

Diacylglycerides as nutrition components or precursors of carcinogens: a critical view on an ambular question

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ABSTRACT

Obesity is considered as a noninfectious epidemic worldwide. Metabolic disorders associated with the accumulation of adipose tissue lead to obesity-associated diabetes mellitus and cardiovascular diseases. Diet is a component of treatment of diseases associated with obesity. The most commonly used diets are caloric restriction by reducing fat in the diet. Over the years, there have been several attempts to use diacylglycerides (DAGs) as components of dietary oils owing to its ability to suppress the accumulation of visceral fat and reduce postprandial levels of triacylglycerol and cholesterol and glucose in the blood serum. However, in 2009, it was found that when oil was processed at high temperatures during physical refining, DAG-enriched oil had the highest levels of potentially harmful glycidyl esters compared to conventional refined fats and oils. The study of the negative effects of glycidyl esters has prompted the food industry, which has traditionally used oil, to focus on strategies in preventing or mitigating these effects by changing the refining process or modifying deodorization equipment to reduce or eliminate process contaminants.

Keywords: diacylglycerol; glycidol; oil; nutrition; obesity.

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Диацилглицериды как важные компоненты питания или предшественники канцерогенов: критический взгляд на неоднозначный вопрос

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АННОТАЦИЯ

Ожирение признано неинфекционной эпидемией во всём мире. Метаболические нарушения, связанные с накоплением жировой ткани, приводят к прогрессированию ассоциированных с ожирением сахарного диабета, сердечно-сосудистых заболеваний. Одним из компонентов лечения патологий, связанных с нарушением метаболизма, является диета. Наиболее часто применяются диеты с ограничением калорийности пищи за счёт уменьшения в рационе питания жиров. За последние несколько десятилетий проведено большое количество попыток использовать диацилглицериды (ДАГ) в качестве компонентов масел для диетического питания. Это связано со способностью ДАГ подавлять накопление висцерального жира, снижать постпрандиальный уровень триацилглицеридов и холестерина, глюкозы в сыворотке крови. Однако в 2009 году было обнаружено, что при высокотемпературной обработке в процессе физической рафинации масло, обогащённое ДАГ, характеризуется наиболее высоким уровнем образования потенциально опасных глицидоловых эфиров по сравнению с обычными рафинированными жирами и маслами. Изучение негативных эффектов глицидоловых эфиров побудило пищевую промышленность, в которой традиционно использовалось масло, сосредоточить внимание на стратегиях предотвращения или смягчения этих последствий путём изменения процесс са рафинации или модифицирования оборудования для дезодорации, чтобы уменьшить или устранить технологические контаминанты.

Ключевые слова: диацилглицериды; глицидол; масло; питание; ожирение.

Как цитировать

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BACKGROUND

Obesity is gaining alarming proportions and is recognized as a noncommunicable epidemic of the XXI century. Thus, reports from the World Health Organization clearly demonstrate that in 2012, over 250 million people were obese globally (>7% of the population), and 1 billion people were overweight (>30% of the population). In 2016, 650 million patients were obese (13% of the total population), and >1.9 billion people were overweight (39% of the global population). By 2025, the number of patients with obesity is foreseen to increase from 30% to 50% in economically developed countries [1].

Along with obesity, the incidence of associated insulin resistance and diabetes mellitus (DM) is increasing, which are the result of metabolic disorders that are inextricably linked with the accumulation of adipose tissue. Obesity, along with arterial hypertension, smoking, type 2 DM, and dyslipidemia, is a risk factor for cardiovascular diseases. In addition, an association was established between obesity and progression of chronic heart failure, musculoskeletal system diseases, and certain cancers [2].

