Original Article

Effects of phytosterols in diacylglycerol as part of diet therapy on hyperlipidemia in children

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Background: The incidence of hyperlipidemia in children is increasing in Japan, but drug therapy for such children is limited. The ingestion of 4% phytosterols-containing diacylglycerol (PS/DAG) decreases serum total cholesterol and low density lipoprotein cholesterol (LDL-C) concentrations in adults. In the present study, we examined the effect of PS/DAG as part of a diet therapy in pediatric patients with hyperlipidemia. Methods: Pediatric patients with hyperlipidemia with ≥ 5.18mmol/L (200 mg/dL) serum total cholesterol and/or ≥1.70mmol/L (150 mg/dL) triglycerides (N = 22) ingested bread containing PS/DAG (total daily intake, 10g) for 6 months. Blood chemistry was examined prior to and 2, 4, 6 months after the initiation of ingestion, and 4 months after the ingestion period. Results: No significant differences in energy intake or cholesterol intake during the study period were found. After 4 months of ingestion of PS/DAG, LDL-C, lipoprotein(a) [Lp(a)], free fatty acids and total ketone bodies decreased significantly. In seven patients with familial hypercholesterolemia, total cholesterol and remnant-like lipoprotein particles (RLP)–cholesterol also significantly decreased in addition to LDL-C and Lp(a). Conclusions: PS/DAG improves serum lipid metabolism in pediatric patients with hyperlipidemia for whom drug therapy is limited, suggesting that PS/DAG may reduce the risk of developing various diseases induced by hyperlipidemia.

Key Words: diacylglycerol, phytosterols, cholesterol, lipoprotein(a), children

Introduction

The relation between pediatric hyperlipidemia and arteriosclerosis has attracted attention for several decades in America, and countermeasures for hyperlipidemia in children have been published by the National Cholesterol Education Program (NCEP) in 1992. The suggested countermeasures, based on low density lipoprotein cholesterol (LDL-C) levels, are education on hyperlipidemia and diet therapy when the LDL-C values are 110-129 mg/dl, and diet therapy when the values are ≥130 mg/dL, with LDL-C < 110 mg/dL as the goal. [1]

In the Bogalusa Heart Study, the formation of arteriosclerotic lesions started in childhood, and was strongly correlated with serum total cholesterol, LDL-C, and triglyceride levels. [2] The causes of hyperlipidemia in children are similar to those in adults, but familial or hereditary factors are more likely in children. Familial hyperlipidemia includes familial hypercholesterolemia (FH), and has a prevalence of 1 in 500 worldwide. LDL-C in patients with heterozygous FH reaches values that are 2- to 3-fold higher than that in healthy individuals, and the risk of ischemic heart disease is high. [3]

Therapies for hyperlipidemia in children are mainly diet therapy and exercise therapy because drug therapy is limited in such subjects. Dietary intervention and positive changes in lifestyle are the main recommended treatments for children with hyperlipidemia. The recommended diet is usually based on restriction of total and saturated fat and dietary cholesterol intake, but is insufficient to lower cholesterol levels into the desired target range. The NCEP recommends drug therapy for children older than 10 years for whom diet therapy fails to reduce high LDL-C levels. [1] However, the use of HMG CoA reductase inhibitors, or statins, is controversial in children, and is related to concerns regarding long-term safety, cost-effectiveness, and efficacy in term of reducing clinical disease or events, although there are now several studies that document the effectiveness of lipid lowering and short-term safety. [4-9]

Many plant oils contain small amounts of phytosterols, which decrease serum cholesterol. Phytosterols inhibit the absorption of cholesterol in the small intestine by competing with cholesterol for the formation of bile acid

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Manuscript received 12 December 2005. Initial review completed 24 February 2006. Revision accepted 12 April 2006.

micelles. [10-13] Since phytosterols are almost insoluble in water and oil, their application has been limited. Thus far, Pelletier et al., showed that 700mg/day of phytosterols mixed with butter fat lowered serum cholesterol in normocholesterolemic subjects. [14]

In addition, Hendriks et al also reported that a spread containing 830 mg /day of phytosterols had a cholesterol lowering effect in normocholesterolemic and mildly hypercholesterolemic subjects. [15] These reports demonstrate the efficacy of phytosterols at low doses.

