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Relationship among Nutritional Intake, Duration of Outdoor Activities, Vitamin D Status and Bone Health in High School Girls*

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In this study, we examined the effects of dietary 1,3-diacylglycerol (DG) compared to conventional triacylglycerol (TG) oil on the postprandial response of total and chylomicron TG, glucose, insulin, and free fatty acid (FFA). This study was conducted using a cross-over design. Ninety subjects participated in the high-fat meal tolerance test where they were randomly assigned to consume two experimental sandwiches containing mayonnaise with TG or DG oil with a seven-day interval. Blood samples were collected before ingestion and at 2, 3, 4 and 6 hr time point after ingestion and analyzed for total and chylomicron TG, glucose, insulin, FFA and phospholipid fatty acid composition. Both TG and DG ingestion had similar effects on postprandial TG response, but a different response from chylomicron TG. Compared with the TG group, TG levels were significantly lower only at 6 hr time point in the DG group. On the other hand, chylomicron TG rose steeply at 2 hr time point and decreased faster in this group. Also, the adjusted value to fasting levels was the same as the unadjusted level. Fasting levels and net differences in insulin were significantly lower at 3 hr time point where chylomicron TG levels were significantly lower in the DG group. But those of glucose and FFA in the TG and DG groups did not differ significantly. Fasting and postprandial levels of fatty acid composition in serum phospholipids in the two groups did not differ significantly. In conclusion, this study indicated that one could reduce the magnitude of postprandial lipemia without influencing glucose metabolism by consuming DG oil as a substitute for TG oil. Based on the correlation of coronary artery disease and postprandial lipemia, dietary DG ingestion might have a beneficial effect in treating such a disease. Further studies are required to clarify the long-term effects of dietary DG on blood lipid levels in humans.

Key words : 1,3-Diacylglycerol, Postprandial lipemia, Total TG, Chlyomicon TG, High fat meal

INTRODUCTION

Most of us eat regular meals and spend our lives in the postprandial state.[1,2] Recent studies have shown that repeated episodes of exaggerated or perturbed alimentary lipemia are linked to multiple disturbances of lipoprotein metabolism related to premature coronary atherosclerosis.[3] These include abnormal cholesteryl ester enrichment in VLDL, abnormal LDL composition reflected by a predominance of small dense LDL or triglyceride accumulation in the dense LDL fraction, and an increased propensity of LDL to undergo oxidative modification. There is a possibility that we can reduce the risk of coronary artery disease and atherosclerosis by reducing the plasma triglyceride (TG) level during the postprandial phase.

Numerous studies on factors that influence postprandial lipemia have been performed in healthy subjects[2,4-6] and patients suffering from various diseases.[7-9] Dietary fiber,[10,11] soybean protein[12] and exercise[13,14] have been demonstrated to affect the magnitude of postprandial lipemia. Several recent studies have also examined the acute effects of dietary fatty acids on postprandial lipemia.[15-18]

Diacylglycerol (DG) oil has been commercially produced by removing a free fatty acid of triacylglycerol through the action of lipase. Specifically, 1,3-DG possessing fatty acid moeities only in the 1 and 3 position on the glycerol backbone has characteristics similar to those of TG oil (kcal per gram, taste, color

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etc), which has been newly recognized as a good substitute for conventional edible oil in a sensible daily diet and as a new tool for maintaining a healthy weight.[19] Nagao *et al.*[20] showed that DG suppresses the accumulation of fat in the visceral adipose and subcutaneous tissue and reduces fat in the liver, as compared to TG of the same fatty acid composition. Therefore, dietetic treatment using DG as a cooking oil would prevent diseases associated with lipid metabolism and might be more effective in treating such diseases than conventional dietetic treatment using TG.

The objective of this study was to examine the effects of dietary DG on the postprandial response of total and chylomicron TG, glucose, insulin and FFA in healthy human subjects.

SUBJECTS and METHODS

1. Subject Information

Study subjects were recruited from volunteers who responded to an advertisement for a nutrition study conducted by the Clinical Nutrition Research Team at Yonsei University in 2003. The subject selection criteria demanded that participants have a BMI greater than 25 kg/m^2 and fall within the normal range on the glucose tolerance test and electrocardiograms. Excluded was anyone who had experienced weight changes within the past 6 months, was taking medication, or had any type of disease such as diabetes mellitus (DM), cardiovascular disease (CVD) including coronary heart disease, stroke, peripheral vascular disease or cancer. Ninety people (male: 62, female: 28) were selected as study participants. Written informed consent was obtained from all of the study subjects and the protocol was approved by the Ethical Committee of Yonsei University. Subjects were asked to refrain from doing strenuous exercise or drinking alcoholic beverages 24 hours prior to the meal tolerance test. They were also instructed to avoid eating or drinking anything except water during the experimental period.

