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Addition of conjugated linoleic acid in whole milk improves lipid profile in high fat diet induced hypercholesterolemia of rats

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ABSTRACT

Conjugated linoleic acid (CLA) is an isomer of linoleic acid that has been shown to have many beneficial effects in prevention of atherosclerosis, hypertension, cardiovascular diseases and improve immune function. Although majority of CLA in the diet are derived from dairy product such as milk, however, the content of CLA in milk is affected by cow's diet. The aim of this study was to investigate the beneficial effect of CLA supplementation in milk for improving lipid profile in high fat diet of rats. Twenty four male Sprague Dawley rats aged 8 weeks were given high fat diet for 3 weeks to induce hypercholesterolemia. Six rats were maintained in standard diet as control. Rats then were divided into 4 groups i.e. normal control, negative control, high fat diet + CLA 0.5%, high fat diet + CLA 0.5% supplemented skim milk, and high fat diet + CLA 0.5% supplemented whole milk. Blood sample was drawn after high fat diet induced hypercholesterolemia and after 4 weeks of treatment for total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDL cholesterol), and high density lipoprotein cholesterol (HDL cholesterol) analysis. Body weight was measured each week. Results showed that body weight was significantly increase in all groups received high fat diet ($p < 0.05$). There was no significant difference in body weight between treatment group ($p > 0.05$). Total cholesterol, triglyceride, and LDL cholesterol was significantly decrease in whole milk followed by significant increase in HDL cholesterol level. Skim milk supplemented with CLA had only modest effect on triglyceride and HDL cholesterol level. In conclusion, CLA supplementation in whole milk improves lipid profile in high fat diet.

ABSTRAK

Asam linoleat terkonjugasi adalah isomer asam linoleat yang terbukti mempunyai manfaat dalam mencegah aterosklerosis, hipertensi, penyakit kardiovaskuler dan meningkatkan sistem imun. Meskipun sebagian besar asam linoleat dalam diet berasal dari produk makanan sehari-hari seperti susu, namum demikian kandungan asam linoleat dalam susu dipengaruhi oleh diet sapi penghasil susu. Penelitian ini bertujuan untuk mengkaji manfaat asam linoleat dalam suplemen susus dalam memperbaiki profil lipid pada tikus diet lemak tinggi. Dua puluh empat tikus jantan Sprague Dawly berumur 8 minggu diberi diet lemak tinggi selama 3 minggu untuk menginduksi hiperkolesterolemia. Enam ekor tikus diberi diet makan standar sebagai control. Tikus kemudian dibagi menjadi empat kelompok yaitu kelompok kontrol normal, kelompok kontrol negatif, kelompok diet tinggi lemak dengan

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asam linoleat 0.5%, kelompok diet tinggi lemak dengan asam linoleat 0.5% dalam suplemen susu skim, kelompok diet tinggi lemak dengan asam linoleat 0.5% dalam suplemen susu susu murni. Sampel darah diambim setelah diet induksi hiperkolesterolemia dan setelah empat minggu perlakuan untuk ditetapkan kadar kolesterol total, trigliserida, *low density lipoprotein cholesterol* (kolesterol LDL) dan *low density lipoprotein cholesterol* (kolesterol HDL). Berat badan tikus diukur setiap minggu. Hasil penelitian menunjukkan berat badan meningkat secara nyat di semua kelompok ($p<0,05$). Tidak terdapat perbedaan berat badan tikus di semua kelompok ($p>0,05$). Kadar kolesterol total, trigliserid dan kolesterol LDL menurun secara bermakna pada kelompok yang diberi susu murni dengan asam linoleat, sedangkan kadar kolesterol HDL meningkat secara nyata ($p>0,05$). Kelompok yang diberi susu skim dengan asam liinoleat mempunyai pengaruh moderat terhadap kadar trigliserida dan kolesterol HDL. Dapat disimpulkan, suplemen asam linoleat dalam susu murni dapat memperbaiki profil lipid tikus yang diberi diet tinggi lemak.

