DIACYLGLYCEROL OIL: AN ANTIOBESITY DIETARY SUPPLEMENT

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ABBREVIATIONS

ACC: Acetyl CoA carboxylase

ATGL: Adipose triglyceride lipase

BAT: Brown adipose tissue

DAG: Diacylglycerol

DEXA: Dual-energy X-ray absorptiometry

FAS: Fatty acid synthase

GAPDH: Glyceraldehyde-3-phophate dehydrogenase

HSL: Hormone sensitive lipase

LC-TAG: Long-chain triacylglycerol

LDL-C: Low-density lipoprotein cholesterol

MAG: Monoacylglycerol

MCE-DAG: Medium-chain enriched diacylglycerol

MCFA: Medium-chain fatty acid

MC-TAG: Medium-chain triacylglycerol

MLC-TAG: Medium- and long-chain triacylglycerol

MUFA: Monounsaturated fatty acid

PUFA: Polyunsaturated fatty acid

SFA: Saturated fatty acid

SREBP1: Sterol regulatory element binding protein

TAG: Triacylglycerol

TC: Total cholesterol

UCP1: Uncoupling protein 1

WAT: White adipose tissue

INTRODUCTION

There is a common misconception that all fats and oils are bad for health, when in actuality the right types of oils and fats will provide an effective energy source for the body, as well as enhance the texture, taste and aroma of many foods. Various types of oils and fats are commonly used in the preparation of many types of foods by methods such as cooking, baking and frying, and are also an important component in fillings, icings, toppings and coatings. The key to a healthy diet is to stay away from foods and oils with high levels of trans and hypercholesterolemic fats. Unfortunately, a high-fat diet is often linked to obesity, which is subsequently related to various diseases (Krawczyk, 2000). In the past two decades, the image of fats and oils has shifted from bad to healthful (Kennedy, 1991).

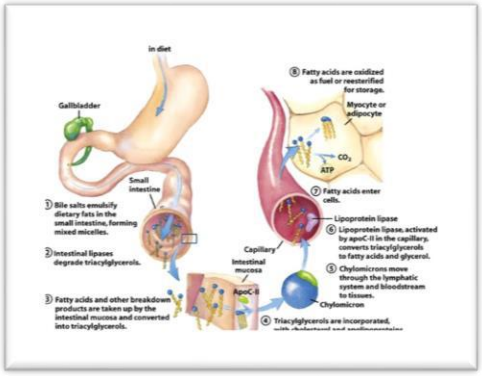
DIETARY FATS

In nutrition, biology, and chemistry, fat usually means any ester of fatty acids, or a mixture of such compounds, most commonly those that occur in living beings or in food. The term often refers specifically to triglycerides (triple esters of glycerol), that are the main components of vegetable oils and of fatty tissue in animals; or, even more narrowly, to triglycerides that are solid or semisolid at room temperature, thus excluding oils. The term may also be used more broadly as a synonym of lipid—any substance of biological relevance, composed of carbon, hydrogen, or oxygen, that is insoluble in water but soluble in non-polar solvents. In this sense, besides the triglycerides, the term would include several other types of compounds like mono- and diglycerides, phospholipids (such as lecithin), sterols (such as cholesterol), waxes (such as beeswax) and free fatty acids, which are usually present in human diet in smaller amounts.

Fats are one of the three main macronutrient groups in human diet, along with carbohydrates and proteins and the main components of common food products like milk, butter, tallow, lard, salt pork, and cooking oils. They are a major and dense source of food energy for many animals and play important structural and metabolic functions, in most living beings, including energy storage, waterproofing, and thermal insulation. The human body can produce the fat it requires from other food ingredients, except for a few essential fatty acids that must be included in the diet. Dietary fats are also the carriers of some flavor and aroma ingredients and vitamins that are not water-soluble. The quantity and quality of dietary fats will influence the physiological functions in our body.

METABOLISM OF FAT

Digestion is the first step to lipid metabolism, and it is the process of breaking the triglycerides down into smaller monoglyceride units with the help of lipase enzymes. Digestion of fats begin in the mouth through chemical digestion by lingual lipase. Ingested cholesterol is not broken down by the lipases and stays intact until it enters the epithelium cells of the small intestine. Lipids then continue to the stomach where chemical digestion continues by gastric lipase and mechanical digestion begins (peristalsis). The majority of lipid digestion and absorption, however, occurs once the fats reach the small intestines. Chemicals from the pancreas (pancreatic lipase family and bile salt-dependent lipase) are secreted into the small intestines to help breakdown the triglycerides, along with further mechanical digestion, until they are individual fatty acid units able to be absorbed into the small intestine's epithelial cells. It is the pancreatic lipase that is responsible for signalling for the hydrolysis of the triglycerides into separate free fatty acids and glycerol units.

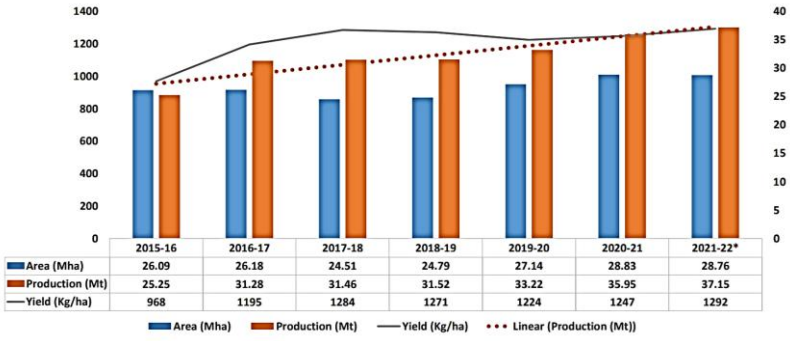


The second step in lipid metabolism is absorption of fats. Short chain fatty acids can be absorbed in the stomach, while most absorption of fats occurs only in the small intestines. Once the triglycerides are broken down into individual fatty acids and glycerols, along with cholesterol, they will aggregate into structures called micelles. Fatty acids and monoglycerides leave the micelles and diffuse across the membrane to enter the intestinal epithelial cells. In the cytosol of epithelial cells, fatty acids and monoglycerides are recombined back into triglycerides. In the cytosol of epithelial cells, triglycerides and cholesterol are packaged into bigger particles called chylomicrons which are amphipathic structures that transport digested lipids. Chylomicrons will travel through the bloodstream to enter adipose and other tissues in the body.

Due to the hydrophobic nature of membrane lipids, triglycerides and cholesterols, they require special transport proteins known as lipoproteins. The amphipathic structure of lipoproteins allows the triglycerides and cholesterol to be transported through the blood. Chylomicrons are one sub-group of lipoproteins which carry the digested lipids from small intestine to the rest of the body. The varying densities between the types of lipoproteins are characteristic to what type of fats they transport. For example, very-low-density lipoproteins (VLDL) carry the synthesized triglycerides by our body and low-density lipoproteins (LDL) transport cholesterol to our peripheral tissues. A number of these lipoproteins are synthesized in the liver, but not all of them originate from this organ.

Lipids are stored in white adipose tissue as triglycerides. In a lean young adult human, the mass of triglycerides stored represents about 10–20 kilograms. Triglycerides are formed from a backbone of glycerol with three fatty acids. Free fatty acids are activated into acyl-CoA and esterified to finally reach the triglyceride droplet.

Trends of area, production and yield of Oilseeds in India (2015-16 to 2021-22)



	2015-16	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22*
Area (Mha)	26.09	26.18	24.51	24.79	27.14	28.83	28.76
Production (Mt)	25.25	31.28	31.46	31.52	33.22	35.95	37.15
Yield (Kg/ha)	968	1195	1284	1271	1224	1247	1292

Source: Ministry of Agriculture & Farmers Welfare, Govt. of India (19th April, 2022)

The graph above depicts that from 2015-16 to 2021-22,the production of oilseed has breen increased upto 37.15 MT.

EDIBLE OIL SECTOR

Year	Edible oil for available for consumption (MT)	Domestic Production (MT)	Imports (MT)	Value of import (Rs. in crore)	Dependency on imports (%)	Per capita Consumption (kg/yr)
1986-87	5.34	3.87	1.47	700	28.0	6.2
1994-95	7.54	7.19	0.35	300	5.0	7.3
2014-15	21.36	8.63	12.73	64,894	59.6	18.3
2019-20	25.06	10.60	14.46	68,576	57.7	18.7
2020-21	25.82	12.47	13.35	79,190	54.9	18.2

Source: Department of Sugar & Vegetable Oils; DG, CI&S, Dept. of Commerce, Kolkata

ICMR recommendation i.e. 30 g/person /day : 12 kg/person/annum

(Longvah *et al.*, 2017)

Total import of edible oils: 13.35 million tons

Palm oil (56%), Soybean oil (27%), Sunflower oil (16%), Others (1%)

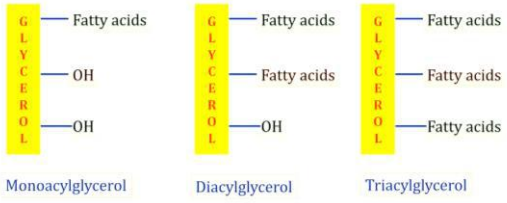
In the edible oil sector, it is observed that domestic production is highest in the tear 2021-22 (12.47 MT) and per capita consumption from 2014 to 2021 is almost similar with the little fluctuation yearly. ICMR recommends consumption of 30g fat/person/day.

India is the 4th largest oilseeds producer in the world. It has 20.8 per cent of the total area under cultivation globally, accounting for 10 per cent of global production. The country produces groundnut, soybean, sunflower, sesamum, niger seed, mustard and safflower oilseeds. Nearly 72 per cent of the oilseeds area is restricted to rainfed farming done by small farmers which led to poor productivity. However, a breakthrough was realized in oilseed production through introducing latest crop production technologies. Consequently, the oilseed production grew to 365.65 tones in 2020-21 from 108.3 lakh tonnes in 1985-86.

The production of oilseeds in India has been growing for the last five years. In 2020-21, the production the country was 365.65 lakh tonne which was a 10% increase from that of the previous year. From the year: 2015-16 to 2020-21, the compound annual growth rate (CAGR) of production was 7.7 per cent. This was achieve due to implementation of various programs like special programmes on mustard & rapeseed during cluster demonstrations of improved technology by the Government of India.

The largest oilseed-producing states in India include Andra Pradesh, Gujarat, Haryana, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, Uttar Pradesh, West Bengal. Out of these states, Rajasthan, Gujarat, Madhya Pradesh and Maharashtra are the top producers with a share of about 20, 20, 19 and 16 per cent of the total production, respectively.

STRUCTURE OF ACYLGLYCEROL



The composition of edible oil has two main constituents one is acylglycerol and one more is fatty acids. An acylglycerol is ester of glycerol and fatty acids that occur naturally in fat and fatty oils. Glycerol has 3 hydroxyl

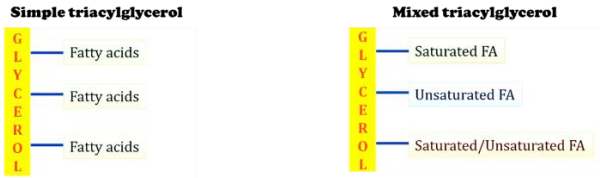
functional groups which can be esterified with one fatty acid to form monoacylglycerol, two fatty acids – diacylglycerol and three fatty acids triacylglycerol.

