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The Effect of CLA on Obesity of Rats: Meta-Analysis

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1. Introduction

Obesity is the noticeably fat mass, and an excessive nutrient intake is a cause of that. When the energy in body exceeds healthy limit, the much energy is stored as fat or in fatty tissue. Obesity was accelerated by eating fatty foods, insufficient exercise, and accumulation of stress.

Obesity has come to be recognized as a critical global health issue. Rates of obesity in North America and in most European countries have more than doubled in the last 20 years and over half the adult population are now either overweight or obese (1). According to the report which was released by Korea National Health Insurance Corporation (NHIC) in 2004, the obesity rate for subjects of the survey in 2003 was 56%, and increased in all age groups from 10s to 60s. In addition, the study by Oh *et al*. in 2005 reported that obesity was strongly associated with risk of some cancers, such as skin, liver, large intestine, thyroid gland, biliary tract, and uterus. It is well known that obesity is one of the causes that generate heart problems, diabetes, and other disorders (2).

Conjugated linoleic acid (CLA) is one of trans fatty acids and found mainly in the meat and dairy products of ruminants (3). CLA has received attention for its antioxidant and anti-cancer properties (4). Studies of CLA show that CLA supplementation tends to reduce body fat, improve serum lipid profiles, and decrease whole-body glucose uptake. However, the results of some studies on rats suggest that CLA supplement was not effective in reducing the fat accumulation (5). Though studies done on CLA have increased, they do not all show uniform results, showing it is necessary to summarize and analyze them.

Meta-analysis is a tool for summarizing the results of studies with related research hypotheses. It has three steps; deciding the association measure for detecting difference between groups, summarizing the association measure in assumed model, and identifying publication bias. The association measures in meta-analysis are the measure of related effects from the primary research such as the standardized mean difference, the mean difference, the risk difference, and an odds ratio. The decided measure is combined using only within variation of studies on the assumption that the results are homogeneity and

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uniformly distributed, and then it is tested on whether the homogeneity assumption is plausible. If the results are heterogeneity, the measure is recombined using within variation with the estimated the between variations of the studies. The former model is called the fixed effect model and the latter is the random effect model. Because significant results are more likely to be submitted or accepted than insignificant ones and the combined estimates are calculated by only published researches, the bias easily occurs in a small number of studies and is called the publication bias. If the publication bias is doubtable, the combined estimate is adjusted or the additional studies are required (6, 7).

The purpose of this study was to summarize the studies about effect of CLA on factors related to the anti-obesity in experimental rats by meta-analysis.

2. Method

2.1 Preparation of dataset for meta-analysis

The studies used in this meta-analysis were searched on the ScienceDirect in English, the DBpia database, and the KISS (Koreanstudies Information Service System) in Korean. The search keywords used were CLA, weight, health, or fat and the research was limited to the experimental rat studies. About 50 studies were collected and 12 studies were finally selected after omitting some studies with insufficient information, such as no sample variance or no sample size (5, 8-18).

The factors used to investigate the effect of CLA were collected from target studies, if it was studied in at least 2 studies. The selected factors were body fat (%), epididymal fat (g/100g), fat cell size (μm), final body weight (g), food intake (g/d), leptin (pg/ml), liver-TC (mg/g), liver-TG (mg/g), plasma-TC (mg/dl), plasma-TG (mg/dl), and weight gain (g/d). The unit of each factor was uniformly changed.

In each study, there were two groups. One group of rats was treated with fat source such as beef tallow, coconut oil, corn oil, fish oil, safflower oil or soybean oil (FR), and the other group of rats was treated with fat source supplemented with CLA (FRC). Because the size of studies after grouping by treated fat source was not enough large, the each fat source has similar effect on obesity.

