Therapeutic Application of Diacylglycerol Oil for Obesity: Serotonin Hypothesis

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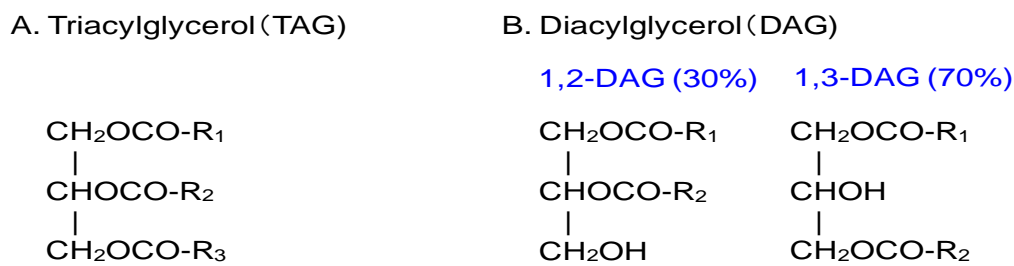
ABSTRACT:

Characteristics for the serum lipid abnormalities in the obesity/metabolic syndrome are elevated fasting, postprandial triglyceride (TG), and decreased high-density lipoprotein-cholesterol (HDL-C). Diacylglycerol (DAG) oil ingestion has been reported to ameliorate postprandial hyperlipidemia and prevent obesity by increasing energy expenditure, due to the intestinal physiochemical dynamics that differ from triacylglycerol (TAG). Our study demonstrated that DAG suppresses postprandial increase in TG-rich lipoprotein, very low-density lipoprotein (VLDL), and insulin, as compared with TAG in young, healthy individuals. Interestingly, our study also presented that DAG significantly increases plasma serotonin, which is mostly present in the intestine, and mediates thermogenesis, proposing a possible mechanism for a postprandial increase in energy expenditure by DAG. Our other study demonstrated that DAG suppresses postprandial increase in TG, VLDL-C, and remnant-like particle-cholesterol, in comparison with TAG in an apolipoprotein C-II deficient subject, suggesting that DAG suppresses postprandial TG-rich lipoprotein independently of lipoprotein lipase. Further, to understand the molecular mechanisms for DAG-mediated increase in serotonin and energy expenditure, we studied the effects of 1-monoacylglycerol and 2-monoacylglycerol, distinct digestive products of DAG and TAG, respectively, on serotonin

release from the Caco-2 cells, the human intestinal cell line. We also studied effects of 1- and 2-monoacylglycerol, and serotonin on the expression of mRNA associated with β-oxidation, fatty acids metabolism, and thermogenesis, in the Caco-2 cells. 1-monoacylglycerol significantly increased serotonin release from the Caco-2 cells, compared with 2-monoacylglycerol by approximately 40%. The expression of mRNA of acyl-CoA oxidase (ACO), fatty acid translocase (FAT), and uncoupling protein-2 (UCP-2), was significantly higher in 1-MOG-treated Caco-2 cells, than 2-MOG-treated cells. The expression of mRNA of ACO, medium-chain acyl-CoA dehydrogenase, FAT, and UCP-2, was significantly elevated in serotonin-treated Caco-2 cells, compared to cells incubated without serotonin. In conclusion, our clinical and in vitro studies suggested a possible therapeutic application of DAG for obesity, and obesity-related metabolic disorders.

Key words: Diacylglycerol, intestine, obesity, serotonin, thermogenesis

INTRODUCTION:

Triacylglycerol (TAG) is the main form of lipid in most common edible oils. Diacylglycerol, DAG, is present in small quantities in vegetable oils [1-3]. DAG can be synthesized enzymatically with the reverse reaction of 1,3-specific lipase. The chemical structure of TAG and DAG is shown in Figure 1. The ratio of the 1,3-DAG to 1,2-DAG in DAG oil is approximately 7 to 3 [4].
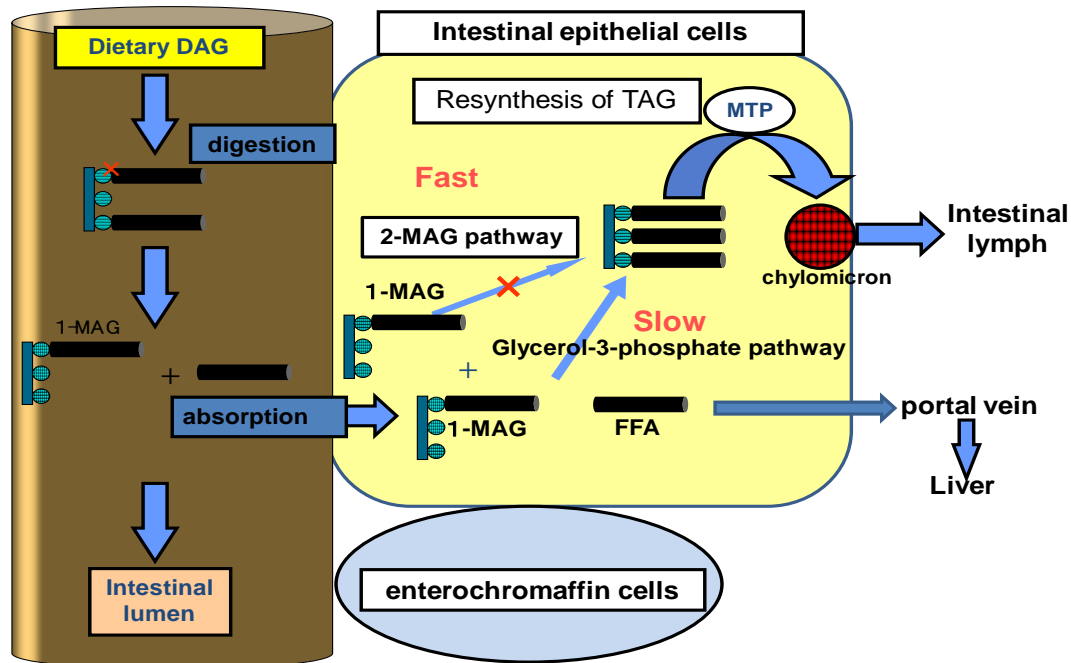
A. Triacylglycerol（TAG）   B. Diacylglycerol（DAG）

                                           1,2-DAG (30%)   1,3-DAG (70%)

CH_2OCO-R_1 CH_2OCO-R_1 CH_2OCO-R_1

| | |

$CHOCO-R_2$ $CHOCO-R_2$ $CHOH$

CH_2OCO-R_3 CH_2OH CH_2OCO-R_2

Figure 1. The chemical structure of triacylglycerol and diacylglycerol. R_1, R_2 and R_3 indicate fatty acids.

TAG is hydrolyzed by lipase to free fatty acids (FFA), and 2-monoacylglycerol (MAG), in the intestinal lumen, to then be absorbed by intestinal cells. In intestinal cells, TAG is re-synthesized from 2-MAG and FFA, predominantly by the 2-MAG pathway [1,5]. Monoacylglycerol acyltransferase and diacylglycerol acyltransferase work in the 2-MAG pathway [6,7].

In the case of DAG, the metabolic pathway is different from that of TAG. The main form of DAG, 1,3-DAG is hydrolyzed to 1-MAG and FFA [1,8]. 1-MAG cannot be the substrate for both monoacylglycerol acyltransferase and diacylglycerol acyltransferase.

Therefore, TAG can be re-synthesized via the glycerol-3-phosphate pathway, which is less active than the 2-MAG pathway (Figure 2) [1,9]. Such a slower re-esterification to TAG in small intestinal cells is supposed to be the underlying mechanism for DAG-mediated amelioration in postprandial hyperlipidemia.

Figure 2. The underlying mechanisms for diacylglycerol-mediated ameliorating postprandial hyperlipidemia. FFA, free fatty acid; MAG, monoacylglycerol; MTP, microsomal triglyceride transfer protein; TAG, triacylglycerol.

DAG ingestion has also been reported to increase the postprandial energy expenditure, in comparison with TAG [10,11]. The increase in postprandial energy expenditure is supposed to be one of the mechanisms for the anti-obesity effect of DAG [12]. The up-regulated expression of mRNA associated with □-oxidation, thermogenesis, and FA metabolism, in the small intestine, by DAG, may be the explanation for the part of the mechanisms responsible for increased postprandial energy expenditure [13,14]. Thus, the small intestine is likely to be the key organ to make differences in lipid and energy metabolism between TAG and DAG.

