

HEALTH PROMOTION

Conjugated linoleic acid and disease prevention: a review of current knowledge.

MacDonald HB J Am Coll Nutr 2000 Apr;19(2 Suppl):111S-118S

Dairy Farmers of Canada, Montreal, Quebec.

Conjugated linoleic acid (CLA), a derivative of a fatty acid linoleic acid (LA), has been reported to decrease tumorigenesis in animals. CLA is unique because unlike most antioxidants which are components of plant products, it is present in food from animal sources such as dairy foods and meats. CLA concentrations in dairy products typically range from 2.9 to 8.92 mg/g fat of which the 9-cis, 11-trans isomer makes up to 73% to 93% of the total CLA. Low concentrations of CLA are found in human blood and tissues. In vitro results suggest that CLA is cytotoxic to MCF-7 cells and it inhibits the proliferation of human malignant melanoma and colorectal cancer cells. In animal studies, CLA has inhibited the development of mouse epidermal tumors, mouse forestomach cancer and rat mammary cancer. Hamsters fed CLA collectively had significantly reduced levels of plasma total cholesterol, non-high-density lipoprotein cholesterol, (combined very-low and low-density lipoprotein) and triglycerides with no effect on high-density lipoprotein cholesterol, as compared to controls. Dietary CLA modulated certain aspects of the immune defense but had no obvious effect on the growth of an established, aggressive mammary tumor in mice. It is now thought that CLA itself may not have anti-oxidant capabilities but may produce substances which protect cells from the detrimental effects of peroxides. There is, however, insufficient evidence from human epidemiological data, and very few of the animal studies have shown a dose-response relationship with the quantity of CLA feed and the extent of tumor growth. Further research with tumor models is needed to test the efficacy and utility of CLA in cancer and other disease prevention and form the basis of evaluating its effect in humans by observational studies and clinical trials.

Mechanisms of action of conjugated linoleic acid: evidence and speculation.

Pariza MW, Park Y, Cook ME Proc Soc Exp Biol Med 2000 Jan;223(1):8-13

Food Research Institute, Department of Food Microbiology, University of Wisconsin-Madison, 53706, USA.

Conjugated linoleic acid (CLA) has been shown to inhibit carcinogenesis and atherosclerosis, enhance immunologic function while protecting against the catabolic effects of immune stimulation, affect body composition change (reducing body fat gain while enhancing lean body mass gain), and stimulate the growth of young rats. We discuss possible biochemical mechanisms that underlie these physiological effects. We emphasize the importance of considering the effects, both individually and combined, of the two CLA isomers (cis-9, trans-11 CLA and trans-10, cis-12 CLA) that have been shown to exhibit biological activity and which appear to exert their effects via different biochemical mechanisms.

CANCER

GENERAL

Newly recognized cytotoxic effect of conjugated trienoic fatty acids on cultured human tumor cells.

Igarashi M, Miyazawa T *Cancer Lett* 2000 Feb 1;148(2):173-9

Laboratory of Biodynamic Chemistry, Tohoku University Graduate School of Life Science and Agriculture, Sendai, Japan.

We investigated the cytotoxic effect of conjugated trienoic fatty acids on various human tumor cell lines: DLD-1, colorectal; HepG2, hepatoma; A549, lung; MCF-7, breast; and MKN-7, stomach. Conjugated linoleic acid (CLA) and conjugated linolenic acid were prepared from linoleic acid (18:2, n-6) and alpha-linolenic acid (18:3, n-3), respectively, by treatment with 6.6% or 21% potassium hydroxide. Spectrophotometric readings at 235 nm for the conjugated diene formation, and at 268 nm for the conjugated triene, were confirmed for the respective conjugated fatty acids. In addition, tung oil (*Aleurites fordii*) fatty acids consisting principally of a conjugated triene (eleostearic acid, approximately 80% of total fatty acids) were prepared using an alkaline saponification procedure. All tumor cells were incubated for 24 h with 5-100 microM of the conjugated fatty acids, and MTT dye reduction was measured to verify the cell viability. Among the conjugated fatty acids examined, conjugated linolenic acid and tung oil fatty acids exhibited the most intense cytotoxic effects on DLD-1, HepG2, A549, MCF-7 and MKN-7 cells, while CLA was not cytotoxic to the tumor cells. These results demonstrate that conjugated trienoic fatty acids are more cytotoxic to human tumor cells than the conjugated dienoic fatty acid, CLA.

