rate of helium gas was 1.6 ml/min, and the oven, injector and detector temperatures were 200°C, 250°C and 250°C, respectively. To compare the molecular percentage of diacyl phospholipid with EPL, the total fatty acid amount of the diacyl subclass was divided by two because only one fatty acid is derived from EPL [34,35]. To examine the recovery ratio of three subclasses from the initial CPG and EPG fraction through purification and separation processes, 10% of CPG and EPG fractions were used for analyzing the total content of fatty acids in CPG and EPG. The remaining 90% of each fraction was used for EPL analysis. The average recovery ratio of three subclasses of CPG and EPG was 75% (data not shown).

2.5. Statistical analysis

The values were shown as means±S.D. from seven animals for each diet group. Significant differences between the control group and experimental groups were determined by one-way analysis of variance (ANOVA) with SAS (version 8.2, SAS Institute, Cary, NC). Effects of diet treatment were determined by a Duncan multiple range test at a significance level of $P<.05$, $P<.01$, $P<.001$ or $P<.0001$.

3. Results

3.1. Animal growth and intestinal mucosa

Initial body weight of animals and weight after 2 weeks of diet treatment were not significantly different between experimental and control groups. Intestinal mucosal weight and length were not affected by dietary treatment. Food consumption was not influenced by diet treatment either (data not shown).

Table 2
Fatty acid content of alkenylacyl-, alkylacyl- and diacyl-GPC and GPE from intestinal mucosa of animals fed experimental diets[1]

Subclass (μg/g tissue)	Control	SM	GG	Diet effect ($P<$)
Total EPL-GPC[2]	31.8±6.7[b]	37.8±8.7[a,b]	43.3±8.2[a]	.05
Alkenylacyl-GPC	14.0±3.6[b]	17.0±3.0[a,b]	21.8±7.2[a]	.05
Alkylacyl-GPC	17.8±3.9	20.8±7.5	21.5±4.8	
Diacyl-GPC	1860±340[a]	1610±470[a,b]	1220±310[b]	.01
Total EPL-GPE	68.1±7.9[c]	97.1±15[b]	113±20[a]	.001
Alkenylacyl-GPE	34.4±7.7[b]	45.2±15[b]	60.9±12[a]	.001
Alkylacyl-GPE	33.7±4.4[b]	51.9±11[a]	51.8±11[a]	.001
Diacyl-GPE	774±99	731±290	801±170	

[a,b,c] Within the row, values with different superscript letters are significantly different at $P<.05$, $P<.01$, $P<.001$, or $P<.0001$.

[1] Means±S.D. from seven animals for each diet group.

[2] Fatty acid content of total EPL (alkenylacyl and alkylacyl together) in CPG or EPG.

Table 3
Molecular percentage of alkenylacyl-, alkylacyl- and diacyl-GPC and GPE from intestinal mucosa of animals fed experimental diets[1]

Subclass (%)	Control	SM	GG	Diet effect ($P<$)
Total-EPL-GPC[2]	3.7±0.8[b]	4.8±1.1[b]	6.5±1.8[a]	.01
Alkenylacyl-GPC	1.6±0.4[b]	2.2±0.8[a,b]	3.3±1.3[a]	.05
Alkylacyl-GPC	2.1±0.5[b]	2.6±0.6[a,b]	3.2±0.8[a]	.05
Diacyl-GPC	96.3±0.8[a]	95.2±1.1[a]	93.5±1.8[b]	.01
Total-EPL-GPE	14.7±1.6[b]	20.4±5.4[a]	23.4±3.2[a]	.001
Alkenylacyl-GPE	7.4±1.5[c]	10.2±2.1[b]	12.5±1.4[a]	.0001
Alkylacyl-GPE	7.3±1.1[b]	10.2±3.7[a,b]	10.9±2.5[a]	.05
Diacyl-GPE	85.3±1.6[a]	79.6±5.4[b]	76.6±3.2[b]	.01

[a,b,c] Within the row, values with different superscript letters are significantly different at $P<.05$, $P<.01$, $P<.001$, or $P<.0001$.

[1] Means±S.D. from seven animals for each diet group. Molecular percentage is presented as relative percentage of total fatty acid amount in the alkenylacyl, alkylacyl or diacyl phospholipid. To compare the molecular percentage of diacyl phospholipid with ether phospholipid, total fatty acid amount of the diacyl subclass was divided by two because only one fatty acid is derived from ether phospholipid.

