DIACYLGLYCEROL OIL: AN ANTIOBESITY DIETARY SUPPLEMENT

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INTRODUCTION

A widespread misconception suggests that all fats and oils are detrimental to health. In reality, specific types of fats and oils offer valuable energy sources for the body and enhance the sensory aspects of numerous foods, including texture, taste, and aroma. Diverse oils and fats are integral to the preparation of various dishes, employed through cooking, baking, frying, and they also play a significant role in fillings, icings, toppings, and coatings. The foundation of a healthy diet lies in steering clear of foods and oils rich in trans and hypercholesterolemic fats. Unfortunately, diets high in fats have often been associated with obesity, which, in turn, links to various illnesses (Krawczyk, 2000). Over the past two decades, perceptions surrounding fats and oils have evolved from negative to beneficial (Kennedy, 1991).

DIETARY FATS

In the realms of nutrition, biology, and chemistry, the term "fat" generally refers to any fatty acid ester or a combination of such compounds. Frequently, it pertains specifically to triglycerides—triple esters of glycerol—constituting the primary constituents of vegetable oils and the fatty tissues in animals. Alternatively, it can be narrowly defined as triglycerides that exist in solid or semisolid forms at room temperature, excluding liquid oils. The term can also have broader connotations as a synonym for lipids—substances of biological significance composed of carbon, hydrogen, or oxygen, insoluble in water but soluble in non-polar solvents. In this broader sense, besides triglycerides, the concept encompasses other compounds like mono- and diglycerides, phospholipids (such as lecithin), sterols (like cholesterol), waxes (such as beeswax), and free fatty acids, which are typically present in smaller quantities within the human diet.

Among the three primary macronutrient categories in human diets—carbohydrates and proteins being the others—fats assume a vital role. They form the backbone of common food items such as milk, butter, tallow, lard, salt pork, and cooking oils. Fats serve as substantial and concentrated sources of dietary energy for numerous organisms, fulfilling essential roles like energy storage, waterproofing, and thermal insulation. Although the human body can synthesize necessary fats from other dietary components, a few essential fatty acids must be sourced from the diet itself. Additionally, dietary fats act as vehicles for fat-soluble vitamins and certain flavor and aroma compounds that are insoluble in water. The quantity and nature of dietary fats have a direct impact on various physiological functions within the body.

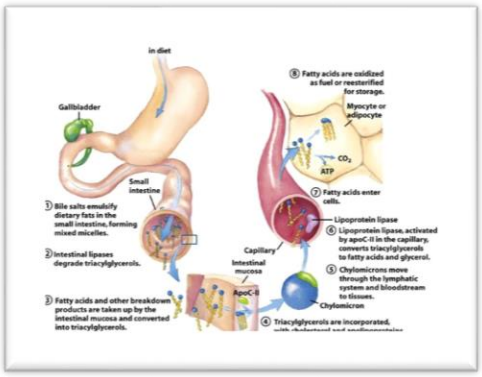
METABOLISM OF FAT

The metabolism of fats begins with digestion, which marks the initial phase of lipid metabolism. This process involves the breakdown of triglycerides into smaller monoglyceride units, facilitated by lipase enzymes. Fat digestion commences in the mouth, where lingual lipase initiates chemical digestion. Notably, lipases do not affect ingested cholesterol, which remains intact until it reaches the epithelial cells of the small intestine.

As the journey through the digestive tract continues, lipids progress to the stomach. Here, gastric lipase contributes to further chemical digestion, while mechanical digestion is initiated through peristalsis. However, the most significant portion of lipid digestion and absorption takes place in the small intestines. The pancreas releases chemicals such as the pancreatic lipase family and bile salt-dependent lipase into the small intestines.

These substances collaborate to break down triglycerides, aided by ongoing mechanical digestion. The outcome of this process is the transformation of triglycerides into individual fatty acid units, which are then primed for absorption into the epithelial cells lining the walls of the small intestine.

A pivotal role is played by pancreatic lipase, which is instrumental in signaling the hydrolysis of triglycerides. This enzymatic activity triggers the breakdown of triglycerides into distinct free fatty acids and glycerol units. This process is vital for converting triglycerides into their component parts, thereby enabling their efficient absorption by the epithelial cells within the small intestine.



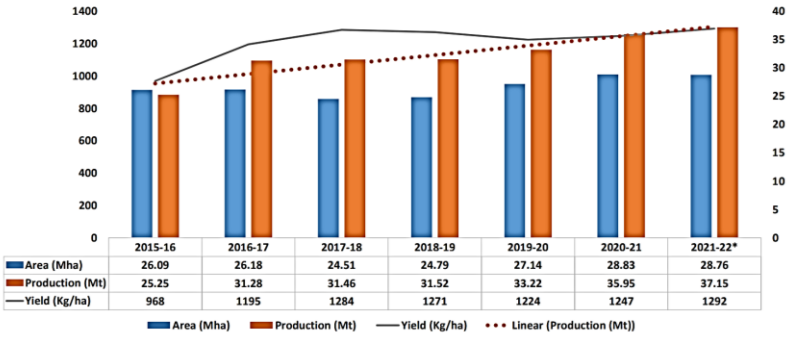
Following the initial digestion, the second stage of lipid metabolism involves the absorption of fats. While short-chain fatty acids can be absorbed in the stomach, the primary absorption of fats occurs exclusively within the small intestines. Once triglycerides are broken down into individual fatty acids, glycerols, and cholesterol, they aggregate into structures known as micelles. Subsequently, fatty acids and monoglycerides exit the micelles and diffuse across the membrane to enter the intestinal epithelial cells.

Inside the cytosol of these epithelial cells, fatty acids and monoglycerides reunite, reconstituting triglycerides. Similarly, triglycerides and cholesterol combine to form larger particles called chylomicrons. These chylomicrons, amphipathic structures, function as carriers that transport digested lipids. They embark on a journey through the bloodstream, eventually reaching adipose and other tissues throughout the body.

Given the hydrophobic nature of membrane lipids, triglycerides, and cholesterol, specialized transport proteins, known as lipoproteins, are required for their transportation. The amphipathic structure of lipoproteins enables efficient transport of triglycerides and cholesterol through the bloodstream. Among these lipoproteins, chylomicrons constitute one subgroup responsible for conveying digested lipids from the small intestine to other bodily regions. Variations in density characterize different types of lipoproteins, determining the type of fats they transport. For instance, very-low-density lipoproteins (VLDL) carry synthesized triglycerides, while low-density lipoproteins (LDL) transport cholesterol to peripheral tissues. The liver synthesizes several of these lipoproteins, although their origins are not solely confined to this organ.

Triglycerides find their storage in white adipose tissue. In the body of a lean young adult, the mass of stored triglycerides typically ranges from 10 to 20 kilograms. Formed from a glycerol backbone and three fatty acids, triglycerides are produced by activating free fatty acids into acyl-CoA. This process culminates in the esterification of fatty acids, leading to their incorporation into 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 been increased up to 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%)

The edible oil sector in India has seen significant developments. Domestic production reached its peak in the year 2021-22, with a production of 12.47 million metric tons (MT). Per capita consumption of edible oil has remained relatively consistent from 2014 to 2021, with minor fluctuations each year. The Indian Council of Medical Research (ICMR) recommends a daily consumption of 30 grams of fat per person.

