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ORIGINAL ARTICLE

Dietary effects of diacylglycerol rich mustard oil on lipid profile of normocholesterolemic and hypercholesterolemic rats

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Abstract Several recent studies have established that diacylglycerol (DAG) rich oils significantly reduce the body weight. The present study was conducted to evaluate the dietary effects of DAG- rich mustard oil on normal and hypercholesterolemic rats. DAG- rich mustard oil (45.5% DAG) was prepared in the laboratory by enzymatic glycerolysis process. For the feeding experiment, 32 rats were taken and divided into four groups (average body weight 130 g) and body weight gain, food efficiency ratio, lipid profile of plasma, liver, mesentery and erythrocytes membrane (EM), HMG Co-A reductase activity and plasma leptin content were measured and compared with the normal TAG-rich diet. The dietary DAG rich mustard oil significantly decreased body weight and FER compared to TAG rich mustard oil both in normal and hypercholesterolemic rats. The total cholesterol content was decreased with significant increase in HDL- cholesterol by feeding DAG rich diet. Total lipid and TAG content of both liver and mesentery were significantly decreased in DAG diet group compared to control group. Liver HMG CoA: mevalonate ratio was also found to be significantly decreased in the DAG group. Blood leptin level significantly reduced with DAG rich diet compared to the TAG rich dietary groups.

Keywords Diacylglycerol · Lipid profile · Triacylglycerol · Cholesterol · Leptin · HMG-CoA reductase

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Introduction

Numerous scientific reports have shown the effectiveness of diacylglycerol (DAG) oil in preventing body fat accumulation and obesity related disorders. (Nagao et al. 2000; Yamamoto et al. 2001; Hibi et al. 2011). At the current growth rate of obese population throughout the world, the necessity for oils with health beneficial effects will be high and it can be expected that the global market demand for DAG oil will increase in the future. The long-term ingestion of dietary DAG composed mainly of 1, 3-DAG decreased both body weight and visceral fat mass in humans, in comparison with ingestion of TAG (Rudkowska et al. 2005). Moreover, several studies with humans have demonstrated that DAG ingestion reduced postprandial hypertriglyceridemia compared with TAG ingestion (Saito et al. 2010) It has been found that energy values of the TAG oil and DAG oil, when measured in bomb calorimeter, were almost similar and being having more or less equal digestibility also, the reduced fat accumulation by dietary DAG is only caused by differential metabolic fates of both the lipids after absorption in the GI tract (Hibi et al. 2009; Matsuzawa et al. 1995; Pi-Sunyer 1991). A vast population of East Asia use mustard oil as the primary cooking oil. Mustard oil *(Brassica juncea)* contains 8–9% of saturated fatty acid and 88–91% of unsaturated fatty acid in which

48–50% is erucic acid (C22:1). According to various studies, erucic acid takes time to digest in human system which leads to less deposition of lipid in different organs (Kannel et al. 1991). More even mustard oil contains two essential fatty acids (EFA) in appreciable amount and natural antioxidant, tocopherol, in significant amount. Therefore it could be a potential raw material to produce a low calorie, healthful edible oil. In the present study mustard oil (*Brassica juncea)* was converted into DAG rich mustard oil by enzymatic glycerolysis (Dhara and Ghosh 2009). The dietary effects of DAG rich mustard oil in comparison with TAG rich mustard oil on the tissue lipid profile of normal and hypercholesterolemic rats was studied.

Materials and methods

Materials

Mustard oil was extracted from brown mustard seeds at laboratory by solvent extraction process and bleached with bleaching earth and activated carbon and finally physically refined to remove free fatty acid (FFA) and allyl isothiocyanate so that enzymatic esterification can be done properly (John 1976). The 1, 3 specific immobilized lipase *Thermomyces lanuginosus* (TLIM) was a gift of M/s Novozymes India Ltd., Bangalore, India. According to the literature supplied by the manufacturer it is produced by submerged fermentation of genetically modified *Aspergillus oryzae* and possesses activity of 250 IU/g. The product complies with the recommendation purity specifications for food-grade enzymes given by the Joint FAO/WHO. According to literature this liapse is suitable for hydrolysis and esterification (Fernandes et al. 2004). Glycerol and all other reagents used were of analytical grade and were of procured from Merck India Ltd. Mumbai, India. All the enzyme kits used for the measurement of various blood parameters were procured from Merck India Ltd., Mumbai, India.