[2] Percent of total EPL (alkenylacyl and alkylacyl together) relative to total phospholipids in CPG or EPG.

3.2. Fatty acid content corresponding to alkenylacyl-, alkylacyl- and diacyl-GPC and GPE

Animals fed the GG diet significantly increased the content of total fatty acids in alkenylacyl-GPC, alkenylacyl-GPE and alkylacyl-GPE in comparison with control animals (56%, 77% and 54%, respectively; Table 2). In animals fed dietary GG, a significant decrease in diacyl-GPC occurred, whereas no change was observed in diacyl-GPE. Animals fed the SM diet exhibited a similar increase in fatty acid content in EPL corresponding to alkylacyl-GPE, but no change occurred in the other subclasses of GPC and GPE. The GG diet produced a significant increase in the total fatty acid content of EPLs (alkenylacyl and alkylacyl together) by 36% (31.8 vs. 43.3) and 66% (68.1 vs. 113), respectively, in CPG and EPG phospholipids compared to the controls (Table 2). Feeding animals the SM diet increased total EPL in EPG by 42% compared to control animals.

3.3. Molecular percentage of alkenylacyl-, alkylacyl- and diacyl-GPC and GPE

Animals fed the GG diet exhibited higher levels of alkenylacyl-GPC (3.3% vs. 1.6%) and alkylacyl-GPC (3.2% vs. 2.1%) with correspondingly lower levels of diacyl-GPC (93.5% vs. 96.3%) than animals fed the control diet (Table 3). No effect of the SM diet was observed on the relative composition of CPG in the mucosa. Feeding animals the GG diet achieved higher molecular levels of alkenylacyl-GPE (12.5% vs. 7.4%) and alkylacyl-GPE (10.9% vs. 7.3%) with correspondingly lower molecular levels of diacyl-GPE (76.6% vs. 85.3%) than animals fed the control diet. Animals fed the SM diet showed a relative increase in alkenylacyl-GPE, but the effect was less pronounced than in those fed the GG diet. There was no effect of the SM diet on alkylacyl-GPE. Relative to molecular percentage in three alkenylacyl, alkylacyl and diacyl classes, animals fed the GG diet exhibited a 76% increase in CPG and 59% increase in total alkenylacyl- and alkylacyl-EPL (Table 3). Feeding the SM diet resulted in a molecular increase in EPG, but not in CPG.

3.4. Fatty acid composition of alkenylacyl-, alkylacyl- and diacyl-GPC

In comparison with control animals, animals fed the GG diet did not show significant change in the fatty acid

Table 4
Fatty acid composition of alkenylacyl-, alkylacyl- and diacyl-GPC in intestinal mucosa of animals fed control diet or treatment diets[1]

	Alkenylacyl-GPC			Alkylacyl-GPC			Diacyl-GPC		
	Control	SM	GG	Control	SM	GG	Control	SM	GG
C 14:0	6.2±1.6	8.1±5.4	5.0±1.3	5.4±2.1	6.1±2.5	6.4±2.5	0.8±0.2	1.1±0.3	0.9±0.2
C 16:0	33.7±5.8	32.2±8.6	32.3±8.2	34.3±7.3	33.3±6.1	33.0±4.3	21.0±1.7[b]	24.4±2.2[a]	24.3±2.5[a†]
C 18:0	25.5±3.7	23.2±5.9	22.0±6.7	25.8±3.6[a]	17.6±5.7[b]	19.0±4.6[b†]	22.7±1.1[b]	24.8±3.4[b]	27.7±3.2[a†]
C 18:1(9)	8.8±4.3	8.5±4.3	5.9±2.3	8.7±3.1	7.7±1.5	7.4±1.3	11.6±0.8[a]	10.5±0.8[b]	9.2±1.1[c¶]
C 18:2(6)	3.2±1.8	3.2±2.1	2.4±1.6	5.3±4.1	5.5±1.8	5.5±2.9	30.0±1.6[a]	26.6±3.1[b]	23.5±3.4[c‡]
C 20:4(6)	1.8±0.6[a,b]	1.1±0.5[b]	3.1±2.1[a]	5.7±3.7	9.5±4.8	9.3±6.7	9.4±0.4	8.8±2.8	10.2±1.5
C 20:5(3)	1.5±0.9	0.6±0.6	2.2±2.0	0.7±0.6	0.8±0.5	1.0±0.5	0.3±0.1	0.2±0.1	0.3±0.1
C 24:0	2.4±1.5	3.3±1.5	3.2±2.2	1.8±1.0[a]	1.8±1.0[a]	0.6±0.8[b]	0.1±0.0	0.1±0.0	0.2±0.1
C 22:6(3)	1.2±1.1	0.9±1.1	tr	1.2±1.0[b]	2.7±0.9[a]	4.2±1.8[a‡]	1.4±0.2	1.1±0.4	1.3±0.2
C 24:1(9)	5.3±2.9	7.5±5.4	8.8±3.6	4.0±1.8[a]	3.0±0.7[a,b]	1.8±1.4[b]	0.1±0.0	0.1±0.1	0.2±0.1
Others[2]	10.4±2.4	11.5±5.6	15.1±6.2	7.2±2.0	12.2±4.2	11.8±3.3	2.6±0.2	2.4±0.4	2.2±0.4
Total	100	100	100	100	100	100	100	100	100
SFA	69.5±7.3	70.4±10.9	66.6±6.6	69.6±10.6	61.8±9.3	61.7±7.8	45.2±2.3[b]	50.7±5.5[a]	53.6±5.8[a†]
MUFA	19.1±4.8	19.4±7.3	18.7±2.6	14.6±2.5	13.2±1.9	12.4±2.2	12.8±0.9[a]	11.5±0.9[b]	10.2±1.1[c¶]
PUFA	11.4±3.6	10.2±5.2	14.8±4.9	15.8±8.9[b]	25.0±7.6[a]	25.9±6.6[a]	42.0±1.7[a]	37.7±5.7[a,b]	36.2±4.8[b]