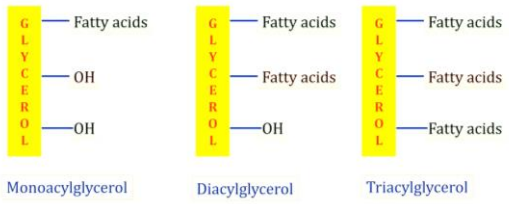
India ranks as the 4th largest oilseeds producer globally. It commands a substantial 20.8% of the total global cultivation area, contributing around 10% of the worldwide oilseeds production. The country cultivates a variety of oilseeds, including groundnut, soybean, sunflower, sesamum, niger seed, mustard, and safflower. The majority of oilseeds cultivation (approximately 72%) occurs under rainfed conditions, largely managed by small-scale farmers, which often results in lower productivity.

However, a breakthrough was achieved through the introduction of advanced crop production technologies. This led to significant growth in oilseed production, rising from 108.3 lakh tonnes in 1985-86 to 365.65 tonnes in 2020-21.

Over the past five years, oilseed production in India has been on the rise. In the year 2020-21, the country produced 365.65 lakh tonnes of oilseeds, reflecting a 10% increase from the previous year. The compound annual growth rate (CAGR) of production from 2015-16 to 2020-21 stood at 7.7%. This growth was facilitated by the

implementation of various government programs, such as special initiatives focused on mustard and rapeseed, along with the demonstration of improved agricultural technologies.
Leading oilseed-producing states in India encompass Andhra Pradesh, Gujarat, Haryana, Karnataka, Madhya Pradesh, Maharashtra, Rajasthan, Tamil Nadu, Uttar Pradesh, and West Bengal. Among these, Rajasthan, Gujarat, Madhya Pradesh, and Maharashtra are the key contributors, collectively accounting for approximately 75% of the total production, with each state holding shares of around 20%, 20%, 19%, and 16% respectively.

STRUCTURE OF ACYLGLYCEROL



Edible oil is composed of two primary components: acylglycerol and fatty acids. Acylglycerol refers to the natural ester formed from glycerol and fatty acids, commonly found in fats and fatty oils. Glycerol features three hydroxyl functional groups, enabling the formation of different compounds through esterification with fatty acids. This results in monoacylglycerol when esterified with one fatty acid, diacylglycerol with two fatty acids, and triacylglycerol with three fatty acids.

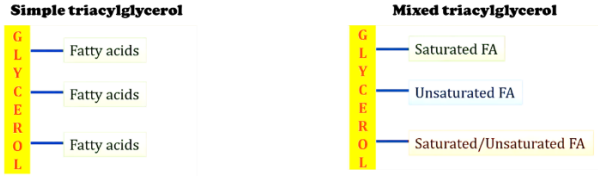
SIMPLE LIPIDS: TRIACYLGLYCEROL (TAG)

A triglyceride (abbreviated as TG, also known as triacylglycerol, TAG, or triacylglyceride) is an ester formed through the combination of glycerol and three fatty acids, resulting in its name that includes "tri-" for three and "glyceride" for glycerol. It possesses a nonpolar nature and is commonly referred to as neutral fats due to its lack of charge. Its primary role lies in serving as a reservoir for energy storage. These fats and oils, which are prevalent in both plant and animal organisms, are chemically classified as Triacylglycerols.

An excessive consumption of energy leads to the accumulation of TAG in adipose tissue. Triglycerides are the predominant constituents of body fat not only in humans but also in other vertebrates, as well as in vegetable fats. They are present in the bloodstream, facilitating the bidirectional transfer of adipose fat and blood glucose from the liver. Furthermore, they constitute a significant portion of the oils found in human skin.

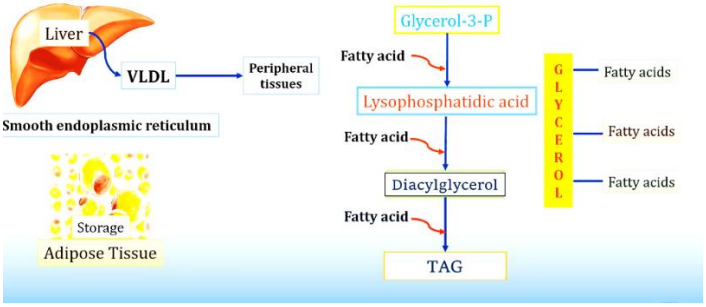
Triglycerides exhibit various forms, with one classification distinguishing between saturated and unsaturated types. Saturated fats lack carbon-carbon double bonds (C=C groups), while unsaturated fats contain one or more of these double bonds. The presence of double bonds in unsaturated fats results in a lower melting point compared to their saturated counterparts, causing them to typically exist in a liquid state 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

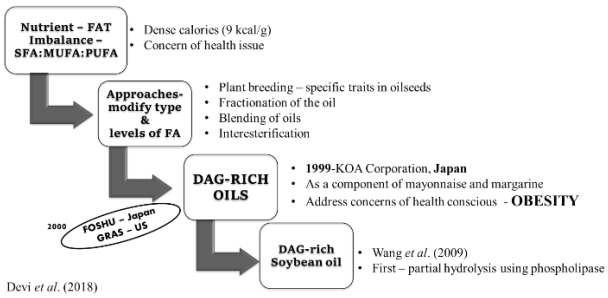


Lipogenesis denotes the biochemical process of converting fatty acids and glycerol into fats. This metabolic pathway involves the transformation of acetyl-CoA into triglycerides, which are subsequently stored as adipose tissue. The scope of lipogenesis encompasses the synthesis of both fatty acids and triglycerides. In the latter process, fatty acids are combined with glycerol to form triglycerides, which are then assembled into very-low-density lipoprotein (VLDL) for transport.

Fatty acids are generated within cell cytoplasm by iteratively appending two-carbon segments to acetyl-CoA. On the other hand, the synthesis of triacylglycerol takes place within the endoplasmic reticulum membrane of cells. This involves the bonding of three fatty acid molecules to a glycerol molecule. These intricate processes primarily occur in the liver and adipose tissue, although they also manifest to a lesser extent in other tissues like the gastrointestinal tract and kidneys.

Upon completion of the synthesis, the resultant lipoproteins, enclosed within VLDL, are released directly into the bloodstream from the liver. This mechanism ensures the transportation of lipids to peripheral tissues for their utilization. (Xia *et al*., 2014).