Specific versus non-specific effects of dietary fat on carcinogenesis.

Guthrie N, Carroll KK *Prog Lipid Res* 1999 May;38(3):261-71

Department of Biochemistry, University of Western Ontario, London, Canada.

It will be apparent from this review that dietary fat can exert both specific and non-specific effects on carcinogenesis, at least in experimental animals. The non-specific effects appear to be related primarily to effects of dietary fat on energy balance. Although a positive energy balance can be achieved on a high-carbohydrate low-fat diet, it is much more likely to occur on a high-fat diet because of the high energy density of fat and the fact that dietary fat is less capable of imparting a sense of satiety. A continuing state of positive energy balance leads to obesity which has been associated with increased risk of cancer at a number of sites, including endometrium, postmenopausal breast cancer renal cancer and possibly cancers of the colorectum, pancreas and prostate. Whereas the non-specific effects of dietary fat appear to be deleterious for cancer, the specific effects in some cases can be beneficial. Examples are long-chain n-3 polyunsaturated fatty acids, CLA and tocotrienols. It is still too early to predict whether these may be of value in the prevention and/or treatment of human cancer but they seem worthy of further investigation. Knowledge of their mechanism of action may suggest novel approaches to the cancer problem and, as in the case of vitamins A and D, it may be possible to find analogues with more potent anti-cancer activity.

Inhibition of DNA adduct formation of PhIP in female F344 rats by dietary conjugated linoleic acid.

Josyula S, He YH, Ruch RJ, Schut HA *Nutr Cancer* 1998;32(3):132-8

Department of Pathology, Medical College of Ohio, Toledo 43614-5806, USA.

The dietary mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) is a mammary carcinogen in the female Fischer (F344) rat and a colon carcinogen in the male F344 rat. To exert its carcinogenicity, it is believed that PhIP needs to form adducts with DNA, a process requiring N-hydroxylation of PhIP by cytochromes P-450 1A1 and/or 1A2 (CYP 1A1 and/or 1A2), as well as further esterification of the hydroxylamine thus formed. Dietary conjugated linoleic acid (CLA) inhibits chemical carcinogenesis in various experimental models. We have examined the effect of dietary CLA on PhIP-DNA adduct formation in female F344 rats. Four-week-old animals were maintained on AIN-76A diet without or with CLA (1%, 0.5%, and 0.1% wt/wt) for 57 days. PhIP was added to the diets (0.04% wt/wt) from Days 14-42. Animals were killed (4/group) on Days 43, 50, and 57. DNA isolated from liver, mammary epithelial cells (MEC), colon, and white blood cells (WBC) was analyzed for PhIP-DNA adducts by ³²P-postlabeling assays. On Day 43, CLA inhibited adduct formation in the liver (up to 58%) in a dose-dependent manner. CLA also inhibited hepatic adduct levels (29-39%) on Day 50 (at 1.0% and 0.5% CLA) and on Day 57 (53% at 0.5% CLA). CLA significantly reduced adduct levels in the WBC on Day 50 (63-70%). Adducts in MEC and the colon were not affected by dietary CLA. On Day 57, adduct levels in MEC, liver, colon, and WBC were 0-30.3%, 8.6-41.7%, 21.5-50.7%, and 7.5-11.8%, respectively, of those on Day 43. Northern blot analysis of liver RNA showed that dietary CLA did not affect steady-state levels of CYP 1A1 or 1A2 mRNA. It is concluded that dietary CLA inhibits PhIP-DNA adduct formation in liver and WBC but that those in MEC and the colon are unaffected when a low-level dietary regimen of carcinogen and inhibitor was used. In inhibiting PhIP-DNA adduct formation, CLA does not appear to act by inhibiting CYP 1A1 or 1A2 expression.

Retention of conjugated linoleic acid in the mammary gland is associated with tumor inhibition during the post-initiation phase of carcinogenesis.

Ip C, Jiang C, Thompson HJ, Scimeca JA *Carcinogenesis* 1997 Apr;18(4):755-9

Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY USA.

