Conjugated Linoleic Acid (CLA) and Human Health; A Review of the Current Literature

Michelle Kay McGuire[1], Ph.D. and Mark A. McGuire[2], Ph.D.

[1]Department of Food Science and Human Nutrition, Washington State University
[2]Department of Animal and Veterinary Sciences, University of Idaho

SUMMARY

The potential for conjugated linoleic acid (CLA) to impact human health is strongly supported by a growing literature which suggests that CLA isomers can influence carcinogenesis, growth, immune function, glucose regulation and bone health in animal and cell culture models. The purpose of this paper was to review the current state of the literature pertaining specifically to studies that have been published on the effects of CLA intake (or supplementation) on human health, specifically nutrient partitioning, cancer and immune status. We also provide a review of the literature related to documentation of CLA intake in humans. Data suggest that humans consume anywhere from negligible amounts of CLA to about 500 mg/d from natural sources, but our current databases do not allow accurate distinction among the various CLA isomers in food sources. Documentation of CLA intake is difficult as wide variability in the CLA content among similar foods makes accurate determination of CLA intake from dietary records difficult. These issues create problems for investigators trying to relate CLA intake from natural sources to indicators of health and/or disease and will need to be addressed in future studies. None-the-less, several epidemiologic studies have been conducted to assess the relationship between estimated CLA intake and risk of mammary cancer. In summary, although there exists limited evidence that increased CLA intake might be related to decreased risk of breast cancer, the data are not particularly compelling nor consistent. Currently, to our knowledge, there are no published epidemiologic data concerning the relationship between CLA intake and risk of any other form of cancer. The need for controlled, clinical CLA intervention trials designed to test this hypothesis can not be understated at this time. In fact, there have been several CLA intervention studies designed to determine the potential effects of CLA on growth and nutrient partitioning. In general, their results remain difficult to interpret and suggest that the relationship between CLA intake and nutrient partitioning is complex. We believe that it is possible that the effects of CLA might be most pronounced in the growing human; thus, investigations with young adults at risk of gaining additional weight would be appropriate. Further, appropriate studies should investigate the effects of CLA consumption in free-living individuals with access to unlimited amounts of food for a relatively long period of time. Similarly, there is very little data support a relationship between CLA intake and immune status in humans, although the number of studies is

[1] Corresponding author: Michelle Kay McGuire, PO Box 646376, Pullman, WA 99164, 509-335-3896, Fax 509-335-4815, E-mail: smcguire@wsu.edu

very small at this time. Future studies designed to test this potential physiologic function should focus on effects of specific isomers and should include both sexes as subject participants. Clearly, there remains important research to be conducted to determine whether CLA can influence human health, and study design deserves careful consideration.

INTRODUCTION

Conjugated Linoleic Acid (CLA); Definitions and Brief History

Conjugated linoleic acids (CLA) are those fatty acids possessing 18 carbons, and 2 double bonds separated by one single bond, in contrast to the more commonly occurring unsaturated fatty acids that have two single bonds between each double bond. The most abundant CLA isomer found naturally in foods is consistently reported to be c9,t11-18:2 (given the trivial name of rumenic acid; RA) (Chin et al., 1992); many other isomers are also found (Yurawecz et al., 1998).

Although the presence of various isoforms of CLA in foods, particularly dairy products, has been known for decades (Parodi, 1977), their potential importance to human and animal health was not hypothesized until the late 1980s, when work from Dr. Michael Parizas research group at the University of Wisconsin at Madison suggested that foods containing naturally occurring CLA might decrease the risk of skin cancer (Ha et al., 1987). It has been shown repeatedly that CLA can inhibit many forms of cancer in animals (e.g., Ha et al., 1994, 1997) and can influence other indicators of various chronic diseases like diabetes, obesity and bone disease. Further, an impressive literature suggests that some, but not all, CLA isomers can cause milk fat depression in the lactating dairy cow (Baumgard et al., 2000). Thus, there is considerable interest in determining whether CLA consumption can influence human health and disease.

In addition, recent interest in the potential for CLA to improve human health (see below) has prompted some in the nutritional supplement industry to develop and market CLA supplements for human consumption. In an informal phone survey (McGuire, unpublished data), we found that of 11 General Nutrition Centers located in eastern Washington state, all were currently selling *at least* one form of CLA. In one store (Columbia Center GNC, Kennewick, WA), the manager reported selling 80 bottles (nearly 10,000 capsules) in the month of June, 2001. Clearly, as the supplement industry continues to advertise these products to the general public, supplement consumption will increase. Little is known about differences between supplemental CLA vs. naturally-occurring CLA as to their effects on human health.

Thus, researchers, public health workers, agricultural scientists and industry personnel are currently beginning to pursue the research required to actually determine whether changes in human CLA consumption (especially, that of specific CLA isomers) can impact human health. The purpose of this manuscript is to review the published literature which addresses this issue. For the purposes of this review, we have considered only peer-reviewed, published papers which have reported summary data on human CLA

consumption, CLA *in human tissues* or the effect of CLA supplementation or fortification on various indicators of health *in humans*. Noteworthy is the fact that, although several of these papers (n = 15) were published prior to 2000, the majority of the work concerning CLA and human health began to be published in the new millennium, highlighting the relative infancy of this sort of research.

