Symposium: Dairy Product Components and Weight Regulation

The Conjugated Linoleic Acid (CLA) Isomer, t10c12-CLA, Is Inversely Associated with Changes in Body Weight and Serum Leptin in Subjects with Type 2 Diabetes Mellitus[1,2]

Martha A. Belury,[3] Annie Mahon* and Sebastiano Banni†

*Department of Human Nutrition, The Ohio State University, Columbus, OH, *Department of Foods and Nutrition, Purdue University, West Lafayette, IN and †Department of Experimental Biology, University of Cagliari, Italy*

ABSTRACT Isomers of conjugated linoleic acid (CLA) are found in beef, lamb and dairy products. Diets containing CLA reduce adipose mass in various depots of experimental animals. In addition, CLA delays the onset of diabetes in the ZDF rat model for obesity-linked type 2 diabetes mellitus. We hypothesize that there would be an inverse association of CLA with body weight and serum leptin in subjects with type 2 diabetes mellitus. In this double-blind study, subjects with type 2 diabetes mellitus were randomized into one of two groups receiving either a supplement containing mixed CLA isomers (CLA-mix; 8.0 g daily, 76% pure CLA; $n = 12$) or a supplement containing safflower oil (placebo; 8.0 g daily safflower oil, $n = 9$) for 8 wk. The isomers of CLA in the CLA-mix supplement were primarily c9t11-CLA (~37%) and t10c12-CLA (~39%) in free fatty acid form. Plasma levels of CLA were inversely associated with body weight ($P < 0.05$) and serum leptin levels ($P < 0.05$). When levels of plasma t10c12-CLA isomer were correlated with changes in body weight or serum leptin, t10c12-CLA, but not c9t11-CLA, was inversely associated with body weights ($P < 0.05$) and serum leptin ($P < 0.02$). These findings strongly suggest that the t10c12-CLA isomer may be the bioactive isomer of CLA to influence the body weight changes observed in subjects with type 2 diabetes. Future studies are needed to determine a causal relationship, if any, of t10c12-CLA or c9t11-CLA to modulate body weight and composition in subjects with type 2 diabetes. Furthermore, determining the ability of CLA isomers to influence glucose and lipid metabolism as well as markers of insulin sensitivity is imperative to understanding the role of CLA to aid in the management of type 2 diabetes and other related conditions of insulin resistance. J. Nutr. 133: 257S–260S, 2003.

KEY WORDS: • *conjugated linoleic acid* • *body weight* • *leptin* • *type 2 diabetes mellitus*

Conjugated linoleic acid (CLA) refers to a group of polyunsaturated fatty acids (PUFA) that exist as positional and stereoisomers of conjugated dienoic octadecadienoate (18:2). The predominant isomer in foods is the c9t11-CLA isomer (1,2) (also called "rumenic acid") (3) followed by 7,9-CLA (c/t), 11,13-CLA (c/t), 8,10-CLA (c/t) then t10c12-CLA isomer (1). CLA is found primarily in foods such as beef, lamb and dairy foods (2,4,5). A synthetic mixture of CLA (referred to as CLA-mix) may also be found in nutritional supplements and is composed primarily of the c9t11-CLA and the t10c12-CLA isomers (**Fig. 1**).

Conjugated linoleic acid alters adipose tissue distribution

The conjugated fatty acids, or CLA, reduce adiposity in normoglycemic (nondiabetic) individuals as well as experimental animals including mice, rats, and pigs (6–8). Total adipose tissue mass was reduced by over 50% in mice fed a diet containing CLA-mix (1.0 wt %) compared to mice fed a control diet (without CLA). The reduction of adiposity by dietary CLA could be sustained in mice even after CLA was removed from the diet (9). Subsequent studies in nonobese mice demonstrated that some depots of fat mass were more sensitive than others to the effects of CLA. Diets with CLA-mix were especially effective in reducing adipose tissue mass in retroperitoneal and epididymal white adipose tissues (10–12) as well as brown adipose tissue (11).

There appears to be an isomer-specific effect of CLA on adiposity. t10c12-CLA is much more effective at lowering adipose tissue mass than the c9t11-CLA isomer in mice (13). In addition, t10c12-CLA is the effective isomer for modulating gene expression in cultured 3T3-L1 preadipocytes (14). The ability of CLA to reduce adipose tissue mass occurs

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[3] To whom correspondence should be addressed. E-mail: belury.1@osu.edu.