DIACYLGLYCEROL OIL– THE INGRESS



In the past, the knowledge regarding naturally occurring vegetable oils and fats was centred around the understanding that they primarily consisted of triacylglycerols (TAG). Nonetheless, these oils and fats also contained minor quantities of diacylglycerols (DAG) and monoacylglycerols (MAG). Fats and oils play a crucial role in human nutrition as they serve as significant sources of energy, essential fatty acids, and fat-soluble vitamins. Notably, fat is a nutrient dense in calories, providing 9 calories per gram. The potential surplus of calories from dietary fat has raised concerns among health-conscious individuals, as it has the potential to

contribute to obesity. Additionally, the consumption of imbalanced fats, specifically in terms of saturated, monounsaturated, and polyunsaturated fatty acid levels, can contribute to the development of chronic diseases.
To address these concerns, various strategies have been employed. These include plant breeding to select desirable traits in oilseeds, oil fractionation, oil blending, and oil interesterification. These methods are utilized to modify the composition and amounts of fatty acids within oils. An emerging approach involves oils rich in diacylglycerols (DAG), which has gained attention among health-conscious individuals. Japan introduced an anti-obesity diacylglycerol oil in 1999, marking a significant development in this area. Modifying the ratio of triacylglycerols (TAG) to diacylglycerols (DAG) proved to be a successful study avenue.
This modified DAG oil received recognition as Generally Recognized as Safe (GRAS) by the United States and Food for Specified Health Uses (FOSHU) by Japan in 2000. Initially integrated into products such as mayonnaise and margarine, the DAG oil aimed to address health concerns, particularly those related to obesity. In 2009, Wang *et al*. pioneered the creation of DAG oil from soybeans through partial hydrolysis employing phospholipase. This marked an innovative step in the development of this type of oil.

DIACYLGLYCEROL OIL

Traditional cooking oils typically contain up to 10 percent (w/w) diacylglycerols (DAG), but a new category of healthier cooking oils is gaining attention, claiming to consist of 80 percent (w/w) or more DAG as their primary functional component. These diacylglycerol oils (DAG oils) stand apart from regular cooking oils due to their distinctive composition, where the predominance of diacylglycerols (DAGs) over triglycerides (TAGs) is the key feature. Unlike conventional cooking oils that are rich in TAGs, DAG oils are designed to contain a higher proportion of DAGs.
An illustrative example is vegetable DAG oil, which is composed of 80 percent diacylglycerols (DAGs). Both DAGs and TAGs naturally occur in all vegetable oils. Through enzymatic processes, the concentration of diacylglycerols in a blend of soy and canola oils is significantly elevated. In contrast to triglycerides (TAG), which are stored as adipose tissue, diacylglycerols (DAG) are swiftly utilized as an energy source. The distinctive characteristic of DAG-rich oil, containing more than 80 percent DAG, lies in its reduced storage of oil as body fat compared to conventional TAG-rich oils. It's important to note that regardless of whether it is consumed as DAG or TAG, excess calories ingested by the body are converted into fat and stored.

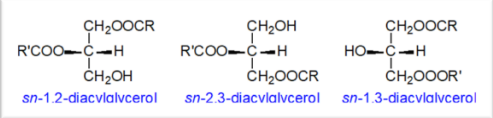
NATURAL SOURCES OF DAG

Unaltered oil will not have a natural DAG (diacylglycerol) content exceeding 10 percent. Palm oil, which we tend to avoid due to its elevated saturated fat levels, inherently possesses greater levels of DAG, specifically around 5.8 percent. This is followed by olive oil and tallow, an animal fat source.

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 represent esters of glycerol, a trihydric alcohol, wherein two hydroxyl groups are esterified with fatty acids. They can take on two distinct structural isomers: 1,2-DAG and 1,3-DAG. These isomers tend to undergo acyl migration to reach an equilibrium ratio of approximately 3–4:7–6 between 1,2-DAG and 1,3-DAG, often facilitated by the presence of an acid, alkali, or heat. Notably, the thermodynamic stability of 1,3-DAG is higher due to molecular steric effects.

In contrast to TAG (triacylglycerol) polymorphism, DAG exhibits two variations of polymorphic forms. Specifically, 1,2-DAG manifests as the α- and β′-forms, lacking a β-form. Conversely, 1,3-DAG lacks an α-form and showcases two varieties of β-forms, denoted as β1 and the less stable β2.

DAG oil serves as a beneficial addition to dietary therapy for managing obesity.

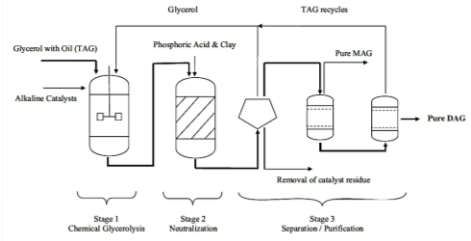
PRODUCTION OF DAG

There are three primary approaches to synthesizing DAG, each involving distinct methods:

1. Glycerolysis: This method involves the reaction of TAG with glycerol, leading to the production of DAG.
2. Esterification: In this process, Free Fatty Acids (FFA) are esterified with either glycerol or MAG (Monoacylglycerol), resulting in the synthesis of DAG along with the formation of water molecules.
3. Hydrolysis: Through the interaction between TAG and water, this method yields MAG, DAG, and FFA as products.

The use of chemicals in these methods results in chemical production of DAG, while employing enzymes leads to enzymatic production of DAG.

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



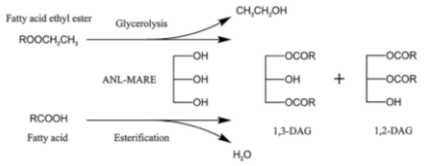
The predominant industrial approach for producing DAG involves chemical glycerolysis. In this technique, glycerol stands as a key feedstock. Glycerol is a co-product of both triglyceride esterification and transesterification, and its primary source lies in the biodiesel industry. With the rapid expansion of the biodiesel sector, the surplus glycerol emerges as a potentially valuable resource for generating useful products from an economical and adaptable reactant. Instead of being disposed of in landfills, the crude glycerol originating from biodiesel production can be harnessed for creating more valuable substances like DAG and MAG, as advocated by Satriana *et al*. in 2016.

On an industrial scale, chemical glycerolysis is typically conducted at temperatures ranging from 210°C to 260°C. This process occurs in the presence of diverse alkaline catalysts like sodium hydroxide (NaOH), potassium

hydroxide (KOH), sodium methoxide (NaOCH3), and potassium acetate (KC2H3O2). The production process is illustrated in a diagram.

However, this method has several drawbacks stemming from the extreme operational conditions employed. Prolonged exposure to high temperatures, especially for heat-sensitive polyunsaturated fatty acids, can lead to the formation of undesirable by-products and the emergence of unfavorable characteristics in the final oil product, affecting sensory qualities, appearance, and aroma. Consequently, a purification process is necessary prior to commercializing the product. Moreover, the harsh conditions also trigger an unwanted side reaction involving acyl migration, resulting in reduced DAG yield and purity. Additionally, the significant energy demands associated with these extreme conditions can notably elevate production costs. A notable limitation for DAG production, particularly through chemical glycerolysis, arises from poor miscibility between the substrates—namely, oil and glycerol. This issue contributes to low mass transfer and an overall sluggish reaction rate.

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



The combined enzymatic catalysis involving glycerolysis of EE (esterified fatty acids) and esterification of FFA (free fatty acids) in a single-step process is an area of limited exploration. DAG (diacylglycerol) oil possesses distinct nutritional qualities, particularly in terms of its impact on body fat metabolism. However, there's a dearth of research delving into the nutritional attributes of DAG-enriched oil derived from SSBO (Soya Sauce Byproduct Oil). The anticipation is that DAG-enriched oil will find utility as a functional oil, as highlighted by Feng *et al*. in 2022.