Obesity is mainly due to the excessive consumption of high-calorie foods, particularly high-fat foods, which causes an imbalance between energy intake and energy expenditure. Restricting energy intake is the primary method of creating a negative energy balance leading to weight loss. However, owing to the different metabolic roles of proteins, fats, and carbohydrates, diets with the same energy content but with different macronutrient distributions may have diverse effects on metabolism, appetite, and therefore the rate of weight loss. Studies examining dietary macronutrient ratios for weight loss have focused on the beneficial role of protein, carbohydrate, and lipid ratios as important factors in weight control [3]. Research is currently focused on the detection of optimal macronutrient distribution to achieve sustainable weight loss using both energy-restricted and non-restricted diets. However, currently, research evaluating the long-term effects of different dietary approaches in people with obesity is insufficient. Recommendations and strategies for the treatment of overweight and obesity in adults highlight the importance of further research to elucidate optimal diet and lifestyle interventions [4]. In recent years, lipids that have undergone chemical or enzymatic modification (so-called structured lipids) have attracted particular interest owing to their ability to reduce the calorie content of foods. Unlike other fat substitutes, structured lipids have nearly the same properties as conventional edible fats but do not compromise the textural and organoleptic properties of food. The most popular of these lipids are diacylglycerides (DAGs), which exist in three stereoisomers, namely, sn-1,2-DAG, sn-1,3-DAG, and sn-2,3-DAG. Some studies have shown that DAG consumption can suppress the accumulation of visceral fat, reduce postprandial levels of

triacylglycerides (TAGs) and cholesterol in the blood serum, and exert beneficial effects on the glycemic profile [5, 6]. Such properties of DAGs are associated with their structural features, so they can be metabolized differently. Among the three stereoisomers of DAGs, sn-1,3-DAG is of particular interest as it can exert the above-mentioned health benefits better than sn-1,2-DAG and sn-2,3-DAG [7]. Unlike ordinary vegetable oil, where the DAG content ranges from 2% to 10%, oil synthesized by structural modification of ordinary fats can be enriched with DAGs up to 80%. However, during the refining process of oils with high-DAG content, by-products that are probable carcinogens can be formed [8].

This study aimed to summarize current data from experimental and clinical studies on the effect of DAGs on carbohydrate and lipid metabolism and body weight and evaluate the possibility of adding them to various food products from the standpoint of health benefits and risks.

SOURCE SEARCH METHODOLOGY

Databases of PubMed, RSCI, MEDLINE, and EMBASE were searched for the period from 1980 to December 2022, and 641 papers were reviewed in total. Search queries were performed using the keywords "obesity," "diacylglycerides," "oil," and "nutrition."

ASPECTS OF CLEAVAGE AND ABSORPTION OF DAGS IN THE DIGESTIVE TRACT

The bulk of lipids in the body are fats, namely, TAGs, which serve as a form of energy storage. Hydrolysis of TAGs in the intestine occurs under the action of a specific pancreatic lipase (sn-1,3 pancreatic lipase), resulting in the formation of 2-monoacylglycerol (2-MAG) and free fatty acids, and its main part is involved in the resynthesis of TAGs. Re-esterified TAGs circulate in the lymphatic vessels and blood as chylomicrons. Although the same gastrointestinal enzymes are involved in the conversion of DAGs, they have different features of absorption and metabolism. First, DAGs do not follow the TAG re-synthesis pathway, which includes the 2-MAG pathway. DAGs, particularly 1,3-DAG, are cleaved to form 1,3-MAG in the small intestine by sn-1,3-specific pancreatic lipase. Compared with 2-MAG, 1,3-MAG is poorly transesterified into TAG. Instead, 1,3-MAG is hydrolyzed into free fatty acid, and only a small portion is resynthesized into TAG. This mechanism prevents the formation of chylomicrons; consequently, the amount of atherogenic lipids in the blood decreases. Second, DAG metabolism is related to its substrate specificity for the enzyme diacylglycerol acyltransferase (DGAT), which is involved in the conversion of DAGs back to TAGs. In experimental studies, 1,3-DAG demonstrated low substrate specificity for DGAT, which prevented the synthesis of new TAGs [9].

EFFECT OF DAGS ON LIPID PROFILE

Experimental and limited clinical studies have shown that DAG consumption (particularly 1,3-DAG) improves the lipid profile, normalizing cholesterol and triglyceride levels. Thus, N. Taguchi et al. revealed that DAG intake was associated with improved blood lipid profile owing to a decrease in cholesterol and chylomicrons in healthy volunteers [10].

Somewhat later, the same authors, using experimental mouse models with DM and apolipoprotein E deficiency, showed that a DAG-enriched diet improves the lipid profile by reducing cholesterol and lipoproteins rich in triglycerides and reduces significantly fat accumulation in the liver and reduces the activity of hepatic microsomal triglyceride transport protein [11].