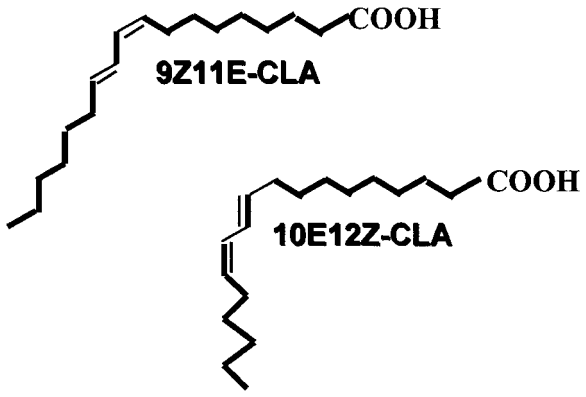

FIGURE 1 Structures of c9t11-CLA and t10c12-CLA.

regardless of food intake or fat level (total fat level, 6.5–20.0 wt %) in growing mice so that feed efficiency is affected (6,9). Furthermore, CLA reduces leptin in diabetic (ZDF) rats (15), nondiabetic mice (11) and humans with type 2 diabetes (Belury, M. A., unpublished data). Because leptin is a hormone secreted by adipose tissue that regulates food intake, it may be of significance to note that dietary CLA reduces food intake in mice and rats (7,16). However, supplementation with CLA in nonobese humans (3.0 g/d) has a modest and transient effect on leptin and had no effect on food intake (17). There is a possibility that a higher dose of CLA and/or longer duration of supplementation of CLA may affect food intake but this is yet to be determined.

Although most studies using nonobese or growing animal models have shown that dietary CLA lowers adipose tissue mass, not all studies show such an inverse relationship between dietary CLA and adipose tissue mass. Obese Zucker rats (8), but not diabetic fatty Zucker (ZDF) rats (12), exhibit an adipose-enhancing effect of dietary CLA-mix (8). In C57BL/6J mice, a mouse model for obesity and insulin resistance, long-term feeding with CLA-mix (1.0% CLA for 8 mo) leads to the formation of lipodystrophy, resulting in complete ablation of brown adipose tissue, increased fat accumulation in the liver and reduced leptin. Eventually, the lipodystrophic mice fed CLA developed insulin resistance (11). However, others have found this effect was transient (18). Mice (C57BLK $Lepr^{dbdb}$/$lepr^{dbdb}$) fed for 12 wk with CLA-mix diets exhibited induction of insulin resistance after 5 wk of feeding but a restoration of insulin sensitivity by 11 wk (18). These data suggest the effect of CLA on adiposity may be dependent on preexisting adiposity and/or insulin sensitivity.

In adult humans, the association of supplementation with CLA-mix and reductions of body weight or adipose tissue mass has been demonstrated in some (19–21) but not all (22,23) studies. In one study, overweight or obese human subjects supplemented with CLA-mix (3.4–6.0 g/d) for 12 wk exhibited a significant reduction of fat mass (20), whereas another study showed no such benefit of CLA supplementation (23). More recent studies have demonstrated that CLA supplementation reduces body weight, leptin and/or body adiposity in people (19,21; Belury, M. A., unpublished data). It is likely that dose, duration (short- vs. long-term) and the isomeric composition of CLA will each impact the ability of CLA to affect obesity in humans. In addition, how strain-, species-, age- and sex-specific effects of various isomers of CLA to influence adipose tissue accumulation, either in obese humans or those seeking to prevent adipose gain, is yet to be determined. Furthermore, a well-controlled study to determine the role of CLA in altering the distribution of adipose tissue (e.g., intraabdominal vs. subcutaneous fat) using validated methods has yet to be reported.

Conjugated linoleic acid affects body weight in human subjects with type 2 diabetes

Several risk factors for developing type 2 diabetes have been identified, including obesity, impaired glucose tolerance, some ethnicities (e.g., African-American, Asian, Pacific Islanders and Native American), advancing age, gestational diabetes, a positive family history of type 2 diabetes and lipid abnormalities. Central to all of these risk factors is the influence of obesity. In fact, lifestyle intervention resulting in a modest reduction of body weight (~7%) was associated with a 58% reduction in the incidence of diabetes in a cohort of people who were considered at high risk for developing this disease (24).