For the synthesis of DAG-enriched oil, a cost-effective SSBO with substantial content of EE and FFA was employed as the raw material. In the capacity of a catalyst, a non-commercial immobilized form of Aspergillus niger lipase (ANL-MARE) was used. Taking advantage of the differing behaviors between the transesterification of EE and the esterification of FFA within a one-pot enzymatic process, a two-step conversion strategy under vacuum conditions was devised. This approach entailed the use of vacuum to eliminate generated water and ethanol through vacuum-assisted air bubbling. This technique resulted in increased conversions of FFA and EE, subsequently leading to higher DAG yields.

In this reaction, EE and FFA extracted from Soya Sauce Byproduct Oil were harnessed as the sources of acyl groups. The lipase displayed varying characteristics between the glycerolysis of EE and the esterification of FFA with glycerol. This methodology showcased potential for synthesizing DAG-enriched oil through an innovative combination of enzymatic transformations.

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 exhibits quicker crystallization in comparison to TAG (triacylglycerol) and possesses a reduced melting rate, resulting in higher solid fat content than TAG. This is attributed to its distinct properties. DAG showcases two distinct types of polymorphism, and its microstructure reveals smaller, more uniform, needle-like crystals. In terms of rheology, DAG demonstrates elevated viscosity and elasticity, contributing to its harder texture.

Applications of MAG and DAG in fat-based food products

DAG oil derived from sources such as corn, palm, soybean, canola, chicken, or mutton fat holds potential as a viable ingredient in the production of MAG/DAG fats and oils, margarine, shortening, emulsifiers, and organogels. Its versatility is underscored by its capability to seamlessly integrate into various food products, owing to its similarity in taste, appearance, and fatty acid composition to conventional oils like 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

Both animal and human studies consistently indicate the absence of detrimental outcomes stemming from the consumption of DAG-rich oil, whether through single doses or prolonged periods. Additionally, the intake of such oil does not impact the status of fat-soluble vitamins. Consequently, oils containing elevated levels of 1,3-DAG are positioned to combat chronic diseases linked to obesity.

RECOMMENDATION

In various human studies, a daily intake ranging from 8 to 20 grams of DAG has been administered to evaluate the effectiveness of DAG in influencing body composition, particularly weight loss, among individuals with obesity, as noted by Scholz *et al*. in 2022.

HEALTH BENEFITS OF DAG OIL

In addition to its recognized anti-obesity attributes, DAG also possesses anti-atherosclerotic, anti-diabetic, and anti-inflammatory properties.

OVERWEIGHT AND OBESITY

Obesity is a medical condition that is often regarded as a disease, characterized by the accumulation of excessive body fat to a degree that it can have adverse impacts on an individual's health. Classification as obese typically occurs when a person's body mass index (BMI), calculated by dividing their weight by the square of their height, exceeds 30 kg/m². The range between 25 and 30 kg/m² is categorized as overweight.

This condition is defined by the excessive buildup of fat within adipose tissue, which functions as an endocrine organ. Adipose tissue serves as a site for energy storage and plays a crucial role in maintaining body weight. In

males, adipose tissue distribution is predominantly above the waist, resulting in an "android" or "apple" body shape, while in females, it's situated below the waist, leading to a "gynoid" or "pear" body shape.

Within the realm of adipose tissue, there are four distinct types of adipocytes: white, brown, beige, and pink adipocytes. White adipose tissue is primarily involved in the process of lipogenesis, while brown adipose tissue is responsible for thermogenesis.

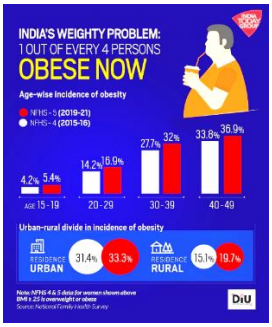
TYPES OF BODY FAT

In the human body, two main types of fat accumulate: visceral fat and subcutaneous fat. Visceral fat is located around internal organs, and its measurement is typically conducted through methods like CT scans and full-body MRIs. Although these approaches are highly accurate, they can be costly. Therefore, estimates based on waist circumference are commonly employed as a more practical alternative.

On the other hand, subcutaneous fat is situated beneath the skin. This type of fat is measured using devices like skinfold calipers, which provide an assessment of the thickness of the subcutaneous fat layer at specific sites on the body.

OBESITY- INDIA'S STATISTICS

A comprehensive analysis of obesity statistics within the Indian population reveals a noticeable upward trajectory from the period 2015-16 to 2020-21. This trend is particularly pronounced among individuals aged 15 to 49 years. Moreover, the prevalence of obesity is notably higher in urban areas when compared to rural regions, as highlighted by the National Family Health Survey in 2022.



CAUSES AND EFFECTS OF OBESITY

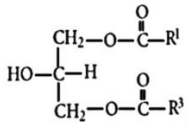
Obesity can be attributed to a variety of causative factors, including emotional triggers, a sedentary lifestyle, overeating, certain medications, smoking, alcohol consumption, insomnia, and genetic predisposition. The consequences of obesity are far-reaching, contributing to conditions such as high blood pressure, cancer, cardiovascular disease, diabetes, infertility, and sleep apnea.

OREXIGENIC AND ANOREXIGENIC FACTORS OF OBESITY

Orexigenic neurons play a role in promoting eating behaviors and are influenced by neuropeptide hormones such as neuropeptide Y (NPY), orexin, and ghrelin. On the other hand, anorexigenic neurons work to suppress appetite and are associated with the production of melanocyte-stimulating hormone (MSH). Anorexigenic hormones include leptin and insulin, as well as peptide-tyrosine-tyrosine (PYY).

DIETARY DAG OIL AS AN ANTI-OBESITY AGENT

A novel approach suggested to counteract weight gain and the accumulation of fat involves incorporating 1,3 DAG–enriched oil into the diet.

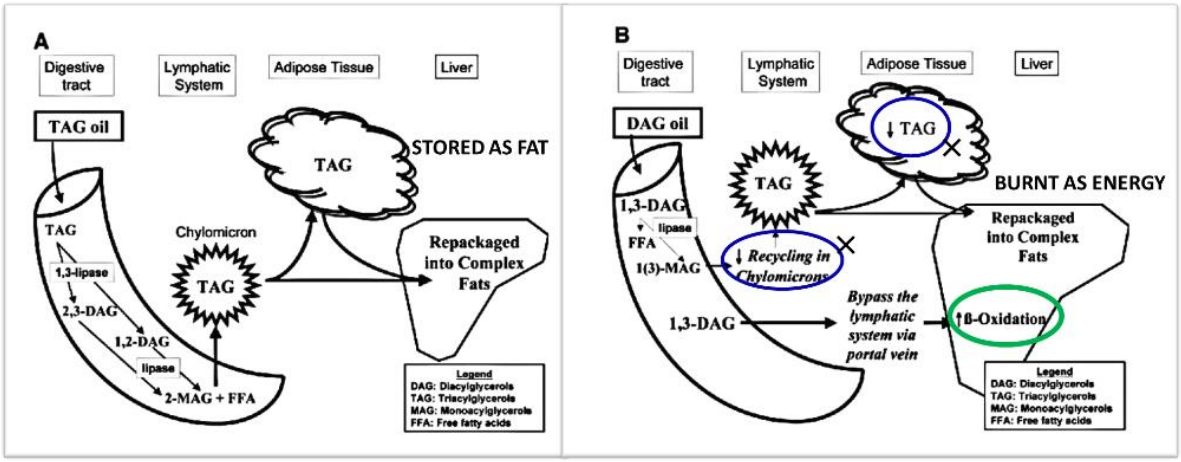


1,3-diacyl-*sn*-glycerol (DAG)

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 distinct structural and metabolic characteristics of diacylglycerol (DAG) as compared to triacylglycerol (TAG) are thought to underlie its capacity to hinder the accumulation of body fat, contribute to body weight loss, and result in lower postprandial serum triglyceride (TG) levels. However, the precise mechanisms driving these effects remain to be fully elucidated. Interestingly, it has been determined that DAG and TAG have almost identical digestibility and caloric values. Bomb calorimetry reveals closely similar energy values for DAG (38.9 kJ/g) and TAG (39.6 kJ/g).

