Original Article

Effect of diacylglycerol on body weight: a meta-analysis

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The effects of diacylglycerol (DAG) on body weight are not consistent in clinical trials. This meta-analysis of randomized controlled trials was conducted to evaluate the efficacy of dietary DAG on body weight. Potential articles were initially searched from the electronic databases of Medline, Embase and Cochrane library using the subject keywords as follows: weight, DAG, triacylglycerol (TAG), reduction and obesity. Inclusion criteria required the trial to be randomized placebo controlled with body weight as an endpoint. Two reviewers independently extracted the information and evaluated the methodological quality using the scoring system developed by Jadad. Meta-analysis was performed with the software of Review Manager 4.2. The robustness of overall analysis was tested by sensitivity analysis and publication bias was visually inspected by funnel plot. Five published trials were included in the statistical pool. The meta-analysis indicated a significant difference in body weight reduction between group receiving DAG and group receiving TAG (weighted mean difference -0.75 kg; 95% CI: -1.11 to -0.39; $p < 0.0001$). Sensitivity analysis corroborated the result of the overall analysis. Linear regression analysis showed that there was significant correlation between daily dose and body weight reduction ($p = 0.044$, $R^2 = 0.889$). In conclusion, this meta-analysis suggested that DAG was efficacious for reducing body weight compared with TAG and this effect was influenced by the daily dose.

Key Words: diacylglycerol, triacylglycerol, body weight, reduction, meta-analysis

INTRODUCTION

During recent decades, the increased prevalence of obesity has become a worldwide phenomenon which affects not only higher socioeconomic countries but also countries considered to be poor.[1-6] Strong evidences showed that obesity is associated with increased risks for serious disorders, in particular, for cardiovascular diseases, diabetes, musculoskeletal problems and cancer.[7] In addition, the economic burden of obesity and obesity related diseases is increasing as the prevalence of obesity increases.[7-9]

1,3-diacylglycerol (DAG), which has been consumed for many years, is a natural component (2-10%) of some edible fats and oils.[10] A cooking oil product manufactured by Kao Corporation containing about 83% (w/w) DAG has been approved as a "Food for Specified Health Use" by the Ministry of Health, Labor and Welfare of Japan since 1999.[11] In 2000, the Food and Drug Administration (FDA) also approved this product a status of generally recognized as safe (GRAS) .[12]

Recent studies suggested that this DAG rich oil (designated as DAG oil) was useful in promoting weight reduction and body fat reduction compared with triacylglycerol (TAG), and it might be used as an adjunct to diet therapy in the management of obesity.[13-15] The results of animal and human studies showed that DAG could decrease postprandial lipemia, inhibit the synthesis of fat and promote the reduction of body weight when TAG was the control.[16, 17] These effects appeared to be related to the influence of DAG on energy expenditure, food intake or both because the energy value and digestibility of DAG is similar to those of TAG.[18]

However, the efficacy of DAG for body weight reduction was not consistent with the results of all past animal and human studies.[19] Katsurage *et al* found that the mean body weight of DAG group increased 0.92% from the baseline after 12-week test period, which was even higher than that of TAG group (0.62%), though this difference was not significant.[20] Furthermore, a 24-month dietary study in rats showed that the body weight and body weight changes in both DAG and TAG groups were similar at the end of the supplementation.[21]

Whether DAG is in fact efficacious for body weight reduction is still a debatable matter. This study was aimed to determine the efficacy of DAG on body weight reduction using meta-analysis of the published and unpublished studies.

METHODS

The inclusion criteria

Before potential articles for analysis could be identified, criteria of inclusion and operational definitions were

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Table 1. Methodological quality assessment system

Options	Score
Generation of allocation sequence	
Computer-generated random numbers	2
Not described	1
Allocation concealment	
Central randomization	3
Sealed envelopes or similar	2
Not described or inadequate	1
Investigator blindness	
Identical placebo tablets or similar	2
Inadequate or not described	1
No double-blinding	0
Description of withdrawals and drop-outs	
Numbers and reasons are described	1
Numbers and reasons are not described	0
Efficacy of randomization	
Pre-treatment variables in tabular form	2
Balance of pre-treatment variables mentioned but not in tabular form	1
No information reported	0

developed. The trials were included if it was randomize control designed with human being as subjects, and took DAG as the only intervention with body weight as one of the endpoints. No restrictions were imposed on the daily dose of test oil and the physical conditions of subjects.