Serotonin, which increases oxygen consumption and body temperature, is present in great quantity in the small intestine [15,16]. Then, we hypothesized that serotonin may be associated with an increased postprandial energy expenditure by DAG (serotonin hypothesis).

Here we will show effects of DAG on serum lipids, insulin and serotonin in lean young healthy males, and the effects of DAG on serum lipids in apolipoprotein C-II deficient

middle-aged males. Furthermore, we will introduce the underlying molecular mechanisms for the anti-obesity effect of DAG.

Effects of DAG on serum lipids, insulin, and serotonin, in lean, young, healthy males:
The effects of DAG on serum lipids and insulin among lean young males as compared with TAG were shown in Table 1.

Table 1. Effects of DAG on serum lipids and insulin as compared with TAG [17,20]

	healthy young lean males (n=7)	apo C-II deficient male (n=1)
FFA	·suppression of increase	·no difference between TAG and DAG
TG	·suppression of increase	·suppression of increase
RLP-cholesterol	·suppression of increase	·suppression of increase
VLDL-cholesterol	·suppression of increase ·a significant difference between TAG and DAG	·suppression of increase ·a significant difference between TAG and DAG
insulin	·suppression of increase ·a significant difference between TAG and DAG	·not determined

Apo C-II, apolipoprotein C-II; DAG, diacylglycerol; FFA, free fatty acids; RLP, remnant-like particle; TAG, triacylglycerol; TG, triglyceride; VLDL, very low-density lipoprotein

To understand the effects of DAG on serum lipids, glucose metabolism, and serotonin, we performed a randomized crossover study with a 2-week washout interval between TAG and DAG oil ingestion [17]. Subjects studied were 7 healthy lean male students. Each subject ingested 30g per square of TAG or DAG oil with 40g of carbohydrate. The measurement of metabolic parameters and serotonin was performed before, at 2, 4, and 6 hours after experimental oil ingestion.

Although there were no significant differences in the changes in serum FFA, remnant-like particle-cholesterol (RLP-C), and triglyceride (TG) levels between TAG and DAG ingestion, significant influences of DAG on changes in serum FFA, RLP-C, and TG were observed as compared with TAG (Table 1). A significant influence of DAG was observed in changes in very low-density lipoprotein (VLDL)-C, and VLDL-C levels were also significantly lower at 2 hours after DAG ingestion, compared with TAG ingestion, suggesting that DAG ingestion suppresses the postprandial increase in VLDL-C, compared with TAG ingestion.

We also observed a higher response and a retarded reduction of insulin after TAG ingestion, compared with DAG ingestion. The area under the curve (AUC) for VLDL-C was positively and significantly correlated with the AUC for insulin. The postprandial increase in VLDL-C may be associated with elevated postprandial insulin. A recent study reported that postprandial hyperlipidemia induces an acute decrease of insulin sensitivity in healthy men [18]. DAG-mediated amelioration in postprandial hyperlipidemia may improve insulin sensitivity, and result in the decrease of insulin secretion.

DAG ingestion significantly increased plasma serotonin, however, TAG did not show any influences on serotonin levels. The serotonin levels at 2 hours after DAG ingestion were significantly higher than those after TAG ingestion by approximately 50%. DAG is hydrolyzed to 1-MAG, which is poorly reesterified into TAG, as compared with 2-MAG, and subsequently is stored in the small intestine. Although the precise mechanism for elevation of plasma serotonin by DAG ingestion remains unknown, the retention of 1-MAG may be associated with serotonin release from the enterochromaffin cells of the small intestine. Serotonin is present in great quantity in the small intestine, and is associated with the increase in oxygen consumption and body temperature [15,19]. An administration of a serotonin 2C receptor agonist, (S)-2-(7-ethyl-1H-furo[2,3-g]indazol-1-yl)-1-methylethylamine significantly increased body temperature and energy expenditure in Zucker rats in a dose-dependent manner, and a selective serotonin receptor antagonist completely inhibited this increase in energy expenditure, suggesting that serotonin regulates thermogenesis and energy expenditure [20]. A single injection of serotonin in rats stimulated resting oxygen consumption; this was reduced by pretreatment with the automatic nerve ganglion blocking agent, hexamethonium, suggesting that serotonin mediates peripheral sympathetic thermogenesis [16]. Therefore, increased plasma serotonin levels may be the underlying mechanism for the increased postprandial energy expenditure after DAG ingestion. Serotonin has been reported to mediate gastrointestinal motility [21], however, none of our subjects reported symptoms of gastrointestinal tract after DAG ingestion. We have to mention that we did not measure energy expenditure in our study, and we should have studied energy expenditure when we measured plasma serotonin. In the future, we will study plasma serotonin levels and energy expenditure in subjects after ingestion of DAG and TAG.