Methods

Glycerolysis reaction

Physically refined mustard oil and glycerol were taken in a molar ratio of 2:1 in round bottom flask and 10% (w/w) enzymes (on the basis of total substrate weight) was added to it. The reaction was carried out at 60 °C with constant stirring of 200 rpm under vacuum for 26 h (Dhara and Ghosh 2009) for optimum DAG production. Final product was filtered to remove enzyme, excess glycerol was separated out by gravity separation and water washing. The product that is DAG rich mustard oil was then vacuum dried, checked for FFA content and stored at refrigerator for feeding experiments.

Estimation of amount of DAG and MAG present in dietary oils

HPTLC analysis was done to estimate MAG and DAG (% w/w) present in the dietary oils (Macala et al. 1983). The extracted lipids were separated by TLC using a Silica gel 60-precoated high-performance TLC (HPTLC) plate (Merck, Germany) and hexane/diethyl ether/acetic acid (80:20:1, by vol) as the development solvent. For quantification of DAG, HPTLC plates were sprayed with 40% sulfuric acid, immediately heated to 180 °C to visualize the lipids, and used for densitometry. The integrated optical density (IOD) of the lipids was measured using a WINCATS-3 software program, CAMAG- HPTLC Scanner 3″ (Scanner 3_ 130214″ S/N 130214). Standards for *sn*-1,2-DAG (99% pure, Sigma) and 1,3-DAG (98% pure, Sigma) and 1 and 2- MAG (prepared in the laboratory) were applied to the plate, and the calibration curves were constructed by plotting the IOD vs the amount of lipid loaded. The standard curve was linear and the value of the IOD of the lipid was interpolated on the corresponding calibration curve.

Gas Liquid Chromatography (GLC)

Gas–liquid chromatography technique was employed for the determination of fatty acid composition of different dietary oils after converting them into methyl esters by Litchfield's method (Litchfield 1972).

Feeding experiment

The animal experiments were performed with the approval of the Ethics Committee for experimental animals of the Department of Chemical Technology (University of Calcutta). The animal experiment was designed on the basis of earlier reports published from this laboratory. Male albino rats (average body weight per gr-170 g) of Charles Foster strain (selected for the authenticity of the strain) were chosen as animal system for feeding experiment. Rats were housed individually in stainless steel cages (27×21×14 cm3) with mesh floors in a room maintained under constant temperature (25–30 °C) and a 10 h light/14 h dark cycle (appropriate light and dark cycle is responsible to maintain the biological rhythm which maintains hormonal cycles that in turn controls the lipid metabolism). Following a 7 days adaptation during which the animals were maintained on a standard diet and water *ad libitum*. Each group of rats received different experimental oil while the other dietary components were same. The rats were fed

balanced diet (Jones and Foster 1942) having the following composition: fat free casein-18% (protein source), fat-20%, starch-55% (carbohydrate source), Salt mixture 4% [composition of salt mixture No. 12 (wt in gm): NaCl-292.5; KH_2PO_4-816.6; $MgSO_4$-120.3; $CaCO_3$-800.8; $FeSO_4$, $7H_2O$-56.6; KCl-1.66; $MnSO_4$, $2H_2O$-9.35; $ZnCL_2$-0.5452; $CuSO_4$, $5H_2O$-0.9988; $CoCl_2$-, $6H_2O$-0.0476]; cellulose-3%; and one multivitamin capsule (Vitamin A I. P. 10,000 units, thiamine mononitrate I.P.5 mg, vitamin B.I. P. 5 mg, calcium pantothenate USP 5 mg, niacinamide I.P. 50 mg, ascorbic acid I.P. 400 units, cholecalciferol USP 15 units, menadione I.P. 9.1 mg, folic acid I.P. 1 mg, and vitamin E USP 0.1 mg) per kg of diet. The diet was adequate in all nutrients. The animals were divided into 4 groups (average body weight 170 g), each consisting of eight animals, naming MO, DAG, MOCh and DAGCh. MO group received normal mustard oil as dietary lipid, DAG group was fed with DAG rich mustard oil as dietary fat. Two groups, MOCh and DAGCh were made hypercholesterolemic by addition of cholesterol (1% of total dietary fat). MOCh group received normal mustard oil and DAGCh group received DAG rich mustard oil respectively. Table 1 describes the details of feeding groups.