Recently, Meguro et al. reported that mayonnaise containing 500 mg/day of phytosterols dissolved in diacylglycerol (DAG) lowered serum cholesterol levels. [16]

DAG oil is used for cooking as a substitute for triacylglycerol (TAG) oil. Recent studies have suggested that, after digestion and absorption in the small intestine, DAG oil is not readily re-synthesized to triglycerides in animals, [17,18] and suppresses postprandial increases in serum triglyceride levels [19] and remnant-like lipoprotein particles (RLP) [20] in humans. It has also been found that DAG oil suppresses the accumulation of visceral fat and decreases body weight compared to TAG oil with the same fatty acid composition. [21,22] In the meantime, DAG is a better solvent for phytosterols than TAG. Dissolved phytosterols are more effective in decreasing serum cholesterol levels because they might be more likely to be incorporated in bile acid micelles. [23,24]

In the present study, pediatric patients with hyperlipidemia ingested 4% phytosterols-containing diacylglycerol (PS/DAG), which decreases serum cholesterol levels in adults, and the efficacy and safety of PS/DAG were investigated by examining serum lipid parameters.

Materials and Methods

Subjects

The subjects were 22 female and male children with hyperlipidemia with ≥5.18mmol/L (200 mg/dL) serum total cholesterol and/or ≥1.70mmol/L (150 mg/dL) triglycerides who were outpatients of the Department of Pediatrics, Fussa Hospital (Fussa, Tokyo), Department of Pediatrics, National Saitama Hospital (Wako, Saitama), and Department of Pediatrics, Ohtawara Red-Cross Hospital (Ohtawara, Tochigi).

There were 11 girls and 11 boys aged 6 to 17 years (mean age: 10 years old). Eleven patients had one parent with hyperlipidemia, 7 patients had type IIa hyperlipidemia, and 9 patients were obese children with a Rohrer index of ≥160. [25]

In addition, seven patients were definitively diagnosed with FH. The criteria for FH according to Mabuchi et al. is hypercholesterolemia accompanied by a tendon xanthoma, or someone with first degree kinship hypercholesterolemia accompanied by a tendon xanthoma. [26] In their study, the mothers of five patients had tendon xanthoma, and the mothers of two patients had mutations in the LDL receptor gene. [27]

Preparation of test oil

The DAG oil (1,3-DAG and 1,2-DAG isomers in a ratio of 7:3, with less than 10% total fatty acids comprised of TAG) was prepared by esterifying glycerol with fatty acids from soybean oil and rapeseed oil using Brigitte's method [28]. The glyceride and fatty acid compositions of DAG are shown in Table 1. The composition of the phytosterols used in this study is shown in Table 2. These phytosterols were dissolved at a concentration of 4% in DAG and the resulting product was used as the test oil (PS/DAG).

Table 1. Glycerides and fatty acids of experimental oil

Glycerides	%
Triacylglycerol	17.2
1(or 3), 2-Diacylglycerol	27.1
1,3-Diacylglycerol	55.0
Monoacylglycerol	ND
Fatty acids	%
C16:0	2.5
C18:0	0.7
C18:1	28.0
C18:2	61.0
C18:3	7.5
C20:0	0.1
C20:1	0.1
C22:0	0.1

Table 2. Plant sterols in experimental oil

beta-Sitosterol (g)	1.78
Stigmasterol (g)	1.16
Campestertol (g)	1.01
Brassicasterol(g)	0.1
Total(g)	4.05

Clinical study

The study was performed in accordance with the principles of the Helsinki Declaration. Prior to the study, informed consent was obtained from the subjects and their parents, and the institutional review board approved the study. Although this study was single-armed clinical test, to keep a certain blind for the subjects (children), the test oil was directly provided to the parents who used it daily for cooking. The parents were instructed to record the contents of meals and snacks in diets for three or more consecutive days before the start of the study, and blood sampling and anthropometric measurements were performed in a morning fasting state immediately before the study. The Rohrer index was calculated from body weight and height.