Selection of studies

Potential articles were initially searched from electronic databases of Medline, Embase and Cochrane library using the subject keywords as follows: "weight, body weight, obesity, fat or fat mass" in combination with "diacylglycerol, triacylglycerol or triglycerides" without time restriction. Then, the references of all located papers were searched for further studies. No language restrictions were imposed.

Extraction of information and the evaluation of methodological quality

Detailed information was extracted independently by two reviewers in a standardized manner according to the predefined criteria. Any discrepancies were investigated in context of the whole article and discussed to resolve them. The methodological quality of including papers was evaluated using the scoring system developed by Jadad (Table 1).[22]

Statistics

The mean extent of body weight change was used as the endpoint to assess the difference between the DAG group and the TAG placebo group. Some studies reported mean and standard deviation (SD) at pre-intervention and post-intervention, but not the SD of the changes. The missing SDs were imputed using the method provided by the Cochrane Handbook.[23] The influence of each study on the result of meta-analysis was weighted by the inverse of the variance, and studies with narrower confidence intervals were given more weight. Random effect model was adopted to calculate the weighted means difference (WMD) and 95% confidence intervals (CI) because of the differences in daily dose, duration of supplementation and initial characteristics of subjects among all studies. Meta-analysis was conducted with the software Review Manager 4.2 (Update Software Ltd, Oxford, England). Sensitivity analysis was performed to test the robustness of the overall analysis. Funnel plot was used to test the publication bias and fail-safe number was used to determine the number of studies with null effects that would have to exist to nullify the reported reduction efficacy.[24] Linear regression was used to test the influence of daily dose on the effect of DAG.

RESULTS

Twenty publications were identified during primary selection and 15 papers were excluded after the evaluation of methodological quality, in which 7 did not take the body weight as the endpoint; 1 was performed without control; 5 were reviews and 2 were not randomized designed. In the study conducted by Yasunaga, the effect of DAG was tested in the males and females independently, so the data of the males and that of females were extracted and included as two independent studies and their influence on the overall result was tested by sensitivity analysis.[15] In the end, 5 papers with 6 independent studies were included in statistical pooling (Table 2).[13-15, 25, 26] In 5 of them, there was no significant difference in energy intake between DAG and TAG groups during the trial period while remaining one did not report the energy intake.

Standard deviations of body weight change from baseline were missing in three studies,[15, 25] so the data of 2

Table 2. Study characteristics

First Author	Quality	Design	Duration	Main Diagnosis	BMI (DAG/TAG)	Age (DAG/TAG)	Dose (g)
Li 2008	4	RDBCPT	120 days	Type 2 diabetes	23.1±2.89/23.8±3.35	54.1±6.70/53.9±6.00	25.0
Maki 2004	4	RDBCPT	24 weeks	Overweight or obese	34.5±3.70/33.9±3.70	45.9±11.4/48.1±11.2	30.0
Yasunaga 2004 (male)	4	RDBCPT	12 weeks	Safety aspects	22.1±2.31/22.2±2.21	34.7±6.10/34.6±6.90	34.4
Yasunaga 2004 (female)	4	RDBCPT	12 weeks	Safety aspects	19.7±2.68/19.8±2.21	31.1±6.80/32.0±6.10	25.6
Nagao 2000	2	RDBCPT	16 weeks	Body fat	24.1±0.40/23.5±0.40	27 to 49 year	10.0
Yamamoto 2001	3	RSBCPT	12 weeks	Hypertriglyceridemia	24.4±1.70/28.1±2.90	56.8±7.30/54.1±18.8	10.6

RD(S)BCPT: Randomized double (single)-blinded controlled parallel trial.