Key words: conjugated linoleic acid – hypercholesterolemia - lipid profile - milk - diet

INTRODUCTION

The pandemic rise of obesity has continued both in economically developed country as well in economically developing country. In United States, the prevalence of obesity among US adult has rise from 23% in 1988 to 30.5% in 2000 and 33.8% in 2008.[1,2] In Australia, the proportion of children with severe obesity has risen significantly from 19.3% in 1985 to 32.0% in 2012.[3] The rise in the prevalence of children with obesity was also observed in Indonesia from 10.3% in 1993 to 16.5% in 16.5% in 2007.[4] High prevalence of obesity has been linked with high risk the development of metabolic syndrome,[5] which is a major risk factor for cardiovascular disease, chronic kidney disease, type 2 diabetes, cancer, and inflammatory bowel disease.[6-8]

Impairment in lipid metabolism and oxidative stress have been reported in obese individual.[9,10] Disturbance in adiposity-releasing hormone or adipokines is one contributing factor in the development of dyslipidemia indicated by elevated level of triglycerides and low HDL cholesterol in obesity.[11] Reducing body weight has been linked with significant improvement in cardiovascular risk factor such as improved glycemia, blood pressure, triglycerides, and HDL cholesterol.[12]

Conjugated linoleic acid (CLA) is a type of isomeric-geometrical group of linoleic acid, which predominantly found in dairy product (milk, butter, yogurt, and cheese), meat, and meat product of ruminant.[13,14] This type of fat can promote several health beneficial effect in preventing several diseases such as aterosclerosis, cancer and diabetes.[15-17] It also has been reported to reduce body weight although the evidence is controversial.[15,18] Here, we evaluated the effect of CLA supplementation in milk in lipid profile and body weight of rats fed high fat diet.

MATERIALS AND METHODS

Preparation of CLA supplemented milk

Skim milk or whole milk was obtained from local market in dry form. Conjugated linoleic acid isomers used is 37-42% cis-9 trans-11 (c9t11) and 37-42% trans-10 cis-12 (t10c12). Supplementation CLA used consisted of 80% pure CLA with the addition of gelatin, glycerin, distilled water, and the color of caramel to 100%. The energy content

of the CLA is 10 kcal/capsule. Conjugated dietary supplement was obtained from Ultima Central Jakarta.

Animal and diet

Thirty male Sprague Dawley rats aged 6 weeks with average weight of 100 g were obtained from Department of Pharmacology and Therapy, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta. Rats were maintained in individual steel cage with 12 hour light cycle. Rats were acclimatized for three days prior to experiment. After adaptation period, 24 rats were subjected to high fat diet for three weeks and six rats were fed standard diet as control. The high fat diet was derived from AIN-93M semi purified diet that consist 20% of lard plus 3 mL of egg yolk through oral gavage/day. The composition of the diet was presented at TABLE 1.

TABLE 1. Diet composition (g/kg diet)

Composition	Standard diet	High fat diet
Cornstarch	465.69	15.69
Dextrinized cornstarch	155.00	155.00
Casein	140.00	140.00
Sucrose	100.00	100.00
Soybean oil	40.00	40.00
Alphacel	50.00	50.00
Mineral mix (AIN-93M)	35.00	35.00
Vitamin mix (AIN-93M)	10.00	10.00
L-cystine	1.80	1.80
Choline bitartrate	2.50	2.50
TBHQ	0.08	0.08
Lard	-	200.00
Calories	3849.96	3849.96

After 3 weeks of high fat diet, rats were divided into 5 groups: standard diet (normal control), high fat diet (negative control), high fat diet + CLA 0.5% (CLA-0.5), high fat diet + CLA supplemented skim milk 0.5% (CLA-Skim 0.5%), high fat diet + CLA supplemented whole milk 0.5% (CLA-Whole 0.5%). Intervention was carried out for 4 weeks. CLA 0.5% was prepared by dissolution of 95.50 mg of CLA with 100 mL of water and homogenized. All CLA and CLA supplemented milk were given by oral gavage in 2 mL of water/milk. Blood sample was taken after high fat diet intervention as pre-test and after 4 weeks of intervention as post-test for lipid profile (total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol) analysis. Body weight was weighed every week. All procedures have been approved by the Medical and Health Research Ethics Committee of Faculty of Medicine, Univesitas Gadjah Mada, Yogyakarta (No. KE/FK/385/EC/2009).