In summary, DAG ingestion suppressed postprandial increase in VLDL-C and insulin, compared with TAG ingestion, which seems to be effective to treat metabolic disorders in obese people. DAG increased plasma serotonin, which mediates peripheral sympathetic thermogenesis [15,16,19,20], suggesting that the increase in serotonin may be the underlying mechanism for DAG-mediated elevation of postprandial energy expenditure.

Effects of DAG on serum lipids on apolipoprotein C-II deficient middle-aged male: We will introduce the therapeutic application of DAG for apolipoprotein C-II deficiency. Apolipoprotein C-II (apo C-II) plays the role of a coenzyme of lipoprotein lipase, which hydrolyzes TG in chylomicron and VLDL [22]. We experienced a 43-year-old man with Apo C-II deficiency [23]. His serum of TG is 2,521 mg/dl by treatment with fenofibrate (201 mg/day) and eicosapentaenoic acid (1800 mg/day). The patient was subjected to a loading test with 10g of DAG or TAG oil after a 15-hour fast, in a cross over style, with a 1-week interval. Blood samples were obtained every 2 hours (hours 0–8), to measure serum lipids. An outline of the effects of DAG on serum lipids in an Apo C-II deficient patient is shown in Table 1. Serum FFA gradually increased after ingestion of both kinds of oil, with no difference between TAG and DAG. Serum TG was remarkably increased by TAG from hour 4, the increase with DAG was almost half of that with TAG, at hours 4 and 6. DAG decreased VLDL-C serum up to hour 6 after oil ingestion, while TAG increased VLDL-C continuously. Although serum RLP-C was linearly elevated by TAG, the increase by DAG was modest.

DAG ingestion suppressed the postprandial increase in serum TG and TG-rich lipoprotein in a subject with Apo C-II deficiency. This means that DAG suppresses postprandial hyperlipidemia, independent of lipoprotein lipase, which is inactivated in patients with metabolic syndrome/obesity [24].


The underlying molecular mechanisms for the anti-obesity effects of DAG: The Caco-2 cells (the human intestinal cell line) have been reported to synthesize and degrade serotonin [25], and serotonin receptors and transporters are expressed in the Caco-2 cells [26,27,28]. Therefore, we studied the effects of 1-MAG and 2-MAG, distinct digestive products of DAG and TAG, respectively, on serotonin release from the Caco-2 cells [29]. We also studied effects of 1- and 2-MAG, and serotonin, on expression of mRNA associated with □-oxidation, FA metabolism, and thermogenesis in the Caco-2 cells. 100-μM 1-MAG significantly increased serotonin release from the Caco-2 cells, compared with 2-MAG, by about 40%. Although a statistical significant difference was not obtained, increased serotonin release was observed in the Caco-2 cells treated with 200-μM 1-MAG, as compared with cells treated with 2-MAG. The expression of mRNA of acyl-CoA oxidase (ACO), fatty acid translocase (FAT), and uncoupling protein-2 (UCP-2) was significantly higher in 1-MAG-treated Caco-2 cells than 2-MAG-treated cells by 13%, 24%, and 35%, respectively. The expression of mRNA of ACO, medium-chain acyl-CoA dehydrogenase (MCAD), FAT, and UCP-2 were significantly elevated in serotonin-treated Caco-2 cells compared with cells incubated without

serotonin. We have to mention that we did not measure fatty acid oxidation (enzymatic activity) after 1-MAG and serotonin treatment, and we should have studied fatty acid oxidation, which will be studied in the future.