Previous studies from our laboratory demonstrated that CLA delays the onset of diabetes in the ZDF rat model. Therefore, we designed a study to elucidate the relationship of CLA to improvements in the management of type 2 diabetes mellitus in humans (Belury, M. A., unpublished results). Criteria for enrollment in this study included the requirement that subjects were not currently using medication for glucose control. The study was double blinded, where subjects were randomized in a block design according to fasting blood glucose values for either CLA supplementation ($n = 11$; 6.0 g/d) or safflower placebo supplementation ($n = 10$) for a duration of 8 wk. The CLA-mix supplement was composed of c9t11-CLA (~37%), t10c12-CLA (~39%), palmitic (6%), stearic (4%), oleic and linoleic (15%) acids in free fatty acid form.

Dietary intake of energy and fat quantity and quality were measured by use of a 3-d diet record followed by four repeated measures using a 24-h recall analysis. Dietary records were analyzed with the Minnesota Database (University of Minnesota, St. Paul, MN). Dietary intake of energy (kcal), fat (% kcal) or fat quality were similar between treatment groups at baseline. Subjects were instructed to maintain a healthy diet using the Food Guide Pyramid as a guide and were asked not to change their diet or activity habits for the 8-wk intervention period. There was no significant change in dietary energy or fat calories between week 0 and week 8 for either group. Compliance of subjects for pill consumption was reported to be >80–100% for pills consumed for all subjects in either group. Through use of a plasma biomarker for compliance, the accumulation of the t10c12-CLA isomer in plasma was significant ($P < 0.05$) for subjects supplemented with CLA (data not shown). In addition to measuring body weight and dietary composition, serum leptin was measured by radioimmunoassay (LINCO, St. Charles, MO). By week 8, supplementation with CLA (6.0 g CLA/d) was associated with decreases in fasting plasma glucose in nine out of 11 (81%) subjects on CLA supplementation and two out of 10 (20%) subjects on safflower supplementation.

When the level of CLA that accumulated in plasma was correlated with the change in body weight, there was a significant inverse correlation ($r = -0.4234$; $P < 0.05$) (**Fig. 2**). In addition, the plasma level of CLA was significantly inversely correlated with serum leptin ($r = -0.4275$; $P < 0.05$). Because the c9t11-CLA isomer is the predominant isomer found in foods such as beef, lamb and dairy foods, we determined the association of this isomer in plasma to changes in body weight or serum leptin. Associations of plasma c9t11-CLA to body weight or serum leptin were not significant ($r = -0.2873$ and $r = -0.3224$, respectively; data not shown). Because the

t10c12-CLA isomer has been shown to be the bioactive isomer to reduce adipose tissue in experimental animals, we determined the correlation coefficient of changes in body weight and leptin vs. t10c12-CLA levels in plasma.

In contrast to findings with the c9t11-CLA levels in plasma, the correlation coefficients for the t10c12-CLA isomer vs. changes in body weight or serum leptin were significant (body weight, $r = -0.4309$; $P < 0.05$; leptin, $r = -0.5260$, $P < 0.02$) (**Fig. 3**). Furthermore, the coefficients were stronger than the relationship for total plasma CLA to either body weight or serum leptin. These data suggest the lower body weights and serum leptin values in the subjects supplemented with CLA are attributed to the accumulation of the t10c12-CLA isomer in the plasma.

Unfortunately, body fat mass and distribution were not measured in this study. However, a recent study suggests a lowering of abdominal adiposity where there was an inverse relationship between supplementation with CLA-mix (4.2 g/d) for 12 wk and sagittal abdominal diameter (25) in overweight subjects. It is possible that the changes in serum leptin values that we observed may simply reflect a reduction in adipose tissue mass; however, leptin secretion may be more highly associated with reduced subcutaneous, not intraabdominal, adipose tissue mass (26). Of further note, a second neuroendocrine hormone, adiponectin, may be highly and inversely correlated to intraabdominal adipose mass (27). Unfortunately, we did not measure abdominal fat mass or adiponectin in this study, although future studies are warranted with such an analysis.