2. Study Design and Meal Tolerance Test

The ninety test subjects took the high-fat meal tolerance test under a randomized cross-over design. On the first day of the experiment, participants were randomly assigned to consume one of two types of sandwich, containing mayonnaise with TG or DG oil. After a seven-day interval, they consumed the kind of sandwich that was not eaten on the first day. On both days, the 6-hr postprandial lipemia response test was conducted starting at 8:30 AM after an overnight fast of more than 12 hours. Experimental sandwitches for the high-fat meal tolerance test were prepared in the metabolic ward. They consisted of white bread, lettuce, ham and mayonnaise (soybean based TG or DG oil).

TG and DG oil composition and contents are presented in Table 1. DG oil was composed of 1,3-DG (58.0%, wt/wt), 1,2-DG (26.0%, wt/wt), TG (16.0%, wt/wt) and TG oil composed of TG (100.0%, wt/wt). The FA content of the DG oil (285.29 wt%) was slightly higher than that of the TG oil (248.86 wt%). The energy content, calculated from the Korean food code, a computerized database based on food composition tables from the National Rural Living Science Institute (6th ed., 2000) in Korea, was 544.5 kcal. Fat represented 50% (29.9 g) of the total calories, carbohydrates made up for 37% (51.5 g) and 13% (17.3 g) was derived from protein. In contrast, the macronutrient composition of the subjects' usual diet derived about 57% of its food energy from carbohydrates, 22% from fat, 16% from protein and 5% from others. The subjects had similar habitual dietary fat intake, physical activity and socioeconomic status.

Table 1. The composition and the contents of TG and DG oil in mayonnaise

	TG oil	DG oil
Acylglycerol Species (wt%)		
Triglyceride	100	16
Diglyceride		84
(1,3-Diglyceride)		(58)
(1,2-Diglyceride)		(26)
Fatty Acid Composition (wt%)		
Palmitic (16:0)	10.75	9.40
Stearic (18:0)	4.39	5.31
Oleic (18:1)	22.00	24.83
Linoleic (18:2)	55.47	57.04
Linolenic (18:3)	7.39	3.42
Saturated Fatty Acid	15.14	14.71
Unsaturated Fatty Acid	84.86	85.29
Monounsaturated	22.00	24.83
Polyunsaturated	62.86	60.46

3. Anthropometric Parameters and Blood Pressure

Body weight and height were measured in the morning, with the subjects in light clothing and shoeless. Body mass index (BMI) was calculated as body weight in kilograms divided by height in meters squared. Waist and hip circumference were combined into the waist to hip ratio (WHR) as an indication of the index of body fat distribution. Blood pressure was read from the left arm while the subjects remained seated. An average of three measurements were recorded for each subject.

4. Blood Collection

Venous blood samples were obtained from the forearm

and collected into plain or EDTA-treated tubes at 0 (baseline) and at 2, 3, 4 and 6 hr after eating the experimental sandwiches. Serum glucose, insulin, FFA and total TG and plasma chylomicron TG were analyzed. Tubes were immediately placed on ice until they arrived at the analytical laboratory (within 1~3 hrs) and stored at -70 ℃ (for glucose, insulin, FFA and total TG) and at 4 ℃ (for chylomicron TG) until analysis.

5. Serum Lipid Profile Measurement

Fasting serum concentrations of total cholesterol and TG were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd. Tokyo, Japan). After precipitation of serum chylomicron, low-density lipoprotein (LDL) and VLDL with dextran sulfate-magnesium, high-density lipoprotein (HDL) cholesterol left in the supernatant was measured using an enzymatic method. LDL cholesterol was estimated indirectly using the Friedewald formula for subjects with serum TG concentrations < 4.52 mol/l (400 mg/mL). Postprandial TG concentration was also measured using the same methods that were used to measure serum TG.

6. Glucose, Insulin and FFA Assessment

Blood glucose was measured based on a glucose oxidase method using the Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA). Insulin was measured by radioimmuno-assay using commercial kits from the Immuno Nucleo Corporation (Stillwater, MN). FFA was analyzed using a Hitachi 7150 autoanalyzer (Hitachi Ltd, Tokyo Japan). Postprandial responses of glucose, insulin and FFA to the fat challenge were calculated using the trapezoidal method as area under curve (AUC).