In summary, the purpose of this report is to review and briefly evaluate the state of the scientific literature related to studies involving the effects of CLA consumption on human health. Specifically, we will review the research to-date (or lack thereof) pertaining to documentation of CLA consumption in humans, as well as the effects of CLA consumption (or supplementation) on (1) risk of cancer, (2) nutrient partitioning and (3) immune function. Clearly, this is not an exhaustive review of the current literature, but is intended to give the reader an encapsulated view of the level of human experimentation that has occurred as well as what research is still needed to fully evaluate the potential for CLA to influence human health and well-being.

ESTIMATION OF DIETARY CLA INTAKE IN HUMANS

Primary to studying the potential impact of dietary CLA intake on humans is the documentation of normal CLA intake in various segments of the population, and studies pertaining to this have been summarized previously by us (McGuire et al., 1999) and some of these data are summarized in Figure 1. Inherent in the accuracy of these estimates is consideration of the methodologies used by the researchers. For example, early estimates utilizing population-based estimates of dairy and beef products suggested CLA intakes of 100-1,000 mg/d (Ip et al., 1994; Parodi, 1994). Similarly, utilizing a national food intake survey in conjunction with values for CLA contents of German foods, Fritsche and colleagues (1998) estimated CLA intakes of 430 and 350 mg/d for German males and females, respectively. Fremann et al. (2002) estimated CLA intake by means of 7 d written record and found that, among college-aged, German students (mean age: 24 yr), average CLA intake was 323 mg/d. Using similar methods, Ens et al. (2001) estimated the average CLA intake for adult Canadians (n = 21; mean age: 31 yr) to be 95 mg/d.

However, the gold standard method for determining the intake of a nutrient is the chemical analysis of representative food duplicate portions of what an individual consumes during a period of 3 days (3-d, food duplicate methodology). Utilizing this methodology, we (Ritzenthaler et al., 2001) have documented total CLA intakes to be 212 and 115 mg/d in healthy, free-living, men (n = 46) and women (n = 47), respectively, living in the inland northwest. On a body weight basis, these intake values are approximately 2-3 mg/kg body weight/d. Interestingly, these values were actually higher ($P < 0.05$) than those estimated by 3-d dietary record methodology (176 and 104 mg/d in men and women, respectively). Approximately 91% of the dietary CLA was found to be RA, although this estimate inherently reflects the availability of CLA isomer data in our food database. Currently, there exists no database which contains the CLA isomer distribution of foods commonly consumed in the U.S., and this information will be important as we continue to pursue the potential health implication of the CLA isomers.

Another important aspect to consider is the potential importance of CLA intake during specific periods of growth and development. Ip et al. (1994) have shown that, in rodents, very early intake of CLA can have long-lasting positive impacts on risk of mammary cancer. Currently, there is no published data documenting CLA intakes during specific periods of the lifecycle in humans. However, we and others have shown that human milk contains significant amounts of CLA (Fogerty et al., 1988; McGuire et al., 1997), and that the CLA concentration can be altered by maternal CLA consumption from either natural foods (Park et al., 1999) or supplementation (Masters et al., 2002). Conversely, infant formula are devoid of CLA (McGuire et al., 1997), clearly indicating that exclusively formula-fed infants do not consume any form of CLA until supplementary foods are introduced. Unpublished data from our laboratory (Harrison et al.) suggest that CLA intake in the exclusively breastfed baby is approximately 108 mg/d, which is about 20 mg/kg body weight/d. Noteworthy is the fact that these figures are similar to, or substantially higher than, those of adult subjects. Additional recent data (Edwards et al., unpublished data) suggest relatively high CLA intake values for school-aged children (5-12 yr) of 130-170 mg/d (approximately 3-5 mg/kg body weight/d). Because it is possible that early nutritional environment can influence long-term health, future research might focus on studying the potential influence of early CLA intake in humans.

In summary, it appears that humans consume anywhere from negligible amounts of CLA to about 500 mg/d from natural sources. Our current databases do not allow accurate distinction among the various CLA isomers in food sources, and these data should be procured in the near future. Further, variability in the CLA content among similar foods makes accurate determination of CLA intake from dietary records somewhat difficult; thus, it is difficult to estimate individual dietary intakes of CLA in survey and epidemiologic studies. Finally, very little is known about CLA intake over the lifespan. These issues should be addressed in future studies.

CLA AND RISK OF CHRONIC DISEASE; HUMAN STUDIES

CLA and Carcinogenesis

Although the anticarcinogenic effect of CLA (most likely RA) on mammary cancer is well established in animal models (e.g., Ip et al., 2002), the possibility that CLA might influence mammary carcinogenesis in humans has not been studied extensively. For example, there have been no CLA supplementation intervention trials initiated to determine the effect of CLA intake on risk of breast cancer; these sorts of studies are *required* to determine whether there is an actual effect of particular nutrient (e.g., CLA) on an outcome variable (e.g., breast cancer). Thus, we will review the epidemiological data that is available on the relationships between (1) likely CLA intake (as reflected by milk and beef intake) and risk of breast cancer and (2) plasma or tissue CLA concentration and risk of breast cancer.