SIMPLE LIPIDS: TRIACYLGLYCEROL (TAG)

A triglyceride (TG, triacylglycerol, TAG, or triacylglyceride) is an ester derived from glycerol and three fatty acids (from tri- and glyceride). It is non polar and also known as neutral fats, Serves as energy storage. Fats, oils that are primarily distributed in both plants and animals are chemically Triacylglycerols. The excess intake of energy leads to an increase in the storage of TAG in adipose tissue.

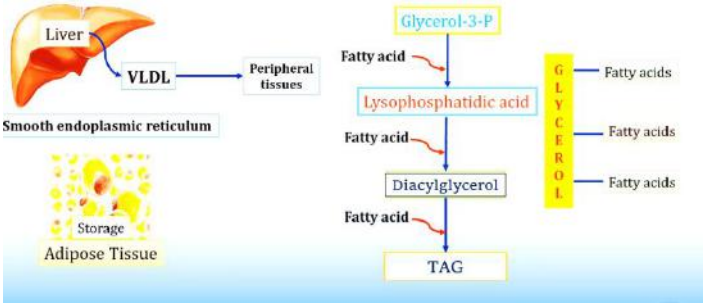
Triglycerides are the main constituents of body fat in humans and other vertebrates, as well as vegetable fat. They are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver, and are a major component of human skin oils. Many types of triglycerides exist. One specific classification focuses on saturated and unsaturated types. Saturated fats have no C=C groups; unsaturated fats feature one or more C=C groups. Unsaturated fats tend to have a lower melting point than saturated analogues; as a result, they are often liquid at room temperature.

TYPES OF TRIACYLGLYCEROL



TAG can be simple with three identical fatty acids (Tripalmitin, Tristearin) or mixed TAG (Animal fats – more saturated fatty acids; Vegetable fats – more unsaturated fatty acids) with two or three different acids.

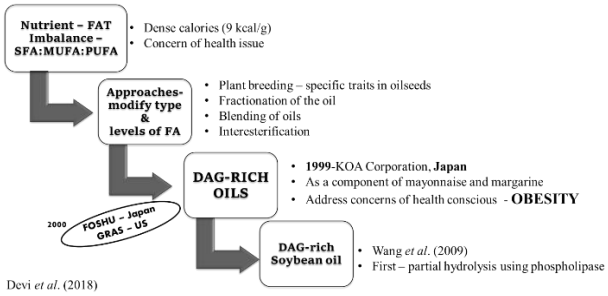
BIOSYNTHESIS OF TAG



Lipogenesis is the conversion of fatty acids and glycerol into fats, or a metabolic process through which acetyl-CoA is converted to triglyceride for storage in fat. Lipogenesis encompasses both fatty acid and triglyceride synthesis, with the latter being the process by which fatty acids are esterified to glycerol before being packaged into very-low-density lipoprotein (VLDL). Fatty acids are produced in the cytoplasm of cells by repeatedly adding two-carbon units to acetyl-CoA. Triacylglycerol synthesis, on the other hand, occurs in the endoplasmic reticulum membrane of cells by bonding three fatty acid molecules to a glycerol molecule. Both processes take place mainly in liver and adipose tissue. Nevertheless, it also occurs to some extent in other tissues such as the gut and kidney.

After being packaged into VLDL in the liver, the resulting lipoprotein is then secreted directly into the blood for delivery to peripheral tissues (Xia *et al*., 2014).

DIACYLGLYCEROL OIL– THE INGRESS



Devi *et al*. (2018)

People during the years only knew that naturally occurring vegetable oils and fats contain triacylglycerols (TAG) as a major constituent. They also contain small amounts of diacylglycerols (DAG) and monoacylglycerols (MAG). Fats and oils are essential ingredients of human food as they are important sources of energy, essential fatty acids and fat-soluble vitamins. Fat is an important nutrient which provides dense calories (9 Kcal/g). The unutilized calories from dietary fat are a concern for health-conscious individuals as it may lead to obesity. The consumption of imbalanced fats with respect to levels of saturated, monounsaturated and polyunsaturated fatty acids may also lead to chronic diseases.

To address these concerns, several approaches such as plant breeding to select specific traits in oilseeds, fractionation of the oil, blending of oils and interesterification of oils are currently being used to modify the type and levels of fatty acids in the oils. DAG-rich oils are also being considered for addressing concerns of health-conscious individuals. The country Japan in the year 1999 introduced world, an anti-obesity diacylglycerol oil. To modify the type and levels of fatty acid many approaches including plant breeding, fractionation of oil, blending of oil, interesterification was done. But when the ratio of TAG and DAG was modified, the study was successful. The DAG oil was recognized as GRAS by United States and FOSHU by Japan in 2000. It was first used as a component of mayonnaise and margarine to address the concern of health issue mainly obesity. Wang *et al*. (2009) were the first to develop DAG oil from Soybean by partial hydrolysis using phospholipase.

DIACYLGLYCEROL OIL

Conventional cooking oil that contains only up to 10 per cent (w/w) DAG, these rather light-tasting healthful cooking oils are claimed to contain 80 per cent (w/w) or more of DAG as the main functional component. Diacylglycerol oil (DAG oil) is a cooking oil in which the ratio of triglycerides, also known as Triacylglycerols (TAGs), to diacylglycerols (DAGs) is shifted to contain mostly DAG, unlike conventional cooking oils, which are rich in TAGs. Vegetable DAG oil, for example, contains 80 per cent DAG DAGs and TAGs are natural components in all vegetable oils. Through an enzymatic process, the DAG content of a combination of soy and canola oils is significantly increased. Unlike TAG, which is stored as body fat, DAG is immediately burned as energy. With DAG-rich oil containing more than 80 per cent DAG, less of the oil is stored as body fat than with traditional oils, which are rich in TAG. Excess calories consumed by the body are converted into fat and stored, regardless if it is consumed as DAG or TAG.

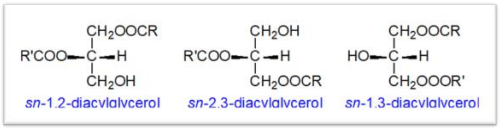
NATURAL SOURCES OF DAG

Unless oil is modified it will not contain DAG >10 per cent naturally. Palm oil, though we avoid consuming because of its high saturated fat content, it naturally contains higher amounts of DAG *i.e.*, 5.8 per cent followed by olive oil and tallow which is an animal fat.

Oil	Monoacylglycerol (% w/w)	Diacylglycerol (% w/w)	Triacylglycerol (% w/w)
Soybean	–	1.0	97.9
Cottonseed	–	3.1	95.0
Palm	–	5.8 (1)	93.1
Corn	–	2.8	95.8
Sunflower	–	2.0	95.6
Safflower	–	2.1	96.0
Peanut	–	2.2	93.3
Sesame	–	2.6	95.0
Olive	0.2	5.5 (2)	93.3
Rapeseed	0.1	0.8	96.8
Cocoa butter	0.2	2.2	96.0
Tallow	–	3.8 (3)	89.6
Lard	–	1.3	97.9

Chiayasit *et al.* (2007)

STERIOISOMERS OF DIACYLGLYCEROL



DAGs are esters of the trihydric alcohol glycerol in which two of the hydroxyl groups are esterified with fatty acids. They can exist in two structural isomers namely, 1,2-DAG and 1,3-DAG. These isomers will undergo acyl migration to form equilibrium at a ratio of 3–4:7–6 between 1,2- and 1,3-DAG (Takano and Itabashi 2002) often in the presence of an acid, alkali, or heat. 1,3- DAG is more thermodynamically stable because of the steric effect of the molecule. Unlike TAG polymorphism, DAG exhibits two types of polymorphic forms. 1,2-DAG exhibits the α- and β′-forms but has no β-form, while 1,3-DAG has no α-form but exhibits two types of β-form, β1 and the more unstable β2. DAG oil, an useful adjunct to diet therapy in the management of obesity.

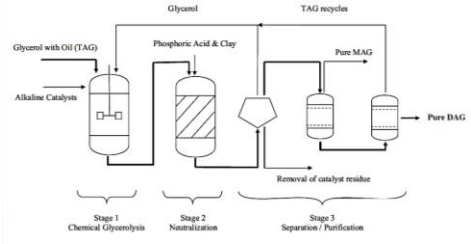
PRODUCTION OF DAG

There are three major methods in which DAG is synthesized and they are as follows;

1. Glycerolysis - TAG is reacted with glycerol to produce DAG
2. Esterification - FFA is esterified with either glycerol or MAG, synthesizing DAG and water molecules
3. Hydrolysis - Reaction between TAG and water, resulting in MAG, DAG and FFA

If chemicals are used in the above methods, it is the chemical production of DAG and if enzymes are used it is enzymatic production of DAG.

Illustration of the chemical glycerolysis for DAG production in an industrial scale

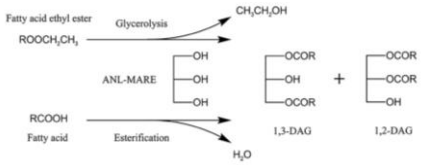


Chemical glycerolysis is the common industrial approach employed for DAG production. In this process, glycerol is used as one of the main feedstocks. Glycerol is produced as a co-product of triglyceride esterification and transesterification. Currently, the largest source of glycerol is the biodiesel industry. As the biodiesel industry

grew up quickly the glycerol surplus can be seen as a potential source to obtain valuable products from a cheap and versatile reactant. Crude glycerol from the biodiesel industry should be utilized for production of more valuable products, such as DAG and MAG, instead of being dumped in the landfills (Satriana *et al*., 2016).

On an industrial scale, chemical glycerolysis is normally carried out at temperatures of about 210–260°C in the presence of various alkaline catalysts such as sodium hydroxide (NaOH), potassium hydroxide (KOH), sodium methoxide ($NaOCH_3$), and potassium acetate ($KC_2H_3O_2$). The production scheme is shown in Fig. This process, however, has many drawbacks related to the extreme operating conditions used. The prolonged exposure to high temperature conditions especially for heat-sensitive polyunsaturated fatty acid might lead to the formation of undesirable by-products and the development of deleterious characteristics such as sensory, appearance, and aroma of the final oil product. As a consequence, a purification process must be employed before product commercialization. On the other hand, the extreme operating conditions also triggers an unwanted acyl migration side reaction which leads to low DAG yield and purity. Additionally, a high energy requirement might significantly increase the production cost. Poor miscibility between the substrates, namely oil and glycerol is a major limiting factor for DAG production especially through chemical glycerolysis. It leads to low mass transfer and overall rate of reaction.

Enzyme catalyzed DAG-enriched oil by a two-step vacuum-mediated conversion



The combination of glycerolysis of EE and esterification of FFA in one-pot enzymatic catalysis is rarely studied. DAG oil has unique nutritional properties concerning lower body fat metabolism. However, to our knowledge, little work investigates the nutritional properties of DAG-enriched oil from SSBO. It is expected that DAG-enriched oil will be used as functional oil (Feng *et al*., 2022).