2.2 Measure and models for combining

Since the mean difference between FR and FRC was used for test on anti-obesity effect of CLA, the association measure was decided as the mean difference (MD) of each factor; mean of FRC minus mean of FR. The combined MD of each factor was calculated by the inverse variance method and the mean weighted by inverse variance in the primary studies. If the homogeneity was accepted by Cochran's Q test, it is assumed that the effect measured in the study population has a single value. The association measure is estimated by using a variation within the studies in the fixed effect model. To reduce the bias caused by heterogeneity, meta-regression models are used to analyze association between treatment effects and study characteristics. The period and amount of CLA supplementation were considered as covariates, and the estimated coefficients of covariates were investigated for possible sources of heterogeneity. If the homogeneity was rejected, the combined MDs of studies were calculated in random effect model in which total variation defined the variation within studies with estimated variation between studies.

2.3 Identification the publication bias

The existence of publication bias was checked by using a funnel plot or Egger's linear regression test. The funnel plot was a scatter plot of the association measure against a inverse of standard error of measure. If the shape of funnel plot about any factor is not funnel or cone around the combined MD, the publication bias was doubtable. Egger's linear regression test was used to test the null hypothesis that the funnel plot was not asymmetry. Egger's linear regression is a linear regression of standard normal deviate (defined as association measure over SE) against the inverse of SE, and there may be publication bias if the estimated intercept is significantly different from 0. A positive intercept indicates that more studies are associated with a bigger effect (19).

2.4 Software used for the meta-analysis

Version 1.5 of MIX program was used for combining MDs, plotting the funnel plots and checking the publication bias. The STATA program was used for meta-regression.

3. Result

3.1 Combined mean difference and homogeneity test of studies (Fat/Weight/Plasma/Liver)

The combined MDs between FR and FRC were presented with p-value to test heterogeneity of results in Table 1. In the fixed effect model, the MDs were significantly different from 0 for body fat, final body weight, liver-TC, plasma-TC, plasma-TG and weight gain (<0.05). Especially the p-values tested for body fat, final body weight, and weight gain were less than 0.01, showing CLA significantly decreased the level of each of them.

	Fixed effect model [a]		Heterogeneity [b]	Random effect model [a]	
	estimate	p-value [c]	p-value	estimate	p-value [c]
Body fat(%)	-1.2504	<0.0001	<0.0001	-1.1239	0.1020
Epididymal fat (g/100g)	-0.0131	0.6988	<0.0001	-0.0470	0.4512
Fat cell size(μm)	-0.6088	0.4707	0.4213	-0.6088	0.4770
Final body weight(g)	-3.5922	0.0004	<0.0001	-3.3114	0.3941
Food intake(g/d)	-0.1639	0.1190	0.1829	-0.2235	0.2634
Leptine(pg/ml)	-6.6161	0.7563	0.0696	33.5415	0.4738
Liver-TC(mg/g)	-0.1859	0.0352	0.0146	-0.5187	0.2483
Liver-TG(mg/g)	-0.2007	0.2689	<0.0001	-0.8575	0.2539
Plasma-TC(mg/dl)	-11.5280	<0.0001	0.0087	-15.0920	0.0009
Plasma-TG(mg/dl)	-3.2202	0.0446	0.0730	-4.0647	0.1287
Weight gain(g/d)	-0.5377	<0.0001	<0.0001	-0.1561	0.5062

Table 1. Combined MDs and Homogeneity test in fixed effect model and random effect model. [a]

The fixed effect model was inadequate in terms of body fat, epididymal fat, final body weight, liver-TC, liver-TG, plasma-TC, plasma-TG and weight gain, because of the heterogeneity of studies for them (p-value<0.05). The MD about heterogeneity factor was regressed against two covariates, amount and period of CLA supplementation, by meta-regression method in Table 2. Only the MD of the final body weight was significantly correlated with the period of CLA supplementation (p-value <0.05), and the coefficient of

period was estimated -0.0698. That meant the MD of the final body weight was estimated to decrease by 0.0698 per unit increase in the period.

The random effect model was used to combine MD on heterogeneity factors; body fat, epididymal fat, final body weight, liver-TC, liver-TG, plasma-TC, plasma-TG, and weight gain. The value of the MD of plasma-TC was significantly negative, but the value of the MD of other factors was not negative.