In a large, case-cohort study designed to look specifically at the relationships between calcium/vitamin D intake on breast cancer risk, Shin et al. (2002) studied 88,691 women in the Nurses Health Study between 1988 and 1996. Food frequency questionnaires were utilized to estimate and categorize dairy intake; CLA intake was not determined. Pooled logistic regression was used to estimate multivariable relative risks (RR) using 2-yr time increments. Data were adjusted for many variables thought to be related to breast cancer risk including family history of breast cancer, height, weight, age at menarche, parity, alcohol intake, fat intake, and total energy intake. These data suggested no relationship between breast cancer risk and dairy product intake in postmenopausal women. Conversely, there was an inverse association ($P < 0.005$) between low-fat dairy food intake and breast cancer risk in premenopausal women. Skim and low-fat milk were the dairy foods found to be most strongly related to breast cancer; women consuming 1 serving/day had a 28% lower risk of cancer as compared to women consuming no skim or low-fat milk. This relationship could not be explained statistically by adjusting for intakes of lactose, calcium, vitamin D or phosphorus, suggesting that these factors were not the causative agents. Although the authors found a similar, but nonsignificant ($P = 0.06$), protective effect of high-fat dairy food intake with premenopausal breast cancer risk, there were highly significant protective effects of total milk (8 oz/day; $P = 0.001$) and total dairy (servings/day; $P = 0.009$) intake on risk of cancer in this group of women. Interestingly, there were many fewer subjects reporting high dairy fat intake, thus resulting in substantially lower statistical power to test relationships involving this variable. None-the-less, these authors concluded that, if there is a protective effect of dairy consumption on breast cancer, that it is due to a bioactive component in the fat-free portion of the food. Calcium and vitamin D intake were found to be consistently related to decreased risk of breast cancer in the premenopausal, but not postmenopausal, women, but as previously stated, these variables did not explain the significant inverse relationships found between dairy intake and premenopausal cancer risk. The authors considered CLA as a likely causative effect explaining milks protective effect, but this possibility was dismissed because CLA intake would not be expected to be high in low-fat dairy products.

Several other studies have investigated the relationship between meat/dairy food consumption and risk of breast cancer and have found a variety of results ranging from protective effects of dairy foods consumption (e.g., Knekt et al., 1996) to negative effects of dairy foods consumption (Gaard et al., 1995). However, statistical sophistication and ranges in dairy fat intakes are clearly disparate among these studies and very well might influence the conclusions that can be drawn from them. In response to this, data from several ($n = 8$) prospective studies were pooled for purposes of conducting a meta-analysis with impressive statistical power ($n = 351,041$; Missmer et al., 2002). In all of these studies, food frequency questionnaires were utilized to obtain information concerning dietary intake; CLA intake was not determined. When data from all studies were modeled as continuous variables, these authors found no significant associations between total dairy fluids or solids and risk of breast cancer; there was also no evidence of effect modification by menopausal status (pre- vs. postmenopausal). There was a protective effect, however, found for consumption of total dairy fluids in one of the studies included in the meta-analysis (the Canadian National Breast Screening Study).

When dairy intake was modeled by quartiles, there was a nonsignificant ($P = 0.09$) trend for a protective effect (7% reduction in risk) for the highest quartile of dairy fluid intake. When these analyses were conducted for specific dairy sub-groups or products, no relationships were found except for when butter was considered; there was an increased risk (2%; $P = 0.04$) associated with the highest quartile of butter consumption. Similar analyses were conducted using measures of meat (red and white) consumption with similar results. These authors concluded that neither beef nor dairy consumption is associated with altered risk of breast cancer.

Clearly, these sorts of epidemiologic studies can be used as a framework for future investigations of the relationships among intakes of specific nutrients (e.g., CLA) and risk of chronic disease (e.g., breast cancer). However, because none of these studies was able to relate CLA *intake* to risk of cancer, interpretation of their results with regard to CLA is limited. To our knowledge, there have been 3 studies designed to actually assess the relationship between either CLA intake or tissue concentrations of CLA and risk of breast cancer. Aro and colleagues (2000) studied dietary and serum CLA in Finnish subjects ($n = 499$) in a case-control investigation between 1990 and 1995. Dietary intakes of CLA, C14:0 (myristic acid), C15:0 (pentadecanoic acid) and *t*11-18:1 (trans-vaccenic acid) were calculated from food frequency questionnaires utilized in conjunction with published data concerning the quantities of these fatty acids in commonly-consumed foods. Noteworthy is the fact that this database was limited and only relied on two reports of the fatty acid composition of foods. Relatively sophisticated analytical methods were used to determine the isomeric fatty acid composition of the serum. Data indicate that, although there were no major differences in the intakes of most nutrients (including fat and energy), CLA intake was lower in postmenopausal cases than in controls (126 vs. 133 mg/d, respectively; $P < 0.05$); these data were adjusted for age and energy intake. Similar, but nonsignificant, trends were observed for the premenopausal women; it should be noted that there were approximately 50% fewer premenopausal women resulting in less statistical power in this group. Odds ratio analysis suggested that postmenopausal women consuming the most CLA (5^{th} quintile; 204 mg/d) had a 60-70% lower risk of breast cancer than those consuming the least (1^{st} quintile; 72 mg/d). This relationship was actually strengthened when the data were adjusted for a variety of variables (e.g., age, age at menarche, family history of breast cancer, body fat content) thought to be related to risk of breast cancer. No similar relationships were found for other fatty acids investigated. Postmenopausal control women were also found to have higher ($P < 0.05$) proportions of *trans*-vaccenic acid and CLA in their serum, as compared to women with breast cancer, supporting the data that they were consuming higher amounts of foods (e.g., dairy products) containing these substances. After adjusting for a variety of important variables, data indicated an 80% lower risk of cancer in women exhibiting the highest (5^{th} quintile) of serum *trans*-vaccenic acid or CLA. These data strongly suggest that CLA and its precursor, *trans*-vaccenic acid, might be involved in physiologic processes inhibiting cancer initiation and/or growth in postmenopausal women.