The parents of the subjects prepared bread containing PS/DAG (10 g/day) using a baking machine. The parents were instructed to record a diet diary from 3 days before the test day and to avoid changes in dietary content and amount of exercise. Blood sampling in a fasting state and anthropometric measurements were performed every 2 months for 6 months, and for 4 months after completion of the study. Girth at the navel level (waist) and maximal girth at the gluteal region (hips) were measured.

Measurements

The serum and plasma were analyzed by Mitsubishi Kagaku Bio-Clinical Laboratories Inc., Tokyo, Japan. Serum lipids and glucose were measured using standard enzyme methods, aspartate aminotransferase (AST) and alanine aminotrasferase (ALT) were measured by the UV method, gamma- glutamyl transpeptidase (GTP) was measured by the enzyme activity measurement method, insulin was

Table 3. Characteristic of subjects

No.	Sex	Age (year)	Height (cm)	Weight (kg)	Waist (cm)	Hip (cm)	Rohrer index
1	M	11	146	41.0	65.0	76.0	131
2	M	11	153	65.5	87.6	93.0	181
3	M	7	130	43.5	78.1	77.2	214
4†	M	9	135	42.0	76.5	80.5	171
5†	M	11	145	73.0	96.0	105.0	238
6†	M	13	156	64.0	80.4	87.3	168
7	M	11	121	22.0	50.7	59.0	126
8	M	11	137	39.5	69.7	77.5	154
9	M	12	158	63.5	86.5	93.6	160
10	M	14	167	76.5	94.4	96.0	163
11	M	9	126	24.5	55.2	63.0	123
12†	F	11	145	41.0	67.7	79.5	136
13†	F	9	141	28.5	58.0	67.5	102
14	F	7	115	20.0	47.0	61.1	133
15†	F	8	120	20.8	49.5	59.5	122
16	F	7	124	22.0	59.0	59.0	115
17†	F	5	108	18.0	45.3	55.2	141
18	F	11	143	59.1	93.0	96.5	201
19	F	9	141	33.3	62.0	73.6	119
20	F	7	131	37.7	75.0	78.0	168
21	F	17	141	34.6	60.0	82.0	124
22	F	12	142	33.6	58.0	77.0	117

FH subjects (n=7) are denoted by † marks

Table 4. Changes in anthropometric parameters

	Baseline	2 month	4 month	6 month	+4 month
Height (cm)	138±14.8	138±14.7**	140±14.9**	141±14.7**	143±15.8**
	(100)	(101±0.5)	(102±0.8)*	(103±0.9)**	(104±1.3)**
Weight (kg)	41.1±18.2	41.7±18.1**	43.5±19.2**	44.1±19.2**	47.0±21.0**
	(100)	(102±2.7)**	(106±2.7)**	(108±3.3)**	(113±4.5)**
Waist (cm)	68.8±16.0	68.9±15.4	68.7±15.8	68.7±15.3	71.3±16.3
	(100)	(100±3.4)	(99.8±4.2)	(100±5.4)	(103±5.7)
Hip (cm)	75.3±14.7	75.4±13.8	76.6±15.6*	78.1±15.2**	80.0±15.5**
	(100)	(101±3.8)	(102±3.2)*	(104±3.5)**	(106±3.9)**
Rohrer index	150±34.0	149±32.6	151±33.2	150±33.7	152±35.1
	(100)	(99.7±2.9)	(101±3.8)	(100±3.5)	(99.4±3.6)

Values are mean ±SD (n=22); Significantly different from the initial value: *<0.05, **<0.01; Percentage of the initial value is shown in parentheses.

measured by enzyme immunoassay, [29] plasma plasminogen activator inhibitor-1 (PAI-1) was measured by enzyme immunoassay according to the method of Declerck et al., [30] and leptin was measured by radioimmunoassay. [31] RLP-C was measured using the method of Nakajima et al. [32] Serum sitosterol, campesterol, and lathosterol, were measured by gas liquid chromatography using the method of Gylling et al. [33]

Statistical analysis

The measurement items are presented as the mean ± standard deviation (SD), and the rates of change, designating the initial values as 100% in individual subjects, were calculated as the mean ± SD. Statistical analyses were performed using SAS Version 8.2 (SAS Institute Inc, Cary, NC, USA). In the analysis of the diets, the data were fitted into a linear model using SAS, and analyzed by ANOVA. The measured values before and after the initiation of the study were analyzed using Wilcoxon's test. A P value of less than 0.05 was considered to be significant.