Factor(unit)	Intercept	Coefficient of amount	Coefficient of period
Body fat(%)	-0.1278	-1.9423	0.0038
Epididymal fat(g/100g)	0.0203	-0.3859	-0.0003
Final body weight(g)	1.7030	4.5770	-0.0698 [b)]
Liver-TC(mg/g)	NA		
Liver-TG(mg/g)	-2.6548	-13.8573	0.0947
Plasma-TC(mg/dl)	-1.2486	13.5180	0.0032
Plasma-TG(mg/dl)	-2.1294	-10.2354	0.0681
Weight gain(g/d)	-0.0165	-0.0914	0.0004

[a)] If it is found to be hetero in the effect of treatment between studies, then meta-regression can be used to analyze associations between treatment effect and covariates in study.
[b)] The estimated coefficient of period was significantly efficient to combined mean difference about final body weight between the FR and the FRC(p-value <0.05), thus the MD of final body weight is estimated to decrease by 0.0698 per unit increase in the period.

Table 2. Estimated intercept and coefficient of covariates in meta-regression.

Factor(unit)	Egger's linear regression	
	Intercept [a)]	p-value [b)]
Body fat(%)	14.1719	0.3649
Epididymal fat(g/100g)	-1.5864	0.0756
Fat cell size(μm)	1.5284	0.5107
Final body weight(g)	-0.0471	0.9677
Food intake(g/d)	-0.1382	0.7718
Leptine(pg/ml)	1.8242	0.2223
Liver-TC(mg/g)	NA	
Liver-TG(mg/g)	-2.4299	0.3152
Plasma-TC(mg/dl)	-2.8889	0.0807
Plasma-TG(mg/dl)	-0.7732	0.2357
Weight gain(g/d)	3.1444	0.5980

[a)] The data were expressed the intercept of Egger's linear regression about factors.
[b)] The expressed p-values were for testing that the estimated intercept is not significantly different with 0. If the p-value is less than 0.05, the combined MD in Table 1 possibly exist the publication bias, and the additional analysis is required.

Table 3. Estimated intercepts and p-values of Egger's linear regression test

3.2 Publication bias

To check the publication of the funnel plot expressed in Fig. 1. The funnel plots of fat cell size, food intake, leptine and plasma-TG were checked in the fixed effect model due to the homogeneity. The dots of fat cell size and the dots of leptine were not symmetric by combined

MD (solid line), so the publication biases were doubtable. The dots of factors in the random effect model were symmetric by solid line, thus the publication bias were ignorable. Since the intercepts of Egger's linear regression by factors in Table 3 were not significant (p-value >0.05), the funnel plot on each factor was symmetric and the publication bias was ignorable.