In a similar, prospective, case-control study conducted in the Netherlands, Voorrips et al. (2002) studied 2,539 women. Dietary information was collected using a

semiquantitative food frequency questionnaire in conjunction with Dutch food composition tables and a separate database which was not described. Thus, it is not possible to evaluate the completeness or accuracy of the CLA data used to estimate CLA intakes in this study. After adjusting for appropriate confounders, data show no differences between cases and controls with regards to any dietary variable studied; CLA intake was approximately 200 mg/d for both groups. However, when analyzed as risk ratios, it was found that the highest quintiles of both *trans*-vaccenic acid and CLA intakes were associated with increased risks of breast cancer (24-40% increased risk; $P < 0.05$). In conclusion, these authors suggest that these data provide no evidence that CLA intake is protective against development of breast cancer; if anything, CLA was found to be a risk factor. The discrepancy between this study and that of Aro et al. (2000) remains puzzling.

Finally, there exist 2 reports (Chajés et al., 2002; Chajés et al., 2003) of a study designed to investigate the relationship between breast adipose CLA concentration in women diagnosed with breast pathologies. This included 241 women with invasive breast carcinomas (cases) and 88 women without carcinoma (controls); no difference in mammary adipose CLA concentration was found between controls and cases. Further, in the women with breast cancer, there were no clear relationships observed between CLA concentration and any prognostic factor (e.g., tumor size), risk of metastsis or risk of death. The authors freely acknowledged several limitations to their study including low statistical power, lack of dietary CLA intake data and inconclusive evidence that adipose tissue CLA concentration is highly correlated to long-term CLA intake. Further, all women studied in this experiment were recruited because they had breast anomalies; future studies should be designed to include a true control group.

In summary, although there is some epidemiologic evidence that increased CLA intake might be related to decreased risk of breast cancer, the data are not particularly compelling nor consistent. Currently, to our knowledge, there are no published epidemiologic data concerning the relationship between CLA intake and risk of any other form of cancer. The need for controlled, clinical CLA intervention trials cannot be understated at this time.

CLA and Regulation of Nutrient Partitioning

As previously mentioned, there exists a large literature relating the effects of CLA consumption on nutrient partitioning (body fat regulation) in experimental and production animal species. Because of the potential public health benefits of regulation of weight and body fat in humans, considerable research has been more recently conducted to test the potential effect of CLA intake on human body fat and related physiologic processes. These studies will be reviewed briefly here and are summarized in Table 1.

One of the first true dietary intervention studies to be conducted to investigate the effect of CLA on human body composition was that published by Zambell et al. (2000). This study was a very well controlled clinical dietary intervention trial conducted on a relatively small group ($n = 17$) of healthy, normal weight, young women (mean age: 28

yr). Subjects were admitted to a metabolic unit (24 h/d; 7 d/wk) for this 94 d, randomized, double-blind, placebo-controlled study and were assigned to one of 2 cohorts: control (n = 7) and CLA (n = 10). All subjects received a placebo capsule (sunflower oil) for 30 day, and either continued with this treatment (controls) or were provided with a CLA supplement (3 g/d; mixed CLA isomers; approximately 1% of calories) for the remaining 64 d intervention period. Subjects were weighed daily, and body composition determined both by total body electrical conductivity (TOBEC) and dual energy x-ray absorptiometry (DEXA). Metabolic rate and respiratory exchange ratio were determined by measuring oxygen consumption and carbon dioxide production. The subjects diets were made equivalent to the American Heart Associations Step II Diet and adjusted if body weight changed.

Data indicate no effect of CLA supplementation on subject weight, body fat, fat-free mass or nutrient intake during the intervention period. Similarly, there was no effect of CLA supplementation on metabolic rate or energy expenditure. In a secondary study to that previously mentioned, Medina et al. (2000) examined the effects of CLA supplementation on ciculating leptin concentrations and appetite in the same women and found no effect of CLA on these parameters. Noteworthy is the fact that these subjects were adults (not growing), clearly not free-living, and that the dietary intake was somewhat controlled and contrived. It would be interesting to determine whether conducting this study in younger subjects who are self-selecting diets might lend different results.

