

Author's response to reviews

Title: Diacylglycerol oil for the metabolic syndrome

Authors:

Hidekatsu Yanai (yanaih@jikei.ac.jp)

Yoshiharu Tomono (tomono@jikei.ac.jp)

Kumie Ito (ito@jikei.ac.jp)

Nobuyuki Furutani (furutani@jikei.ac.jp)

Hiroshi Yoshida (yoshida@jikei.ac.jp)

Norio Tada (tada@jikei.ac.jp)

Version: 3 **Date:** 24 November 2007

Author's response to reviews: see over

The Editor

Nutrition Journal

Dear Sirs,

We will re-submit our revised manuscript entitled “Diacylglycerol oil for the metabolic syndrome (1,537 words, 39 references, 3 table, and 3 figures)” to *Nutrition Journal*.

According to your reviewers’ comments, we completely corrected our manuscript. We will show you the list of modification in the next page.

We have no financial or other contractual agreements that might cause conflict of interest or be perceived as causing conflicts of interest. The corresponding author and all of the authors have read and approved the final submitted manuscript. No portion of the work has been or is currently under consideration for publication elsewhere. No portion of the manuscript other than the abstract has been published or posted on the internet. We will appreciate your consideration of our revised manuscript for publication in the section of “Review” in your journal.

Sincerely yours,

Hidekatsu Yanai, MD, PhD

Assistant Professor

Department of Internal Medicine,

The Jikei University School of Medicine,

The Member of American College of Physicians

The List of Modification

1. Reviewer (Referee 1): Makoto Ayaori

According to Dr. Ayaori's comment "Yasunaga et al. (J Lipid Res. 2007 May;48(5):1108-219) reported that diacylglycerol reduced plasma TG levels, after oral or intravenous DAG, result from more efficient clearance of DAG by both LPL lipolysis and apoE-mediated hepatic endocytosis. The authors should discuss LPL-dependent mechanisms."

We cited Yasunaga's article (J Lipid Res. 2007 May;48(5):1108-219) and added the following sentence: "Recently, Yasunaga K, et al found that DAG oil reduced plasma TG levels, resulting from more efficient clearance of DAG by both LPL-mediated lipolysis and apolipoprotein E-mediated hepatic endocytosis [11]." in page 5.

2. Reviewer (Referee 2): Yuji Hirowatari

1) According to Dr. Hirowatari's comment "The diacylglycerol is natural edible oil, not toxic oil. But, the authors should show the predicted demerit of long-term ingestion or excess ingestion in Introduction or others.(major compulsory revisions 1)"

We added a new paragraph as the followings in page 9-10.

"A safety of chronic consumption of DAG

In c-Ha-ras proto-oncogene transgenic rats, DAG oil administration was associated with a significant increase in the incidence of squamous cell carcinomas of the tongue with

the Cochran-Armitage trend test and also number of tumors in coefficients for linear contrast trend tests [36]. However, 24 months-DAG-treated rats had no higher risk of carcinogenic effects than rats fed on similar feeding regimens with TAG [37]. The potential chronic toxic effects of DAG when administered orally for 12 months were evaluated using Beagle dogs [38]. DAG at dietary concentrations up to 9.5% for one year had no effect on normal dogs growth and developments, in comparison to TAG [38]. In mice, DAG at dietary concentrations up to 6.0% for 24 months produced no signs of systemic toxicity and had no effect on the incidence of neoplastic findings [39]. Most of studies investigating a chronic dietary toxicity of DAG reported that DAG did not produce systemic toxicity and had no effect on the incidence of neoplastic findings. However, we should observe the safety of chronic consumption of DAG carefully.”

2) According to his comment “1) I thought that 1(3)-MAG is 1-MAG or 3-MAG in figure 3. But, the authors did not indicate that. 2) I had found two words of Fig. 2 in figure legends. One of Fig. 2 is mistaken.(Minor Essential Revisions)”

We corrected these mistakes.

3) According to his comment “1) The mechanism of improvements in postprandial hyperlipidemia by DAG ingestion: Although 1,2-DAG is not mainly in form of DAG, the metabolic pathways should be slightly shown.(Discretionary Revisions)”

We mentioned that “1,2-DAG would be hydrolyzed to 2-monoacylglycerol, and TG is synthesized via the 2-monoacylglycerol pathway [2]” (page 5).