Table 2. Continued

First Author	Sample size DAG/TAG	Body weight (DAG) Baseline/Final	Body weight (TAG) Baseline/Final	Control of Lifestyle Factors
Li 2008	(20:36)/(22:28)	61.4±10.57/60.2±10.54	65.2±12.61/64.7±12.59	Usual diet continued
Maki 2004	(25:40)/(25:37)	98.0±12.90/NR	97.6±14.17/NR	Dietary advice
Yasunaga 2004 (male)	(21:0)/(21:0)	65.3±6.83/65.6±6.87	65.6±6.50/66.2±6.66	Dietary advice
Yasunaga 2004 (female)	(0:18)/(0:21)	50.1±6.83/49.8±7.44	49.9±5.55/49.8±5.83	Dietary advice
Nagao 2000	(19:0)/(19:0)	72.1±7.85/69.5±7.41	68.1±5.67/67.0±6.54	Dietary advice
Yamamoto 2001	(3:5)/(4:4)	60.8±7.60/60.7±7.20	74.5±15.50/73.9±15.00	Dietary advice

NR: Not reported
Sample size: (M:F) of the DAG group/(M:F) of the TAG group.

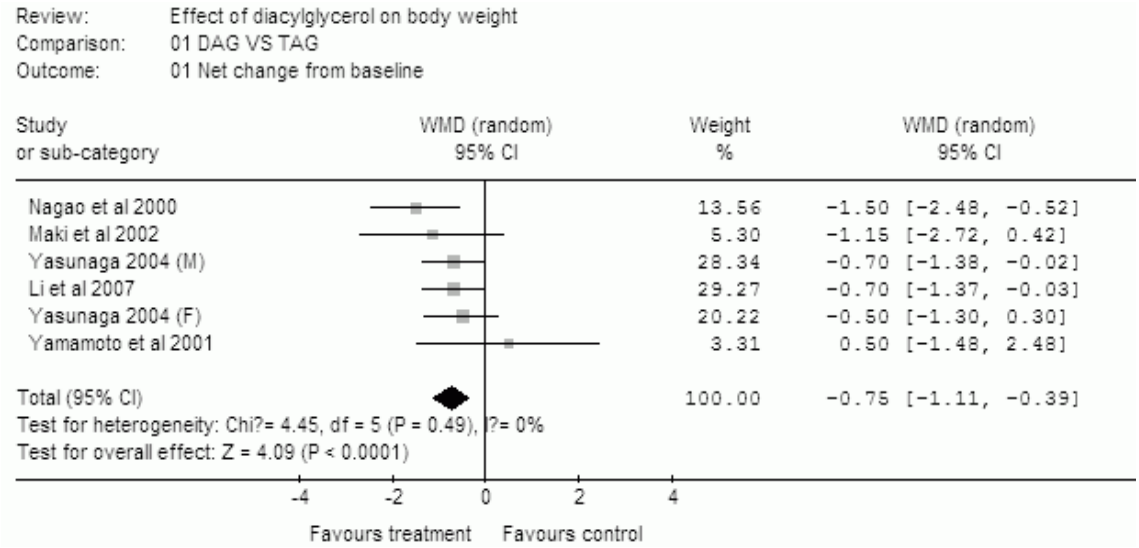

Figure 1. Effect of DAG on body weight (random effect model). The mean differences in the change from baseline were given with 95% confidence intervals. The vertical line represented no difference between DAG and TAG placebo.

included studies that were reported in considerable detail were used to calculate the imputing SD.[23, 27] The correlation coefficients (0.986 and 0.987 for DAG group; 0.991 and 0.969 for TAG group) estimated from these 2 studies were very similar,[13] so the means (0.987 and 0.980 for DAG and TAG groups respectively) of them were used as the correlation coefficient to calculate the missing SD.[23]

The 95% CI of 3 trials overlapped the line of zero effect which indicated no significant differences. Five trials revealed mean differences that favored DAG over TAG on body weight reduction while one did not. Meta-analysis of all trials indicated significant difference in body weight reduction between DAG and TAG groups (WMD -0.75 kg; 95% CI: -1.11 to -0.39; $p < 0.0001$) (Figure 1).

Funnel plot of the mean difference plotted against 1/SE was shown in Figure 2. Visual inspection showed that all trials were distributed in the 95% CI. Analysis of fail-safe number indicated that 31.5 null effect studies were necessary to nullify the reported reduction efficacy of DAG.