Mougious et al. (2001) also studied the effect of CLA supplementation in a randomized, double-blind, placebo-controlled study of younger (mean age: 22 y) healthy, normal weight (BMI < 30 kg/m^2) Greek men and women (n = 24). Participants were given a weekly dietary plan for the duration of the study, but subjects were free-living and able to choose their own foods. Subjects were assigned to consume 0.7 g CLA mixture/d or an identical placebo (soybean oil) for 8 wk. Body weight, height and skinfold thicknesses were measured throughout the study. Additionally, blood samples were monitored for a variety of metabolites and hormones. Data from this study provide limited evidence that the CLA-treated, but not placebo-treated, participants decreased body fat (%) and fat mass (kg) throughout the study. However, although not statistically significant, the CLA group began the study with somewhat higher measurements of body fatness, suggesting that regression to the mean might explain some of the findings presented here. Clearly, a larger sample size and longer intervention period would be preferable for this sort of study.

Blankson et al. (2000) have also published results from a randomized, double-blind, human intervention study designed to determine whether there is a dose-response relationship between CLA supplement intake and body fat in humans. In this study, otherwise healthy, overweight and obese middle-aged men and women (BMI > 25 kg/m^2 and < 35 kg/m^2; average age approximately 44 y; n = 47) were assigned to one of 5 groups: placebo (9 g olive oil/d) and 4 CLA interventions (1.7, 3.4, 5.1 or 6.8 g CLA mixture/d). Body weight, composition (DEXA) and a variety of circulating metabolites were measured throughout this 12 wk trial. Overall, subjects in the CLA supplementation

groups lost ($P < 0.05$) body weight and body fat while gaining lean body mass, while subjects in the placebo group experienced opposite trends. This attenuation of weight gain was most prominent in the group consuming 6.8 g CLA/d. Further, in all CLA treated groups (but not placebo group) there was a significant ($P < 0.05$) reduction in HDL cholesterol concentration; subjects consuming 1.7 or 3.4 g CLA/d also experienced reductions in total cholesterol and LDL cholesterol. Several differences should be noted between this study and that previously mentioned including the fact that these subjects were actively gaining weight and that they were consuming self-selected diets. Further, the upper level of CLA supplementation was substantially higher and the intervention period longer in the study conducted by Blankson et al (2000).

In another randomized, double-blind study designed to test the effects of CLA supplementation in obese Swedish men ($n = 24$) were assigned to either the control group (4.2 g olive oil/) or CLA group (4.2 CLA mixture/d) for a 4 wk intervention period (Risérus et al., 2001). Weight, height, body mass index and sagittal abdominal diameter (SAD), as well as a variety of circulating metabolites and hormones were measured. Consumption of CLA caused a significant reduction of SAD; this parameter did not change in the placebo group. There were no other effects of CLA supplementation documented.

Thom et al. (2001) studied the effects of CLA supplementation (1.8 g CLA mixture/d; 12 wk) in young (mean age: 27.8 y), Norwegian subjects (men and women; $n = 20$) actively involved in a strenuous exercise program (BMI < 25 kg/m^2). Body composition was estimated using near infrared light with the midpoint of the biceps as the measuring site. Lean body mass was not measured. Body fat (%), but not weight, was reduced ($P < 0.01$) in the CLA group (but not the placebo group) at 4, 8 and 12 wk. Interestingly, there was a nonsignificant trend for weight reduction in the CLA treated subjects, but not those consuming the placebo. Because the data indicated a relatively clear effect of CLA supplementation on body fat percent, it is likely that the CLA treated subjects were able to increase their lean body mass during the treatment period. The disparate results between this study and those previously mentioned may be due to different physiologic processes that might mediate the effects of CLA consumption between sedentary and physically active individuals.

However, in a well-controlled randomized, double-blind study of the effects of CLA supplementation (9 g CLA mixture/d; 28 d) in experienced resistance training males ($n = 23$), Kreider et al. (2002) found no effect of treatment on total body mass, body composition or strength. These variables also did not change during the study for the control group. Similar findings were reported in a study of resistance-trained, German athletes ($n = 14$) consuming 7 g CLA mixture/d for 6 mo (von Loeffelholz et al., 2003). Interestingly, in this study, the subjects all lost significant amounts of body fat. Thus, it is possible that the type of physical activity might also mediate potential effects of CLA consumption on nutrient partitioning in active individuals and that individuals engaged in activities designed to reduce body fat would not respond to the effects of CLA supplementation. Further, gender may also play an important role.

In summary, although several studies have now been conducted and published concerning the effects of CLA supplementation on nutrient partitioning in humans, their results remain difficult to interpret and suggest that the relationship between CLA intake and nutrient partitioning is complex. We believe that it is possible that the effects of CLA might be most pronounced in the growing human; thus, investigations with young adults (18-25 y), especially those at risk of gaining additional weight (e.g., college freshmen) would be appropriate. Further, appropriate studies should investigate the effects of CLA consumption in free-living individuals with access to unlimited amounts of food for a relatively long period of time.