The amount of daily diet consumed by each rat and weekly body weight gain were noted. The Food Efficiency ratio (FER) of each rat was calculated by the following equation:-

$$\text{FER} = \text{Body weight gain}/\text{Food consumed}.$$

At the end of 28 days of experimental period rats were fasted over-night for 12 h and then sacrificed under anesthesia using chloroform. The abdomen was opened, blood samples was collected from hepatic vein into clear heparinised centrifuge tube and centrifuge at low speed (3,000 g, 4 °C) for 10 min to isolate the plasma. Plasma was separated as the supernatant layer and was collected carefully without disturbing the rest lower part. The liver, heart and mesentery were immediately excised, cleaned by washing with saline (0.98% NaCl sol), blotted, weighed and stored at deep freeze temperature (−40 °C) for subsequent extraction of tissue lipid.

Lipid analysis

According to the standard methods, the lipid components such as total cholesterol (Allain et al. 1974), and high-density lipoprotein (HDL)-cholesterol (Warnick et al. 1985) and triacylglycerol (TAG) (Bucolo and David 1973) of plasma were analyzed using enzymatic kits Ecoline CHOD PAP method, supplied by Merck India Ltd., Mumbai, India.

Preparation of EM ghost

After plasma separation, the red blood cells (RBC) were washed three times by centrifugation at 3,000 g, for 10 min with three volumes of a cooled isotonic solution containing 0.15 M NaCl and 10^{-5}M EDTA. RBC was haemolysed using hypotonic solution and centrifuged at 20,000 · g for 40 min in a cold centrifuge at 4 °C. The supernatant was removed carefully with a pasteur pipette. The process was repeated two more times. After the last wash step, the supernatant was removed as much as possible and the loosely packed milky-looking membrane pellet was re-suspended at the bottom of the tube using 0.89% NaCl solution. Concentrated membrane solution was taken in 2 ml screw cap vial and stored at −40 °C (Rose and Oklander 1965).

Plasma lipid peroxidation

Plasma lipid peroxidation was measured by the assay of thiobarbituric acid-reactive substances (TBARS) according to the standard method (Wills 1987). The amount of malonedialdehyde formed was calculated by taking the extinction coefficient of malonedialdehyde to be $1.56\times 105\ M^{-1}\ cm^{-1}$.

Liver & mesentery tissue lipid extraction

Total lipids were extracted from an aliquot of tissue homogenate by the method of Bligh and Dyer (Bligh and Dyer 1959).

Table 1 Food composition of different dietary groups

Animal	Groups	Composition of diet	Dose	Duration
Male Albino Rat (Charles foster strain)	Control (MO)	Balanced diet+20% physically refined mustard oil	10 g of food/rat/day for each group	28 days
	Treated (DAG)	Balanced diet+20% DAG rich mustard oil		
	Control (MOCh)	Balanced diet+20% physically refined mustard oil+Cholesterol (1% of dietary oil)		
	Treated (DAGCh)	Balanced diet+20% DAG rich mustard oil+Cholesterol (1% of dietary oil)		

Estimation of total protein

Total protein was estimated by the method of Lowry et al. (Lowry et al. 1951).

Estimation of phospholipid

Phospholipid content in tissue lipid was determined by estimating phosphorous according to the method of Chen et al. (Chen et al. 1956).

Measurement of HMG CoA: mevalonate ratio

To measure the HMG Co A reductase activity an indirect method was used (Rao and Ramakrishnan 1975). 1 g of fresh liver tissue was homogenized in 10 ml saline-arsenate solution and equal volume of diluted perchloric acid was added and kept for 5 min. After centrifugation at 2,000 rpm for 10 min, 1 ml of the filtrate was utilized for HMG-Co A analysis with 0.5 ml alkaline hydroxylamine (pH 5.5) and 1.5 ml ferric chloride and 1 ml of the filtrate was utilized for measuring the mevalonate with 0.5 ml acidic hydroxylamine (pH 2.1) and ferric chloride. The absorbance was measured at 540 nm.

Measurement of leptin

Leptin content was measured using ELISA Kit procured from LINCO Research, MO, USA [Cat. No. E6083-K] by the method provided with the kit.