In 2013. R. Dhara et al. demonstrated that the addition of DAG-added mustard oils to the diet was associated with a significant decrease in body weight in rats with normo- and hypercholesterolemia. Moreover, levels of total cholesterol decreased, whereas those of high-density lipoproteins significantly increased. A decrease in lipid and TAG content was also noted in both the liver and mesentery [12]. K. Tang et al. conducted a more comprehensive study and demonstrated a decrease in the level of low-density lipoprotein (LDL) in the blood serum of mice that received DAG-added oils and low expression of the apolipoprotein B mRNA gene [13].

B. Prabhavathi Devi et al. used a DAG-added diet in combination with a low dose of phytonutrients and showed a decrease in LDL and triglyceride levels in the serum and liver of Wistar rats. However, the authors stated that these effects could be associated with the presence of phytonutrients in the diet, which may also influence the lipid profile [14].

M. Anikisetty et al. revealed that dietary supplementation with DAG-rich sunflower oils and rice bran oils for 12 weeks significantly reduced levels of TAGs and total cholesterol in the serum, liver, and adipose tissue of Wistar rats. The authors concluded that DAG-rich oils reduced the levels of some prognostic biomarkers of cardiovascular diseases, such as serum C-reactive protein, tumor necrosis factor-alpha, platelet aggregation, inducible nitric oxide synthase, cyclooxygenase-2, and vascular cell adhesion molecule-1 [15].

STIMULATION OF BETA-OXIDATION PROCESSES WHEN TAKING DAGS

DAGs can suppress fat accumulation by increasing the beta-oxidation rate of fatty acids in the liver. As a result of beta-oxidation, fatty acids are converted into ketone bodies and then into acetyl-CoA, which is a quick source of energy; therefore, the repeated formations of the TAG molecule are held off [13, 16].

As confirmed by Murase et al., the activity of rat liver acetyl coenzyme, which is involved in beta-oxidation, was

higher than the activity of the enzyme responsible for fatty acid synthesis when fed a DAG-containing diet. The authors concluded that DAGs enhanced oxidation and reduced the synthesis of fatty acids in the liver. In addition, DAG consumption was associated with increased expression of some genes responsible for lipid metabolism, for example, the gene for uncoupling protein 2 (UCP2) in the small intestine [17].

In a clinical study using DAGs, T. Yasukawa and K. Yasunaga concluded that the intake of DAG-containing oils for 14 days stimulates fat oxidation by increasing the metabolic rate. The effect of DAGs on increasing energy expenditure, fat oxidation, and respiratory quotient is greater in individuals who are overweight. Increased rapid fat oxidation after DAG therapy at least partially explains the significant weight loss associated with DAGs. However, these results may not be conclusive because most resting metabolic rate tests were performed over a short period [18].

EFFECT OF DAGS ON BODY WEIGHT

Controlled studies have suggested the beneficial effects of DAG-enriched oil as an adjunctive therapy in the treatment of obesity. Most of these studies have shown a strong correlation between DAG intake and reduction in body weight, subcutaneous and visceral fat distribution, and waist circumference in individuals who were overweight or obese when taking DAGs for 6 weeks to 6 months [19-21]. Some clinical and preclinical studies have shown that DAGs enriched with medium-chain fatty acids are more effective in reducing the body mass index than DAGs enriched with long-chain fatty acids. H. Kim et al. concluded that DAGs added with medium-chain fatty acids are much better at reducing body weight because they are more easily metabolized in the body than those added with long-chain fatty acids [22].

EFFECT OF DAGS ON GLUCOSE METABOLISM

The use of oil with high-DAG contents in food can prevent complications of type 2 DM by reducing fasting insulin levels and index of insulin resistance (homeostatic assessment of insulin resistance) [23, 24]. This effect was directly proportional to the weight reduction noted with DAG consumption, which probably contributed to the improvement in glucose metabolism. However, J. Zheng et al. demonstrated that a decrease in insulin resistance depends on the initial body mass index of a patient with type 2 DM, with maximum severity only in individuals with normal body weight in contrast to patients who were overweight or obese. A 3-year follow-up demonstrated a delay in the progression of renal failure in patients with diabetic nephropathy, who took DAGs for 3 months, most probably due to a decrease in serum TAG levels [24].