DAG-enriched oil was synthesized using a low-cost SSBO containing a high content of EE and FFA as raw material and a non-commercial immobilized Aspergillus niger lipase (ANL-MARE) as a catalyst. Based on the different behaviors between the transesterification of EE and the esterification of FFA in one-pot enzymatic catalysis, a two-step vacuum-mediated conversion was developed for the conversions of EE and FFA to DAG. The vacuum was employed to remove produced water and ethanol in a vacuum-driven air bubbling operation, thereby increasing the conversion of FFA and EE, as well as the yield of DAG. The EE and FFA from Soya Sauce Byproduct Oil were served as acyl group donors in this reaction, while the lipase showed different behaviors between the glycerolysis of EE and the esterification of FFA with glycerol.

DAG CHARACTERISTICS COMPARED TO TAG

Characteristics	Changes in characteristics	References
Crystallization	- Faster crystallization - Higher temperature	(Basso *et al.*, 2010; Saberi *et al.*, 2011; Normah *et al.*, 2014)
Melting	- Slower Melting - Higher temperature	(Basso *et al.*, 2010; Saberi *et al.*, 2011)
Solid fat content	- Higher solid fat content	(Saberi *et al.*, 2012; Ng *et al.*, 2014)
Polymorphism	- β-crystal >> β´-crystal	(Saberi, Tan and Lai, 2011; Saberi *et al.*, 2011; Zhang *et al.*, 2014)
Microstructure	- Smaller crystal - More homogeneous - Needle-like crystals	(Maruyama *et al.*, 2014; Xu *et al.*, 2016; Naderi *et al.*, 2016)
Rheology	- Higher viscosity and elasticity	(Rocha-Amador *et al.*, 2014; Naderi *et al.*, 2018)
Texture (Hardness)	- Harder	(Saberi, Tan and Lai, 2011; Subroto *et al.*, 2019)

DAG crystalizes faster than TAG and has slower melting property with high solid fat content compared to TAG. It exhibits 2 types of polymorphism and its microstructure reveals a more homogenous smaller and needle like crystals. DAG rheology reveals higher viscosity and elasticity whereas its texture is harder.

Applications of MAG and DAG in fat-based food products

DAG oil from corn, palm, soybean, canola, chicken or mutton fat can be used as a source in MAG/DAG fats and oils, margarine/shortening, emulsifier and organogels. It can be easily incorporated into food products as it is similar in taste, appearance and fatty acid composition to other conventional oils including rapeseed, soybean and safflower oil.

Products	Source of MAG/DAG	References
MAG-DAG Fats/Oils	Corn oil	(Zhang *et al.*, 2017)
	Vegetable oils	(Ferretti *et al.*, 2018)
	Palm olein	(Yeoh *et al.*, 2014; Cheong *et al.*, 2007)
	Palm-based oils	(Saberi *et al.*, 2011)
	Mutton tallow and rapeseed oil	(Kowalska *et al.*, 2014)
	Palm stearin and palm olein	(Subroto *et al.*, 2019)
Margarine/shortening	Palm mild fraction	(Latip *et al.*, 2013)
	Sunflower oil, palm kernel olein	(Saberi *et al.*, 2012)
	Canola oil	(Naderi *et al.*, 2018)
	Chicken fat	(Naderi *et al.*, 2016)
Surfactant/emulsifier	Palm based oils/fats	(Fu *et al.*, 2018)
	Palm oils	(Hattori *et al.*, 2015)
	Vegetable oil	(Maruyama *et al.*, 2014)
Organogels	Monostearate	(Ferro *et al.*, 2019)
	Monostearate and monopalmitate	(Rocha-Amador *et al.*, 2014; Lopez-Martínez *et al.*, 2015)
	Saturated fat	(Yilmaz and Öğütcü, 2014; Pérez-Martínez *et al.*, 2019)

SAFETY OF DAG OIL

Animals and human studies reveal that there are no adverse effects from either single-dose or long-term consumption of DAG-rich oil. Fat-soluble vitamins status is not affected by its consumption. As a result, oils containing higher amounts of 1,3-DAG - fight against obesity-related chronic diseases.

RECOMMENDATION

Daily ingestion of 8–20 g DAG has been used in human studies to check the efficacy of DAG on body composition especially weight loss in obese people (Scholz *et al*., 2022).

HEALTH BENEFITS OF DAG OIL

DAG along with anti-obesity property also has anti-atherosclerotic, anti-diabetic and anti-inflammatory properties.

OVERWEIGHT AND OBESITY

Obesity is a medical condition, sometimes considered a disease, in which excess body fat has accumulated to such an extent that it may negatively affect health. People are classified as obese when their body mass index (BMI)—a person's weight divided by the square of the person's height—is over 30 kg/m^2; the range 25–30 kg/m^2 is defined as overweight. Obesity is defined as an excess accumulation of fat in adipose tissue and adipose tissue is an endocrine organ which is the site of energy storage and has importance in maintaining body weight. In male adipose tissue is distributed above the waist leading to android or apple shape and in female it is below the waist with gynoid or pear shape. There are 4 different types of adipocytes such as white, brown, beige and pink adipocytes. White adipose tissue is responsible for lipogenesis and brown adipose tissue is responsible for thermogenesis.

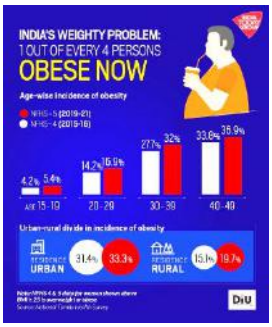
TYPES OF BODY FAT

There are 2 types of fat accumulated in the body namely visceral fat and subcutaneous fat. Visceral fat is surrounding the organs and is measured through CT scans and full-body MRIs are the most precise but expensive

and hence, often use estimates based on waist circumference. Whereas subcutaneous fat is found beneath the skin and measured using skinfold calipers.

OBESITY- INDIA'S STATISTICS

Overview of obesity statistics of Indian population indicates an increasing trend from 2015-16 to 2020-21 and is evident for the age groups between 15 to 49 years, obesity in urban residence is higher compared to rural residence **(**National Family Health Survey, 2022).



CAUSES AND EFFECTS OF OBESITY

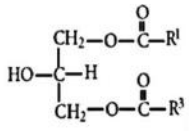
The causative factors for obesity include - emotional factors, sedentary lifestyle, over-eating, medicines, smoking, alcoholism, insomnia and genetic factors. Obesity effects in causing high blood pressure, cancer, cardiovascular disease, diabetes, infertility and sleep apnea.

OREXIGENIC AND ANOREXIGENIC FACTORS OF OBESITY

Orexigenic neurons stimulate eating and are neuropeptide hormones (neuropeptide Y-NPY) represented by orexin, ghrelin. Anorexigenic neurons suppress the appetite, producing melanocyte-stimulating hormone (MSH) represented by leptin and insulin as well as by and peptide-tyrosine-tyrosine.

DIETARY DAG OIL AS AN ANTI-OBESITY AGENT

A new strategy proposed to prevent weight gain and fat deposition is the inclusion of 1,3 DAG–enriched oil into the diet.

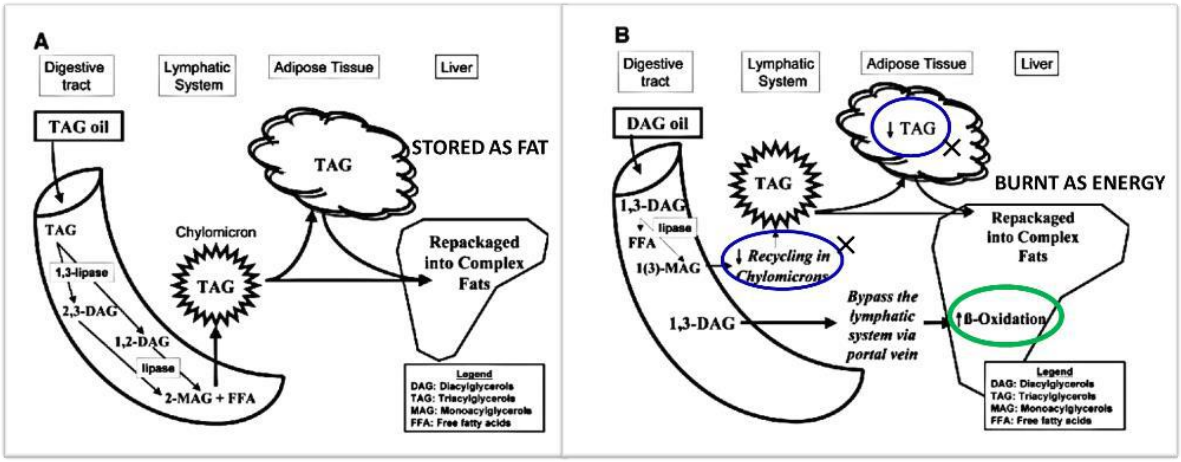


1,3-diacyl-*sn*-glycerol (DAG)

Mechanism of DAG for long-term weight maintenance

1. Reduced chylomicron formation
2. Increased oxidation of fatty acids in small intestine and liver
3. Weight loss

ANTIOBESITY MECHANISM OF DAG OIL



(A) TAG OIL **(B) DAG OIL**

The structural and metabolic characteristics of DAG compared with TAG are believed to be responsible for suppression of body fat accumulation, body weight loss and lower serum TG levels postprandially. However, the underlying mechanisms for these actions remain to be determined. It has been shown that DAG and TAG are almost identical in terms of digestibility and caloric value. Bomb calorimetry energy values are similar for DAG (38.9 kJ/g) vs. TAG (39.6 kJ/g). Beyond the small difference in energy value of both oils, it is the specific structural differences of DAG isomers and not the fatty acid composition of DAG or TAG that explain any differential action on lipid metabolism. During digestion, TAG is hydrolyzed by 1,3-lipases to 1,2-DAG and 2,3-DAG, but not 1,3-DAG, because lipases cleave only at the 1 or 3 position.

Supplementary action of lipase on 1,2-DAG and 2,3-DAG leads to the end products 2-monoacylglycerol and free fatty acids. These are the normal end products of TAG digestion that are absorbed by intestinal mucosal cells and used for reconstruction of circulating chylomicron TGs (Figure 1A). At equilibrium, DAG is composed of 65 to 70 per cent 1,3-DAG and 30 to 35 per cent 1,2-DAG. An enzymatic process can produce DAG, in particular 1,3-DAG oil. The main end products of lipase action on 1,3-DAG are glycerol and free fatty acids, which may be less readily resynthesized to chylomicron TGs.

Moreover, compared with TAG oils, larger amounts of fatty acids from digested DAG may be released into portal circulation rather than being incorporated into chylomicrons. This may also explain why serum TG levels are lower in a fasted state and in the postprandial state in addition to lower TG content of chylomicrons. In addition, this hepatic exposure to fatty acids by increasing DAG intake may lead to greater β-oxidation by the liver than that after TAG intake. Enhanced β -oxidation may lead to increased satiety. Thus, decreasing caloric intake may induce a decrease in weight and fat loss in long-term DAG feedings if the dose of DAG oil is sufficient. This potential mechanism is shown in Figure 1B.