Beyond the minor difference in energy content between these two oils, the distinct structural disparities among various DAG isomers—not the fatty acid composition of either DAG or TAG—account for any differential influence on lipid metabolism. During digestion, triacylglycerols are broken down by 1,3-lipases into 1,2-DAG and 2,3-DAG, but not 1,3-DAG, due to the specific cleavage sites of lipases. Further enzymatic action on 1,2-DAG and 2,3-DAG results in the formation of 2-monoacylglycerol and free fatty acids, which are typical products of TAG digestion. These substances are absorbed by intestinal mucosal cells and contribute to the synthesis of circulating chylomicron triglycerides (TGs).

Within the equilibrium, DAG comprises around 65 to 70 percent 1,3-DAG and 30 to 35 percent 1,2-DAG. Enzymatic processes can produce specific types of DAG oil, especially 1,3-DAG oil. When lipase acts on 1,3-DAG, the primary outcomes are glycerol and free fatty acids, which might be less efficiently reformed into

chylomicron TGs. Additionally, more fatty acids from digested DAG may enter the portal circulation compared to their incorporation into chylomicrons, potentially explaining the lower serum TG levels both in the fasted state and postprandially, as well as the decreased TG content of chylomicrons.

Furthermore, the heightened exposure of the liver to fatty acids through increased intake of DAG might encourage greater β-oxidation in the liver than after TAG consumption. This heightened β-oxidation could result in increased satiety. Consequently, prolonged DAG intake with a sufficient dose might lead to decreased caloric intake, thereby prompting weight loss and fat reduction over the long term. This proposed mechanism is depicted in Figure 1B.

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

Lipid Metabolism: When considering fasting serum lipids, the consumption of DAG oil leads to a reduction in triglycerides, total cholesterol, low-density lipoprotein cholesterol (LDL-C), and an increase in high-density lipoprotein cholesterol (HDL-C). In terms of postprandial serum lipids, DAG oil intake results in decreased levels of triglycerides, remnant-like lipoprotein particle triglycerides, remnant-like lipoprotein particle cholesterol, and chylomicron triglycerides.

β-Oxidation: The consumption of DAG oil triggers an increase in the activity of hepatic enzymes associated with β-oxidation. These enzymes include carnitine palmitoyl transferase, acyl-CoA dehydrogenase, acyl-CoA oxidase, enoyl-CoA hydratase, and 3-hydroxyacyl-CoA dehydrogenase.

Body Composition: The incorporation of DAG oil into the diet results in favorable changes in body composition. This includes reductions in body weight, body fat, visceral fat, subcutaneous fat, hepatic fat, waist circumference, and 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

As of now, there are only two commercially recognized brands of DAG oil available globally: ENOVA oil in the United States and ECONA oil in Japan. Additionally, the Central Food Technological Research Institute (CFTRI) has successfully produced 10 kg of DAG oil after considerable efforts. CFTRI is now prepared to introduce this oil into the market under the label of "anti-obesity oil." This development suggests growing interest and efforts in providing consumers with healthier oil alternatives.

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:

In this study, Refined Rice Bran Oil (RBO) and Sunflower Oil (SFO) were obtained from a local supermarket. The process for creating 1,3-DAG-rich oil involved enzymatic hydrolysis of a mixture of RBO and SFO into fatty acids, followed by the esterification of these fatty acids with glycerol. The resultant DAG-rich oil was then purified using separation techniques.

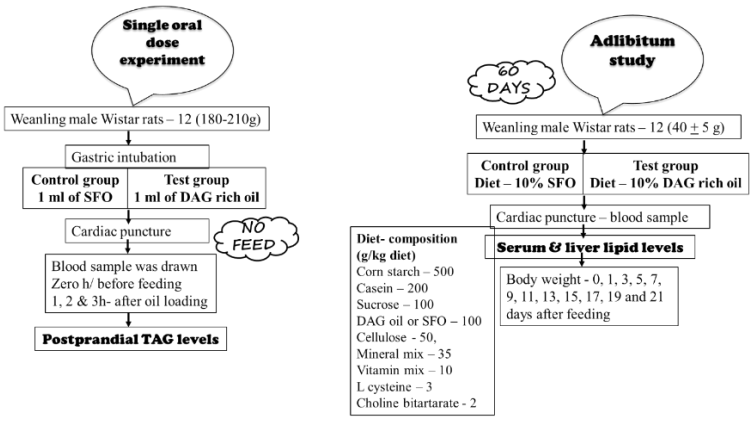
For the experimental phase involving rats, male Wistar rats of the Outbred strain were randomly grouped, with each group consisting of 6 animals. These rats were housed individually in cages within a controlled environment maintained at 25 ± 2°C, with a 12-hour light cycle at the Central Food Technological Research Institute in Mysore, India. The animal experiments were approved by the Institutional Animal Ethical Committee recognized by the Government of India, and the rats were provided with fresh water and food daily.

In a single oral dose experiment, rats with body weights ranging from 180 to 210 grams were orally administered either 1 ml of Sunflower Oil (SFO) or 1 ml of DAG-rich oil via gastric intubation. Blood samples were collected through cardiac puncture before feeding (baseline) and at 1, 2, and 3 hours after the oil administration. The rats did not receive any feed during the experiment.

In a separate experiment, twelve weanling male Wistar rats with initial body weights of 40 ± 2 grams were fed a diet based on the AIN-93 formulation containing either 10 percent Sunflower Oil (SFO) as a control or 10 percent DAG-rich oil as the test oil. The diet composition consisted of various components including corn starch, casein, sucrose, DAG oil or SFO, cellulose, mineral mix, vitamin mix, L-cysteine, and choline bitartrate. The rats were distributed randomly into three groups, each comprising six rats. One group was fed an ad libitum diet containing 10 percent SFO. The rats in the test groups received 50 percent of the feed consumed by the ad libitum group on the previous day. Feed consumption was monitored daily without any feed spillage. Body weights were recorded on specific days throughout the experimental period.

The study aimed to assess the calorific availability of DAG-rich oil by comparing the growth of rats fed diets containing DAG-rich oil with the growth of rats fed diets containing SFO. This comprehensive investigation involved various aspects of oil administration and dietary intake to evaluate the potential effects of DAG-rich oil on the rats' growth and metabolism.