Statistical analysis

The data was expressed as mean±standard error of mean (SEM). One-dway analysis of variance (ANOVA) was also used for statistical analysis between groups. F ratio of one-way ANOVA is significant when p value<0.05. Tukey's multiple range method (Scheffe 1961) was used for comparison. For statistical analysis Origin 7 software was used to calculate the results.

Results and discussions

The physioco-chemical properties of TAG rich mustard oil and DAG rich mustard oil used as dietary oils for the feeding experiment are depicted in Table 1. The fatty acid composition of TAG rich mustard oil was similar with that of DAG rich mustard oil and so both the oils similar in respect to fatty acid composition. The amount of TAG, DAG, MAG, FFA and unsapponifiable matter in original (physically refined) mustard oil and DAG rich mustard oil prepared by enzymatic glycerolysis process are given in Table 2. There was an increase in DAG percentage from 1.18% to 45.5% and decrease in the percentage of TAG from 95.37% to 50.21% in DAG rich mustard oil. There was no significant change observed in amount of MAG, unsapponifiable matter and FFA in both the oils. Fatty acid composition of different dietary DAG (1, 3 DAG and 1,2/2,3 DAG) produced by enzyme catalysed glycerolysis reaction of mustard oil is shown in Table 2. Mean body weight gain observed in the experimental subjects is given in Table 3 and the food efficiency ratio (FER) calculated from body weight gain and food intake of each rat per week is represented by Fig. 1. The FER of DAG group was significantly decreased with respect to the other three dietary groups in all the 4 weeks. In 3rd week the group DAGCh showed a significant lower FER compared to the group MOCh. Consistent with previous reports of Murase et al. (Murase et al. 2002) feeding with the high DAG diet for 1 month resulted in significant decreases in body weight and food efficiency ratio compared with rats fed with TAG rich diet. Taguchi et al. reported that the apparent digestibility of diacylglycerol and TAG oil was identical (96.3%) in rats, and the energy content measured in a bomb calorimeter was similar (38.9 and 39.6 kJ/kg for DAG and

Table 2 Amount of DAG, MAG, TAG, FFA and unsap matter in original mustard oil and DAG rich mustard oil

Oils	TAG[b] (% w/w)	DAG[c] (% w/w)			MAG[d] (% w/w)	FFA[e] (% w/w)	Unsap[f] (%w/w)
		Total	1,3 (% of total)	1,2 (% of total)			
Mustard oil (Physically refined)	95.37±0.56	1.18±0.015	68.12±2.42	30.76±0.75	1.02±0.02	0.33±0.07	1.98±0.25
DAG rich mustard oil[a]	50.21±0.25	45.50±0.35	66.42±0.35	33.58±0.08	2.21±0.02	0.25±0.03	1.83±0.14

[a] Mole ratio between Mustard oil: Glycerol=2:1 and 10% enzyme TLIM was used at 60 °C, 200 r.p.m for 26 h. (all values are mean±SD, n=3)

[b] TAG- Triacylglycerol

[c] DAG- Diacylglycerol

[d] MAG- Monoacylglycerol

[e] FFA- Free fatty acid

[f] unsap- Unsaponifiable matter

Table 3 Mean body weight gain of rats of different dietary groups

Week	Weight gain in gm			
	MO	DAG	MOCh	DAGCh
I	15.55±0.04	8.28±0.04	17.68±0.70	17.0±0.32
II	12.3±0.31	10.26±0.16	14.36±0.10	16.35±0.06
III	10.32±0.26	9.56±0.21	18.35±0.13	15.21±0.13
IV	12.60.14	10.48±0.09	15.55±0.18	14.62±0.15

All values are means±SEM of 8 rats/diet

MO Mustard oil control group; *DAG* DAG rich mustard oil; *MOCh* mustard oil with 1% cholesterol; and *DAGCh* DAG rich mustard oil with 1% cholesterol