Sensitivity analysis was performed to test the robustness of the overall analysis. The first tested whether removing the only single-blinded trial, which was the trial with the lowest sample size,[25] would alter the direction of the overall result. Result showed that the remaining trials corroborated the result of the overall analysis (WMD -0.80 kg; 95% CI: -1.16 to -0.43; $p < 0.0001$). The second tested whether removing those trials with imputed SDs would alter the direction of the overall result.[15, 25] Result showed that the remaining trials was consistent with the overall result (WMD -0.97 kg; 95% CI: -1.49 to -0.46; $p = 0.0002$). The third corroborated the overall result after removing the trial with the lowest score of methodological quality (WMD -0.63 kg; 95% CI: -1.02 to -0.25; $p = 0.001$).[13] Finally, the removal of the two studies from the trial conducted by Yasunaga did not affect the overall result (WMD -0.36 kg; 95% CI: -0.60 to -0.13; $p = 0.002$).

Sub-group analysis tested whether the overall physiological condition and initial BMI influenced the effect of DAG. Firstly, all trials were divided into two sub-groups according to the overall physiological condition. Both meta-analysis of the healthy group (WMD -0.83 kg; 95 % CI: -1.35 to -0.30; $p = 0.002$) and the overweight, obese and type 2 diabetic group (WMD -0.66 kg; 95% CI: -1.24 to -0.07; $p = 0.03$) supported the result of overall analysis.

Secondly, all trials were divided into normal BMI group (BMI < 25) and overweight group (BMI ≥ 25). Meta-analysis showed that DAG could promote the reduction of body weight in subjects with normal BMI (WMD -0.73; 95% CI: -1.11 to -0.35; $p = 0.0002$) but not

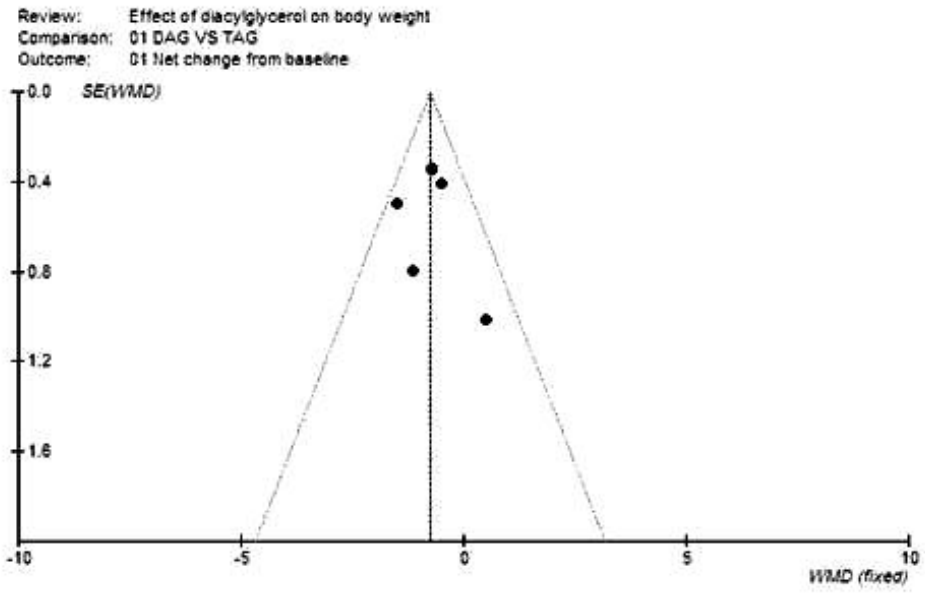

Figure 2. Funnel plot of the mean difference in body weight reduction plotted against 1/SE (2 studies overlapped). The distribution of studies was inspected visually to detect publication bias. An asymmetric funnel indicates the possibility of publication bias. In this figure, the distribution was relatively symmetric which meaning low possibility of publication bias.