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.

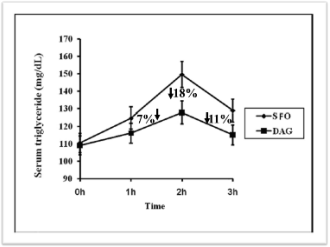


Fig.1. Postprandial serum triglyceride levels in rats given 1 ml of SFO or DAG-rich oil by intubation

In the Sunflower Oil (SFO) used, approximately 95 percent consisted of Triacylglycerols (TAG), with 2.6 percent being Diacylglycerols (DAG). Conversely, the DAG-rich oil created in this specific study had 16 percent TAG and 84 percent DAG content. This distinction in composition could potentially lead to differing effects on postprandial serum triglyceride levels.

To assess this impact, rats were administered 1 ml of either SFO or DAG-rich oil through intubation. The levels of triglycerides in the rats' serum were monitored over various time intervals (as illustrated in Figure 1). It was observed that postprandial serum triglyceride levels in rats given DAG-rich oil were significantly lower compared to rats given an equivalent amount of SFO.

Specifically, the postprandial serum triglyceride levels were reduced by 7 percent, 18 percent, and 11 percent at 1 hour, 2 hours, and 3 hours, respectively, in rats that received DAG-rich oil compared to those that were given SFO. These findings suggest that the consumption of DAG-rich oil might have a beneficial impact on postprandial serum triglyceride levels when compared to conventional oils like 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% ↓)

In order to assess the potential long-term effects of consuming DAG-rich oil, rats were provided with a diet containing 10 weight percent (wt%) fat in the form of either DAG-rich oil or Sunflower Oil (SFO). The results demonstrated significant differences in various serum lipid levels between the two groups.

Rats that were fed a diet incorporating DAG-rich oil exhibited notably lower levels of cholesterol and triglycerides in their serum, showing reductions of 23 percent and 22 percent, respectively, when compared to the rats that were given SFO (as outlined in Table 2). Additionally, levels of Low-Density Lipoprotein (LDL) were reduced by 31 percent in rats consuming DAG-rich oil in comparison to those given SFO. While High-Density Lipoprotein (HDL) showed a marginal decrease of 7 percent in rats fed DAG-rich oil, this difference was not statistically significant.

Moreover, rats consuming DAG-rich oil exhibited significantly lower levels of cholesterol and triglycerides in their liver, with reductions of 21 percent and 20 percent, respectively, compared to those consuming SFO (as also highlighted in Table 2). These findings collectively suggest that DAG-rich oil demonstrated hypolipidemic effects, indicating its potential benefits in positively influencing lipid profiles when consumed as part of a long-term dietary regimen.

Conclusion

The enzymatically produced 1,3-Diacylglycerol (DAG)-rich oil, created by combining rice bran and sunflower oils, offers an opportunity to incorporate beneficial nutraceutical compounds such as γ-oryzanol, tocotrienol, and phytosterols. This specialized blend has the potential to serve as a low-calorie fat source while also possessing anti-obesity properties.

The synergistic effects of these nutraceutical molecules combined with the unique characteristics of 1,3-DAG-rich oil could result in a product that not only provides essential fats but also contributes to weight management efforts. This innovative approach holds promise for developing functional foods that support overall health and address obesity-related concerns.

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

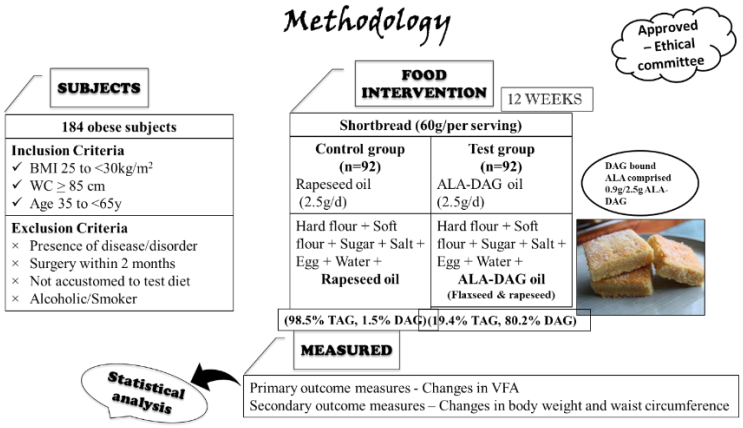
Methodology:

This study was designed as a randomized, double-blind controlled trial using a parallel-group structure. The study spanned 12 weeks of treatment followed by an additional 4-week non-treatment phase. The primary objective

was to assess changes in Visceral Fat Area (VFA), with subjects divided into groups consuming either 2.5 g/d of control Triacylglycerol (TAG) derived from rapeseed oil or ALA-DAG (which included 0.89 g/d of DAG-bound alpha-linolenic acid, or ALA) alongside their regular diet.

Secondary outcomes measured included alterations in body weight, waist circumference, and safety parameters. Participants were instructed to incorporate the test food into one of their daily meals (breakfast, lunch, or dinner) while refraining from consuming it between meals. Nutrient consumption was analyzed based on dietary records from the three days preceding the measurements. Additionally, participants were advised to limit alcohol consumption to less than 30 g/d and to maintain their customary exercise routines throughout the study. Their level of physical activity was monitored by recording the number of daily steps using a pedometer.

The selection of alpha-linolenic acid (ALA) was based on previous human and rodent studies. ALA was chosen due to its propensity to undergo oxidation more readily within the body, in contrast to palmitic, stearic, oleic, and linoleic acids. This choice was grounded in the potential benefits of ALA in the context of the study's objectives and previous research findings.



Analysis

After the 12-week food intervention period, the study evaluated changes in Visceral Fat Area (VFA) as the primary outcome among obese participants. Secondary outcome measures included changes in body weight and waist circumference. The data collected from these measurements was subjected to analysis using appropriate statistical methods.

The purpose of this analysis was likely to determine the effects of the food intervention on key indicators of obesity, such as VFA, body weight, and waist circumference. Statistical tools would have been employed to assess the significance of the observed changes and to draw conclusions about the impact of the intervention on these outcomes. The choice of statistical methods would depend on the study design, the distribution of the data, and the specific hypotheses being tested.

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 cm²							
(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).

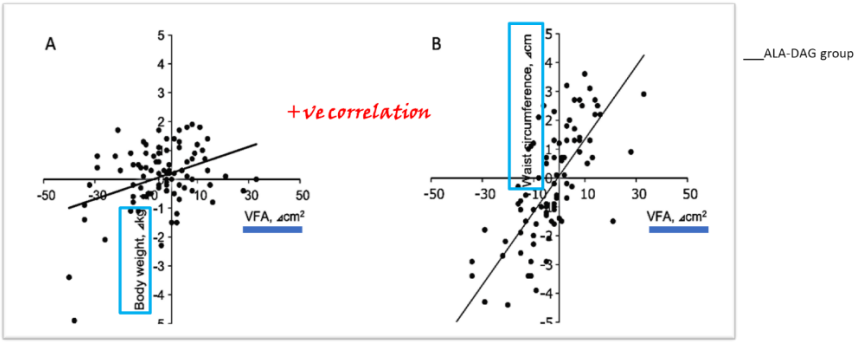


Fig.1. Correlation between changes in (A) VFA and body weight (B) VFA and waist circumference in ALA-DAG groups

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.