TAG respectively) (Taguchi et al. 2001). The plasma lipid profile, LDL and plasma peroxidation of four dietary groups of rats are given in Table 4. The total cholesterol decreased in DAG group in comparison with the other three dietary groups. DAGCh group showed significantly lowered total cholesterol than MOCh group. Plasma HDL cholesterol levels of rats fed with high DAG diet was significantly higher than those of rats fed the high TAG diet and the plasma non-HDL cholesterol and TAG concentrations were significantly lowered in rats fed with high DAG in comparison with the high TAG diet (MO Vs DAG and MOCh Vs DAGCh). The plasma lipid profile indicates that DAG rich mustard oil reduced total cholesterol, TAG, non-HDL cholesterol and raised HDL cholesterol even when cholesterol was supplemented in diet. High DAG diet has shown a reduced plasma peroxidation and LDL peroxidation decreased in the hypercholesterolemic DAGCh compared to the MOCh diet. To further examine the local accumulation of fat, we determined the liver and mesentery lipid profiles that are represented in Table 5. The total lipid accumulation in liver and mesentery were significantly lower in high DAG group (DAG) compared to the high TAG group (MO). Similarly, in 1% cholesterol fed groups the mesentery lipid was high in TAG rich group (MOCh) compared to the DAG rich group (DAGCh) group. The total liver cholesterol content significantly decreased in DAGCh group in comparison with MOCh group and the TAG of liver was decreased with high DAG group than high TAG group (MO). The liver phospholipid content was increased in the DAG group than the MO group. In the mesentery, the total cholesterol and TAG was reduced in DAG groups (DAG and DAGCh) than high TAG groups (MO and MOCh). Measurement of plasma leptin was an excellent index of obesity. Table 6 shows the liver HMG-CoA: Mevalonate ratio and plasma leptin content of different dietary groups. Plasma leptin content was significantly lowered in high DAG fed groups compared to that of high TAG fed groups both without and with cholesterol. During cholesterol biosynthesis 3 hydroxy 3-methyl gluteryl CoA (HMG CoA) is converted into mevalonate by HMG CoA reductase, a rate limiting enzyme for cholesterol synthesis. DAGCh group consuming DAG along with cholesterol showed increased HMG-CoA: Mevalonate ratio than TAG fed MOCh group which in turn illustrates decreased activity of HMG-CoA reductase that indicated decreased synthesis of liver cholesterol in DAGCh group. Lipid profile of erythrocyte membrane (EM) of different dietary groups is presented in Table 7. TAG content decreased in DAG fed group compared to the TAG fed group.

In the present study, we examined the effects of dietary DAG rich mustard oil in normal and hypercholesterolemic rat model. The results of this study indicate that structural differences between DAG and TAG, did not affect the fatty acid composition, has markedly affect nutritional behavior of lipids including body fat accumulation, serum lipid profile and lipid profile of the liver and mesentery and the antiobesity hormone leptin. Many studies have been conducted to determine the preventive or therapeutic effects of various dietary oils on obesity. The decrease in FER and

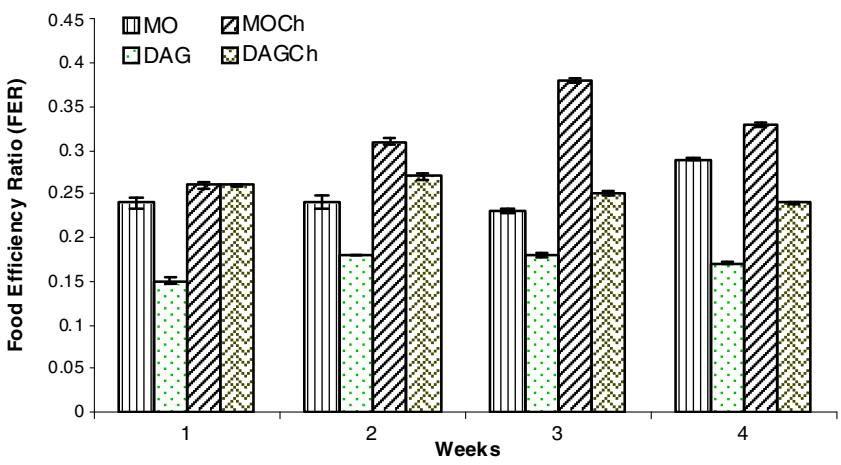

Fig. 1 Food efficiency ratio of different dietary groups of rats. MO: mustard oil control group; DAG: DAG rich mustard oil; MOCh: mustard oil with 1% cholesterol and DAGCh: DAG rich mustard oil with 1% cholesterol. All values are means±SEM of 8 rats/diet

Table 4 Plasma Lipid Profile, Lipid peroxidation and Lipoprotein peroxidation of Rats in different dietary groups