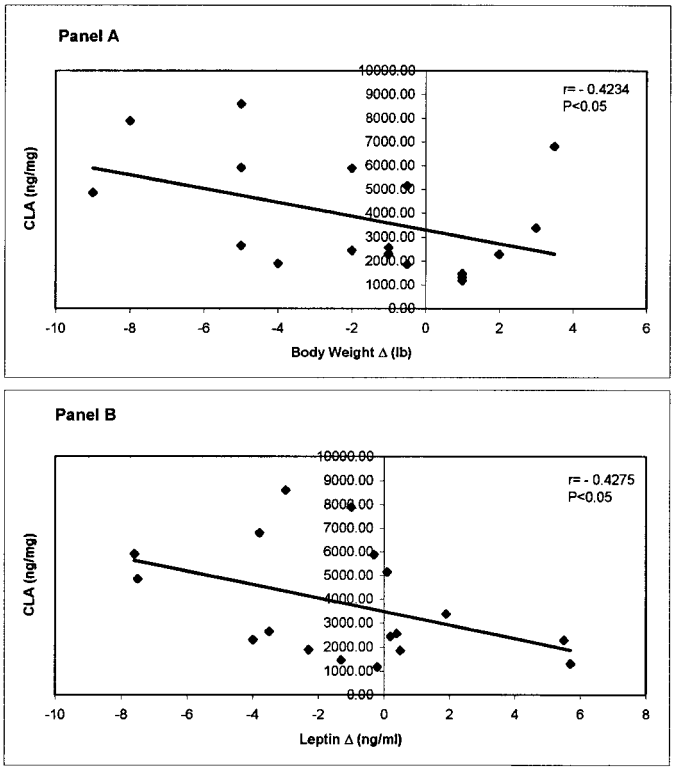

FIGURE 2 Plasma CLA is inversely correlated with (*A*) changes in body weight and (*B*) changes in serum leptin in subjects with type 2 diabetes. Subjects were supplemented with CLA or safflower capsules (8.0 g/d) for 8 wk. The level of CLA was determined by high performance liquid chromatography and gas chromatography as described previously (28,29).

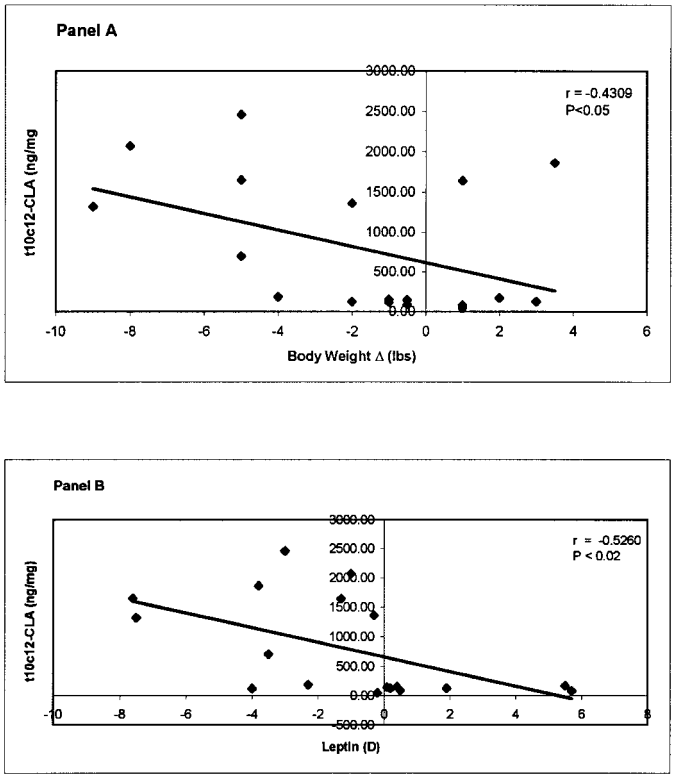

FIGURE 3 Plasma t10c12-CLA is inversely correlated with (*A*) changes in body weight and (*B*) changes in serum leptin in subjects with type 2 diabetes. Methods are as described in Figure 2.

Summary

The intake of dairy foods has been shown to be correlated with reduced body fat and enhanced insulin sensitivity in various cohorts. A potential group of bioactive compounds that could explain these effects might be CLA. However, our data suggest that there is a stronger correlative of the t10c12-CLA isomer than the naturally occurring rumenic acid (c9t11-CLA). Further work is needed to address the specific actions of the t10c12-CLA vs. c9t11-CLA isomers in the management of body weight in subjects with type 2 diabetes. In addition to determining the influence of CLA to reduce intraabdominal adiposity, it is important to determine the extent that favorable modifications of adipose tissue (e.g., reduction and/or redistribution) by CLA or various CLA isomers may affect glucose and lipid metabolism as well as insulin sensitivity in subjects with type 2 diabetes mellitus.

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