EFFECTS OF DAG ON LIPID METABOLISM, β-OXIDATION AND BODY COMPOSITION

Lipid metabolism - In fasting serum lipids consumption of DAG oil lowers triglyceride, total cholesterol, low-density lipoprotein cholesterol and increases high-density lipoprotein cholesterol. In postprandial serum lipids it reduces triglyceride, remnant-like lipoprotein particle triglyceride, remnant-like lipoprotein particle cholesterol and chylomicron triglyceride.

β-oxidation - Increases hepatic enzymes involved in the β-oxidation such as carnitine palmitoyltransferase, acyl-CoA dehydrogenase, acyl-CoA oxidase, enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase.
Body composition – It lowers body weight, body fat, visceral fat, subcutaneous fat, hepatic fat, waist circumferences, skin fold thickness.

PATENT PUBLICATIONS ON PRODUCTION TECHNOLOGIES AND PRODUCT APPLICATIONS OF DAG OIL

1. Oil-in-water type emulsion (O/W) food products - Mayonnaise and salad dressings (Nomura *et al*., 1992)
2. Shortenings - Comprising a nonhydrogenated vegetable oil (Doucet and Olathe, 1999)
3. Fried foods – Fried cakes, french fried potatoes, fried chicken and doughnuts (Sakai *et al*., 2002a)

COMMERCIAL BRAND OF DAG OIL

Till date the only two commercial brands of DAG oil in the world are ENOVA oil in United States and ECONA oil in Japan. After decades CFTRI has produced 10 kg DAG oil and is ready to market it in the name of anti-obesity oil.

RESEARCH PAPERS

STUDY-1 (Devi *et al*., 2018)
Nutritionally enriched 1,3-diacylglycerol-rich oil: Low calorie fat with hypolipidemic effects in rats

Objective: To study the impact of consuming DAG-rich oil as a part of the diet on serum and tissue lipids

Methodology:

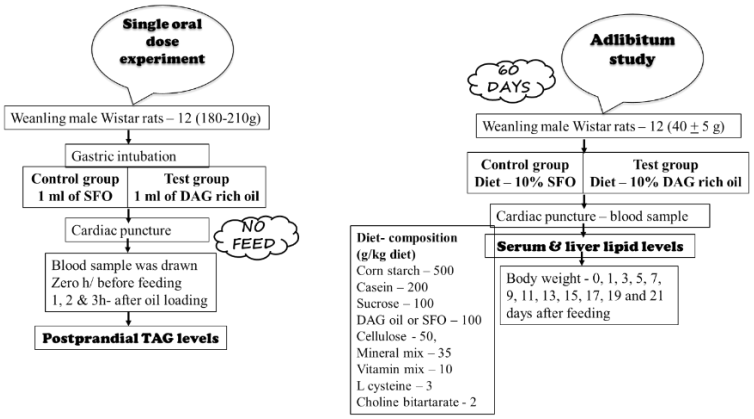
Refined Rice bran oil (RBO) and sunflower oil (SFO) were purchased from local super market. The preparation of 1,3-DAG-rich oil comprises of enzymatic hydrolysis of vegetable oil mixture consisting of RBO and SFO into fatty acids, followed by esterification of the fatty acids with glycerol and purification of DAG-rich oil by separation techniques. Male Wistar rats (Out Bred strain- Wistar rats, IND- cft (2c) (Rattus norvegicus) were grouped by random distribution (n = 6 animals per group) for the experiments. The rats were housed in individual cages in a room whose temperature was maintained at 25 ± 2 °C with lights on for 12 h daily in an animal house facility at Central Food Technological Research Institute, Mysore, India. Approvals for animal experiments were obtained from Institutional Animal ethical committee recognized by Government of India. Fresh tap water and food was provided daily.

The single oral dose experiment was conducted and the weights of the rats used in this experiment were in the range of 180–210 g. The rats were administered orally by gastric intubation 1 ml of SFO or 1 ml of DAG-rich oil. Blood was collected by cardiac puncture before feeding (zero hour) and at 1, 2, and 3 h after oil loading. During the period of experiment no feed was provided to the rats.

Twelve weanling Wistar male rats with initial body weights of 40 ± 2 g were fed AIN- 93 diet (Reaves, Nielsen, & Fohey, 1993) containing 10 per cent SFO as control or 10 per cent DAG-rich oil as a test oil. The basal composition of the diet (g/kg diet) consists of corn starch 500, casein 200, sucrose 100, DAG oil or SFO 100, cellulose 50, mineral mix 35, vitamin mix 10, L cysteine 3 and choline bitartarate 2. After initial acclimatization, the rats were distributed randomly into 3 groups of six rats each. One group of rats was maintained on ad libitum diet containing 10 per cent SFO. The rats in the test groups received 50 per cent of the diet consumed previous day by ad libitum fed groups. Feed consumption was monitored daily. Rats on restricted

diet consumed all feed with no spillage. Body weights were recorded on 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19 and 21 days after feeding SFO or DAG-rich oil containing diets. Calorific availability of DAG-rich oil was determined by comparing growth of rats fed DAG-rich oil with that of rats fed SFO.

Analysis- Serum and liver lipid levels, body weight was recorded. Data obtained was analysed using suitable statistical tools.



RESULTS

Table 1: Effect of glycerol to fatty acid ratio for the preparation of 1,3-DAG-rich oil

Glycerol/ Fatty acid (Molar ratio)	Conversion (wt%)		
	TAG	DAG	MAG
1:1	6.3 ± 1.2	44.5 ± 1.1	49.2 ± 1.0
1:2	19.5 ± 0.5	63.4 ± 1.3	17.1 ± 1.2
1:3	60.1 ± 1.2	32.7 ± 1.6	7.2 ± 1.0
1:4	62.6 ± 1.5	24.3 ± 0.9	13.1 ± 0.6

It was indicated that the formation of DAG was higher when the glycerol to fatty acid molar ratio is 1:2, whereas the formation of TAG was higher when the glycerol to fatty acid molar ratio is ≥1:3 (Table 1). The optimum conditions for the preparation of ~63 per cent of DAG in the crude product is as follows: glycerol to fatty acid ratio, 1:2 wt/wt; dosage of the enzyme, 10% (wt% of substrates); temperature, 68–70 °C and the reaction period, 12 h.

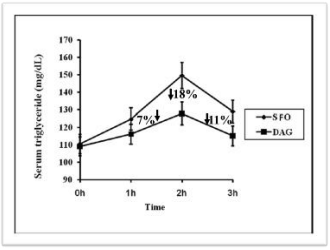


Fig.1. Postprandial serum triglyceride levels in rats given 1 ml of SFO or DAG-rich oil by intubation

The SFO contained about 95 per cent TAG along with 2.6 per cent of DAG, whereas DAG-rich oil prepared in the present study contained only 16 per cent TAG and 84 per cent DAG. This may differentially impact the postprandial serum triglyceride levels. To evaluate this, the rats were given 1 ml of SFO or DAG-rich oil by intubation. The triglyceride level in serum was monitored at different time intervals (Fig. 1). The postprandial triglyceride level in serum of rats given DAG-rich oil by intubation was significantly lower as

compared to rats given equivalent amounts of SFO. The postprandial serum triglycerides observed at 1, 2 and 3 h, was lesser by 7, 18 and 11 per cent respectively in rats given DAG-rich oil as compared to those given SFO.

Table 2: Serum and liver lipid levels in rats given SFO or 1,3-DAG-rich oil

Parameters	Serum lipid		Liver lipid	
	SFO	DAG-rich oil	SFO	DAG-rich oil
Cholesterol (mg/dL)	108.4 ± 3.0[b]	83.9 ± 2.1[a] (23% ↓)	9.0 ± 1.5[b]	7.1 ± 1.2[a] (21% ↓)
HDL cholesterol (mg/dL)	37.2 ± 2.4[a]	34.6 ± 1.5[a] (7% ↓) NS	n.d	n.d
LDL + VLDL (mg/dL)	71.2 ± 3.7[b]	49.3 ± 3.1[a] (31% ↓)	n.d	n.d
Triglycerides (mg/dL)	149.5 ± 7.6[b]	116.9 ± 4.5[a] (22% ↓)	19.2 ± 1.2[b]	15.3 ± 1.0 [a] (20% ↓)

To evaluate long term implications of consuming DAG-rich oil, rats were fed a diet containing 10 wt% fat in the form of DAG-rich oil or SFO. Rats fed a diet containing DAG-rich oil showed significantly lower amounts of cholesterol and triglycerides in serum by 23 and 22% respectively as compared to the rats given SFO (Table 2). LDL levels in rats given DAG-rich oil were reduced by 31% as compared to those given SFO. HDL was marginally reduced by 7% in rats given DAG-rich oil which was statistically not significant. Rats fed with DAG-rich oil showed significantly lower amounts of cholesterol and triglycerides in liver by 21 and 20% respectively as compared to those given SFO (Table 2). Thus DAG-rich oil exhibited hypolipidemic effects.

Conclusion

Enzymatically developed 1,3-DAG-rich oil using a blend of rice bran and sunflower oils with nutraceutical molecules such as γ-oryzanol, tocotrienol and phytosterols can be exploited as low-calorie fat with anti-obesity potential.

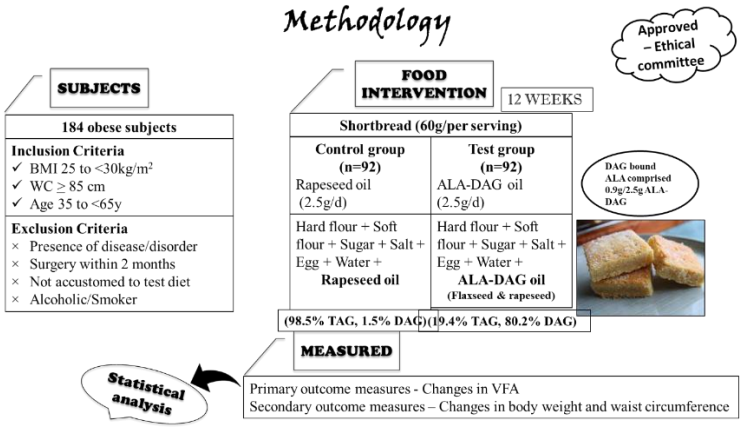
STUDY-2 (Saito *et al.*, 2016)

Consumption of alpha-linolenic acid enriched diacylglycerol reduces visceral fat area in overweight and obese subjects

Objective: To investigate the suppressive effects of ALA-DAG consumption on VFA in obese people

Methodology:

This was a randomized, double-blind controlled, parallel-group designed study with a 12-wk treatment period and a subsequent 4-wk non-treatment period. Changes in VFA as the primary outcome were measured in all subjects during the consumption of 2.5 g/d of control TAG (rapeseed oil) or ALA-DAG (containing 0.89 g/d DAG-bound ALA) in addition to their daily diet for 12 wk. Secondary outcomes were changes in body weight, waist circumference, and safety parameters. Subjects were instructed to incorporate the test food into their daily diet (breakfast, lunch, or dinner) and not to consume between meals. Based on dietary records for the 3d before the measurements, the amounts of nutrients consumed were analyzed. The subjects were instructed to limit their alcohol consumption to less than 30 g/d and to maintain their usual exercise habits during the study. To confirm their physical activity level, the subjects recorded their number of daily steps measured using à pedometer. Author here has chosen alpha-linolenic acid based on human and rodent studies because it easily oxidizes by the body than palmitic, stearic, oleic and linoleic acid.