Groups	Total cholesterol (mg/dL)	HDL-cholesterol (mg/dL)	Non-HDL cholesterol (mg/dL)	Triacylglycerol (mg/dL)	Plasma lipid peroxidation (n mole of MDA/ml of plasma)	LDL-peroxidation (n mole of MDA/mg of non-HDL cholesterol)
MO	85.54±3.06	22.86 ±1.22[a]	62.68±2.07[b]	97.74±1.33[t]	5.46±0.40	14.76±1.66
DAG	83.18±1.94	28.71±1.31[a]	54.17±2.74[b]	86.27±2.49[t]	3.34±0.30	12.98±2.54
MOCh	114.16±1.61**	22.48 ±0.47	60.24 ±1.60[bb]	126.72±2.64[tt]	4.11±0.34	[aa]22.9±1.06
DAGCh	103.50±1.73**	20.84±0.76	81.64±1.66[bb]	92.5 ±2.44[tt]	3.34±0.34	[aa]17.51±1.83

MO Mustard oil control group; *DAG* DAG rich mustard oil; *MOCh* mustard oil with 1% cholesterol; and *DAGCh* DAG rich mustard oil with 1% cholesterol

All values are means±SEM of 8 rats/diet, **MOCh vs. DAGCh, [a] MO vs DAG, [b] MO vs DAG, [bb] MOCh Vs DAGCh, MO vs DAG, [tt] MOCh vs DAGCh, [aa] MOCh vs. DAGCh

Significant F ratios for total cholesterol (**p=0.0492) HDL-cholesterol ([a]p=0.0091), non-HDL-cholesterol ([b]p=0.0082, [bb]p=0.0279) and triacylglycerol ([t]p=0.02418, [tt]p=0.0360) total LDL peroxidation ([a a]p=0.023)

body weight gain in DAG rich oil group suggests that there is reduced accumulation of energy derived from dietary DAG. In control feeding studies diacylglycerol prevented the accumulation of body weight and fat associated with the high fat and high sucrose diet in obesity prone mice (Murase et al. 2001). These effects do not appear due to the poor digestibility or reduced energy content of DAG. The apparent digestibility of DAG and TAG oil was identical (96.3%) in rats, and the energy content was similar (Taguchi et al. 2001). Therefore, decreased body weight is due to energy expenditure and food or both (Maki et al. 2002). Watanabe et al. (Watanabe and Tokimitsu 2004) found that in comparison with a triacylglycerol control, oxygen consumption in rats increased 1 ml.kg-1 min−1 during the 90 min after DAG administration, suggesting a short term increase in energy expenditure. Studies also suggested that, fatty acids released from dietary 1,3 diacylglycerol (DAG) oil are not effectively incorporated into chylomicron after absorption from the intestinal lumen, resulting in greater fatty acid oxidation in the small intestine (Murase et al. 2002) and liver (Mori et al. 2005; Murase et al. 2001; Murata et al. 1997; Nagao et al. 2000) which leads to lower postprandial plasma triglyceride (PPTAG) level (Bauer et al. 2006; Umeda et al. 2006) and lower body weight with DAG enriched diets. Lower postprandial TAG has also been observed (Tada et al. 2001; Taguchi et al. 2000) people with type –II diabetes mellitus. Consumption of DAG oil also reduces glycosylated hemoglobin ($HbA1_C$) in diabetic patient (Yamamoto et al. 2001). Another important difference between DAG and TAG metabolism is the substrate specificity of the diacylglycerol acyltransferase (DGAT) enzyme, DGAT-1 and DGAT-2 in the small intestines (Cases et al. 1998) DGAT has low substrate specificity toward 1, 3 DAG and therefore does not significantly convert 1,3 DAG to TAG (Lehner and Kuksis 1993). There are three possible causes for the slightly lower recovery of fatty acids originated from 1,3 DAG in lymph. First, a part of the fatty acids from DAG might be transferred to the portal vein preferentially for β oxidation in liver (Watanabe et al. 1997). Second,