Lipid profile analysis

Blood sample was taken and immediately centrifuged at 4,000 rpm to obtain serum. Total cholesterol and triglyceride were assessed

using enzymatic colorimetric method using total cholesterol and triglyceride kit according to manufacturer protocol. LDL cholesterol and HDL cholesterol were precipitated prior to the analysis.

Statistical analysis

Data were presented as mean ± standard deviation (SD) and analysed by analysis of variance (ANOVA) followed by paired t-test. A $p < 0.05$ was considered to be statistically significant. All data analysis were performed by using SPSS 17.0 (SPSS Inc).

RESULTS

Impact on CLA-supplemented milk on body weight

Body weight did not significantly different ($p>0.05$) between groups during high fat diet-induced obesity as well as in week 1 intervention. Significant difference ($p<0.05$) in body weight was observed only in control group compared with other during week 2 up to week 4 intervention (FIGURE 1).

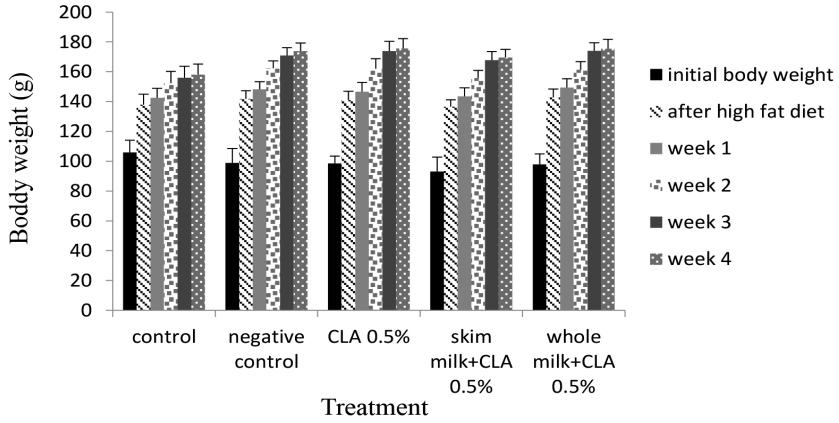

FIGURE 1. Effect of CLA supplemented milk on body weight of rats

Effect of CLA-supplemented milk on lipid profile

Serum total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol were not significantly different ($p<0.05$) among high fat induced obesity rats. After 4 weeks of intervention, whole milk supplemented with 0.5% CLA group had the lowest total cholesterol, triglyceride and LDL cholesterol level as well as highest HDL cholesterol compared with other treatment groups ($p<0.05$). The cardiovascular index risk as assessed by the LDL/HDL ratio was significantly different among the group after 4 weeks of intervention ($p<0.05$). The negative control group had higher risk of cardiovascular disease as indicated by higher LDL/HDL ratio. Among treatment group, whole milk supplemented with CLA 0.5% had the lowest LDL/HDL ratio followed by CLA 0.5% and skim milk supplemented with CLA 0.5% (TABLE 2).