DAGS AND BONE TISSUE PATHOLOGY

H.S. Choi et al. reported that DAGs increased bone mineral density and improved the bone microstructure [25]. DAGs can reduce glucose and lipotoxicity, which eases the severity of oxidative stress in bone cells. This increases the activity of osteoblasts and decreases the activity of osteoclasts, thereby preventing bone resorption. However, the possible beneficial effects of DAGs on bone tissue require further study, such as the current study by H.S. Choi et al.

USE OF DAGS IN FOOD PRODUCTS

The above-demonstrated effects of DAGs in relation to weight normalization have aroused great interest among technologists in terms of its possible addition to various food products [26, 27]. DAGs were approved as a food ingredient in 2006 for use in vegetable oils, salad dressings, mayonnaise, beverages, and baked goods. Several patents have been filed for DAG-based products such as nutritional drinks and bars and baked goods (cupcakes, muffins, brownies, bread, cookie dough, and butter). In the food industry, among other properties, DAGs are most often used as an emulsifier. Nowadays, DAGs, along with MAGs, dominate 70% of the global market for food emulsifiers. However, a problem associated with the use of DAGs in food products is the high price because fats and oils must undergo an additional modification process to obtain DAGs. Some consumers may also question the "naturalness" of high-DAG oils compared with conventional vegetable oils.

DAG SAFETY

Given that DAGs are present in several common foods and have a long history of use, the Food and Drug Association considered them a candidate for the generally recognized as safe (GRAS) list. Initially, two categories of DAG use were claimed for the GRAS list, namely, as a component of butter and margarine for use in preparing food at home. Subsequently, for several years, the DAG use categories for GRAS were expanded to include home cooking oils, margarine spread, bakery products, seasonings, and instant foods.

The caloric value of DAGs is equivalent to ordinary vegetable oils, which is 120 kcal per serving [28]. However, from the perspective of physiologists, biochemists, and others, DAG metabolism differs from that of TAG. For a common consumer who uses oil for frying or as the main ingredient in preparing foods, these are different products. The first advertising slogan for DAG-containing oils was "Less likely to be stored as fat." When sufficient evidence was obtained, another slogan was developed, "When using DAGs as a component of a rational diet, normal weight can be maintained." Later studies of DAGs formed the basis of postulate 3, that is, "Blood triglycerides decrease with DAG intake." The above statements are indeed based on the results of preclinical and clinical studies, which have demonstrated convincing evidence that DAGs reduce the risk of diseases associated with metabolic disorders and lifestyle characteristics.

The Ministry of Health, Labor and Welfare of Japan approved DAGs as a food product for specified health use (FOSHU) in 1999. After receiving FOSHU status, the professional association of Japanese doctors also suggested that DAG consumption is beneficial to human health. In the 2000s, DAG sales in Japan accounted for approximately 80% of premium oils, which equated to approximately 14% of the entire Japanese edible oil market of 10 billion yen. Subsequently, high-DAG oils received marketing authorization in various countries worldwide, such as the United States, the European Union, Canada, Australia/New Zealand, and Brazil [29]. At that time, studies on DAGs regarding their carcinogenicity, genotoxicity, and general toxicity, conducted on animals and humans over a short or long period, showed that DAGs were safe for consumption [30, 31]. In addition, oil DAG-added oils do not have a mutagenic or genotoxic effect, regardless of the degree of its heating during cooking food [32]. However, it was not until September 2009 that Kao Corporation (Japan) suspended the sale of DAG oils after the German Federal Institute for Risk Assessment expressed concern about glycidol esters (GEs) detected in processing DAG-added edible oils. A study suggested that the free form of glycidol, resulting from the hydrolysis of GEs in the body, is a potential carcinogen [33]. Because DAGs are believed to be one of the precursors for GE formation (during high-temperature processing of oils during physical refining), DAG-enriched oils are characterized by the highest level of GE formation compared with conventional refined fats and oils. Another study showed that the GE content in DAG-added oils is approximately 10-40 times higher than that of ordinary vegetable oil, being approximately 269 and 6.7–22.8 µg/g, respectively [34]. Esters of 2,3-epoxy-1-propanol (glycidol) were originally used as raw materials and stabilizers in the production of polymers in cosmetic and pharmaceutical industries. GEs were subsequently discovered in refined edible oils and fats or products containing them [35-38]. A study reported that heat treatment and particularly deodorization during oil processing led to GE formation [39]. R. Inagaki et al. demonstrated high concentrations of GEs in meat samples heated at high temperatures. The composition of thermoformed GEs was consistent with the fatty acid composition of unheated samples, indicating that fatty acids and triglycerides in foods can be converted to GEs [40]. Carcinogenicity studies have shown that subcutaneous administration of glycidyl oleate or stearate did not significantly increase the incidence of local tumors in mice [41, 42]. Based on the results of these studies,