[1] Means±S.D. (% w/w) in three subclasses from seven animals for each diet group. [a,b,c] Within a row, values with different superscript letters are significantly different at $P<.05$. Superscript letters with †, ‡ and ¶ are significantly different at $P<.01$, $P<.001$ and $P<.0001$, respectively.

[2] Minor fatty acids were pooled as others but were included in SFA, MUFA and PUFA.

composition of alkenylacyl-GPC (Table 4). Animals fed the GG diet showed an increase in 20:4n-6 compared to animals fed the SM diet. Decrease in alkylacyl-GPC content of 18:0, 24:0 and 24:1 occurred for animals fed the GG diet as well as an increase by 2.5-fold in 22:6n-3 ($P<.001$) compared to control animals. The SM diet produced a decrease in 18:0 and an increase in 22:6n-3 content. In diacyl-GPC, higher levels of 16:0 and 18:0 and lower levels of 18:1n-9 and 18:2n-6 were observed in animals fed the GG diet compared to control animals. Similar trends in 16:0, 18:1n-9 and 18:2n-6 were observed in animals fed the SM diet.

3.5. Fatty acid composition of alkenylacyl-, alkylacyl- and diacyl-GPE

The fatty acid composition of alkenylacyl-, alkylacyl- and diacyl-GPE in intestinal mucosa of animals fed experimental diets is illustrated (Table 5). Animals fed dietary GG showed higher levels of 22:4n-6 in alkenylacyl-GPE (100% increase, $P<.001$) and 22:6n-3 (71% increase, $P<.001$), and lower levels of 16:0 and 18:0 compared to feeding the control diet. Animals receiving the SM diet exhibited a similar change in 22:4n-6, 22:6n-3 and 16:0 fatty acid content, but the effect was smaller than observed for animals fed the GG diet. In alkylacyl-GPE, higher content of 20:4n-6, 22:4n-6 and 22:6n-3 (36%, 87% and 77% increases, respectively) was observed in animals fed the GG diet with a considerable reduction in saturated fatty acids (SFA), specifically 16:0, 18:0 and 24:0 relative to animals fed the control diet. Dietary GG increased the content of 20:4n-6 by 36% in diacyl-GPE, relative to control diet ($P<.01$).

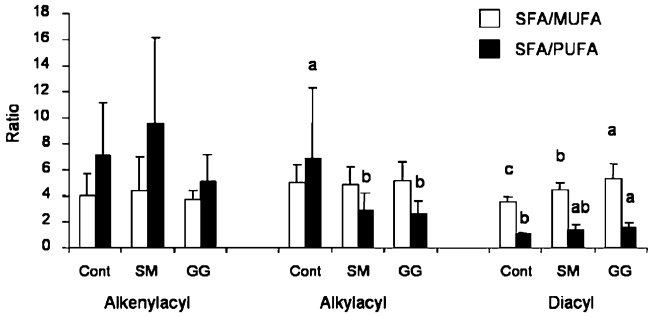

Fig. 1. Ratio of SFA to MUFA (white columns) and SFA to PUFA (black columns) in alkenylacyl-, alkylacyl- and diacyl-GPC in intestinal mucosa of animals fed control or treatment diets. Values are means±S.D. of seven animals for each diet group. Letters represent a significant difference between groups at $P<.05$, except for the ratio of SFA to MUFA in the diacyl subclass at $P<.001$.