Analysis: After food intervention period of 12 weeks obese people were measured for changes in VFA as a primary outcome and changes in body weight and waist circumference as secondary outcome measures. The obtained data was then analysed using suitable statistical tools.

RESULTS

Table 1: Changes in VFA in the FAS and in the subjects with VFA > 140 cm2

Group	wk 0	wk 4	wk 8	wk 12	wk +4	⊿ at wk 12
FAS						
(N=92) TAG	127 ± 27	127 ± 29	129 ± 29	127 ± 29	123 ± 29	+0 ± 11
(N=92) ALA-DAG	128 ± 26	129 ± 27	129 ± 28	124 ± 27	124 ± 30	−4 ± 14*
≥ 140 cm^2						
(N=24) TAG	163 ± 18	163 ± 23	161 ± 21	163 ± 22	158 ± 21	+0 ± 13
(N= 25) ALA-DAG	162 ± 15	160 ± 19	161 ± 20	154 ± 20	161 ± 23	−9 ± 15*

Data are mean ± SD. *$p < 0.05$ between groups.

Changes in VFA at 12-wk from baseline were significantly lower in the ALA-DAG group than in the TAG group, both in the FAS samples and in subjects with VFA ≥ 140 cm^2 who were in the top quartile (Table 2). A greater effect was found in the subjects with VFA ≥ 140 cm^2 and the reduction of VFA was significantly correlated with the baseline VFA in the ALA-DAG group, but not in the TAG group.

Table 2: Changes in body weight (BW) and waist circumference (WC) in FAS

Group	wk 0	wk 4	wk 8	wk 12	wk +4	⊿ at wk 12
BW, kg						
TAG	71.5 ± 7.8	71.7 ± 7.8	71.8 ± 7.8	72.0 ± 7.7	72.0 ± 7.7	+0.6 ± 0.1
ALA-DAG	71.1 ± 7.5	71.4 ± 7.4	71.4 ± 7.4	71.2 ± 7.5	71.6 ± 7.5	+0.1 ± 1.1*
WC, cm						
TAG	95.8 ± 4.8	96.0 ± 5.2	96.0 ± 5.3	96.0 ± 5.1	95.2 ± 4.9	+0.2 ± 1.5
ALA-DAG	95.8 ± 5.3	96.2 ± 5.3	96.0 ± 5.7	95.4 ± 5.9	95.3 ± 5.8	−0.4 ± 2.3*

Data are mean ± SD. *$p < 0.05$ between groups.

In the FAS, changes in body weight and waist circumfer- ence were significantly lower in the ALA-DAG group com- pared with the TAG group (Table 2).

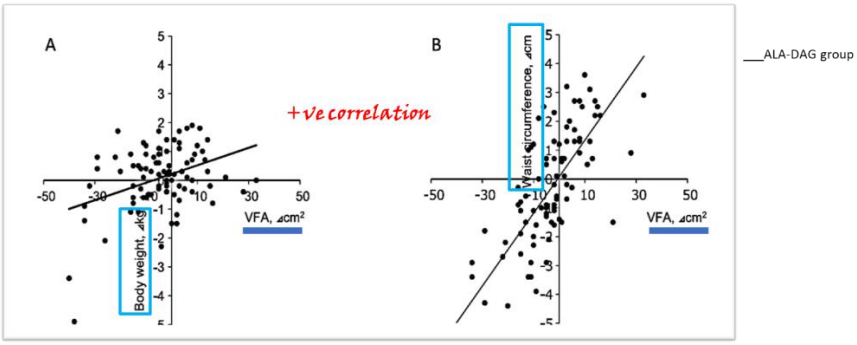


Fig.1. Correlation between changes in (A) VFA and body weight (B) VFA and waist circumference in ALA-DAG groups

Body weight in the ALA-DAG group did not change, however, and the change in body weight differed significantly between groups. Additionally, changes in the VFA and body weight (Fig. 1) were significantly correlated in the ALA-DAG group, suggesting that ALA-DAG suppressed weight gain, possibly *via* reduction in VFA.

Conclusion

ALA-DAG could be useful for reducing VFA and concomitantly suppressing weight gain with no side effects. Hence, it can be used as an anti-obesity dietary supplement.

<u>STUDY-3</u> (Mengke *et al*., 2018)

Diacylglycerol-enriched oil from hydrolysis of soybean oil with *Rhizopus oryzae* lipase against high-fat diet-induced obesity in mice

Objective: To investigate the impact of intake of DAG from soybean oil on the reduction of fat accumulation and its long-term effect on the development of obesity

Methodology:

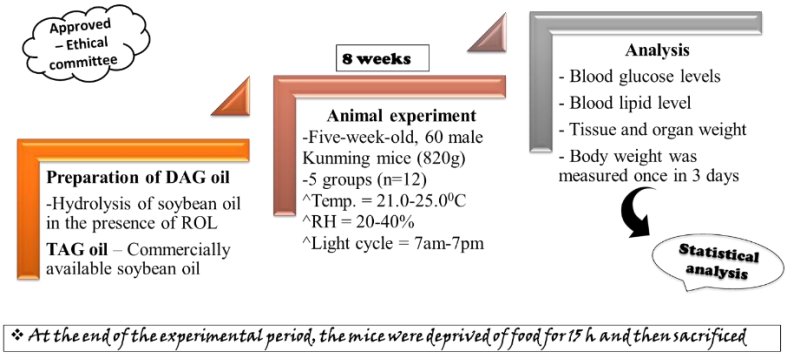


Table 1: Experimental groups

Group	Treatment	Dosage (g/kg BW)	Number of male animals
Control	Equal volume of distilled water	-	12
Soybean oil low-dose	Soybean oil	2.5	12
Soybean oil high-dose	Soybean oil	10	12
Modified oil low-dose	Modified soybean oil	2.5	12
Modified oil high-dose	Modified soybean oil	10	12

Five-week-old male Kunming mice, weight 820 g, were obtained from PLA Military Academy of Medical Sciences Laboratory Animal Center (Beijing, China). All mice (n = 12 in each group) were maintained under a 12 h light/dark cycle (light cycle: 7 AM to 7 PM). The mice were kept in an animal room in which the temperature was maintained at 21.0–25.0^0 C, the relative humidity was 20–40 per cent and the air in the animal room was changed at 12 h intervals. The mice were given a 1 week acclimation period to stabilize the metabolic conditions and then randomly divided into five groups (n = 12) shown in Table 1.

In each group, the mice were administered their respective test oils by gavage once per day for 8 weeks, in addition to basic forage. Basal animal feed was provided by Beijing Vital River Laboratory Animal Feed Science and Technology Co., Ltd. (Beijing, China). The mice were given free access to the basic forage and water. During the trial period, the animals were cared for in accordance with the principles for the use of animals for research and education, following the Statement of Principles adopted by the FASEB Board. The weight of the mice and the food intake were measured once every 3 days. At the end of the experimental period, the mice were deprived of food for 15 h and then sacrificed.

Analysis- Blood glucose level, serum lipid levels, mass index of tissues and organs. The data obtained was analysed using suitable statistical tools.

RESULTS

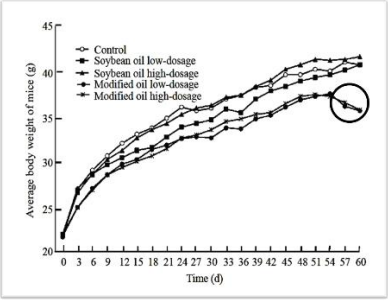


Fig. 1. The effect of different oil diets on the body weight of mice

The changes in body weight gain and food intake in mice fed with the test oils for 8 weeks are shown in Fig. 1. It showed that the initial body weights of mice were similar in each group. All groups experienced a gain in body weight. The weight of mice in the soybean oil low-dose group was significantly lower than that of mice in the control group ($P < 0.05$). However, the group fed for 8 weeks with the soybean oil high-dose diet was similar in body weight to the mice in the control group. The body weight gain was markedly reduced by administration of the modified oil diets compared with the control group ($P < 0.01$), whereas no significant differences were observed between the groups administered the high-dose and low-dose of the modified oil.

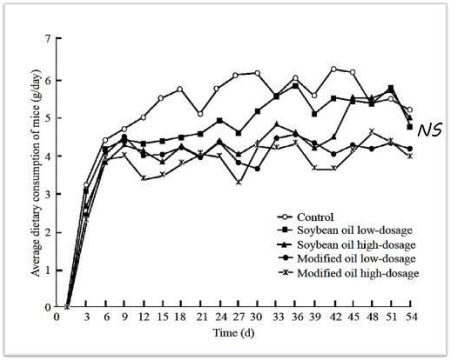


Fig. 2. The effect of different oil diets on the average dietary consumption of mice

The explanation of the discrepancy between DAG oil and TAG oil on body fat accumulation is related to the different effects on energy expenditure and energy intake exerted by these two test oils. No significant differences in food consumption between the mice fed different diets were observed in the present study, as shown in Fig. 2. As the apparent energy value of DAG oil was almost identical to that of conventional oil, the energy intake was not significantly different in the soybean oil and modified oil groups. Thus, the differences in weight and body fat loss observed from DAG oil consumption may result from the increased energy expenditure.

Table 2: Effects of different diets on the blood sugar of normal mice

Group	Dosage (g/kg BW)	Number (*n*)	Blood sugar (mmol/L)		
			Fasting 3 h after 30 days	Delivery after 58 days at random	Fasting 5 h after 59 days
Control	-	12	6.67 ± 1.06	7.90 ± 0.90	7.12 ± 0.70
Soybean oil	2.5	12	8.76 ± 1.14^{cd} ↑	8.88 ± 0.65^{b} ↑	7.90 ± 0.61^{b} ↑
Soybean oil	10	12	8.64 ± 0.72^{cd} ↑	9.04 ± 1.08^{a} ↑	8.03 ± 0.61^{b} ↑
Modified oil	2.5	10	8.39 ± 1.50^{b} ↓	7.21 ± 1.69^{f} ↓	6.33 ± 1.16^{d} ↓
Modified oil	10	12	7.85 ± 1.05^{ad} ↓	6.98 ± 1.36^{f} ↓	5.80 ± 1.50^{ae} ↓

Note: The values are means ± SD. [a] denotes $P < 0.05$, [b] denotes $P < 0.01$, [c] denotes $P < 0.001$ *vs* control group; [d] denotes $P < 0.05$, [e] denotes $P < 0.01$, [f] denotes $P < 0.001$ *vs* the original soybean oil.

After 30 days, the blood sugar level of mice in both the soybean oil groups was significantly higher than the control group ($P < 0.001$) (Table 2). In addition, the increase in the levels of fasting glucose after consumption of modified oil was smaller ($P < 0.05$) compared with that after consumption of soybean oil. After 60 days, the glucose and fasting plasma glucose in the soybean oil groups were still significantly higher than control group ($P < 0.05$ and $P < 0.01$), whereas the random blood glucose and fasting blood glucose of the modified oil groups were lower than normal control group and significantly lower than the soybean oil groups ($P < 0.001$); a dose-response relationship was also observed. The serum glucose levels in soybean oil groups were markedly higher ($P < 0.05$) than the control group, whereas the modified oil diet resulted in a reduction in glucose level compared with the soybean diet.