7. Chylomicron TG Aassessment

To collect chylomicron TG, 1.006 g/mL density solution was added to plasma. Centrifugation was carried out in a Beckman 50.4 Ti rotor at 14,000 rpm, 4 ℃ for 30 minutes using a Beckman LE8 ultracentrifuge. 1 mL of the chylomicron was carefully removed from the top of each tube using a drawn out glass pipette. After separating the chylomicron from the plasma, chylomicron TG was measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd. Tokyo, Japan).

8. Serum Phospholipid Fatty Acid Composition

Serum phospholipid FA composition was analyzed using the modified method of Folch,[21] and gas chromatography (Hewlett Packard 6890, Wilmington, DE, USA). The temperature of the injection and detector ports were set at 280 ℃/min and retention time was 40 minutes. A flame ionization detector (FID) was used and helium was the carrier gas at 0.7 mL/min. Peaks were identified by comparison to a known standard mixture (Supelco, Bellefonte, PA, USA). Individual fatty acids were calculated as a relative percentage with the elevated fatty acids set at 100% with Chemstation software.

9. Statistical Analysis

Statistical analysis was performed with Win SPSS ver 10.0 (Statistical Package for the Social Science, SPSS Ins., Chicago, IL, U.S.A.). An independent t-test was used for differences between the TG and DG groups. Frequency distributions were tested using the chi-square test.

Each variable was examined for normal distribution and significantly skewed variables were log-transformed and then tested. For descriptive purposes, mean values were presented on untransformed and unadjusted variables. Results were expressed as mean±SE. A two-tailed value of $p<0.05$ was considered statistically significant and $p<0.1$ was considered to show tendency.

RESULTS

1. General Characteristics of the Subjects

The general characteristics and lipid profile of the 90 participants are shown in Table 2. The mean age and BMI of the subjects were 29±0.58 yrs and 22.2±0.29 kg/m^2, respectively. Mean values of blood pressure and lipid profile were in the normal range.

Table 2. General characteristics and lipid profile of the subjects (n=90)

Age (yrs)	29.1±0.59
Men/Woman (n)	62/28
Body weight (kg)	63.9±1.19
Height (cm)	169.4±0.78
Body mass index (kg/m^2)	22.2±0.29
PIBW (%)	102.1±1.28
Waist (cm)	79.3±0.89
Waist/hip ratio	0.83±0.01
Body fat (%)	22.0±0.61
Lean Body Mass (kg)	49.3±0.94
Blood pressure	
Systolic BP (mmHg)	118.4±1.37
Diastolic BP (mmHg)	71.2±1.12
Triglyceride (mg/dl)	107.7±7.84
Total cholesterol (mg/dl)	182.4±4.15
LDL-cholesterol (mg/dl)	105.1±3.32
HDL-cholesterol (mg/dl)	53.1±1.57
Tobacco consumption (cigarettes/d)	8.15±1.36
Alcohol intake (g/d)	20.7±3.81

Values are mean±S.E

2. Postprandial Responses of Serum Total Triglyceride and Plasma Chylomicron TG to a High TG or DG Meal

Fig. 1 shows postprandial response and net differences in serum total TG and chylomicron TG to the high TG or DG meals. Both groups showed similar responses in terms of postprandial TG levels. Serum total TG levels peaked at 2 hr time point and steadily declined until 6 hr time point after the meal ingestion, at which time both the TG and DG groups had nearly returned to the initial values. There was no difference between the two groups in terms of TG levels except at 6 hr time point, at which time serum TG concentrations in the DG group were significantly lower than those in the TG group.

Chylomicron TG levels also peaked at 2 hr time point and steadily declined until 6 hr time point in both the TG and DG groups. However, the patterns of response in the two groups differed significantly. In adjusted values to fasting levels of total and chylomicron TG, both groups showed a similar response to the unadjusted ones.