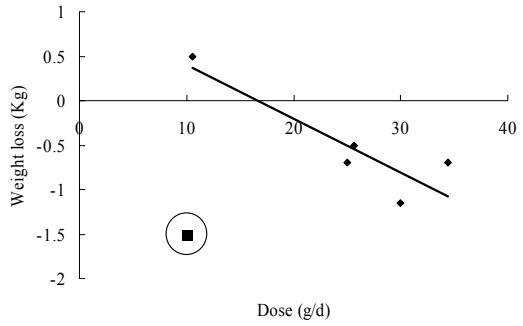

Figure 3. The dose-response between dose and weight reduction examined by linear regression. The trial in circle referred to the study by Nagao[13] which deviated from the trend line greatly. Before excluding this trial, regression equation Y = -0.364 – 0.014X (p = 0.697, R^2 = 0.205) had been obtained. After excluding this trial, regression equation Y = 1.018 – 0.061X (p = 0.044, R^2 = 0.889) had been obtained.

in overweight and obese subjects (WMD -1.15; 95% CI: -2.72 to 0.42; p = 0.15). Linear regression analysis showed that there was no significant correlation between the daily dose of DAG and weight reduction (p = 0.697, R^2 = 0.205). After excluding the study conducted by Nagao[13] in which the strongest effect size was obtained with the smallest dose, the dose-response relationship reached the significant level (p = 0.044, R^2 = 0.889) (Figure 3).

DISCUSSION

These results suggested that DAG was efficacious for body weight reduction compared with TAG placebo. Sensitivity analysis showed a robust significant effect. This result was consistent with those studies which showed that DAG was an effective treatment option for reducing body weight.[13-15]

It was conceivable that some trials were not uncovered although systematic efforts were made to locate and retrieve them. The distorting effects arising from publication bias and location bias have repeatedly been reported.[28-30] For instance, some European journals are not indexed in major medical databases.[31] There is also evidence for the tendency of positive findings to be published in English language journals.[32] In this study, although the fail-safe number was greatly larger than the number of including studies, the vacuum at the left bottom of the funnel plot indicated the potential possibility of publication bias which might overestimate the effect size of DAG. In addition, the number of including studies was small and more studies were needed to illuminate the correlation between DAG oil and weight reduction.

As far as this study was concerned, six studies with good methodological quality were included in this meta-analysis. Analysis of fail-safe number showed that 31.5 null effect studies which were larger than 5 times of the inclusion number were necessary to nullify the reported reduction efficacy of DAG. While most of the trials tested the effect of DAG on the blood relevant variables, energy expenditure and body fat etc,[20, 33] body weight was tested as one of the secondary outcomes. This might reduce the possibility of publication bias to some extent and the robust positive result was educed.

All trials were randomized double-blinded placebo controlled except for one single-blinded trial. Four trials achieved a high score of four on the methodological quality. Although the subjects were assessed under various conditions, factors related to body weight measurements were controlled well in most of the trials. For example, dietary advices were given to subjects in five studies; dietary intake was assessed by food diaries or questionnaire in all six trials; physical activity was monitored in three trials. All these well controlled factors might con-

firm the overall result of this study that the DAG is efficacious for body weight reduction compared with TAG. The sensitivity analysis also corroborated the overall meta-analysis well.

Sub-group analysis showed that DAG could promote the body weight reduction significantly in subjects with normal BMI but not in overweight subjects compared with TAG. Only 1 study tested the effect of DAG in overweight subjects.[14] The non-significant effect might due to the limited number and the large SDs of included study because the effect size of this subgroup was larger than that of the subgroup with normal BMI.

Linear regression analysis showed that there was no significant dose-response between dose and weight reduction. But in the study conducted by Nagao, the strongest effect was obtained with the smallest dose.[13] In this study, the test oil diet was provided to the subjects only as breakfast. But in other studies, the test oil diet was provided as lunch and dinner, or not defined. Animal studies showed that feeding time affected the apparent digestibility of nutrients in rainbow trout, fish fed at dawn had a higher postprandial metabolic rate than those fed at night.[34,35] These results indicated that subjects in the study conducted by Nagao tended to have a higher absorption rate of DAG and TAG which enhanced the healthy effect of DAG. However, more research is needed to prove the effect of DAG intake time on its digestibility in humans. As shown in figure 3, this study deviated from the trend line greatly. After excluding this study, linear regression showed a significant correlation between dose and weight reduction, which indicated that more weight reduction could be achieved while more dietary TAG was replaced with DAG.