Table 3: Effects of different diets on the blood lipids of normal mice

Group	Dosage (g/kg BW)	Number (*n*)	Serum lipids (mmol/L)		
			Serum TG	Total cholesterol	HDL-cholesterol
Control	-	12	1.74 ± 0.49	3.38 ± 0.30	2.99 ± 0.26
Soybean oil	2.5	12	1.87 ± 0.28	3.79 ± 0.48[a]	3.26 ± 0.42
Soybean oil	10	12	1.95 ± 0.32	4.48 ± 0.66[c]	3.81 ± 0.54[c]
Modified oil	2.5	10	1.65 ± 0.34	4.13 ± 0.68[b]	3.47 ± 0.57[a]
Modified oil	10	12	1.38 ± 0.24[a]	4.44 ± 0.63[c]	3.71 ± 0.63[a]

The final concentrations of plasma triacylglycerol, total cholesterol, low density lipid cholesterol, and high-density lipid cholesterol in mice fed the experimental diets for 8 weeks were compared (Table 3). The plasma total cholesterol levels of mice fed the soybean oil and modified oil were higher than control group, and a dose-response relationship was observed ($P < 0.01$ and $P < 0.001$). However, the serum total cholesterol levels of mice fed the modified oil diet were significantly lower than those in the soybean oil high-dose group (Table 3). In addition, the serum triacylglycerol levels were higher in the mice fed soybean oil than in the control group. In contrast, elevated levels of serum triacylglycerol, caused by the soybean oil diet, were significantly suppressed by the modified oil diet. In addition, the serum HDL level of the soybean oil high-dose group and both modified oil groups were markedly higher ($P < 0.001$ and $P < 0.05$) than that of the control group, with an obvious dose-response relationship observed. In contrast, there also was a significant improvement in LDL concentrations in the modified oil groups compared with soybean oil groups ($P < 0.05$).

Table 4: Absolute tissue and organ weights in an 8-week study in mice

Group	Dosage (g/kg BW)	Number (*n*)	Weight (g)			
			Liver	Kidney	Spleen	Epididymal white adipose
Control	-	12	2.226 ± 0.260	0.692 ± 0.072	0.131 ± 0.070	0.802 ± 0.192
Soybean oil	2.5	12	2.094 ± 0.225	0.671 ± 0.103	0.115 ± 0.020	0.728 ± 0.264
Soybean oil	10	12	2.088 ± 0.238	0.653 ± 0.092	0.129 ± 0.022	0.829 ± 0.427
Modified oil	2.5	10	1.771 ± 0.239[b]	0.561 ± 0.100[b]	0.096 ± 0.022	0.664 ± 0.339
Modified oil	10	12	1.643 ± 0.211[c]	0.538 ± 0.091[c]	0.096 ± 0.024	0.642 ± 0.208

Note: The values are means ± SD. [a] denotes $P < 0.05$, [b] denotes $P < 0.01$, [c] denotes $P < 0.001$ *vs* control group.

As shown in Table 4, no significant changes in organ weights were observed in the soybean oil groups compared with the control group. However, the weights of liver and kidney in the modified oil low-dose and high-dose groups were dramatically lower than in the control group ($P < 0.01$ and $P < 0.001$). In contrast, body weight is not a good indicator of obesity as it does not measure the total fat accumulation in the body; therefore, the epididymis was dissected for further observations. The weight of epididymis white adipose tissue was not found to be different between the groups. However, a lower mass index of epididymis white adipose tissue (relative to the final body weight) in the modified oil high-dose group was observed compared with the control group ($P < 0.01$).

Conclusion

The DAG oil from soybean oil can be used as potent functional oil for the suppression of high-fat diet-induced obesity and cardiovascular diseases as its consumption reduced body weight gain, plasma total cholesterol, triacylglycerol and glucose compared with a TAG oil.

STUDY-4 (Lu *et al.*, 2020)
Effects of diacylglycerol and triacylglycerol from peanut oil and coconut oil on lipid metabolism in mice

Objective: To investigate the effects of DAG on body fat, blood lipids, lipid metabolism related enzymes in the liver and adipose tissue of C57BL/6J mice

Methodology:

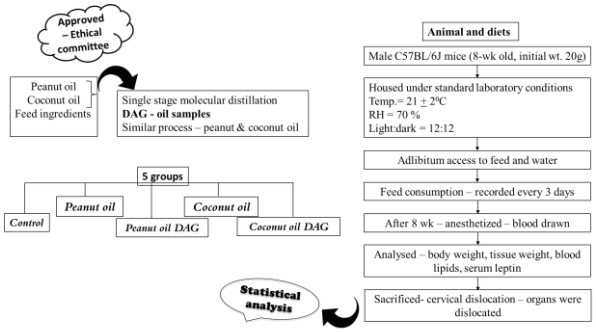


Table 1: Composition of different diets

Ingredient	Normal diet	Peanut oil	Peanut oil DAG	Coconut oil	Coconut oil DAG
Corn starch (%)	58	43	43	43	43
Casein (%)	20	20	20	20	20
Oil (%)	5 (peanut oil)	20	20	20	20
Sucrose (%)	10	10	10	10	10
Cellulose (%)	2	2	2	2	2
Vitamin premix (%)	1	1	1	1	1
Mineral premix (%)	3.5	3.5	3.5	3.5	3.5
L-cystine (%)	0.3	0.3	0.3	0.3	0.3
Choline chloride (%)	0.2	0.2	0.2	0.2	0.2
Dietary fat (g/100 g diet)	5	20	20	20	20
Kcal/100g	397	472	472	472	472
DAG content (g/100 g diet)	0	0	12	0	12

Male C57BL/6J mice ranging from 8 weeks old, with the initial body weights of 20 g, were purchased from Hunan Slack Jingda Experimental Animal Co., Ltd (Hunan, China). The mice were housed under standard laboratory conditions of 21 ± 2°C, relative humidity of 70%, and 12:12 hr light–dark cycle maintained during the experiment. Animals were kept in the animal laboratory of the Animal Science Department of Nanchang University, and were allowed ad libitum access to water and food. The compositions of the experimental diets are listed in Table 1. The mice were randomly divided into five groups with 10 mice each, including control group, peanut oil group, peanut oil DAG group, coconut oil group, and coconut oil DAG group.

Feed consumption was recorded every 3 days during the experiment. After 8 weeks of feeding, all mice were weighed and anesthetized with ether to take blood. Then the mice were sacrificed by cervical dislocation and their organs were dislocated, including the heart, kidney, liver, spleen, and epididymal fat pad. The protocols were performed in strict accordance with the guideline for animal experiments in research laboratories and the PR China legislation. The experiments were approved by the Animal Ethics Committee of Nanchang University. All efforts were made to minimize mice suffering and to reduce the number of mice used.

Table 2: Effects of different oils on body and tissue weight and food intake of C57BL/6J mice

($n = 10$)

	Normal diet	Peanut oil	Peanut oil DAG	Coconut oil	Coconut oil DAG
Initial weight (g)	20.12 ± 0.91	20.16 ± 0.92	20.18 ± 0.89	20.09 ± 1.2	20.04 ± 0.75
Final weight (g)	23.12 ± 0.93^{a}	25.5 ± 1.00^{d}	24.05 ± 0.95^{bc}	24.81 ± 0.91^{cd}	23.73 ± 0.80^{ab}
Heart (g)	0.13 ± 0.01^{a}	0.15 ± 0.02^{b}	0.12 ± 0.01^{a}	0.13 ± 0.02^{ab}	0.12 ± 0.02^{a}
Liver (g)	0.79 ± 0.06^{a}	0.89 ± 0.08^{b}	0.82 ± 0.09^{ab}	0.86 ± 0.12^{ab}	0.84 ± 0.08^{ab}
Spleen (g)	0.08 ± 0.01^{ab}	0.08 ± 0.01^{ab}	0.09 ± 0.01^{b}	0.08 ± 0.01^{ab}	0.07 ± 0.01^{a}
Kidney (g)	0.37 ± 0.03^{bc}	0.4 ± 0.03^{c}	0.35 ± 0.03^{ab}	0.39 ± 0.04^{c}	0.33 ± 0.03^{a}
Epididymal fat pad (g)	0.35 ± 0.07^{a}	0.46 ± 0.06^{b}	0.35 ± 0.07^{a}	0.37 ± 0.05^{a}	0.38 ± 0.03^{a}
Daily feed consumption (g/per mice)	3.88 ± 0.23	3.83 ± 0.12	3.66 ± 0.33	3.85 ± 0.15	3.70 ± 0.34

Table 2 shows that in all groups body weight was increased but in peanut oil and coconut oil DAG group it was significantly lower. Compared to peanut oil, coconut oil DAG group gained less weight. The organ and tissue weight of heart, liver, spleen, kidney and epididymal fat tissue were less in modified oil compared to refined oil. No significant difference was observed in feed consumption.

Table 3: Effects of different oils on blood lipids in C57BL/6J mice

($n = 10$)

Index	Normal diet	Peanut oil	Peanut oil DAG	Coconut oil	Coconut oil DAG
Serum					
TG (mmol/L)	1.38 ± 0.32^{a}	1.82 ± 0.50^{b}	1.70 ± 0.30^{ab}	1.44 ± 0.40^{a}	1.41 ± 0.25^{a}
TC (mmol/L)	3.46 ± 0.26	3.41 ± 0.40	3.52 ± 0.28	3.56 ± 0.24	3.52 ± 0.33
HDL-C(mmol/L)	3.07 ± 0.40^{ab}	2.88 ± 0.44^{a}	3.29 ± 0.35^{ab}	3.44 ± 0.48^{b}	3.32 ± 0.41^{b}
LDL-C (mmol/L)	0.39 ± 0.08	0.36 ± 0.08	0.36 ± 0.04	0.36 ± 0.10	0.45 ± 0.17

The TG level of peanut oil group was higher than other groups and was significantly lower in peanut oil DAG groups (Table 3). The HDL cholesterol in coconut oil and coconut oil DAG groups were significantly higher than other groups.

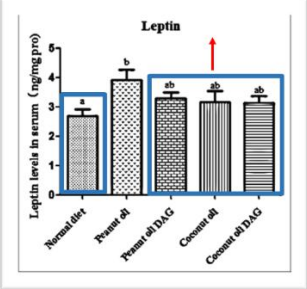


Fig.1. Effect of different oils on serum leptin content in C57BL/6Jmice

The serum leptin content (Fig.1) in peanut oil group was significantly higher than that in control group ($P < 0.05$, Figure 1). The serum leptin content of peanut oil DAG group (3.28 ± 0.54 ng/mg), coconut oil group (3.17 ± 0.87 ng/mg) and coconut oil DAG group (3.15 ± 0.57 ng/mg) were much lower than that of peanut oil group (3.93 ± 0.86 ng/mg), but higher than that of the control group (2.70 ± 0.53 ng/mg).