STUDY-3 (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:

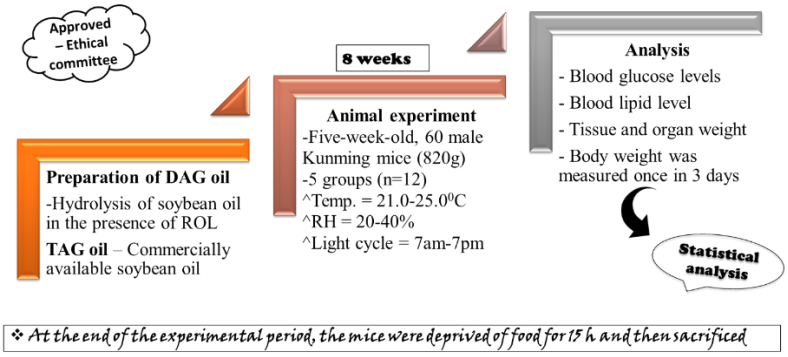


Table 1: Experimental groups

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

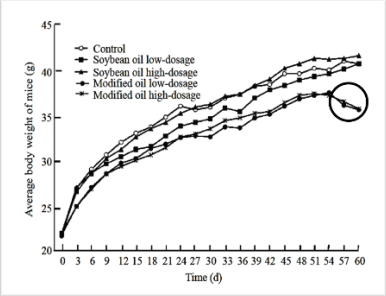


Fig. 1. The effect of different oil diets on the body weight of mice

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.

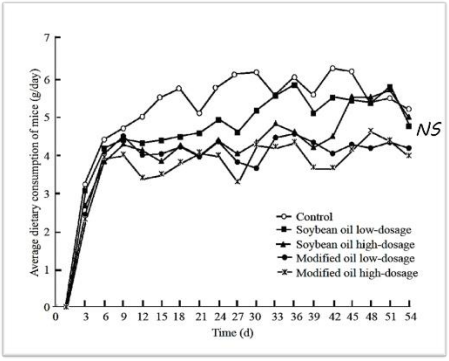


Fig. 2. The effect of different oil diets on the average dietary consumption of mice

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:

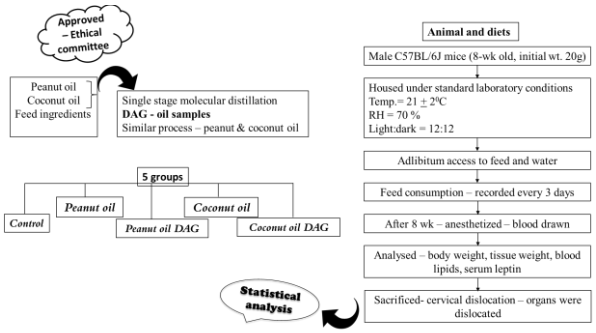


Table 1: Composition of different diets

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.

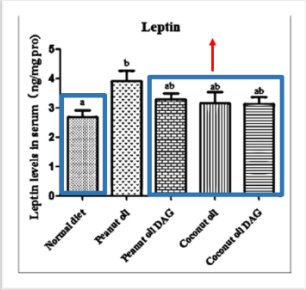


Fig.1. Effect of different oils on serum leptin content in C57BL/6Jmice

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.

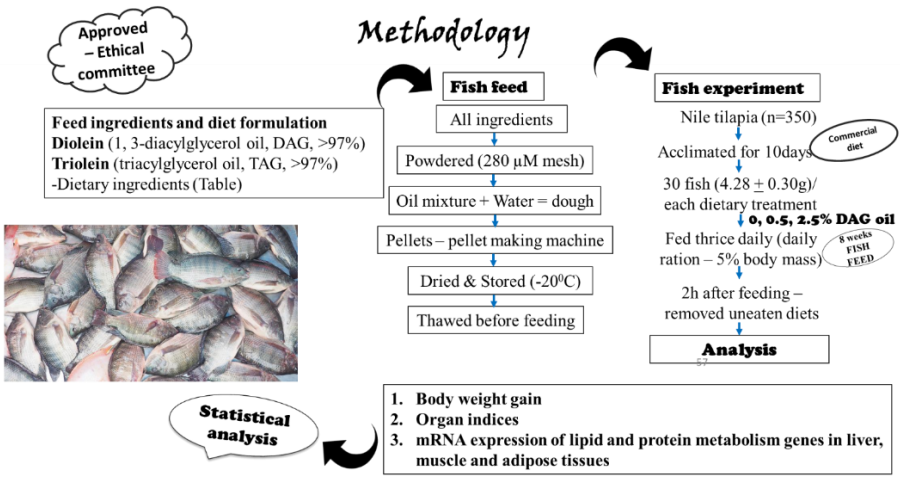


Table: List of dietary ingredients

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

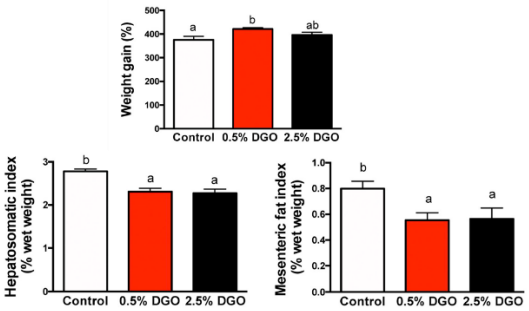


Fig. 1. Growth performance (weight gain), hepatosomatic index and mesenteric fat index (organ indices) of *O. niloticus*

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.

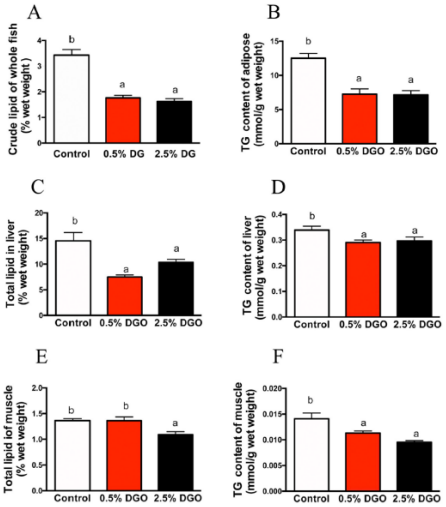


Fig. 2. Total lipid and TG content of *O. niloticus* fed with 0.5% and 2.5% DGO diet

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).

