

Effects of Conjugated Linoleic Acid on Body Composition and Selected Biochemical Parameters in Obese Women

Habibe Sahin¹, Fatma Uyanik² and Neriman Inanc¹

¹Department of Nutrition and Dietetics, Ataturk Health School, University of Erciyes, 38039, Kayseri, Turkey

²Department of Biochemistry, Faculty of Veterinary, University of Erciyes, 38090, Kayseri, Turkey

Abstract: This study was performed to investigate the effects of conjugated linoleic acid (CLA) on body composition and selected biochemical parameters in obese women. Twenty women, aged between 22 and 48 years, with body mass index (BMI, kg/m²) over 25 received 1.8 g CLA/day for 8 weeks. Basal metabolic rate (BMR), anthropometric and selected biochemical parameters as well as serum insulin, leptin and ghrelin were measured at baseline and 8 weeks. Significant decreases were found in body weight, body mass index (BMI), waist and hip circumferences ($p < 0.05$) while no effect of CLA was determined on BMR, waist/hip ratio, fat content and lean body mass (LBM). Significant decreases were found in serum triglycerides (TG), total cholesterol (TC) ($p < 0.05$), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and plasma leptin ($p < 0.01$) levels. Serum glucose level decreased but remained within normal range while insulin levels of subjects increased ($p < 0.01$). Slight but not significant increases were found in plasma ghrelin and serum high density lipoprotein (HDL) levels. The results of this study have shown that supplementation of 1.8 g CLA/day for 8 weeks affect lipid and carbohydrate metabolisms and reduce body weight, waist and hip circumferences which are the indicators of abdominal obesity that is a risk factor for coronary heart disease.

Key words: Anthropometric measurements, conjugated linoleic acid, obesity

Introduction

Obesity, a chronic condition will most likely be needed a long term treatment (Udall *et al.*, 2005). Dietary supplements to treat obesity appeal to many patients who desire a 'magic bullet' for weight loss (Saper *et al.*, 2004). Approximately 50 individual dietary supplements and more than 125 commercial combination products are available for weight loss (Lenz and Hamilton, 2004). Conjugated linoleic acid (CLA) compounds are considered as such products (Joyal, 2004). Conjugated linoleic acid is a group of isomers conjugated octadecadienoic acid that occur naturally in foods, mostly in dairy products (Terpstra, 2004). Ritzenhaler *et al.* (2001) reported that the intake of total CLA as measured with the food duplicate method was 212 mg/day for men and 151 mg/day for women and 60% of the CLA intake was derived from dairy products and 37% from meat products. The cis-9, trans-11 CLA isomer, also called rumeric acid, accounted for >90% of the total CLA intake. Commercial CLA preparations are produced by isomerization of linoleic acid and contain predominantly cis-9, trans-11 and trans-10, cis-12 octadecaenoic acid in a 1:1 ratio (Terpstra, 2004). The body fat-lowering effect of CLA in experimental animals has led to the idea that CLA could be used as a tool in body weight management in humans (Park *et al.*, 1997, Yamasaki *et al.*, 2003, Park and Pariza, 2001). However, consistent and conflicting effects of CLA on body composition have been documented in human

(Blankson *et al.*, 2000; Zambell *et al.*, 2000; Riserus *et al.*, 2002).

In some previous studies (Blankson *et al.*, 2000; Smedman and Vessby, 2001), CLA has been shown to reduce body fat in human in contrast, no effects of CLA on body composition were reported in some other studies (Zambell *et al.*, 2000; Riserus *et al.*, 2002). Similarly, inconsistent results were reported concerning the effects of CLA on biochemical parameters related to lipid and carbohydrate metabolisms (Riserus *et al.*, 2002; Smedman and Vessby, 2001). Furthermore, to the author's knowledge, there is no study investigating the effects of CLA on obesity related hormones such as ghrelin and limited studies on leptin (Medina *et al.*, 2000; Nagao *et al.*, 2003). Thus, the metabolic effects of CLA are not clear yet. The effects of CLA in human have been also investigated mostly in the people who were on a controlled diet or exercise programmes (Zambell *et al.*, 2000). Therefore, this study was performed to investigate the effects of CLA on body composition and on recently discovered hormones related to obesity as well as other biochemical parameters related to carbohydrate and lipid metabolisms in obese women who were neither on a diet nor on an exercise programme.