Conclusion

The DAG with different fatty acid chain lengths were synthesized from peanut and coconut oils. Compared with TAG (peanut oil and coconut oil), medium-chain DAG exhibited more potent effect than long- chain DAG.

STUDY-5 (Zhang *et al*., 2019)

Diacylglycerol oil reduces fat accumulation and increases protein content by inducing lipid catabolism and protein metabolism in Nile tilapia (*Oreochromis niloticus*)

Objective: To evaluate the role of DAG oil on lipid utilization and protein deposition in Nile tilapia

Methodology

The use of animals in this research was approved by the Animal Ethics Committee of East China Normal University. Diolein (1, 3-diacylglycerol oil, DGO, >97%), triolein (triacylglycerol oil, TGO,>97%) and other feed ingredients were procured from local market. The total amount of dietary oil was fixed as 5 per cent by weight. The oil in three diets was prepared by incorporating different contents of DGO and TGO (W/W) in the diets: control (0% DGO+100% TGO), 0.5% DGO (10% DGO+90% TGO, 0.5% DGO in diet) and 2.5 per cent DGO (50% DGO+50% TGO, 2.5% DGO). All ingredients were ground into fine power through 280 μM mesh and completely mixed by hand and then by machine. Oil mixture and water were then added to make a dough. Pellets were made by a pellet-making machine. After drying for 3 days, all diets were stored in plastic bags at −20 °C and thawed at room temperature just before feeding.

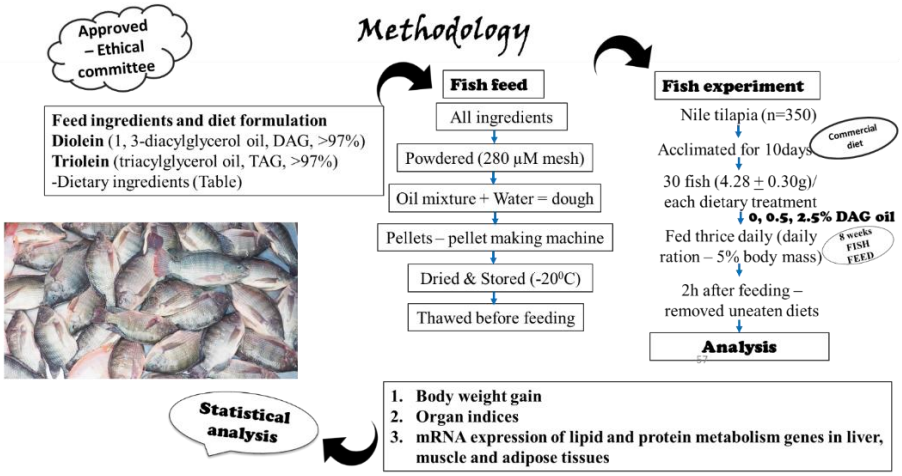


Table: List of dietary ingredients

Ingredients	0% DGO (control)	0.5% DGO	2.5% DGO
Casein	280	280	280
Gelatin	70	70	70
TGO[a]	50	45	25
DGO[b]	0	5	25
Corn starch	320	320	320
Vitamin pre-mix[c]	5	5	5
Mineral pre-mix[d]	5	5	5
Carboxymethyl cellulose	50	50	50
Cellulose	204.75	204.75	204.75
Choline chloride	5	5	5
Butylated hydroxytoluene	0.25	0.25	0.25
$CaHPO_4 \cdot 2H_2O$	10	10	10
Total	1000	1000	1000

Three hundred and fifty Nile tilapia were bought from Yueqiang Company (Guangzhou, China) and acclimated in 4 tanks (500 L) for 10 days. Fish were supplied with compressed air via air-stones from air pumps at a 10 h/14 h light/dark cycle and water quality parameters in the range suitable for Nile tilapia growth and survival. During this period, the fish were hand-fed using a commercial diet (33% protein and 5% lipid, Guangzhou, China). After 10 days of acclimation, 30 fish (4.28 ± 0.30 g) were randomly distributed in nine 300 L tanks with three replicates of each dietary treatment. To determine the effect of DGO on lipid accumulation in Nile tilapia, all fish were fed thrice daily (at 0800,1200 and 1700) with the daily ration of 5% body mass using diets containing different doses of DGO (0, 0.5%, 2.5%) for 8 weeks. Two hours after each feeding, uneaten diets were removed by siphon, dried and weighed to determine feed conversion ratio (FCR). All fish were cultured in a recirculating aquaculture system with dissolved oxygen>6.5 mg/L.

Analysis – Fish were then analysed for gain in body weight, tissue and serum biochemical indices, mRNA expression of lipid and protein metabolism genes in liver, muscle and adipose tissue. The data obtained was then analysed using suitable statistical tools.

RESULTS

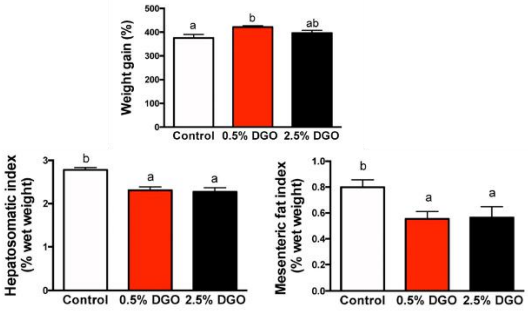


Fig. 1. Growth performance (weight gain), hepatosomatic index and mesenteric fat index (organ indices) of *O. niloticus*

The Nile tilapia fed 0.5% DGO showed a higher weight gain (WG) compared to the control ($P < .05$). The HSI and MFI in fish fed both DGO doses were reduced significantly in comparison to the control but there was no significant difference between these two doses.

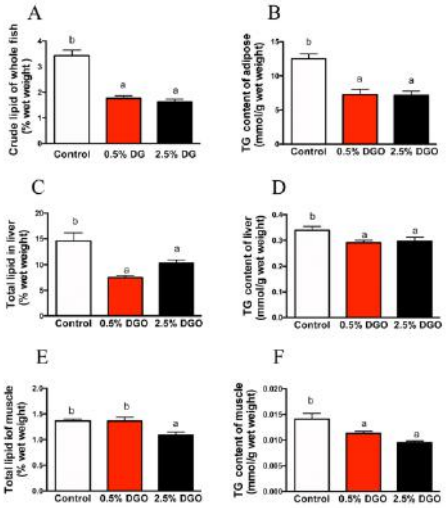


Fig. 2. Total lipid and TG content of *O. niloticus* fed with 0.5% and 2.5% DGO diet

Total lipid of (A) whole fish (C) liver tissue (E) muscle tissue
TG content of (B) adipose (E) liver (F) muscle tissues

The DGO diets significantly decreased the total lipid in whole fish, liver and muscle tissues ($P < .05$; Fig. 2 A, C and E). Similarly, the TG contents in adipose, liver and muscle tissues were significantly decreased after fed with 0.5% DGO but were not significantly different between both doses (Fig. 2 B, D and F).

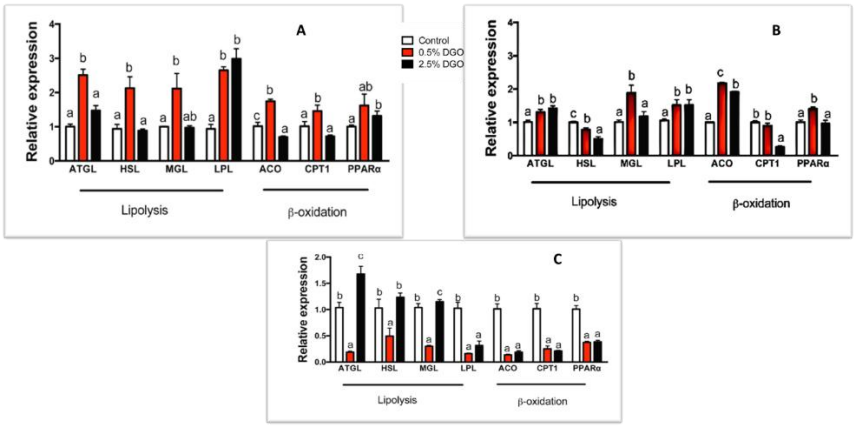


Fig. 3. The mRNA expression of lipid metabolism genes in liver, muscle and adipose tissues of *O. niloticus* (A) liver (B) muscle (C) adipose tissues

In both liver and muscle tissue (Fig. 3. A and B) genes responsible for lipolysis and β-oxidation were up-regulated in 0.5 per cent DGO fed fish group whereas opposite effect was noticed in fish fed with 2.5 per cent DGO. But in adipose tissue (Fig.3. C), fish fed with 2.5 per cent DGO exhibited up-regulation of gene responsible for lipolysis and β-oxidation compared to 0.5 per cent DGO fed groups.

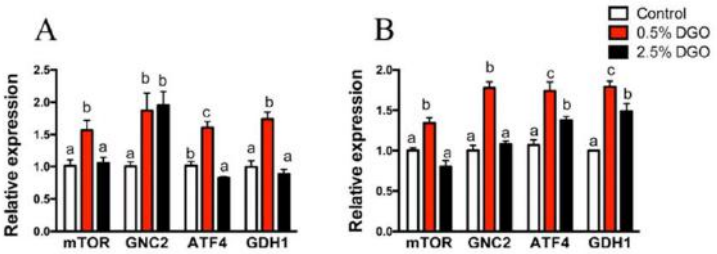


Fig. 4. The protein metabolism mRNA relative expression of *O. niloticus* (A) liver (B) muscle tissues

In protein metabolism genes, mechanistic target of rapamycin kinase (mTOR), eukaryotic translation initiation factor 2 alpha kinase 4 (GCN2IF2), activating transcription factor 4 (ATF4) and glutamate dehydrogenase 1 (GDH1) were increased significantly in the liver and muscle tissues of the fish fed on 0.5 per cent DGO ($P < .05$; Fig. 4 A and B). However, in the 2.5 per cent DGO treatment, mTOR and GDH1 in the liver and muscle did not differ from the control, and GCN2 in the muscle was not different from the control either ($P > .05$; Fig. 4 A and B).

Conclusion

The proper dose of dietary DGO can improve lipid utilization, promote protein deposition and could be a potential additive in aquaculture feeds. These fish hence, when consumed by humans will benefit with anti-obesity property.