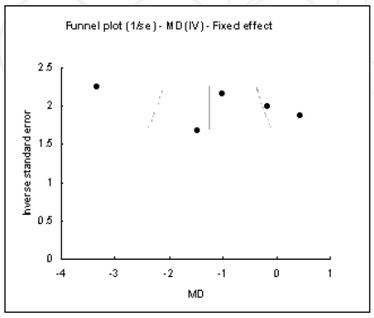
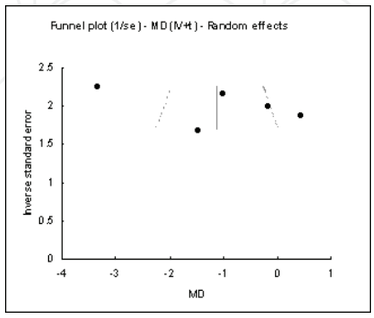
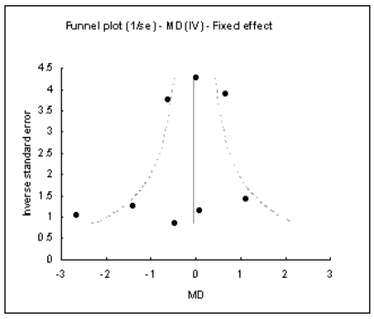
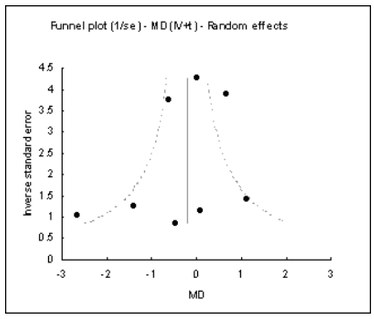
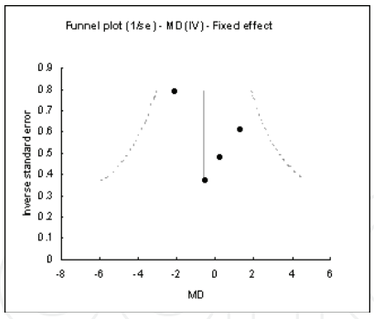
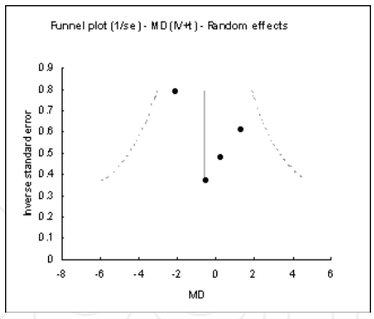
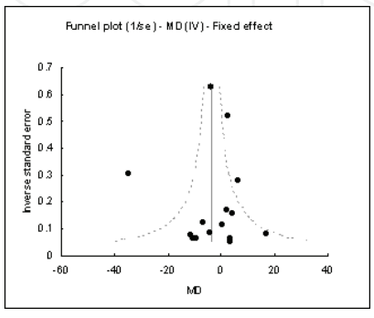
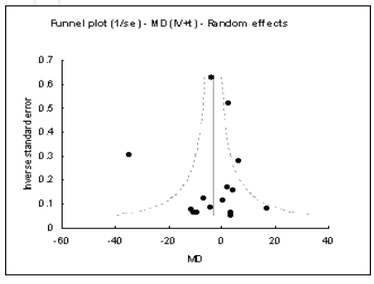
Factor	Fixed effect model	Random effect model
Body fat		
Epididymal fat		
Fat cell size		
Final body Weight		

Fig. 1. Funnel plot: In fixed effect model and random effect model

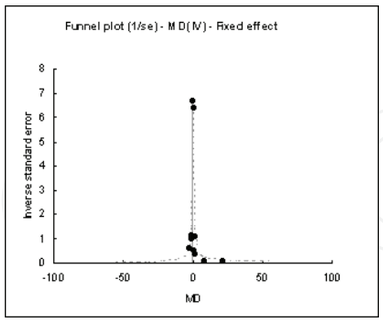
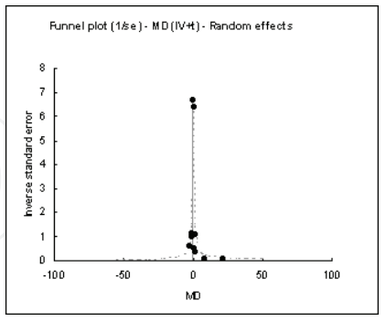
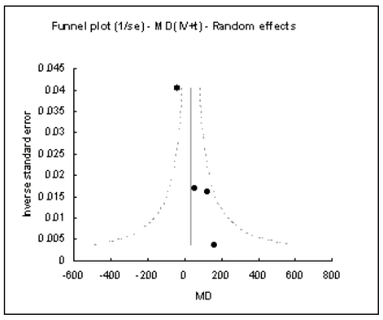
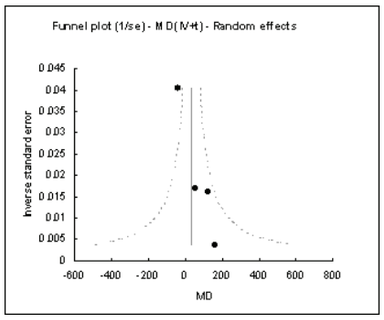
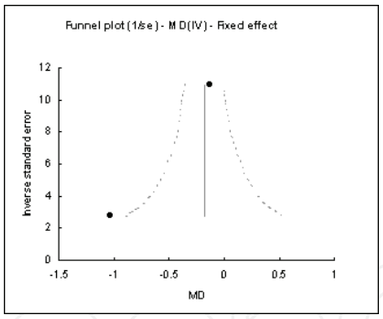
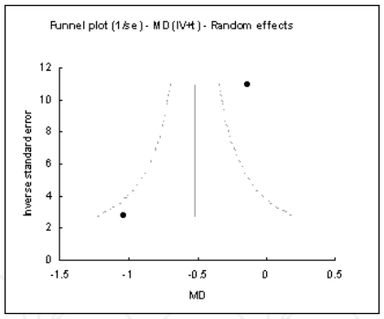
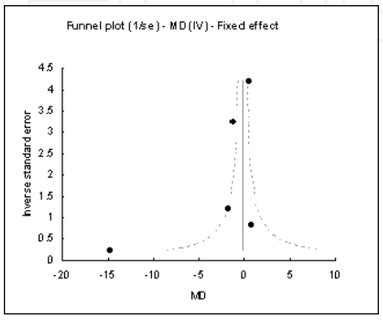
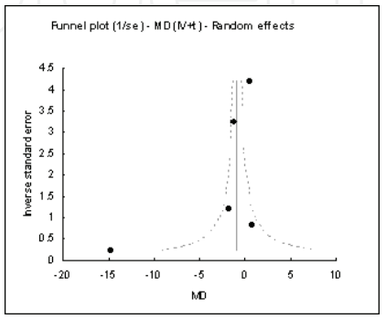
Factor	Fixed effect model	Random effect model
Food intake		
Leptine		
Liver-TC		
Liver-TG		