CLA and Immune Function

Because results of several animal studies suggested that CLA intake could influence immune status, some investigators have begun to study this potential effect in human subjects. The first of these studies was previously described (Zambell et al., 2000) and involved 17 young, normal weight American women enrolled in an investigation of 93 d (Benito et al., 2001; Kelley et al., 2000; Kelley et al., 2001). Subjects were studied in a metabolic research unit and were assigned to either CLA (3.9 g CLA mixture/d; n = 10) or placebo (sunflower oil; n = 7). Supplementation began on d 31 of the study. Subjects were immunized on d 65 with an influenza vaccine, and blood samples were collected on d 15, 22, 29, 78 and 92 of the study. A variety of immunologic parameters and responses were measured. Data suggest no effect of CLA supplementation on any immunologic variable studied. Considering the extreme care taken to control this study for potential confounding variables, its relatively long duration and the relatively high dose of CLA used, these data represent convincing evidence that CLA supplementation does not influence immune function in this particular population.

However, in a more recent report, Albers et al. (2002) studied older (approximate mean age: 52 yr), Dutch men (n = 71) enrolled in a double-blind, randomized, placebo-controlled, 12 wk study. Subjects were free-living and assigned to either CLA 50:50 (CLA isomer mixture with approximately equal amounts of t10,c12- and c9,t11-18:2; 1.7 g/d), CLA 80:20 (CLA isomer mixture with 80% c9,t11-18:2 and 20% t10,c12-18:2; 1.6 g/d) or placebo (sunflower oil). A variety of immune parameters were measured at baseline and at the end of the study. There were trends toward an effect of CLA supplementation for several variables (e.g., antibody titers, hepatitis B-specific lymphocyte proliferation), especially for the CLA 50:50 treatment, but none of these were statistically significant. Noteworthy is the fact that, although there were substantially more subjects in this study than the previously mentioned CLA/immunity study, it is questionable whether there was sufficient statistical power to really test these effects given the variability inherent in many of these parameters.

In summary, although there is considerable animal literature suggesting an effect of CLA on immune function, there is very little data to support this effect in humans. Future studies designed to test this potential physiologic function should focus on effects of specific isomers and include both sexes as subject participants. Further, because of the

complexity of the immune system, a variety of outcome variables should be considered simultaneously.

CONCLUSIONS

As previously described, the purpose of this report was to review the published studies designed to experimentally investigate the relationship between CLA consumption and risk of mammary cancer, nutrient partitioning and immune function. We also reviewed the literature related to the documentation of human CLA intake. To date, only epidemiologic studies have considered this issue with regard to mammary cancer, and their conclusions are mixed. Clearly, long-term human intervention trials will need to be conducted to better evaluate the potential that CLA might influence the risk of this disease. In contrast, there have been several well conducted, randomized, placebo-controlled, double-blind studies designed to determine the effects of CLA supplementation on nutrient partitioning and, except for one of these, no study provides evidence that CLA influences body weight or composition in humans. Similarly, there is very limited evidence that CLA supplementation has an effect on the human immune system.

However, without question, the level of this research remains in a very preliminary state. For example, it is possible that these effects may be modulated by a variety of factors such as physiologic state, age and sex. Further, there are no studies published which have investigated the effects of individual CLA isomers on these parameters. Investigators interested in studying these potential effects must also consider whether the subjects have unlimited access to self-selected during the experimental period, as this factor might easily influence the likelihood of the subject to actually gain or lose weight (or body fat) during the study.

REFERENCES

Baumgard, L. H., B. A. Corl, D. A. Dwyer, A. Saebø, and D. E. Bauman. 2000. Identification of the conjugated linoleic acid isomer that inhibits milk fat synthesis. Am. J. Physiol. 278:R179-R184.

Benito, P.; G. J. Nelson, D. S. Kelley, G. Barolini, P. A. Schmidt, and V. Simon. 2001. The effect of conjugated linoleic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. Lipids. 36:221-227.

Chin, S. F., W. Liu, J. M. Storkson, Y. L. Ha, and M. W. Pariza. 1992. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J. Food Comp. Analysis. 5:185-197.

Ens, J. G., D. W. L. Ma, K. S. Cole, C. J. Field, and M. T. Clandinin. 2001. An assessment of c9,t11 linoleic acid intake in a small group of young Canadians. Nutr. Res. 21:955-960.

Fogerty, A. C., G. L. Ford, and D. Svoronos, 1988. Octadeca-9,11-dienoic acid in foodstuffs and in the lipids of human blood and breast milk. Nutr. Reports Inter. 38:937-944.

Freeman, D., J. Linseisen, and G. Wolfram, 2002. Dietary conjugated linoleic acid (CLA) intake assessment and possible biomarkers of CLA intake in young women. Public Health Nutr. 51:73-80.

Fritsche, J.; and H. Steinhart. 1998. Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. Z. Lebensm. Unters. Forsch. A. 2065:77-82.

Gaard, M., A. Tretli, and E. B. Loken, 1995. Dietary fat and the risk of breast cancer: a prospective study of 25, 892 Norwegian women. Inter. J. Cancer 63:13-17.