3.6. Total SFA, MUFA and PUFA content of alkenylacyl-, alkylacyl- and diacyl-GPC

The fatty acid content of SFA, monounsaturated fatty acids (MUFA) and PUFA in alkenylacyl-, alkylacyl- and diacyl-GPC is shown (Table 4). Feeding animals the GG diet increased total PUFA content in alkylacyl-GPC by 63% compared to animals fed the control diet. No changes occurred in MUFA and SFA for this lipid subclass. Animals fed the GG diet had lower content of PUFA and MUFA and increased content of SFA in diacyl-GPC than observed for control animals. For animals fed the SM diet, a higher level of PUFA in alkylacyl-GPC was found. These animals also exhibited lower MUFA and higher SFA contents in diacyl-GPC than control animals.

Table 5
Fatty acid composition of alkenylacyl-, alkylacyl- and diacyl-GPE in intestinal mucosa of animals fed control diet or treatment diets[1]

	Alkenylacyl-GPE			Alkylacyl-GPE			Diacyl-GPE		
	Control	SM	GG	Control	SM	GG	Control	SM	GG
C 14:0	3.9±1.3	3.1±1.1	3.1±1.2	4.4±1.5	3.0±1.9	2.5±1.5	0.7±0.4	0.4±0.1	0.5±0.2
C 16:0	18.9±3.6^{a}	15.4±1.7^{b}	14.2±2.3^{b†}	18.7±5.4^{a}	12.7±3.2^{b}	11.4±1.9^{b†}	9.8±2.5	8.5±1.8	8.1±1.9
C 18:0	17.5±4.7^{a}	14.4±4.1^{a,b}	11.6±2.7^{b}	14.8±3.2^{a}	11.2±4.0^{b}	9.8±2.1^{b}	47.0±8.6	43.5±7.4	42.6±4.3
C 18:1(9)	8.9±2.0	8.2±1.2	7.3±0.9	12.0±3.1	11.6±1.9	9.2±1.3	12.2±2.9	13.1±2.1	12.8±1.5
C 18:2(6)	4±1.3	4.6±0.5	4.1±0.7	5.4±1.0	6.4±0.6	5.6±0.8	11.8±3.3	14.7±3.0	14.2±1.8
C 20:4(6)	16.8±5.7	21.3±3.9	23.1±4.9	18.5±6.1^{b}	21.9±4.6^{a,b}	25.1±2.6^{a}	11.5±3.6^{b}	14.0±3.5^{a,b}	15.7±2.6^{a†}
C 20:5(3)	0.6±0.3	0.4±0.3	0.5±0.2	0.8±0.3	0.7±0.4	0.6±0.3	0.3±0.1	0.3±0.2	0.2±0.2
C 22:4(6)	4.9±2.8^{b}	7.8±1.6^{a}	9.9±2.1^{a‡}	5.8±2.3^{b}	8.3±2.9^{a,b}	10.9±2.2^{a†}	0.3±0.2	0.4±0.2	0.6±0.5
C 24:0	2.3±1.9	1.4±0.7	0.9±0.5	1.4±0.4^{a}	1.6±0.7^{a}	0.7±0.5^{b}	0.2±0.1	0.2±0.1	0.3±0.2
C 22:6(3)	7.3±2.6^{c}	10.1±0.9^{b}	12.7±1.8^{a‡}	6.3±1.9^{b}	9.2±2.3^{a}	11.2±1.2^{a‡}	2.0±1.0	2.3±1.1	2.7±0.5
C 24:1(9)	2.5±1.4	2.3±1.5	1.5±1.1	1.3±0.7	2.6±1.6	2.4±2.3	0.6±0.5	0.3±0.1	0.2±0.1
Others[2]	12.4±3.9	11.1±2.0	11.1±1.1	10.6±1.9	10.8±2.4	10.7±1.9	3.6±0.9	2.3±0.7	2.2±0.6
Total	100	100	100	100	100	100	100	100	100
SFA	44.7±9.7^{a}	35.7±5.9^{b}	31.2±5.5^{b†}	41.6±9.8^{a}	30.2±7.1^{b}	26.7±4.4^{b†}	58.6±9.8	53.3±8.8	50.4±4.9
MUFA	15.7±4.2	15.2±2.5	13.2±2.1	17.2±3.0	17.9±2.8	15.2±2.4	13.6±2.8	13.9±2.1	13.4±1.1
PUFA	39.4±11.3^{b}	49.1±5.4^{a}	55.6±7.4^{a†}	41.2±11.5^{b}	51.9±9.4^{a}	58.1±4.2^{a†}	27.8±7.4^{b}	32.8±7.0^{a,b}	36.2±4.2^{a}