Fig. 1. Funnel plot: In fixed effect model and random effect model (Continuation)

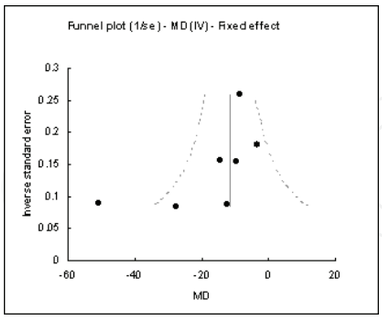
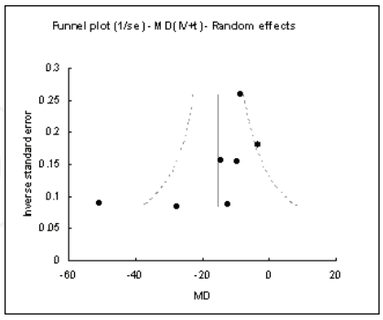
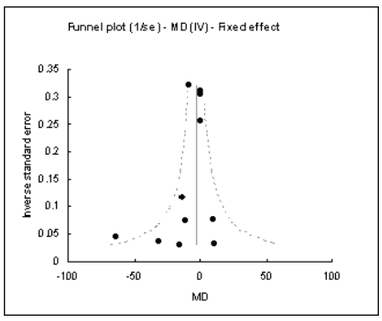
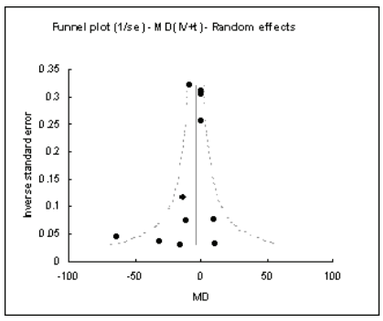
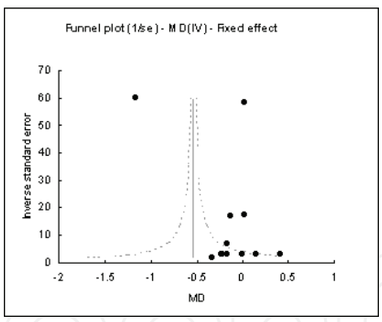
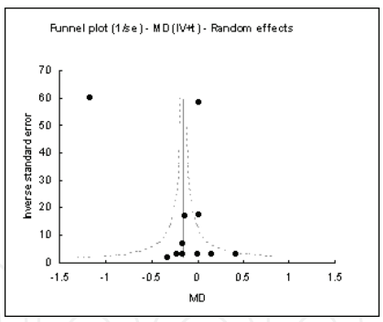
Factor	Fixed effect model	Random effect model
Plasma-TC		
Plasma-TG		
Weight gain		