Our study demonstrated that a hydrolytic product of DAG, and 1-MAG, increases serotonin release from the Caco-2 cells, and enhances expression of genes associated with □-oxidation (ACO), FA metabolism (FAT), and thermogenesis (UCP-2), as compared with a hydrolytic product of TAG, 2-MAG (Figure 1). The present study also demonstrated that serotonin up-regulates the expression of genes associated with □-oxidation (ACO, MCAD), FA metabolism (FAT), and thermogenesis (UCP-2). The Caco-2 cells express various serotonin receptors and the serotonin transporter, which may be associated with the expressions of these genes [26, 27, 28]. Our result proposes a new molecular biological mechanism for the DAG-mediated anti-obesity effect (serotonin hypothesis).

CONCLUSIONS:

The underlying mechanisms for diacylglycerol-mediated ameliorating postprandial hyperlipidemia and preventing obesity were shown in Figure 3.
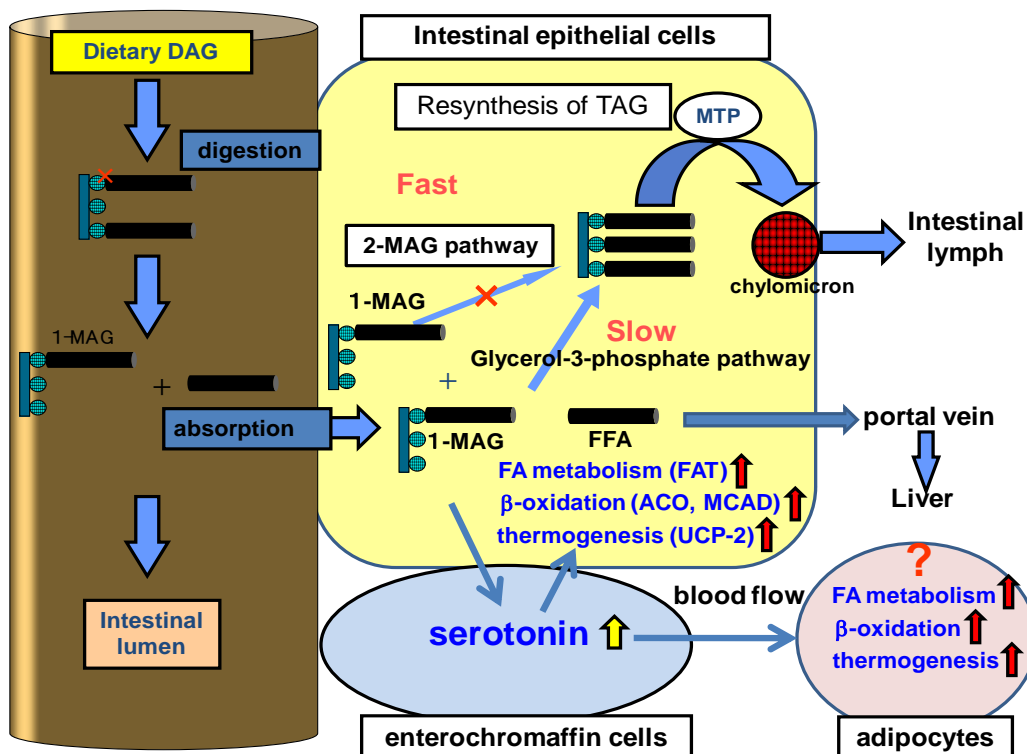
Figure 3. The underlying mechanisms for diacylglycerol-mediated ameliorating postprandial hyperlipidemia and preventing obesity. ACO, acyl-CoA oxidase; DAG, diacylglycerol; FAT, fatty acid translocase; FFA, free fatty acid; MAG, monoacylglycerol; MCAD, medium-chain acyl-CoA dehydrogenase; MTP, microsomal triglyceride transfer protein; TAG,

triacylglycerol; UCP-2, uncoupling protein-2

Our clinical studies showed DAG-mediated amelioration in postprandial TG-rich lipoprotein and insulin, indicating the therapeutic application of DAG for obesity-related metabolic disorders. Further, our study demonstrated that DAG ingestion significantly increases plasma serotonin, which mediates peripheral sympathetic thermogenesis. Our in vitro study demonstrated that 1-MAG, a digestive product of DAG, increases serotonin release from the Caco-2 cells, and enhances expression of genes associated with β-oxidation, FA metabolism, and thermogenesis, and that serotonin increases expression of these genes, proposing a novel molecular mechanism for DAG-mediated anti-obesity effect (serotonin hypothesis).

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