Ip, C., Y. Dong, M. M. Ip, S. Banni, G. Carta, E. Angioni, E. Murru, S. Spada, M. P. Melis, and A. Saebo. 2002. Conjugated linoleic acid isomers and mammary cancer prevention. Nutr. Cancer. 43:52-58.

Ip, C., J. A. Scimeca, and H. Thompson. 1994. Conjugated linoleic acid, a powerful anticarcinogen from animal fat sources. Cancer. 74:1050-1054.

Ip, C., M. Singh, H. J. Thompson, and J. A. Scimeca. 1994. Conjugated linoleic acid suppresses mammary carcinogenesis and proliferative activity of the mammary gland in the rat. Cancer Res. 54:1212-1215.

Kelley, D. S., V. A. Simon, P. C. Taylor, P. Benito, G. J. Nelson, B. E. Mackey, and K. L. Erickson. 2001. Dietary supplementation with conjugated linoleic acid increased its concentration in human peripheral blood mononuclear cells, but did not alter their function. Lipids. 36:669-674.

Knekt, P., R. Jarvinen, R. Seppanen, E. Pakkula, and A. Aromaa. Intake of dairy products and the risk of breast cancer. Bri. J. Cancer. 73:687-691.

Kreider, R.B., M. P. Ferreira, M. Greenwood, M. Wilson, and A. L. Almada. 2002. Effects of conjugated linoleic acid supplementation during resistance training on body composition, body density, strength and selected hematologic markers. J. Strength Conditioning Res. 16:325-334.

Masters, N., M. A. McGuire, K. A. Beerman, N. Dasgupta, and M. K. McGuire. 2002. Maternal supplementation with CLA decreases milk fat in humans. Lipids. 37:133-138.

McGuire, M. K., M. A. McGuire, K. Ritzenthaler, and T. Shultz. 1999. Dietary sources and intakes of conjugated linoleic acid intake in humans. Pages 369-377 in Advances in Conjugated Linoleic Acid Research. Volume 1, AOCS Press, Champaign, Illinois.

Medina, E. A., W. F. Horn, N. L. Keim, P. J. Havel, P. Benito, D. S. Kelley, G. J. Nelson, and K. L. Erickson. 2000. Conjugated linoleic acid supplementation in humans: effects on circulating leptin concentrations and appetite. Lipids. 35:783-788.

Missmer, S. A., S. A. Smith-Warner, D. Spiegelman, S. S. Yaun, H. O. Adami, W. L. Beeson. 2002. Meat and dairy food consumption and breast cancer: a pooled analysis of cohort studies. Inter. Epidemiol. Assoc. 31:78-85.

Mougios, V., A. Matsakas, A. Petridou, S. Ring, A. Sagredos, A. Mslissopoulou, N. Tsigilis, and M. Nikolaidis. 2001. Effect of supplementation with conjugated linoleic acid on human serum lipids and body fat. J. Nutr. Biochem. 12:585-594.

Park, Y., M. K. McGuire, R. Behr, M. A. McGuire, M. A. Evans, and T. D. Shultz. 1999. High-fat dairy product consumption increases ?9c,11t-18:2 (rumenic acid) and total lipid concentrations of human milk. Lipids 34:543-549.

Parodi, P.W. 1977. Conjugated octadecadienoic acids of milk fat. J. Dairy Sci. 60:1550-1553.

Parodi, P.W. 1997. Cows milk fat components as potential anticarcinogenic agents. J. Nutr. 127:1055-1060.

Risérus, U., L. Berglund, and B. Vessby. 2001. Conjugated linoleic acid (CLA) reduced abdominal adipose tissue in obese middle-aged men with signs of the metabolic syndrome: a randomised controlled trial. Inter. J. Obesity. 25:1129-1135.

Ritzenthaler, K. L., M. K. McGuire, R. Falen, T. D. Shultz, N. Dasgupta, M. A. McGuire. 2001. Estimation of conjugated linoleic acid intake by written dietary assessment methodologies underestimates actual intake evaluated by food duplicate methodology. J. Nutr. 131:1548-1554.

Shin, M. H., M. D. Holmes, S. E. Hankinson, K. Wu, G. A. Colditz, and W. C. Willett. 2002. Intake of dairy products, calcium and vitamin D and risk of breast cancer. J. Nat. Canc. Inst. 94:301-1311.

Thom, E., J. Wadstein, O. Gudmundsen. 2001. Conjugated linoleic acid reduces body fat in healthy exercising humans. J. Int. Med. Res. 29:392-396.

Von Loeffelholz, C., J. Kratzsch, and G. Jahreis. 2003. Influence of conjugated linoleic acids on body composition and selected serum and endocrine parameters in resistance-trained athletes. Eur. J. Lipid Sci. Technol. 105:251-259.

Yurawicz, M. P., J. A. G. Roach, N. Sehat, M. M. Mossoba, J. K. G. Kramer, J. Fritsche, H. Steinhart, and Y. Ku. 1998. A new conjugated linoleic acid isomer, 7 trans, 9 cis-Octadecadienoic acid, in cow milk, cheese, beef and human milk and adipose tissue. Lipids. 33:803-809.