4) According to his comment “How dose of DAG is high effective for obesity prevention (Discretionary Revisions).”

We added the following sentences in page 9. “Dairy ingestion of 8-20 g DAG has been used in human studies on the effect of DAG on body composition [35]. However, it remains unknown how dose DAG is high effective for preventing excess adiposity, which should be investigated in the future.”

Reviewer (Referee 3): Francisco Tinahones

1) According to Dr. Tinahones’s comment “There are only three references in the literature cited for the authors. However, in the last two years, the authors would find more than twenty reports including diacylglycerol oil as key words.”

We cited 15 references about diacylglycerol in the last two years.

2) According to his comment “The second paragraph is very redundant”

We deleted redundant expressions.

3) According to his comment “In this section, it is not included any date about the metabolism of glucose.”

We added the following sentences in page 5-6. “Further, DAG ingestion was reported to

prevent the high-sucrose-diet-induced development of impaired glucose tolerance compared with TAG oil ingestion, in male Wistar rats [14].” And “In the subjects who consumed daily 10g of DAG for 12 weeks, serum TG levels were decreased by 39.4%, and serum hemoglobin A1c levels were also decreased by 9.7%, compared with subjects who consumed TAG, suggesting that DAG ingestion ameliorates glucose metabolism [19].”

4) According to his comment “The reference 19 is overly developed.”

We changed our expressions.

From

“The apolipoprotein C-II is a cofactor of lipoprotein lipase (LPL), which hydrolyzes TG of CM and VLDL [18]. We have a therapeutic experience with DAG oil to a patient with apolipoprotein C-II deficiency, a rare autosomal recessively-inherited disease [19]. Serum TG was remarkably increased from hour 4 (4 hour after experimental oil ingestion) by TAG, and the increment by DAG was almost half of that by TAG at hours 4 and 6. Serum VLDL-cholesterol was decreased by DAG up to hour 6 after oil ingestion, while TAG increased VLDL-cholesterol continuously. Serum RLP-C was linearly elevated by TAG from hour 2 to 8, but the increment by DAG was modest. Our study demonstrated that DAG ingestion suppressed postprandial increase in serum TG, and TG-rich lipoprotein-cholesterol in a subject with apolipoprotein C-II deficiency, suggesting that DAG decreases TG-rich lipoprotein independent of lipoprotein lipase.”

To

“The apolipoprotein C-II is a cofactor of LPL, which hydrolyzes TG of CM and very-low-density lipoprotein (VLDL) [23]. We have a therapeutic experience with DAG oil to a patient with apolipoprotein C-II deficiency, a rare autosomal recessively-inherited disease [24]. In a patient with apolipoprotein C-II deficiency, DAG ingestion suppressed increase in serum TG, VLDL-C, and RLP-C levels compared with TAG ingestion, suggesting that DAG can decrease TG-rich lipoprotein, also independent of LPL.”

5) According to his comment “The authors do not contribute enough results to conclude that the DAG would be a promising therapeutic item for the metabolic syndrome. The authors only mentioned the decrease of triglycerides against DAG.”

We changed our expressions.

From

“DAG ameliorates fasting and postprandial TG-rich lipoproteins, the central lipoprotein abnormality observed in the metabolic syndrome, independent of lipoprotein lipase, which is functionally defective in the metabolic syndrome. DAG may be a promising therapeutic item to the metabolic syndrome.”

To

“In summary, DAG ameliorates fasting and postprandial TG-rich lipoproteins, and glucose metabolism, which may be favorable for metabolic disorders observed in the metabolic syndrome.”

6) According to his comment “The authors should know that a higher postprandial energy expenditure does not mean antiobesity.”

We changed our expression from “The mechanism of anti-obesity by DAG ingestion” to “The mechanism to promote negative caloric balance by DAG ingestion”.

7) According to his comment “ The authors do not support enough data to improve the metabolism of glucose.”

We added the following sentence in page 5-6. “Further, DAG ingestion was reported to prevent the high-sucrose-diet-induced development of impaired glucose tolerance compared with TAG oil ingestion, in male Wistar rats [14].” And “In the subjects who consumed daily 10g of DAG for 12 weeks, serum TG levels were decreased by 39.4%, and serum hemoglobin A1c levels were also decreased by 9.7%, compared with subjects who consumed TAG, suggesting that DAG ingestion ameliorates glucose metabolism [19].”