Table 5 Liver and mesentery lipid profile of rats of different dietary groups

Groups	Total lipid (mg/g of tissue)		Total Cholesterol (mg/g of tissue)		TAG (mg/g of tissue)		Phospholipid (mg/g of tissue)	
	Liver	Mesentery	Liver	Mesentery	Liver	Mesentery	Liver	Mesentery
MO	[b]51.52±0.64	[$]231.75±1.25	3.81±0.86	[M]37.33±2.42	[a]32.52±2.00	[@]174.44±4.57	[p]15.16±1.47	17.95±1.13
DAG	[b]45.2±0.80	[$]196.62±2.26	2.88 ±0.27	[M]31.92±1.09	[a]24.25±1.26	[@]151.0±1.30	[p]22.01±1.55	14.24±0.41
MOCh	65.3 ±1.84	* 88.25±1.18	**4.17±0.55	[MM]55.21±3.21	37.27±1.01	[bb]206.62±1.25	25.23±1.52	26.81±0.92
DAGCh	63.36±2.30	*262.27±1.42	**2.36±1.01	[MM]46.79±4.43	34.0±1.05	[bb]188.37±2.04	24.10±2.53	24.05±0.52

MO Mustard oil control group; *DAG* DAG rich mustard oil; *MOCh* Mustard oil with 1% cholesterol; and *DAGCh* DAG rich mustard oil with 1% cholesterol

All values are mean±SEM of 8 rats/diet

[b] MO vs DAG, *MOCh vs. DAGCh, **MOCh vs. DAGCh [a] MO vs DAG, [p] MO vs DAG, [$] MO vs. DAG, [M] MO vs. DAG, [M M] MOCh vs. DAGCh, [@] MO vs.DAG, [bb] MOCh vs.DAGCh,. Significant F ratios for total lipid content ([b]p=0.04917) total cholesterol (**p=0.01396, [MM]p=0.00001) and triacylglycerol ([a]p=0.024) total liver phospholipid, ([p]p=0.02) for total mesentery lipid content ([$]$p$=0.037), triacylglycerol ([@]p=0.0001, [b b]p=0.020),cholesterol ([M]p=0.001, [M M]p=0.002)

Table 6 Liver HMG CoA-Mevalonate ratio and Leptin content (ng/mL) in different dietary groups

Groups	HMG CoA-Mevalonate ratio	Leptin content (ng/mL)
MO	2.29±0.33*	[a]1.45±0.149
DAG	2.86±0.86*	[a]0.87±0.03
MOCh	2.17±0.29**	[b]2.5±0.085
DAGCh	3.75±0.86**	[b]1.61±0.065

MO: mustard oil control group; DAG: DAG rich mustard oil; MOCh: mustard oil with 1% cholesterol;

and DAGCh: DAG rich mustard oil with 1% cholesterol

All values are means±SEM, n=8., **MOCh vs DAGCh, [a] MO vs DAG, [b] MOCh vs DAGCh Significant F ratios for liver HMG CoA-Mevalonate ratio (**p=0.02456), total plasma leptin content ([a] p=0.003, [b] p=0.007)

there is a possibility that a part of the fatty acids from DAG was oxidized in intestinal cells. Murase et al. (Murase et al. 2002) reported that when DAG was fed to mice for 10 days mRNA expression of all β oxidation enzymes like acyl CoA oxidase, medium chain acyl coA dehydrogenase and uncoupling protein −2 in intestine were increased.