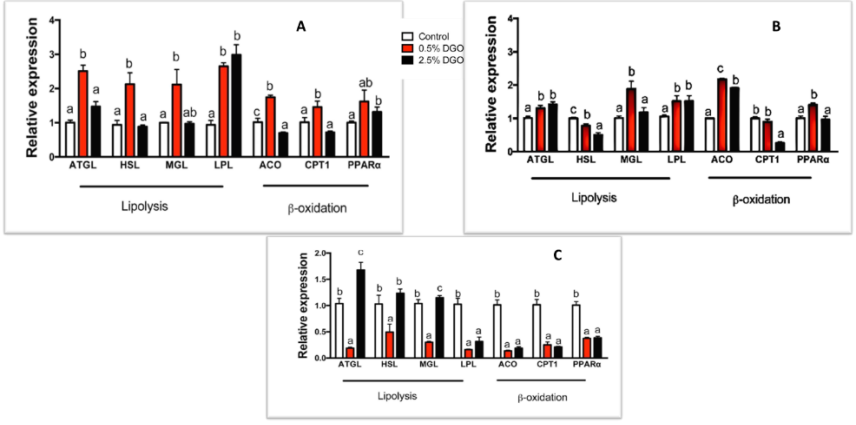


Fig. 3. The mRNA expression of lipid metabolism genes in liver, muscle and adipose tissues of *O. niloticus* (A) liver (B) muscle (C) adipose tissues

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.

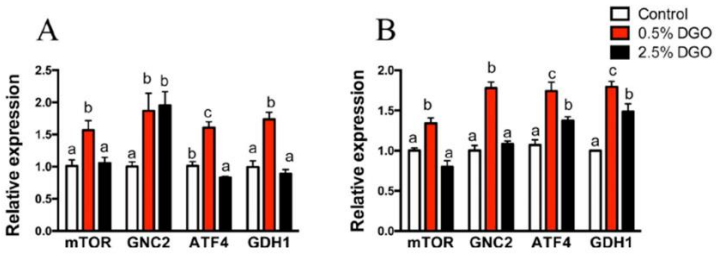


Fig. 4. The protein metabolism mRNA relative expression of *O. niloticus* (A) liver (B) muscle tissues

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.

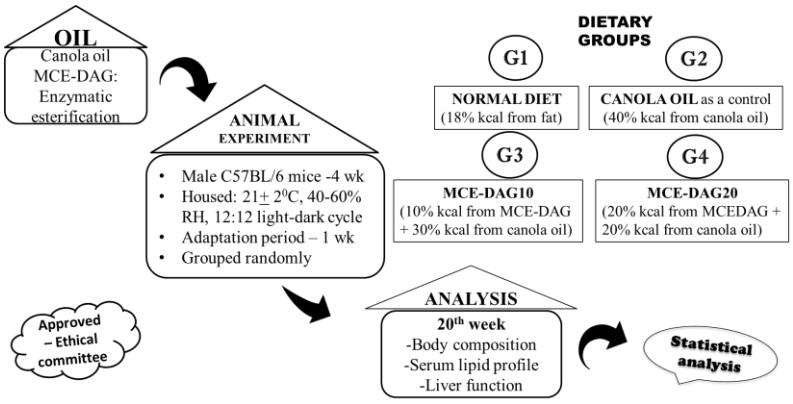
STUDY-6 (Kim *et al*., 2017)

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

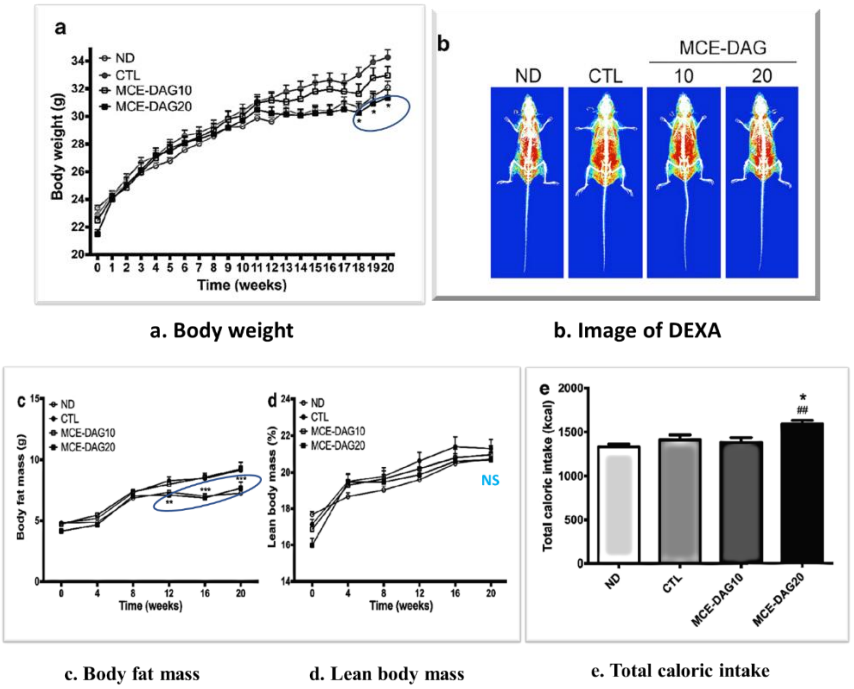
Methodology

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.

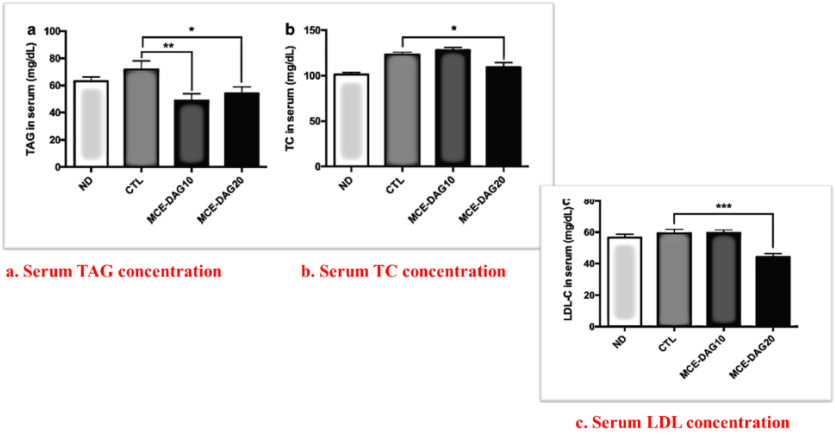


Fig. 2. Serum lipid profiles in mice

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).

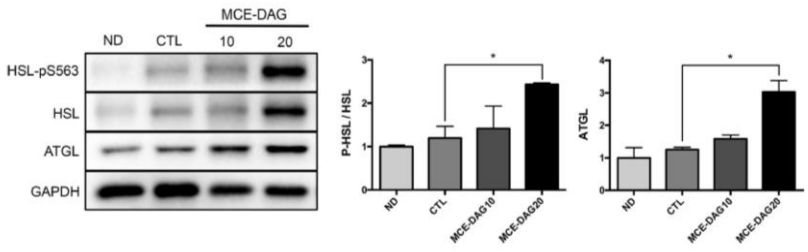


Fig. 3. Changes in the expression profile of proteins involved in lipolysis of WAT in mice

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 ($P < 0.05$).

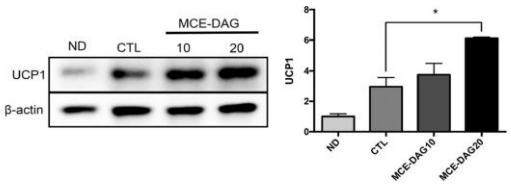


Fig. 4. Changes in the expression profile of protein involved in thermogenesis of BAT in mice

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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