Fig. 1. Funnel plot: In fixed effect model and random effect model (Continuation)

4. Discussion

4.1 The effects of CLA on the fat depositions

The factors related with fat deposition were body fat and epididymal fat, and our study showed that CLA supplement did not affect the fat deposition. The results of studies on body fat and epididymal fat were significantly heterogeneous. In fixed effect model, body fat was significantly affected by CLA. Under the heterogeneity of studies on body fat, the effect of CLA was not significant in random effect model. We could not check the condition of energy expenditure, and that was one of reasons for explaining about heterogeneity.

4.2 The effects of CLA on the weights of body

The results of studies done on the final body weight and weight gain were very heterogeneous (p-value < 0.001), and the result of efficiency test of CLA in fixed effect model differed from one in random effect model. This situation happened due to extreme one of combined results. The extremely negative MD had a small standard error, so the high influence of them on combination made significantly negative value in fixed effect model but not in random effect model. After omitting the extreme value in analysis, the homogeneities results on final body weight and weight gain were accepted (p-value >0.05), and the effects of CLA on them were not significant.

4.3 The effects of CLA on the plasma lipid

The level of plasma-TC was significantly decreased in FR than in FRC regardless of the homogeneity of the factors. Only in fixed effect model, the level of plasma-TG was significantly decreased in FR. According to Hwang and Kang, the reduction effect of CLA on plasma-TG was significant in the case rats that ware fed with beef tallow for 1 and 4 weeks and in the case rats that was fed with fish oil only for 4 weeks (20). The anti-obesity effect of CLA was assured like Hwang and Kang's study.

5. Conclusion

In this study, the researchers studied about effect of CLA on factors, such as body fat (%), epididymal fat (g/100g), fat cell size (μm), final body weight (g), food intake (g/d), leptin (pg/ml), liver-TC (mg/g), liver-TG (mg/g), plasma-TC (mg/dl), plasma-TG (mg/dl), and weight gain (g/d). The CLA supplement was significantly effective on reduction of body fat (%), final body weight (g), liver-TC (mg/g), plasma-TC (mg/dl), plasma-TG (mg/dl), and weight gain (g/d) with homogeneity assumption between studies. However, only plasma-TG was significantly decreased with the random effect model on heterogeneity factors. Further study should focus on what the effect of CLA depend on fat source.

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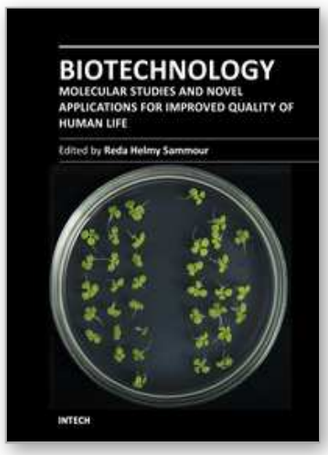

Biotechnology - Molecular Studies and Novel Applications for Improved Quality of Human Life

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This book deals with the importance of application of molecular biology as an approach of biotechnology for improvement of the quality of human life. One of the interesting topics in this field, is the identification of the organisms that produce bioactive secondary metabolites. It also discusses how to structure a plan for use and preservation of those species that represent a potential source for new drug development, especially those obtained from bacteria. The book also introduces some novel applications of biotechnology, such as therapeutic applications of electroporation, improving quality and microbial safety of fresh-cut vegetables, producing synthetic PEG hydro gels to be used as an extra cellular matrix mimics for tissue engineering applications, and other interesting applications.

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