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the International Agency for Research on Cancer IARC has designated glycidyl oleate and stearate as substances "not classifiable with respect to their carcinogenicity to humans." Genotoxicity studies of glycidyl linoleate using the Ames test on five strains, a chromosomal aberration test in Chinese hamster lung cells, and micronucleus formation in mice suggested that glycidyl linoleate should be considered non-genotoxic because a positive result in the Ames test was associated glycidol release [43]. Mutagenic effects of glycidol in vitro have been identified in tests with and without metabolic activation, as well as a wide range of genotoxic effects in mammalian cells (genetic mutations, chromosomal aberrations, sister chromatid exchange, and unscheduled DNA synthesis). In vivo genotoxicity tests were less conclusive. Some positive micronucleus assay results were reported in mice after intraperitoneal administration of glycidol [44]. After confirmation of carcinogenicity in mice and rats, the IARC rated glycidol as "probably carcinogenic to humans" (category 2A); however, epidemiological data were still lacking.

In 2012, a clinical study that was participated by employees of Kao Corporation (Japan) to examine the effects of DAG intake did not find a significant difference in the blood concentrations of the glycidol compound N-(2,3-dihydroxypropyl) valine (diHOPrVal) before and after DAG use. This result suggests a minimal possibility of the release and subsequent absorption of glycidol from GEs during the digestion of DAGs in intestines [45]. However, this study did not definitively conclude that DAGs are truly safe for consumption because of the relatively small sample size (15 patients in the study group and 42 patients in the control group) and the sample was limited to employees of Kao Corporation. In addition, individuals who had previously consumed DAG-added oils but had discontinued their intake at least 4 months before enrollment in the study could participate in the study and therefore may already have some amount of diHOPrVal in their body.

Some studies have revealed that DAGs do not affect the development of precancerous and neoplastic lesions in the gastrointestinal tract [46] and of the embryo or fetus in experimental animal models [47]. On the contrary, the positive effects of DAGs described earlier could be the result of a relatively short duration of preclinical studies; as a result, GEs may not have had time to exert their possible negative effect.

In 2018, the European Union introduced the standardization for the content of GEs (in terms of free form) in edible oils and oils used for the production of infant formula at the level of 1.0 and 0.5 mg/kg of oil, respectively [48]. In Russia, the same indicators were included in the Unified Sanitary-Epidemiological and Hygienic Requirements for Products (Goods) Subject to

Sanitary-Epidemiological Surveillance (Control) by Decision of the Board of the Eurasian Economic Commission dated August 6, 2019 No. 132 [49].

To date, the safety of DAGs has still not been proven, and they are not being brought back into the market worldwide. The main question remains about the health status of people who previously used DAGs [50]. So far, no comprehensive research has been conducted on the effects of their use in food. A study of the effects of GEs has prompted the food industry, which has traditionally used oils and fats, to focus on strategies at preventing or mitigating these effects by modifying the refining process or modifying deodorization equipment to reduce or eliminate process contaminants.

CONCLUSION

Current diets used to reduce body weight and normalize carbohydrate and lipid metabolism are based on limiting the calorie content of foods by reducing their fat content. For many years, scientists have been changing the structure of fats and oils because structured lipids can reduce the calorie content of foods. When vegetable oils containing 80% of DAGs were approved for sale in Japan and the USA, consumers wanted to try these new products in the forefront of publicity about their positive properties. In some countries, DAG-containing foods have been labeled in such a way as to inform the consumer of the potential benefits and health effects. However, the industry did not last long until the identification of GEs as technological contaminants in refined edible oils, including DAG-added oils, in 2009-2010. Concerns have been raised about the possible effects of GEs, which are carcinogenic in animal studies, in humans. A unique challenge for chemical technologists is to examine the contribution of oil composition and structure to GE formation. Thus, until innovative techniques are introduced to prevent GE formation from DAG-added oils, the possibility of their use as preferred fats in the diet of people with metabolic disorders remains uncertain.

ADDITIONAL INFORMATION

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