^{a,b,c} Within a row, values with different superscript letters are significantly different at $P<.05$.

[1] Means±S.D. (% w/w) in three subclasses from seven animals for each diet group. Superscript letters with †, ‡ and ¶ are significantly different at $P<.01$, $P<.001$, and $P<.0001$, respectively.

[2] Minor fatty acids were pooled as others but were included in SFA, MUFA and PUFA.

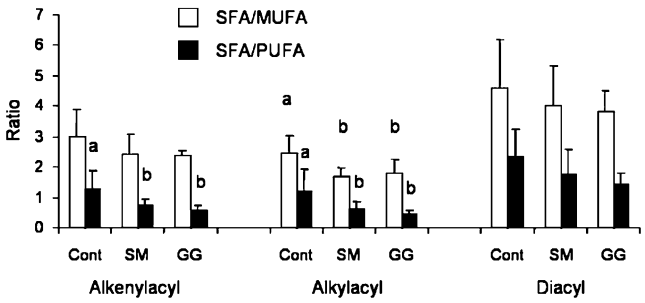

Fig. 2. Ratio of SFA to MUFA (white columns) and SFA to PUFA (black columns) in alkenylacyl-, alkylacyl- and diacyl-GPE in intestinal mucosa of animals fed control or treatment diets. Values are means±S.D. of seven animals for each diet group. Letters represent a significant difference between groups at $P<.01$, except for the ratio of SFA to PUFA in the alkylacyl subclass at $P<.05$.

3.7. Total SFA, MUFA and PUFA content of alkenylacyl-, alkylacyl- and diacyl-GPE

Animals fed the GG diet exhibited increased content of PUFA in the three subclasses of EPG compared to control animals (increase of 41% in alkenylacyl, 41% in alkylacyl and 30% in diacyl-GPE; Table 5). The increase in PUFA content was accompanied with a decrease in SFA content in alkenylacyl- and alkylacyl-GPE. No change was observed in MUFA in alkenylacyl-, alkylacyl- or diacyl-GPE. Feeding of the SM diet also resulted in increased content of PUFA and decreased content of SFA in alkenylacyl-GPE and alkylacyl-GPE compared to controls. No effect of the SM diet was detected in diacyl-GPE.

3.8. Ratio of SFA/MUFA, and SFA/PUFA in alkenylacyl-, alkylacyl- and diacyl-GPC and GPE

As PUFA levels in alkylacyl-GPC increased in animals fed the GG diet, there was a concomitant decrease in the ratio of SFA to PUFA in alkylacyl-GPC (2.6 vs. 6.8, $P<.05$; Fig. 1). In contrast, in diacyl-GPC fractions, animals fed the GG diet exhibited an increase in the ratio of SFA to MUFA (5.3 vs. 3.6, $P<.001$) and SFA to PUFA (1.5 vs. 1.1, $P<.05$).

Changes in relative amounts of SFA, MUFA and PUFA in the three subclasses of EPG are illustrated (Fig. 2). In the GG diet group, alkenylacyl-GPE and alkylacyl-GPE exhibited significantly lower ratios of SFA to PUFA compared to the controls (0.6 vs. 1.3 for alkenylacyl-GPE, $P<.01$; and 0.5 vs. 1.2 for alkylacyl-GPE, $P<.05$). There was also a decrease in the SFA/MUFA ratio (1.8 vs. 2.5) in the alkylacyl group. Animals fed the SM diet exhibited a lower SFA/PUFA ratio in both EPL subclasses than animals fed the control diet. There was no effect of SM or GG treatments observed on the ratio of SFA/MUFA or SFA/PUFA in the diacyl-GPE class.