Dietary high DAG significantly changed the plasma lipid profiles. The decrease in plasma TAG with DAG rich oil as observed in the present study may be due to distinct metabolic pathways of the ingested TAG and DAG. 1, 3 DAG is digested in the metabolic tract to 1-MAG or 3-MAG, those are poorly re-esterified into TAG in the intestinal mucosa (Osaki et al. 2005; Tomonobu et al. 2006). For 1-MAG to follow this pathway, it must be hydrolysed to glycerol, which releases a free fatty acid (FFA) that could be available for TAG re-synthesis or potentially for energy utilization. As a result, postprandial elevations in TAG-rich lipoproteins were lowered significantly after consumption of DAG rich oil (Tada et al. 2001; Taguchi et al. 2000). Experimental studies in animals and humans showed that diacylglycerol (mainly 1, 3 DAG) decreases postprandial triglyceridemia in comparison with a triacylglycerol control (Taguchi et al. 2000; Wang et al. 2010). It was reported that dietary DAG had anti-obesity activity and prevented postprandial hypertriacylglycerolemia in experimental animals and humans (Ikeda and Yanagita 2004; Murase et al. 2002, 2001). Ikeda and Yanagita (Ikeda and Yanagita 2004) suggested that delayed absorption of DAG compared to TAG may be the important determinant in preventing body fat accumulation. A major contributor to the clearance of TAG rich lipoproteins from plasma is lipoprotein lipase (Ikeda and Yanagita 2004; Masui et al. 2001; Kokie et al. 2001). Murata et al. showed that in vitro, DAG emulsions were better substrates for lipoprotein lipase mediated lipolysis. Therefore, efficient hydrolysis of DAG by lipoprotein lipase may attribute to the decreased plasma triacylglycerol level. DAG feeding may be utilized preferentially for fatty acid oxidation rather than for body fat accumulation (Murata et al. 1997). A report also revealed that the ingestion of DAG significantly decreased the respiratory quotient (RQ), which is calculated from consumed oxygen and expired carbon dioxide, and increased lipid oxidation compared with that of TAG in a human clinical study (Kamphuis et al. 2003) and in animals (Kimuru et al. 2003). A decrease in RQ from the baseline indicates an increament in fat utilisation as an energy source. In our study we observed that the DAG rich mustard oil is capable of reducing arteriosclerotic factors like total cholesterol and non-LDL cholesterol and increase antiatheroscleotic factor such as HDL cholesterol. The results are consistent with the previous report of Masui et al. and Koike et al. (Kokie et al. 2001; Masui et al. 2001). Our DAG rich mustard oil contains about 45% of DAG which reduced the liver total cholesterol in hyperlipidemic rats reducing the HMG-CoA reductase activity (Frayn 2002), with net uptake of fatty acids in the postprandial state and net release between meals.

Leptin is a protein hormone a product of ob gene containing 167 amino acids with important effects in regulating body weight, metabolism and reproductive function. It is secreted from white adipose tissues and decreased hunger and food intake by inhibiting gene expression of neuropeptide Y (NPY). Leptin is a product of ob gene with important effects in regulating body

Table 7 Lipid profile of erythrocyte membrane (EM) ghost of different dietary groups

Groups	TG (mg/mg of protein)	Cholesterol (mg/mg of protein)	Phospholipid (mg/mg of protein)
MO	0.24[a]±0.01	0.13±.07	0.60±0.03
DAG	0.15[a]±0.08	0.20±0.07	0.64±0.06
MOCh	0.22**±0.07	0.33[$]±0.02	0.56*±0.01
DAGCh	0.19**±0.09	0.24[$]±0.08	0.44*±0.06

MO Mustard oil control group; *DAG* DAG rich mustard oil; *MOCh* mustard oil with 1% cholesterol; and *DAGCh* DAG rich mustard oil with 1% cholesterol

All values are means±SEM, n=8, [a] MO vs. DAG, **MOCh vs. DAGCh, [$] MOCh vs. DAGCh, *MOCh vs. DAGCh; Significant F ratios for EM TAG ([a] p=0.025, **p=0.0002), cholesterol ([$] p=0.0038), phospholipids (*p=0.0025)

weight, metabolism and reproductive function. Leptin's effects on body weight are mediated through effects on hypothalamic centres that control feeding behaviour and hunger, body temperature and energy expenditure. The body weight reduction in the DAG dietary groups possibly due to the reduced leptin synthesis by the adipose tissues compared to TAG rich dietary oil fed rats. We can propose another mechanism in prevention of body fat accumulation of DAG. Mustard oil contains 45–50% of erucic acid (22:1). After a meal the increase in blood glucose is followed by insulin secretion. When TAG is consumed with meal both blood glucose and post prandial TAG level are increased.

When glucose is exhausted insulin level decreased and due to slow absorption of high molecular weight fatty acid like erucic acid, preferentially used for fatty acid oxidation for energy expenditure rather than to be stored as triglyceride. Oxidation of fatty acids leads to satiety and the food intake between meals is inhibited. Therefore, the amount of chylomicron triglycerides entering the blood stream after mustard oil DAG consumption is less than that for TAG because lymphatic transport of triglyceride is delayed after feeding of DAG.

Conclusions

Thus the present study demonstrates that the DAG rich mustard oil prepared from normal mustard oil reduces body weight as evidenced by food efficiency ratio, lowering of plasma leptin content and reduced atherosclerotic factors such as plasma TAG, Non-LDL cholesterol.

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