Results

Changes in anthropometric values

Table 4 shows changes in anthropometric parameters. Height and body weight increased with time after the initiation of the study. Hip size also significantly increased after ingestion for 4 months. Waist size and the Rohrer index, however, did not change significantly during the study period.

Diet analysis

Table 5 shows changes in food intake. Changes in food intake during the study period were fitted to a linear model using SAS and analyzed by ANOVA. Lipids and carbohydrates tended to increase for 6 months after the start of ingestion, but there were no significant changes in energy intake or the intake of protein, lipids, carbohydrates, dietary fibers, and cholesterol during the study period.

Changes in serum lipids and total ketone bodies

Table 6 shows changes in serum lipids. There were no

Table 5. Changes in nutrient intake

	Baseline	2 months	4 months	6 months	+4 months
Protein (g)	69.7±12.1	69.3±12.3	69.8±7.0	68.8±11.7	69.6±11.7
Lipid (g)	67.9±18.2	56.7±11.3	65.7±16.2	70.7±22.7	69.1±21.8
Carbohydrate (g)	257±38.0	264±40.4	256±36.3	274±52.7	275±52.6
Fiber (g)	10.5±2.9	12.2±2.6	12.1±2.3	12.4±1.5	11.2±2.6
Cholesterol (mg)	317±108	268±53.3	287±81.0	285±110	284±85.7
Total Energy (MJ)	8.24±1.35	7.91±1.24	8.12±1.14	8.60±1.81	8.61±1.24

Values are mean ±SD (n=22)

significant changes in total cholesterol after the initiation of the study in any of the patients, but a subclass analysis of patients with FH revealed that total cholesterol was significantly lower than the initial value after ingestion for 4 months.

LDL-C was significantly lower than the initial value after ingestion for 4 months in all patients (Figure). A ≥10% decrease was observed in patients with FH. LDL-C was significantly higher 4 months after completion of the ingestion period than at the completion of the ingestion period. In contrast, there was no significant change in HDL-C after the initiation of the study.

RLP-C did not change significantly after the initiation of the study. RLP-C was significantly lower than the initial value after ingestion for 6 months in patients with FH, and was significantly higher 4 months after the completion of the ingestion than at the completion.

Lathosterol reached a peak after ingestion for 2 months, then decreased with time. The initial value and the level after initiation of ingestion tended to be higher in FH patients than in all patients.

Lipoprotein(a) [Lp(a)] was significantly lower two and 4 months after ingestion compared to the initial value for all patients, and the level was still significantly lower 4 months after completion of the ingestion period (Figure). The rate of change was greater in FH patients than in all patients.

There was no significant change in serum triglycerides after the initiation of the study.

Free fatty acids tended to increase for 2 months after ingestion and thereafter decreased, and the value was significantly lower than the initial value after ingestion for 4 months. The value was still significantly lower 4 months after completion of the ingestion period.

Total ketone bodies were lower during the ingestion period than the initial value, and significantly lower after ingestion for 4 months.

Changes in blood glucose, insulin, PAI-1, leptin, and liver function

Table 7 shows changes in blood glucose, insulin, PAI-1, leptin, and liver function (AST, ALT, and gamma-GTP). Blood glucose levels did not change after the initiation of the study in any patient. In FH patients, however, blood glucose levels were significantly lower than the initial value, and significantly lower 2 months after starting the ingestion period.

Insulin and leptin levels did not change during ingestion. PAI-1 were significantly increased 2 months after the ingestion, but were clinically within the standard ranges.

AST was significantly lower after ingestion for 6 months compared to the initial value, and was further significantly decreased 4 months after completion of the ingestion period. ALT was significantly transiently higher 2 months after ingestion compared to the initial value in FH patients, but decreased thereafter. gamma-GTP began to decrease significantly after 4 months of ingestion, and in FH patients, the value was significantly lower compared to the initial value after ingestion for 6 months. The value was significantly increased 4 months after completion of the ingestion period compared to that at the end of the ingestion period.