4. Discussion

The present study demonstrates that dietary SPL increases the content of EPL in developing rat intestinal mucosa due to two possible mechanisms. First, it is assumed that hexadecanal, a derivative of SPL, is directly utilized for synthesis of EPL as a precursor of ether-linked fatty alcohols [7,8]. Unexpectedly, a 10 times higher SPL dose in the SM diet than that of the GG diet was less effective at enhancing EPL content. This may suggest that different molecular structures of ceramide between GG and SM may have different ceramidase activity which produces sphingosine. The present study did not determine whether increased EPL resulted from GD3, a major GG in the GG diet. It is not known either whether other GGs are involved in EPL accumulation. Further experiments are needed to determine how SM and GGs regulate enzymes related to EPL biosynthesis.

Secondly, it is possible that cholesterol reduction in intestinal cells caused by dietary SPL may increase EPL accumulation or uptake. Earlier studies show that a decrease in cholesterol content increases EPL uptake in human leukemia cell lines [27]. Since experiments in our laboratory have shown a cholesterol reduction in the intestine of animals fed the SPL diet [6], it is logical to assume that dietary SPL may increase EPL uptake by decreasing intestinal cholesterol content. A recent study demonstrates that intestinal (I)-fatty acid binding protein (FABP) and liver (L)-FABP increase EPL content in CPG and EPG [38]. It is suggested that dietary GG may activate this expression of two FABP in the intestine.

Elevated EPL level could alter inflammatory response. In this regard, alkylacylglycerol, an analogue of diacylglycerol, which is derived from EPL by phospholipase C, is known to have a potent inhibitory effect on lipoxygenase [39] and cytosolic phospholipase A_2 ($cPLA_2$) activity [40], both of which stimulate an inflammatory response. Increased EPL content may enhance anti-oxidative effects on lipid peroxidation [25,26] induced by many food-borne toxic or oxidative materials.

Higher levels of PUFA in EPL in animals fed the GG diet may have resulted from a decrease in total cholesterol content in intestinal mucosa, which is known to cause a corresponding increase of Δ-5 and Δ-6 desaturase activities [28]. Increased PUFA in EPL may be due to inhibition of PLA_2 activity since GG inhibits arachidonic acid-specific PLA_2 activity [41] especially plasmalogen-selective PLA_2 [42]. The activity of plasmalogen-selective PLA_2 is significantly inhibited by GGs or *N*-acetyl neuraminic acids derived from GGs [43,44]. In rat tissues, intestinal brush border has a higher activity of calcium-independent cytosolic phospholipase A_2 by 4- to 16-fold than stomach and spleen and by about 50- to 90-fold than lung, liver, brain, kidney and heart [45]. The present result showing increased 20:4n-6 in animals fed the GG diet may have resulted from attenuation of PLA_2 activity [41,42] or activation of I-FABP and L-FABP expression [38] in the intestine. I-FABP and L-FABP preferentially increase 20:4n-6 in CPG and 20:4n-6 and 22:6n-3 in EPG, respectively. The present finding that dietary GGs promoted

a higher level of 20:4n-6, 22:4n-6 and 22:6n-3 in EPG raises the question of whether dietary GG enhances FABP expression during gut development.

Polyunsaturated fatty acid was preferentially incorporated into alkenylacyl- and alkylacyl-GPE compared to both classes of CPG. Animals fed the GG diet showed an increase in total PUFA content in both classes of EPG compared to control animals (Table 5, $P<.01$). This result agrees with previous studies demonstrating more PUFA in EPL in EPG than in CPG [37,46]. The effect of dietary GG on total PUFA content was different between diacyl-GPC and diacyl-GPE. Animals fed dietary GG exhibited a decrease in total PUFA in diacyl-GPC and an increase in diacyl-GPE. This observation suggests that dietary GG induces incorporation of more SFA into CPG, which is mostly localized at the outer membrane, and of PUFA into EPG situated at the inner membrane. Increase in total PUFA content resulted in a decrease in the ratio of total SFA/PUFA in EPL (Figs. 1 and 2).

The present study demonstrates that dietary GG increases total and relative percentage of EPL and the PUFA content of EPL in intestinal mucosa during early development. These results suggest that dietary GG influences gut development and protection by enhancing EPL content, which has a preventative role in carcinogenesis [21,22,47], inflammation [39–41,48] and lipid oxidation [25,26,49]. The rate of metabolic conversion from GG to EPL in the intestine is not known. Future investigations are needed to determine the conversion rate during age-dependent gut development and whether dietary GG affects FABP expression in developing intestine.

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