Materials and Methods

Subjects: This study was conducted at Erciyes University, Kayseri, Turkey between June and August

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Table 1: Effects of conjugated linoleic acid supplementation on basal metabolic rate and anthropometric measurements of obese women

| | After CLA | | P |
|--------------------------|-------------|-----------------|---|
| | Baseline | supplementation | |
| BMR (kcal) | 1533±37.22 | 1540±35.36 | - |
| Weight (kg) | 78.54±3.22 | 75.38±3.74 | * |
| Height (cm) | 161.08±1.13 | 161.08±1.13 | |
| BMI (kg/m ²) | 30.41±1.50 | 29.05±1.75 | * |
| Waist circumference (cm) | 94.58±2.91 | 90.58±4.01 | * |
| Hip circumference (cm) | 114.50±3.23 | 111.58±3.63 | * |
| Waist/hip ratio | 0.81±0.01 | 0.80±0.02 | - |
| Body fat (%) | 38.16±2.03 | 36.13±3.17 | - |
| LBM (%) | 62.06±2.11 | 63.71±3.14 | - |

*p<0.05

2005. Twenty female subjects with BMI (kg/m²) over 25 were included in the study. Subjects were between 22 to 48 years of age, pre-menopausal, not taking any dietary, vitamin and mineral supplements, not on weight loss and/or exercise programmes and with no history of chronic or acute diseases. All subjects gave written and verbal informed consent. Subjects were asked not to alter their own dietary habits and life style during the course of the study except taking the CLA supplement. This study was approved by The Ethics Committee of Faculty of Medicine, University of Erciyes (Approval date and no. 06.07.2004, 04/240).

Experimental design: The subjects were asked to take 3 capsules/day during the study. All measurements were conducted baseline and after CLA supplementation. The subjects received gelatine capsules containing 600 mg CLA per capsule (Fatty acid specification C18:2, conjugated (CLA) relative area 80-84 %, C18:2 conjugated c9, t11 (CLA isomer) relative area 37-42%, C18:2 conjugated t10, c12 (CLA isomer) relative area 37-42 %, Skip, Sweden). The baseline values and changes after supplementation were compared.

Anthropometric measurements: Body weight and height were measured and BMI of subjects were calculated. Waist and hip circumferences of subjects were measured three times and waist/hip ratio was calculated with the mean value of three measurements. Body fat content (%), LBM (%) and BMR (kcal) were measured by Bioelectrical Impedance (Bodystat 1500 Tanita). Subjects avoided coffee and alcohol consumption as well as excess physical activity before the blood sampling and body composition measurements.

Sample collection: Venous blood samples were collected after overnight fasting. Blood samples were incubated 1 hour at room temperature and sera were separated then stored at -20°C until biochemical analysis. Blood samples with EDTA Na₂ were immediately centrifuged and plasmas were separated, stored at -70°C until leptin and insulin analysis. For

ghrelin analysis, whole blood was directly drawn into centrifuge tubes that contain 500 IU of aprotinin and 1.25 mg of EDTA Na₂ per 1 ml of blood and samples were kept on ice to prevent/minimize the breakdown of ghrelin and tubes were immediately centrifuged at 1500 g for 15 minutes at 4°C for separating plasma. Then 10 µL of 1 mol HCl was added to per ml of plasma and plasma samples were stored at -70°C until ghrelin analysis.

Biochemical analysis: Serum glucose, (Chema Diagnostica, Italy), TG, TC, HDL (Valtek, Chile) concentrations were determined with commercial kits by a Shimadzu 1208 UV/VIS spectrophotometer. The HOMA index which, is an indicator of insulin resistance, was calculated by the following formula described elsewhere. HOMA = Fasting insulin concentration (µU/mL) X Fasting glucose concentration (mmol/L)/22.5 (Matthevs *et al.*, 1985).

Low density lipoprotein concentration was calculated using the Friedewald's formula (LDL = TC-(HDL+TG/5)) and VLDL concentration was calculated using the formula TG/5 (Mahley, 1993). Plasma insulin (Roche Elecsyma E-170, Roche Diagnostics D 68298 Mannheim, U.S.A.), leptin (Mouse Leptin ELISA kit, Linco Research Inc., Missouri, U.S.A.) and ghrelin (Active Ghrelin ELISA kit, Linco Research Inc., Missouri, U.S.A.) concentrations were determined with commercial ELISA kits by Modular Analytics E 170 Module, Roche Diagnostics (Indianapolis, U.S.A.).

Statistical analysis: Data were analyzed by SPSS 10.0 version for Windows. Paired sample t test was used for determination of the difference between the baseline values and values determined after CLA supplementation. The level of significance was set at 0.05 for overall statistical analyses. Data were expressed as means ± standard error of means (SEM).

Results

Significant decreases were found in body weight, BMI, waist and hip circumferences (p<0.05) after CLA supplementation. Conjugated linoleic acid had no effect on BMR, waist/hip ratio, fat content (%), LBM (%) (Table 1). Conjugated linoleic acid reduced TG, TC, LDL, VLDL (p<0.05) and leptin levels (p<0.01). Serum glucose level decreased but remained within normal range while insulin levels of subjects increased (p<0.01). Slight but not significant increases were found in plasma ghrelin and serum HDL levels (Table 2).