Zambell, K.L., N. L. Keim, M. D. Van Loan, B. Gale, P. Benito, D. S. Kelly, G. J. Nelson. 2000. Conjugated linoleic acid supplementation in humans: effects on body composition and energy expenditure. Lipids. 35:777-782.

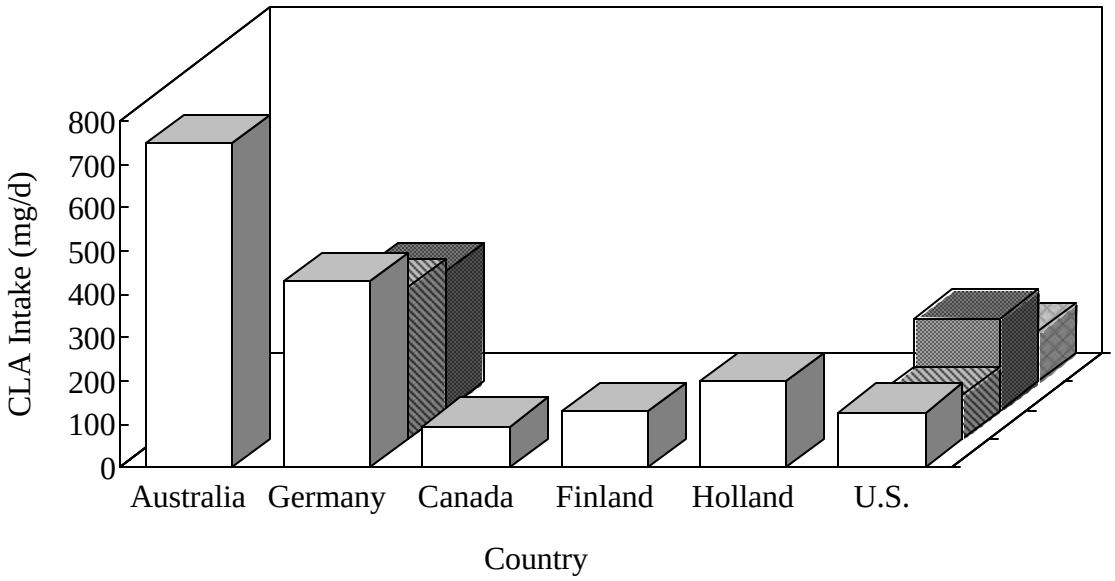

Figure 1 Published estimates of dietary CLA intake in a variety of locations world-wide. Data summarized from Aro et al., 2000, Ens et al., 2001, Fritsche and Steinhart, 1998, Parodi, 1994, Ritzenthaler et al., 2001, Voorips et al., 2002.

Table 1 Summary of published studies described here which were designed to determine the effect of CLA supplementation on human body composition.

Nation	Subjects	Study Design	Outcome Variables	Results	Reference
U.S.	Normal weight women (n = 17)	Randomized, placebo-controlled, double blind study (64 d intervention); 3 g CLA/d or placebo (sunflower oil)	Body composition (DEXA), energy expenditure, fat oxidation, respiratory exchange ratio, appetite, leptin, immune parameters	No effect of CLA supplementation	Zambell, 2000; Medina, 2000
Greece	Normal weight, young men and women (n = 24)	Randomized, placebo-controlled, double blind study (56 d intervention); 0.7 g CLA/d or placebo (soybean oil)	Weight, height, skinfold thickness, variety of metabolites and hormones	Very limited evidence of decreased body fat and fat mass in CLA-treated group	Mougious, 2001
Norway	Overweight men and women (n = 47)	Randomized, placebo-controlled, double blind study (84 d intervention); 1.7, 3.4, 5.1 or 6.8 g CLA/d or placebo (olive oil)	Body composition (DEXA), body weight and a variety of circulating metabolites	Control subjects gained weight and body fat, while CLA-treated subjects lost body weight and body fat; greatest effect with 6.8 g CLA/d; CLA-treatment caused reduction in blood cholesterol	Blankson , 2000
Sweden	Obese men (n = 24)	Randomized, placebo-controlled, double blind study (28 d intervention); 4.2 g CLA/d or placebo (olive oil)	Weight, height, body mass index, sagittal abdominal diameter and circulating metabolites and hormones	CLA treatment decreased sagittal abdominal diameter	Risérus, 2001
Norway	Normal weight men and women involved in strenuous exercise (n = 20)	Randomized, placebo-controlled, double blind study (84 d intervention); 1.8 g CLA/d or placebo	Body composition, weight	CLA treatment decreased body fat (%)	Thom, 2001
U.S.	Normal weight, experienced resistance training men (n = 14)	Randomized, placebo-controlled, double blind study (28 d intervention); 9 g CLA/d or placebo (olive oil)	Body composition, weight and strength	No effect of CLA supplementation	Kreider, 2002
Germany	Normal weight, novice and trained athletes (n = 14)	Randomized, placebo-controlled, double blind study (6 mo intervention); 9 g CLA/d or placebo (olive oil)	Body mass index, body composition and circulating lipid and leptin concentrations	No effect of CLA supplementation; both groups lost significant amounts of body fat	von Loeffelholz , 2003