Changes in serum phytosterols

Table 8 shows changes in serum phytosterols. Sitosterol

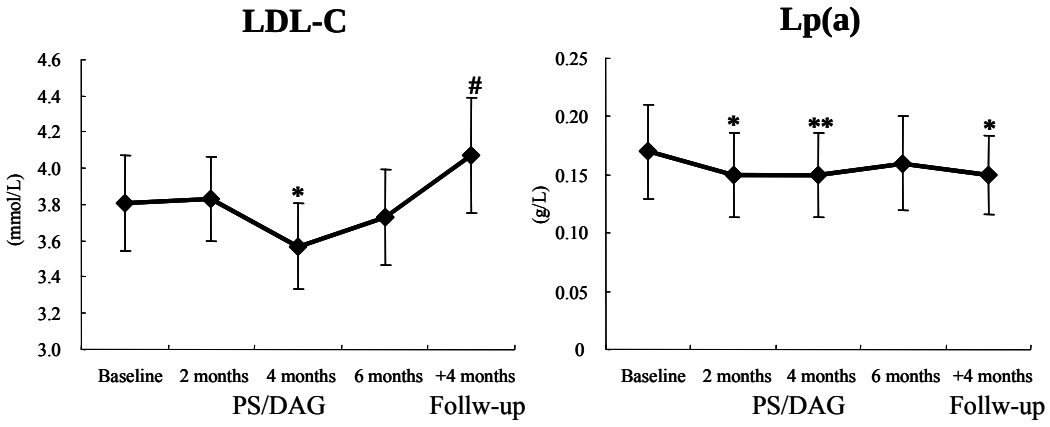

Figure. Changes in serum LDL-cholesterol and Lp(a) during and after ingestion of PS/DAG. Significantly different from the initial value: *p<0.05, *p<0.01; Significantly different from the value after completion of ingestion: #p<0.05

Table 6. Changes in serum concentrations of lipids

	Baseline		2 months		4 months		6 months		+4 months	
	All	FH	All	FH	All	FH	All	FH	All	FH
T-Cholesterol（mmol/L）	5.88±1.01	6.76±1.32	5.93±1.32	6.50±1.55	5.75±0.98	6.35±1.40*	5.70±0.88	6.14±1.27	5.75±1.24	6.81±1.74#
	(100)	(100)	(101±8.3)	(96.0±10.4)	(97.9±9.7)	(93.8±4.2)*	(97.2±8.5)	(91.3±9.4)	(97.5±9.7)	(100±10.6)
LDL-C（mmol/L）	3.81±1.24	4.90±1.50	3.83±1.09	4.64±1.48	3.57±1.11*	4.35±1.42*	3.73±1.24	4.53±1.63	4.07±1.50#	5.28±1.94#
	(100)	（100）	(103±10.6)	（94.6±10.0）	(95.4±14.6)	（88.5±4.9）*	(99.1±15.5)	（91.9±15.6）	(107±15.8)#	（107±12.9）#
HDL-C（mmol/L）	1.58±0.52	1.48±0.21	1.58±0.54	1.37±0.26	1.61±0.49	1.42±0.34	1.61±0.57	1.45±0.31	1.50±0.41	1.48±0.23
	(100)	（100）	(102±14.8)	（91.8±11.1）	(103±14.7)	（95.7±9.4）	(101±11.5)	（96.9±9.3）	(98±15.4)	（100±11.6）
RLP-C(mmol/L)	0.17±0.06	0.16±0.02	0.18±0.10	0.21±0.12	0.16±0.07	0.16±0.04	0.18±0.19	0.12±0.02*	0.18±0.05	0.20±0.06#
	(100)	（100）	(112±40.8)	（126±59.4）	(102±28.9)	（94.2±19.1）	(107±61.5)	（76.9±8.5）*	(117±41.2)	（124±31.1）#
Lathosterol（μmol/L）	8.55±3.11	10.9±11.7	9.84±5.96	14.0±8.55	8.81±4.66	11.7±5.18	6.99±3.11*	8.81±2.85*	7.77±3.11	9.07±3.11*
	（100）	（100）	（116±37.8）	（122±54.4）	（106±34.8）	（105±30.5）	（85.8±27.7）*	（81.9±17.8）*	（98.9±60.8）	（78.6±16.0）*
Lp(a) (g/L)	0.17±0.19	0.11±0.10	0.15±0.17*	0.08±0.07	0.15±0.17**	0.09±0.08*	0.16±0.19	0.10±0.01	0.15±0.16*	0.09±0.08
	(100)	（100）	(93±22.5)*	（84.3±14.5）	(88±18.6)*	（83.4±14.5）*	(92±25.4)	（91.2±22.8）	(93±28.4)	（90.6±27.7）
Triglyceride（mmol/L）	1.27±0.72	0.94±0.29	1.38±0.85	1.56±1.02	1.33±0.87	1.21±0.75	1.29±1.22	0.85±0.31	1.32±0.64	1.18±0.70
	(100)	（100）	（120±62.0）	（157±78.8）	（108±43.2）	（124±51.2）	（101±41.9）	（91.7±21.1）	（121±64.4）	（124±47.8）
Free fatty acids（mmol/L）	0.74±0.18	0.82±0.15	0.74±0.20	0.86±0.24	0.60±0.18**	0.67±0.24	0.60±0.17*	0.57±0.13*	0.60±0.21**	0.56±0.24*
	(100)	（100）	（105±31.2）	（107±33.5）	（83.8±25.0）**	（84.3±34.4）	（85.3±28.1）*	（72.5±22.2）*	（84.1±26.3）*	（67.6±23.9）*
T-Ketone bodies（μmol/L）	86±64	92±55	88±96	77±95	47±34**	66±52	48±30*	53±44	73±93	71±111
	(100)	（100）	（96.2±75.3）	（73.5±45.1）	（72.0±42.6）*	（77.7±52.1）	（84.7±65.9）	（63.4±35.6）	（107±113.6）	（83.8±119.4）