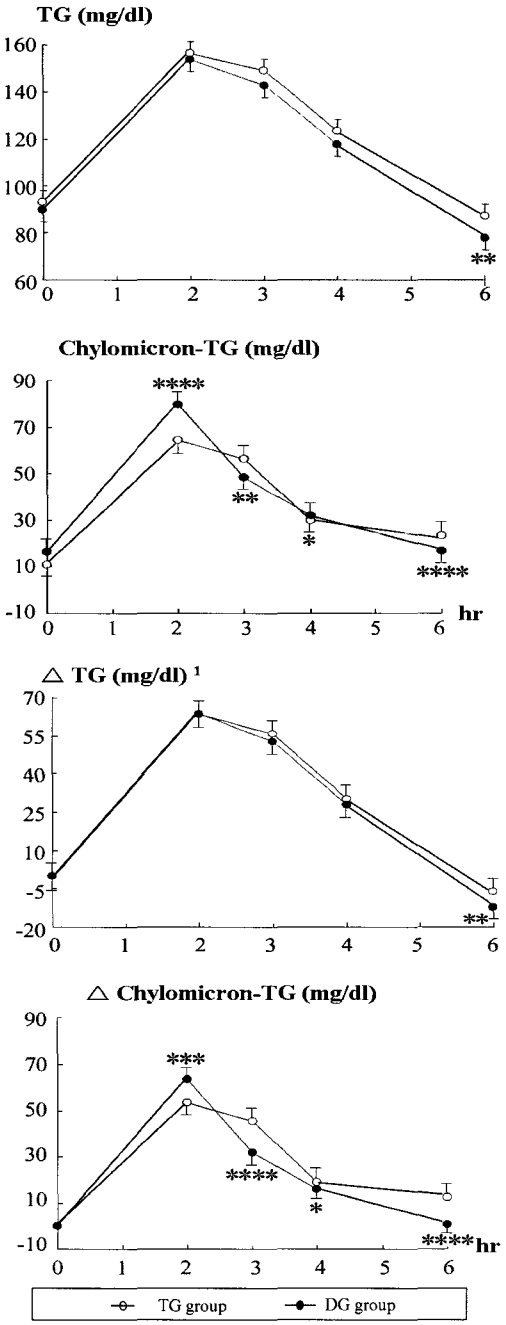

Fig. 1 Total serum TG and chylomicron TG levels during meal tolerance test in healthy subjects

Mean±S.E.
*P<0.1, **P<0.05, ***P<0.01, ****P<0.001 compared with TG group.
[1]△: net difference to fasting level

3. Postprandial Response of Serum Glucose, Insulin, Free Fatty Acid, and Fatty Acid Composition of Phospholipids to a High TG or DG Meal

Table 3 shows the postprandial responses of glucose, insulin and FFA to a high-fat meal. Serum glucose and insulin peaked at 2 hr time point and returned almost to the baseline. On the other hand, FFA levels declined until 2 hr time point as a result of the fasting then gradually increased by the 6 hr time point to almost two times the initial concentrations. The increased level at 6 hr time point might be due to the extended time interval between breakfast and lunch. There were no significant

Table 3. Postprandial glucose, insulin, FFA levels to a high TG or DG meal

		TG group (n=90)	DG group (n=90)
Glucose (mg/dl)	0 hr	85.7±0.97	86.7±2.23
	2 hr	88.8±1.25	89.3±2.30
	3 hr	84.6±0.77	83.2±0.81*
	4 hr	81.3±0.71	81.4±0.72
	6 hr	81.1±0.68	81.2±0.75
Insulin (μU/mL)	0 hr	7.27±0.49	7.32±0.46
	2 hr	16.4±1.21	16.6±1.23
	3 hr	11.9±1.15	8.23±0.87**
	4 hr	5.88±0.40	5.85±0.41
	6 hr	5.31±0.37	5.02±0.34
Free fatty acid (μEq/L)	0 hr	437.8±23.6	423.2±23.5
	2 hr	296.7±15.5	315.7±16.3
	3 hr	426.5±19.2	439.7±18.1
	4 hr	522.0±23.1	536.3±21.5
	6 hr	745.9±25.7	782.4±34.9
Response area			
Glucose (mg/dl×hr)		506.6±4.39	507.3±6.69
Insulin (μU/mL×hr)		61.8±4.09	57.9±3.77
Free fatty acid (μEq/L×hr)		2838±97	2923±107

Values are Mean±S.E.
*p<0.1, **p<0.001 compared with TG group

differences between the two groups in terms of these values, except for the tendency to show lower glucose and insulin levels at 3 hr time point in the DG group. Net differences of glucose, insulin and FFA to fasting levels were found to be similar to the unadjusted values (Data are not shown).

Fasting and postprandial fatty acid composition in serum phospholipids between the two groups did not differ significantly (Table 4).