TABLE 2. Effect of CLA supplementation on lipid profile of rats after high fat diet induced hypercholesterolemia

Group	Cholesterol		Triglyceride		LDL cholesterol		HDL cholesterol		LDL/HDL ratio	
	Pre test	Post test	Pre test	Post test	Pre test	Post test	Pre test	Post test	Pre test	Post test
Control	104.25 ± 2.44[a]	107.14 ± 3.25[a]	75.49 ± 1.90[a]	78.35 ± 2.44[a]	11.16 ± 1.08[a]	14.31 ± 2.52[a]	77.99 ± 1.21[a]	77.16 ± 1.39[a]	0.14 ± 0.01[a]	0.19 ± 0.03[a]
Negative control	207.04 ± 4.58[b]	225.79 ± 5.33[b]	112.50 ± 2.83[b]	128.91 ± 3.08[b]	139.12 ± 4.20[b]	163.11 ± 4.95[b]	45.42 ± 3.58[b]	36.91 ± 2.94[b]	3.08 ± 0.28[b]	4.45 ± 0.41[b]
CLA 0.5%	210.76 ± 6.15[b]	172.49 ± 3.78[c]	115.19 ± 2.78[b]	107.87 ± 3.21[c]	142.63 ± 4.75[b]	96.04 ± 5.11[c]	45.09 ± 3.89[b]	54.87 ± 1.87[c]	3.18 ± 0.29[b]	1.75 ± 0.14[c]
Skim milk+ CLA 0.5%	206.91 ± 3.25[b]	176.85 ± 3.15[c]	117.28 ± 4.89[b]	115.25 ± 3.21[d]	139.55 ± 3.41[b]	109.10 ± 4.44[d]	43.90 ± 2.88[b]	44.69 ± 3.42[d]	3.19 ± 0.25[b]	2.46 ± 0.29[d]
Whole milk+ CLA 0.5%	210.49 ± 5.55[b]	157.01 ± 3.93[d]	116.05 ± 4.57[b]	97.91 ± 2.07[e]	141.87 ± 3.68[b]	72.88 ± 4.02[e]	45.42 ± 2.76[b]	64.55 ± 3.87[e]	3.13 ± 0.16[b]	1.13 ± 0.12[e]

[a,b,c,d,e] :different notation indicates $p<0.05$

DISCUSSION

The role of food in the prevention of disease had been gained attention since ~2500 years ago when Hipocrates said "Let food be thy medicine and medicine be thy food".[19] Indeed, several bioactive compounds have been identified in food and have linked with the prevention of lipid disorder, cardiovascular disease and cancer.[20-22] One of the bioactive compound that has been studied in the prevention of several diseases was CLA.

Some studies have reported the beneficial effect of CLA in the prevention of cardiovascular disease in animal model received high fat diet through improvement in lipid profile such as lower level of total cholesterol, triglyceride and LDL cholesterol as well higher level of HDL cholesterol in blood after treatment with CLA.[23,24] Our result also proved the impact of CLA, both in supplemented milk or not, could reduce total cholesterol by 14.5% - 25.4%, triglyceride level by 1.73 - 15.63%, LDL cholesterol by 21.82 - 48.63% and increase HDL cholesterol level by 1.80% - 42.12%. We also observed decreased LDL/HDL ratio, a cardiovascular risk index,[25] when animal fed CLA in all treatment groups.

Despite the possitive effect on the lipid profile improvement, we did not observe any impact of CLA supplementation in body weight whether it is supplemented in milk or not. This result can be explained by the type of CLA isomer ingested in the body. It was reported that CLA isomer trans-10, cis-12, has been linked with negative effect on health such as impairment in glucose tolerance and reduced insulin sensitivity as well.[26,27] The isomer trans-10, cis-12 CLA has been associated with lower weight reducing effect compared with the cis-9, trans-11 CLA due to the ability of the trans-10, cis-12 CLA in modulating several genes involved in lipolysis and fatty acid oxidation.[5,27,28]

Furthermore, the dose of CLA used in this study is not met the physiological dose to promote human benefit. The CLA dose was 106 mg in this study, whereas the estimated recommended dose of CLA that would benefited health would fall from 530 mg to 3 g of CLA/day.[29] Higher dose of CLA is required to induce body weight reduction in obesity state.[30]

CONCLUSION

In conclusion, CLA improves lipid profile in high fat induced hypercholesterolemia although no effect is observed on body weight. Further research is required to clarify the physiological mechanism of CLA in improving lipid profile in high fat induced hypercholesterolemia.

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