Values are mean ±SD (n=22, FH subjects n=7); Significantly different from the initial value: *<0.05, **<0.01; Significantly different from the value after completion of ingestion: #<0.05; Percentage of the initial value is shown in parentheses.

Table 7. Changes in glucose, insulin, PAI-1, leptin, and liver function

	Baseline		2 months		4 months		6 months		+4 months	
	All	FH	All	FH	All	FH	All	FH	All	FH
Glucose（mmol/L）	5.00±0.33	5.00±0.33	4.77±0.44	4.61±0.56*	5.00±0.44	4.83±0.33	5.05±0.44	5.05±0.39	5.11±0.44	4.83±0.28#
	（100）	（100）	（96.0±10.9）	（91.6±9.1）*	（101±10.0）	（96.9±6.3）	（102±9.6）	（101±7.5）	（103±12.3）	（96.9±4.9）#
Insulin（pmol/L）	70.1±50.4	72.9±58.3	78.5±72.9	93.1±105.6	74.3±42.4	62.5±33.8	72.2±45.1	62.5±50.2	84.0±50.4	85.4±72.9
	（100）	（100）	（114±44.3）	（113±52.4）	（123±59.1）	（94.8±25.4）	（119±53.8）	（91.6±32.9）	（140±73.0）**	（116±31.7）
PAI-1（mg/L）	22±13	20±16	29±20*	36±26*	23±11	24±13	23±15	18±15	23±20	17±13
	（100）	（100）	（157±146.5）**	（229±244.5）*	（121±64.9）	（146±83.5）	（121±89.2）	（92.5±45.2）	（126±180.3）	（91.3±49.1）
Leptin（mg/L）	9.15±6.84	10.1±8.70	9.75±6.94	11.5±8.60	9.80±7.16	11.1±8.30	8.80±6.82	9.40±7.40	8.82±5.79	9.70±6.10
	（100）	（100）	（111±33.7）	（114±37.8）	（113±30.0）	（112±30.6）	（105±40.4）	（95.1±26.7）	（103±27.1）	（108±33.7）
AST(IU/L)	27±11	30±18	27±10	33±15	27±10	32±15	25±10*	29±15	25±11**	28±15
	（100）	（100）	（102±16.0）	（114±16.5）	（99.9±15.9）	（109±16.3）	（94.1±18.9）	（98.2±12.6）	（92.7±9.9）**	（94.3±8.9）
ALT(IU/L)	21±14	19±14	21±12	24±14*	20±11	21±15	18±12	19±16	19±16	19±19
	（100）	（100）	（109±32.5）	（136±41.5）*	（103±28.7）	（114±36.4）	（94.4±30.8）	（94.7±11.2）	（92.1±22.8）	（92.3±17.8）
gamma-GTP(IU/L)	28±42	47±73	26±31	41±52	25±33*	40±58	22±27**	34±46*	25±33#	39±57#
	（100）	（100）	（104±24.8）	（104±18.7）	（94.5±17.5）	（90.7±12.2）	（89.4±19.2）*	（81.6±8.5）*	（96.5±21.2）#	（94.3±11.7）#