The initial products of digested TAG are free fatty acids (FA) and 2-monoacylglycerol (MAG) and those for 1,3-DAG are mainly FA and 1(3)-MAG.[36, 37] The resynthesis of TAG in small intestinal epithelial cells from 1(3)-MAG is slower than from 2-MAG because the former involves the phosphatidic acid pathway, which is a slower turnover pathway than 2-MAG pathway. So the FA released from DAG were transported to liver and β-oxidized. Murata et al reported that the activity of carnitine palmitoyltransferase, acyl-CoA dehydrogenase, acyl-CoA oxidase, enoyl-CoA hydratase, 3-hydroxyacyl-CoA dehydrogenase, 2,4-dienoyl-CoA reductase and δ3, δ2-enoyl-CoA isomerase which are involved in FA β-oxidation pathway were increased after DAG supplementation.[38] In addition, mRNA expressions of FA translocase and FA binding protein associated with FA transport, acyl-CoA oxidase and medium-chain acyl-CoA dehydrogenase associated with β-oxidation, uncoupling protein-2 associated with thermogenesis in the small intestine were upregulated after the supplementation of DAG in mice.[39] These results indicated that DAG oil supplementation tended to produce a higher postprandial energy expenditure compared with TAG oil which leaded to the reduction of body weight.[40] Compared with TAG oil, DAG oil supplementation decreased the resynthesis of TAG in small intestinal epithelial cells because of the slow phosphatidic acid pathway, which resulted the decrease of postprandial TAG concentration. Some evidences showed that the serum TAG concentration was positively correlated with visceral obesity.[41] DAG oil consumption also decreased the synthesis of TAG in liver by decreasing the activity of FA synthetase, malic enzyme and glucose 6-phosphate dehydrogenase which are involved in FA synthesis.[38] The decrease of FA resynthesis in small intestinal and synthesis in liver also resulted the reduction of body weight.

Although there was statistically significant difference in the weight reduction between DAG and TAG groups, this difference (WMD -0.75 kg) was not clinically significant. But as far as all included studies were concerned, the only intervention was the replacement of all or proportion of dietary TAG with DAG which possesses the same energy value and digestibility with TAG. Further, 66.4% of included subjects possessed normal BMI. Both above conditions might result in the non-significant clinical effect. It is apparent that more clinical research is needed to test the effect of DAG on body weight in subjects with various physiological conditions, especially in overweight and obese subjects.

In conclusion, the evidence from randomized controlled trials included in this meta-analysis suggested that DAG was efficacious for reducing body weight in both healthy and diabetic subjects and this effect might be influenced by the daily dose of DAG oil.

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AUTHOR DISCLOSURES

Tongcheng Xu, Xia Li, Zhiguo Zhang, Xiaohang Ma and Duo Li, no conflicts of interest.

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Original Article

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甘油二酯对体重影响之后设分析

在临床试验中，关于甘油二酯对体重影响的研究结果尚不一致。本文旨在通过后设分析，进一步评价甘油二酯与体重之间的关系。首先，以体重、甘油二酯、甘油三酯、降低和肥胖为关键词，在 Medline、Embase 和 Cochrane 图书馆三个数据库中进行检索，并将所得随机对照实验纳入本文统计库。然后，由两名审稿人独立提取相关数据，并按 Jadad 评分系统对文章进行评价。采用软件 Review Manager 4.2 进行后设分析，利用敏感性分析检验所得结果的可靠性，通過倒漏斗图分析检验发表偏倚的可能性。最终，有 5 篇文章纳入统计分析。后设分析结果显示，甘油二酯摄入组受试者的体重降低水平显著高于甘油三酯摄入组的体重降低水平（加权均数差：-0.75 公斤；95%置信区间：-1.11~-0.39；$p < 0.0001$）。敏感性分析结果显示，上述结果的可靠性较高。线性回归分析结果显示，甘油二酯的功效与剂量之间存在显著相关性（$p = 0.044$, $R^2 = 0.889$）。结论：与甘油三酯相比，甘油二酯的摄入可以显著降低受试者的体重，且降低作用受日摄入量的影响。

关键词：甘油二酯、甘油三酯、体重、降低、后设分析