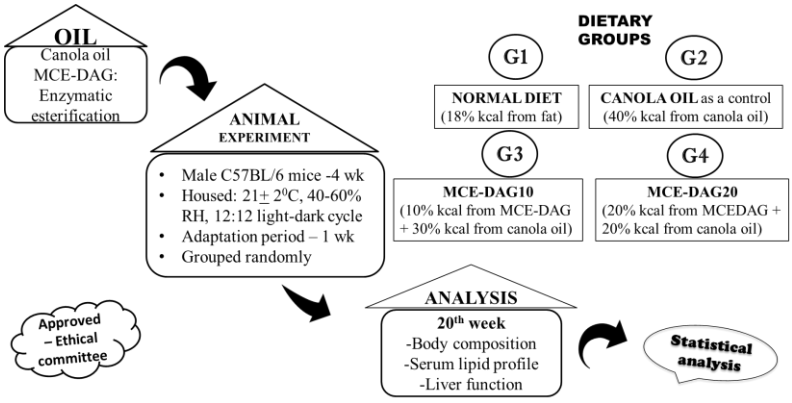
STUDY-6 (Kim *et al*., 2017)

Medium-Chain Enriched Diacylglycerol (MCE-DAG) Oil Decreases Body Fat Mass in Mice by Increasing Lipolysis and Thermogenesis in Adipose Tissue

Objective: To investigate the effects of partial replacement of conventional canola oil with dietary MCE-DAG oil on body weight, body fat mass, lipid profiles in serum, lipolysis and thermogenesis

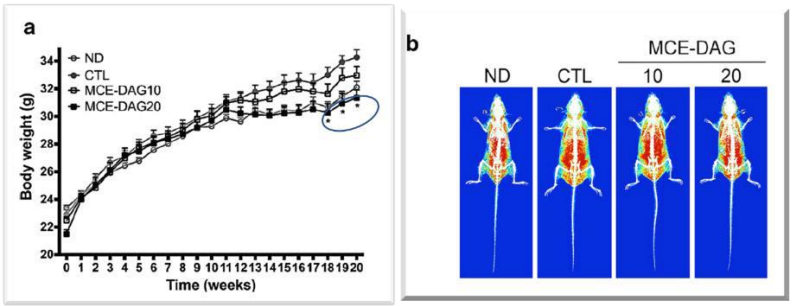
Methodology

Male C57BL/6 mice (4 weeks old) were obtained from Daehan Bio Link (Chung-buk, Korea). Mice were maintained in individually ventilated cages at 21 ± 2 °C, 40–60 per cent relative humidity and a 12:12 h light–dark cycle. After an adaptation period of 1 week, mice were randomly assigned to four groups as follows: (1) normal diet (18% kcal from fat), (2018S, Harlan Laboratories, IN, USA), (2) canola oil as a control (40% kcal from canola oil), (3) MCE-DAG10 (10% kcal from MCE-DAG + 30% kcal from canola oil) and (4) MCE-DAG20 (20% kcal from MCEDAG + 20% kcal from canola oil). Canola oil was used as a control in that it is widely consumed for edible oil.

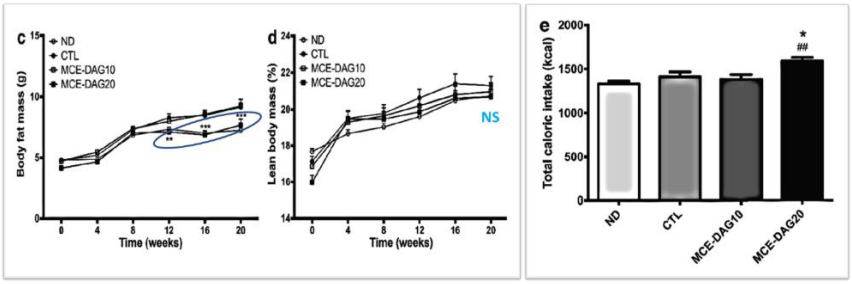


Mice had access to feed and water ad libitum. Feed intake and animal weight were measured every week. In week 20, mice were anesthetized after an overnight fast. Blood was collected from the retro-orbital sinus to determine the levels of plasma biomarkers. Collected blood was settled at room temperature for 30 min, and the serum was separated by centrifuging at 2000rpm for 15 min at 4 °C. Tissues including liver, subcutaneous WAT, quadriceps femoris muscle, and interscapular BAT were dissected, and the samples were snap frozen, followed by storage at −80 °C until further analysis. The animal protocol was approved by the Institutional Animal Care and Use Committee of Kookmin University (KMU-2015-1).

Results



a. Body weight b. Image of DEXA



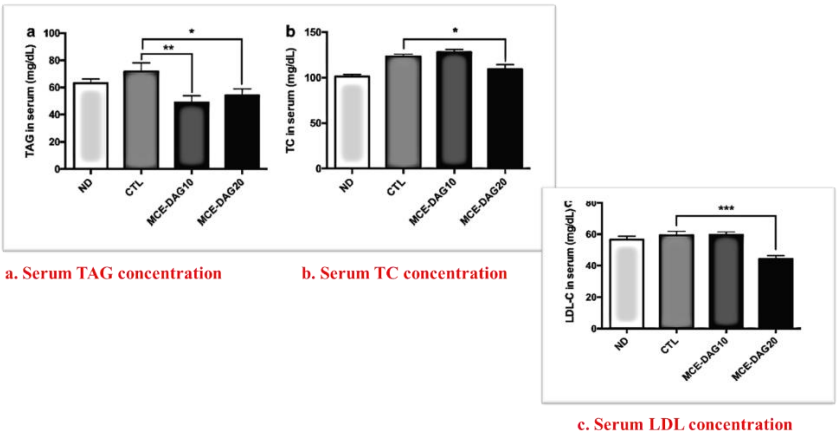
c. Body fat mass d. Lean body mass e. Total caloric intake

Fig. 1. Growth performance and body composition in mice

Body weight was measured for 20 weeks (Fig. 1a). Initial body weights of mice in the four groups were not significantly different. The body weights of MCE-DAG20 group mice differed considerably from those of the CTL group from 18 weeks onward ($P < 0.05$). The final body weights of MCE-DAG20 group mice were significantly lower than those of the CTL group ($P < 0.05$). However, when data was examined as per cent change in body weight, there was no significance. MCE-DAG10 had no meaningful effect on body weight over 20 weeks. There was no interaction between age and diet on body weight in all groups ($P = 0.645$).

Body composition, including body fat mass and lean body mass, was measured using DEXA. Red spots on the DEXA image indicated fat depots. The representative DEXA images were visualized in Fig. 1b. Body fat mass from all animals was quantified in Fig. 1c. MCE-DAG20-supplemented mice showed clearly reduced body fat compared with the CTL group at 20 weeks ($P < 0.001$). Interestingly, body fat in MCEDAG20-fed mice was comparable to that in the normal diet group over the course of the experiment.

There was no difference in lean body mass among groups during the entire experimental period (Fig. 1d). Feed intake was measured over 20 weeks (Fig. 1e). There was no significant difference in energy consumption, calculated by feed intake × energy density, among all groups, except for MCE-DAG20. The caloric intake of MCE-DAG20 was higher than the ND and CTL ($P < 0.01$ and $P < 0.05$, respectively). These results indicated that suppression of body weight gain and body fat accumulation was not caused by reduced energy input from diets.



a. Serum TAG concentration b. Serum TC concentration c. Serum LDL concentration

Fig. 2. Serum lipid profiles in mice

Serum lipid profiles of mice were analyzed by enzymatic methods to test the effect of MCE-DAG oil on lipid biochemistry. The concentration of TAG in serum was reduced in the MCE-DAG10 ($P < 0.01$) and MCE-DAG20 groups ($P < 0.05$) compared with the CTL (Fig. 2a). Mice fed MCE-DAG20 had decreased TC

concentrations compared with CTL mice ($P < 0.05$, Fig. 2b). LDL-C in MCEDAG20 was dramatically decreased by 25% relative to the CTL ($P < 0.001$, Fig. 2c).

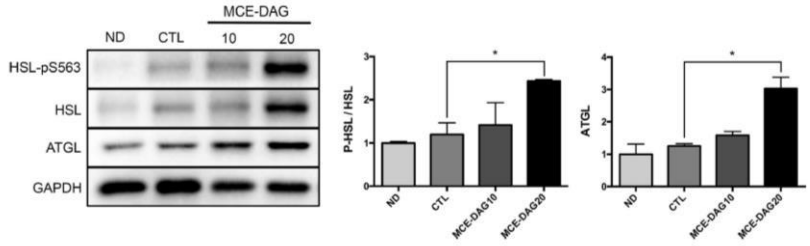


Fig. 3. Changes in the expression profile of proteins involved in lipolysis of WAT in mice

Activation of HSL followed by ATGL was required to initiate intracellular degradation of lipid droplets. In this study, major enzymes related to lipolysis in abdominal WAT were investigated by immunoblotting (Fig. 3). The activated form of HSL, phosphorylated at serine 563, was increased in MCE-DAG20 mice ($P < 0.05$). Another key enzyme in lipolysis is ATGL, which was elevated in MCEDAG20 groups ($P < 0.05$).

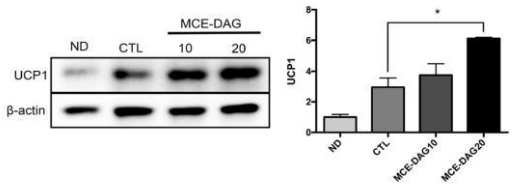


Fig. 4. Changes in the expression profile of protein involved in thermogenesis of BAT in mice

UCP1 is the main modulator of thermogenesis in BAT. Up-regulation of UCP1 directly activates thermogenesis in BAT, resulting in burning of excessive fat in the body. In accordance with lipolysis in WAT, mice fed MCEDAG20 exhibited an increased expression of UCP1 than did mice fed canola oil ($P < 0.05$, Fig. 4).

Conclusion

The decrease in body fat was obtained with increased expression of lipolysis related proteins in WAT and thermogenesis related protein in BAT. MCFA-enriched DAG oil would therefore act as functional lipids in the management of obesity.

SUMMARY

- DAG Oil - A cooking oil in which the ratio of TAGs to DAGs are modified to contain 80% or more of DAG as the main functional component.

- Mechanism of DAG for long-term weight maintenance

-Reduced chylomicron formation
-Increased oxidation of fatty acids in small intestine and liver
-Weight loss

- Enzymatically developed 1,3-DAG-rich oil using a blend of rice bran and sunflower oils with nutraceutical molecules such as γ-oryzanol, tocotrienol and phytosterols can be exploited as low calorie fat with anti-obesity potential.

- ALA-DAG could be useful for reducing VFA and concomitantly suppressing weight gain with no side effects. Hence, it can be used as an anti-obesity dietary supplement.

- The DAG oil from soybean oil can be used as potent functional oil for the suppression of high-fat diet-induced obesity and cardiovascular diseases as its consumption reduced body weight gain, plasma total cholesterol, triacylglycerol and glucose compared with a TAG oil.

- The DAG with different fatty acid chain lengths were synthesized from peanut and coconut oils. Compared with TAG (peanut oil and coconut oil), medium-chain DAG exhibited more potent effect than long- chain DAG.

- The proper dose of dietary DGO can improve lipid utilization, promote protein deposition and could be a potential additive in aquaculture feeds. These fish hence, when consumed by humans will benefit with anti-obesity property.

- The decrease in body fat was obtained with increased expression of lipolysis related proteins in WAT and thermogenesis related protein in BAT. MCFA-enriched DAG oil would therefore act as functional lipids in the management of obesity.

- DAG oil, is a useful adjunct to diet therapy in the management of obesity.

CONCLUSION

DAG oil is a novel technology in the edible oil sector which can be easily incorporated in regular diet as a beneficial constituent against body fat accumulation. So, replacing TAG oil with DAG oil as a functional ingredient with therapeutic application will help to fight against obesity and related chronic disease like CVD and diabetes. Let us not stop eating fat, let us start choosing fat.

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