Values are mean ±SD (n=22, FH subjects n=7); Significantly different from the initial value: *<0.05, **<0.01; Significantly different from the value after completion of ingestion: #<0.05; Percentage of the initial value is shown in parentheses.

Table 8. Changes in serum concentrations of plant sterols

	Baseline		2 months		4 months		6 months		+4 months	
	All	FH	All	FH	All	FH	All	FH	All	FH
Sitosterol（μmol/L）	8.92±4.34	9.64±3.62	9.16±4.10	9.64±3.13	9.88±4.34	11.3±4.34	8.92±4.10	9.16±3.13	7.71±3.86	9.16±3.86
	（100）	（100）	（111±37.0）	（101±12.7）	（124±62.0）	（120±29.2）	（112±58.0）	（96.4±16.6）	（96.7±43.8）	（99.2±17.6）
Campesterol（μmol/L）	17.8±8.00	20.8±7.75	19.8±7.25*	21.8±6.50	21.3±9.00**	25.0±9.75	18.8±7.50	21.3±6.50	16.0±9.00	17.3±6.25*
	（100）	（100）	（119±30.0）*	（107±16.5）	（129±45.5）**	（121±25.8）	（114±41.0）	（105±24.6）	（95.8±38.0）	（86.6±12.1）*

Values are mean ±SD (n=22, FH subjects n=7); Significantly different from the initial value: *<0.05, **<0.01; Percentage of the initial value is shown in parentheses.

and campesterol were significantly increased for 4 months after ingestion compared to the initial values, but decreased thereafter. In FH patients, the peaks were higher than those in all patients, but the rates of decrease thereafter were markedly greater in FH patients. Changes in serum phytosterols were clinically within the standard ranges in the present study.

Discussion

The efficacy of PS/DAG as part of a diet therapy was investigated in children with hyperlipidemia for whom drug therapy is limited. Meguro et al. confirmed the efficacy of PS/DAG in adults [16], and in the present study, a similar efficacy in children was found without any effects on growth. Phytosterols are more soluble in DAG than in TAG due to the structural characteristics of DAG. Meguro et al. investigated the effects of phytosterols dissolved in DAG and phytosterols dispersed in TAG on serum cholesterol concentrations, and reported that total cholesterol and LDL-C were significantly lower in the DAG group than in the TAG group [16].

In the present study, LDL-C was decreased in children, similar to adults. In addition, Lp(a) was significantly decreased, which was not observed in adults. Lp(a) is hereditarily controlled in many cases, and is less likely to be affected by food and drugs. Although a drug containing nicotinic acid, niceritrol, has been reported to decrease Lp(a), its mechanism of action is considered to be a decrease in Lp(a) synthesis in the liver, rather than the promotion of catabolism. [34] Teramoto et al. recently reported that 3-month ingestion of DAG reduced the mount of abdominal fat and Lp(a) in free-living hemodialysis patients. [35] There has been no report of a decreasing in Lp(a) induced by phytosterols, and so the reduction of Lp(a) Tients. [35] There has been no report of a decreasing in Lp(a) induced by phytosterols, and so the reduction of Lp(a) observed in the present study might be occurred by DAG. However, further studies will be required to clarify the mechanism of the reduction. In the general population, high Lp(a) levels as well as LDL-C have been associated with an increased incidence of atherosclerosis cardiovascular risk. [36] Although not demonstrated, it is assumed that the reduction in these parameters is important to reduce cardiovascular risk.