Table 4. Postprandial responses of fatty acid composition in serum phospholipids to a high TG or DG meal

		TG group (n=90)	DG group (n=90)
Palmitic acid (%)	0 hr	30.5±0.81	29.5±0.97
	2 hr	29.3±0.71	29.3±1.06
	3 hr	29.9±0.82	29.2±0.67
	4 hr	29.8±0.65	29.0±0.87
	6 hr	30.1±0.78	29.9±0.82
Stearic acid (%)	0 hr	15.2±0.87	14.8±0.88
	2 hr	14.1±0.68	15.5±0.69
	3 hr	14.4±0.76	14.8±0.70
	4 hr	15.1±0.83	15.8±0.77
	6 hr	14.9±1.39	15.2±0.71
Oleic acid (%)	0 hr	11.0±0.68	11.0±0.57
	2 hr	11.1±0.58	11.7±0.64
	3 hr	11.7±0.66	11.5±0.55
	4 hr	10.6±0.57	11.8±0.70
	6 hr	14.5±0.65	11.2±0.55
Linoleic acid (%)	0 hr	17.8±0.83	17.7±0.79
	2 hr	19.0±0.77	19.1±0.87
	3 hr	19.3±0.96	20.4±0.68
	4 hr	19.1±0.72	19.5±0.67
	6 hr	18.5±1.15	19.7±0.68
Linolenic acid (%)	0 hr	0.53±0.07	0.43±0.06
	2 hr	0.49±0.04	0.48±0.11
	3 hr	0.52±0.07	0.50±0.11
	4 hr	0.70±0.11	0.41±0.05
	6 hr	0.49±0.05	0.52±0.13
Response area			
Palmitic acid (%×hr)		176.0±3.98	179.1±3.45
Stearic acid (%×hr)		91.8±3.97	88.3±4.32
Oleic acid (%×hr)		69.1±3.40	66.4±3.37
Linoleic acid (%×hr)		115.8±3.70	112.7±4.11
Linolenic acid (%×hr)		2.77±0.32	3.40±0.28

Values are Mean±S.E.
There were no significant differences between two groups.

DISCUSSION

In this study, we examined the effects of functional oil DG compared with commonly used TG oil on the postprandial responses of total and chylomicron TG, glucose, insulin and FFA. It was found that DG ingestion might have a more beneficial effect on clearance of TG or chylomicron TG without influencing glucose metabolism.

Both TG and DG ingestion showed a similar response to that of postprandial serum TG, but showed a different response in chylomicron TG. Serum TG levels were significantly lower only at 6 hr time point in the DG group compared with those of the TG group. On the other hand, chylomicron TG rose more steeply until 2 hr time point and decreased more quickly in the DG group. Also, the pattern of adjusted values to fasting levels were the same as the unadjusted levels. This outcome is consistent with the results reported by Murata M *et al.*[22,23] that the release of TG to the lymphatic system and the lymphatic transport of chylomicron TG in rats declined significantly after the loading of DG emulsion compared with what occurred after loading TG of the same fatty acid composition.

TG is thought to metabolize into free fatty acids (FFA) and 2-mono-acylglycerol (2-MG) through digestion before being absorbed into the epithelial cells of the small intestine.[24] After being absorbed, FFA and 2-MG are re-formed into 1,2-DG and then regenerated into TG or so-called chylomicron TG. Ingested 1,2-DG, like TG, metabolizes into 1-MG and FFA through digestion and is thought to go through the same process. 1,3-DG also metabolizes into 1-MG and FFA through digestion but experiences difficulty in re-forming into 1,2-DG or 2,3-DG because the affinities of 1-MG and 3-MG to monoacylglycerolacyltransferase in the epithelial cells of the small intestine are lower than that of 2-MG.[24] Related to this mechanism, our results indicated that ingestion of 1,3-DG instead of TG might be more effective in terms of clearance of total and chylmicron TG and much more effective in terms of clearance of chylomicron TG than total TG in the postprandial state.

Chylomicron remnants are known to be formed by the action of lipoprotein lipase (LPL) on the vascular wall.[25] In addition, the activation of LPL is controlled by the concentration of insulin. In this study, chylomiron TG levels at 3 hr time point fell sharply in the DG group, which might be a result of sharply decreased insulin levels at that time. In terms of fasting levels and net differences in glucose and FFA, there were no significant differences between the TG and DG groups. This corresponds with the findings of Taguchi H *et al.*[19] and Nagao T *et al.*.[20]

In conclusion, this study indicated that one can reduce the magnitude of postprandial lipemia without influencing glucose metabolism by consuming DG oil in place of TG oil. Based on the correlation of coronary artery disease and postprandial lipemia, dietary DG ingestion

might have a beneficial effect on treating such diseases. Further studies are required to clarify the long-term effects of dietary DG on lipid metabolism in humans.

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