Discussion

Many studies have been performed to demonstrate the effect of CLA on body composition but inconsistent results were obtained (Blankson *et al.*, 2000; Zambell *et al.*, 2000; Smedman and Vessby, 2001; Matthevs *et al.*, 1985). In this study, 1.8 g CLA/d reduced body weight and BMI significantly but had no effect on BMR as in the

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Table 2: Effects of conjugated linoleic acid supplementation on leptin, ghrelin and selected biochemical parameters of obese women

| Parameters | After CLA supplementation | | P |
|---------------------------|---------------------------|-----------------|---|
| | Baseline | supplementation | |
| Triglycerides (mg/dl) | 116.13±12.62 | 97.72±12.62 | * |
| Total cholesterol (mg/dl) | 180.06±9.54 | 158.97±8.60 | * |
| HDL (mg/dl) | 61.60±6.94 | 63.95±5.55 | - |
| LDL (mg/dl) | 95.23±10.62 | 75.47±12.27 | † |
| VLDL (mg/dl) | 23.22±2.52 | 19.54±1.61 | * |
| Glucose (mg/dl) | 82.98±3.37 | 73.99±4.22 | * |
| Leptin (ng/ml) | 36.82±3.24 | 29.80±3.51 | † |
| Ghrelin (fmol) | 15.74±1.86 | 17.45±3.92 | - |
| Insulin (mg/dl) | 8.24±1.59 | 14.34±2.89 | † |
| HOMA | 1.71±0.33 | 2.52±0.46 | † |

* p<0.05. † p<0.01

study of Zambell *et al.* (2000) who suggested no effect of 3 g CLA/d on body composition and energy expenditure in adult women.

Smedman and Vessby (2001) found a 3.8 % of reduction in body fat and no changing body weight, BMI and abdominal diameter after 12 week of treatment with 4.2 g CLA/day and Blankson *et al.* (2000) also determined reductions in body fat with 3.4 g and 6.8 g CLA/day in obese subjects. However, in the present study, although statistically not significant, a slight reduction occurred in body fat with 1.8 g CLA/day.

Lack of the effect of 1.8 g CLA consumption on LBM in the present study confirms the results of Blankson *et al.* (2000) who observed no significant difference in LBM with the consumption of CLA between 1.7 g and 6.8 g/day in obese subjects.

Statistically significant decreases were observed in waist and hip circumferences, which are the indicators of abdominal obesity (Misra *et al.*, 2005). Regional body fat distribution has an important influence on metabolic and cardiovascular risk factors (Carr and Brunzell, 2004). Increased abdominal fat accumulation is strongly related to clinical as well as subclinical coronary heart diseases (Nasir *et al.*, 2005). Waist circumference can be used as a complementary measurement to identify health risks (Wannamethee *et al.*, 2005). In the present study, none of the subjects had clinic or subclinic history of coronary artery diseases, but they were still under risk of such diseases because they were obese (Joyal, 2004; Bhattacharya *et al.*, 2005). The reduction in waist and hip circumferences thus the reduction in abdominal obesity and TG, TC, LDL, VLDL and despite being not statistically significant, a slight increase in HDL may show that CLA reduces risk of coronary heart disease.

In a study of Riserus *et al.* (2002), 3.4 g of CLA supplementation tended to increase insulin concentration as in the present study, in contrast to the finding of these authors, serum glucose concentration decreased but it was still within the normal range. The high HOMA index determined in this study was paralleled with the increased insulin level. The HOMA index is accepted an indicator of insulin resistance with the cut off point of 2.5 for adults (Matthevs *et al.*, 1985). It may be speculated from these findings that CLA may have some undesirable effects such as insulin resistance and increased plasma insulin (Terpstra, 2004).

Ghrelin is a recently identified, a 28 amino acid peptide, which is produced mainly in the stomach and circulates in blood (Akamizu *et al.*, 2004). Ghrelin an orexigenic hormone (Muccioli *et al.*, 2002) that may be involved in body weight regulation (Cummings *et al.*, 2002), is reduced in obesity (Morpurgo *et al.*, 2003, Soriano-Gullen *et al.*, 2004; Tschop *et al.*, 2001). It has been suggested that circulated ghrelin concentration is influenced by body fat distribution (Malik *et al.*, 2004) and negatively correlates with body fat (Fagerberg *et al.*, 2003). In this study, because CLA caused no effects on body fat content, the lack of effect of CLA on ghrelin concentration is not surprising.

Leptin is secreted by adipocytes in proportion to the amount of lipid stored and may act as a signal of body energy stores to the brain (Terpstra, 2004). Leptin concentrations in humans are increased with obesity (Bennett *et al.*, 1997). Fat tissue proportion can be measured accurately by determining the blood leptin concentration (Bates *et al.*, 2004). Medina *et al.* (2000) and Riserus *et al.* (2002) found no effects of the consumption of CLA for 9 and 12 weeks on leptin concentration in human. However, in this study, reductions in blood lipid and leptin levels, body weight and although not significant reduction in body fat confirm the existence of a relationship between blood leptin concentrations and body fat proportion (Shibata *et al.*, 2003; Bates and Myers, 2004; Maffei *et al.*, 1995).

In summary decreases in body weight, waist and hip circumferences, blood lipid and plasma leptin levels and slight decreases in body fat may show that supplementation of CLA for 8 weeks improves body composition and reduce abdominal obesity in obese women.

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