A separate analysis of FH patients was performed. Because LDL-C is congenitally very high in FH patients, atheroma formation occurs at an early stage. [37] Thus, serum cholesterol concentrations should be controlled beginning in early childhood, but it is difficult to restrict the diet of growing children. Becker et al. administered phytosterols to children with FH, and reported that sitosterol and sitostanol markedly decreased serum cholesterol levels. [38] Gylling et al. esterified sitostanol for application in margarine. [13] The dissolution of phytosterols in DAG, not by esterification, permits their application to various food products, and an amount of phytosterols smaller than that previously reported was found to be effective in FH patients.

The cholesterol-lowering effect was higher in FH patients than for all patients. The characteristics of FH patients in this study were a high initial value of a typical cholesterol precursor, lathosterol, and the levels of serum sitosterol and campesterol were slightly higher than those in all patients. Naoumova et al. reported that cholesterol synthesis ability is higher in FH patients than in non-FH patients. [39] Moreover, Gylling et al. reported that the LDL-C-lowering effect of sitostanol is greater in FH patients with a high lathosterol concentration. [13] Tilvis et al. noted that because the serum campesterol concentration was significantly positively correlated with the amount of cholesterol absorption, the effect of phytosterols was higher in patients with a high concentration of campesterol. [40] The greater reduction of cholesterol in FH patients observed in the present study is consistent with these reports. The present study demonstrated the effectiveness of PS/DAG on lipid metabolism in children with hyperlipidemia even though the small-sized trial. Further large scale studies with randomised double blind manner would be conducted for the precise evaluation.

In the DELTA-1 study, Ginsberg et al reported that the Step 1 diet, advocated by the American Heart Association, the NCEP, and the American Diabetes Association, gradually increased Lp(a) levels, although it reduced total and LDL cholesterol levels. [41] PS/DAG decreased serum cholesterol by phytosterols and Lp(a) by DAG without strict dietary restrictions in children with hyperlipidemia, for whom drug therapy is limited, indicating that PS/DAG as part of diet therapy may reduce the risk of developing various diseases induced by hyperlipidemia.

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Original Article

Effects of phytosterols in diacylglycerol as part of diet therapy on hyperlipidemia in children

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以雙醯甘油中的植物固醇做為飲食治療的一部份對高脂血症兒童的影響

背景：日本兒童高脂血症的發生率逐漸上升，但是藥物治療對於這些兒童是有限的。成年人攝取含有 4%植物固醇的雙醯甘油(PS/DAG)可降低血清總膽固醇及低密度脂蛋白膽固醇(LDL-C)濃度。在本研究，我們評估以 PS/DAG 當做飲食治療的一部份，其對患有高血脂症的小兒科病人的影響。方法：小兒科病人其血清膽固醇≥ 5.18mmol/L (200 mg/dL)或是三酸甘油酯≥1.70mmol/L (150 mg/dL)的高脂血症患者，連續攝取含有 PS/DAG(每天總攝取量 10 公克)的麵包六個月。評估受試者在開始攝取之前及攝取後的 2、4、6 個月和攝取週期之後的 4 個月之血液生化值。結果:熱量攝取或膽固醇攝取在研究週期間都沒有顯著差異。攝取 PS/DAG 4 個月後，LDL-C、lipoprotein(a) [Lp(a)]、游離脂肪酸及總酮體量顯著降低。七名家族性高膽固醇血症病人，除了 LDL-C 及 Lp(a)外，總膽固醇及脂蛋白粒子剩餘體-膽固醇也顯著降低。結論：PS/DAG 可改善藥物治療受到限制的高脂血症小兒科病人的血清脂質代謝，本研究建議 PS/DAG 可能可以降低各種受到高脂血症誘導而發展的各種疾病危險性。

關鍵字:雙醯甘油、植物固醇、膽固醇、脂蛋白(a)、兒童。