Conjugated linoleic acid (CLA) has been reported to have significant activity in inhibiting mammary carcinogenesis. A major objective of this study was to evaluate how changes in the concentration of CLA in mammary tissue as a function of CLA exposure/withdrawal were correlated with the rate of occurrence of mammary carcinomas. Rats treated with a single dose of dimethylbenz[a]anthracene (DMBA) at 50 days of age were given 1% CLA in the diet for either 4 weeks, 8 weeks or continuously following carcinogen administration. No cancer protection was evident in the 4 or 8 week-CLA treatment groups. Significant tumor inhibition was observed only in rats that were given CLA for the entire duration of the experiment (20 weeks). Analysis of CLA in the mammary gland showed that the incorporation of CLA was much higher in neutral lipids than in phospholipids. When CLA was removed from the diet, neutral lipid- and phospholipid-CLA returned to basal values in about 4 and 8 weeks, respectively. The rate of disappearance of neutral lipid-CLA (rather than phospholipid-CLA) subsequent to CLA withdrawal paralleled more closely the rate of occurrence of new tumors in the target tissue. It appears that neutral lipid-CLA may be a more sensitive marker of tumor protection than phospholipid-CLA. However, the physiological relevance of CLA accumulation in mammary lipids is unclear and remains to be determined. A secondary goal of this study was to investigate

whether CLA might selectively inhibit clonal expansion of DMBA-initiated mammary epithelial cells with wild-type versus codon 61 mutated Ha-ras genes. Approximately 16% of carcinomas in the control group (without CLA) were found to express codon 61 ras mutation. Although continuous treatment with CLA reduced the total number of carcinomas by 70%, it did not alter the proportion of ras mutant versus wild-type carcinomas, suggesting that CLA inhibits mammary carcinogenesis irrespective of the presence or absence of the ras mutation.

BREAST

Morphological and biochemical status of the mammary gland as influenced by conjugated linoleic acid: implication for a reduction in mammary cancer risk.

Thompson H, Zhu Z, Banni S, Darcy K, Loftus T, Ip C *Cancer Res* 1997 Nov 15;57(22):5067-72

Division of Laboratory Research, AMC Cancer Research Center, Denver, Colorado, USA.

Previous research showed that treatment with conjugated linoleic acid (CLA) during the period of active mammary gland morphogenesis was sufficient to confer a lasting protection against subsequent mammary tumorigenesis induced by methylnitrosourea. The present study was designed to characterize certain morphological and biochemical changes of the mammary gland that might potentially render it less susceptible to cancer induction. Female Sprague Dawley rats were fed a 1% CLA diet from weaning until about 50 days of age. The mammary gland parameters under investigation included (a) the deposition of neutral lipid, (b) the identification and quantification of CLA and its metabolites, (c) the density of the epithelium, and (d) the proliferative activity of various structural components. Our results showed that CLA treatment did not affect total fat deposition in the mammary tissue nor the extent of epithelial invasion into the surrounding fat pad but was able to cause a 20% reduction in the density of the ductal-lobular tree as determined by digitized image analysis of the whole mounts. This was accompanied by a suppression of bromodeoxyuridine labeling in the terminal end buds and lobuloalveolar buds. The recovery of desaturation and elongation products of CLA in the mammary gland confirmed our prior suggestion that the metabolism of CLA might be critical to risk modulation. The significance of the above findings was investigated in a mammary carcinogenesis bioassay with the use of the dimethylbenz[a]-anthracene model. When CLA was started at weaning and continued for 6 months until the end of the experiment, this schedule of supplementation produced essentially the same magnitude of mammary tumor inhibition in the dimethylbenz[a]anthracene model as that produced by 1 month of CLA feeding from weaning. The observation is consistent with the hypothesis that exposure to CLA during the time of mammary gland maturation may modify the developmental potential of a subset of target cells that are normally susceptible to carcinogen-induced transformation.

The efficacy of conjugated linoleic acid in mammary cancer prevention is independent of the level or type of fat in the diet.

Ip C, Briggs SP, Haegele AD, Thompson HJ, Storkson J, Scimeca JA *Carcinogenesis* 1996 May;17(5):1045-50

Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY USA.

The objective of the present study was to investigate whether the anticarcinogenic activity of conjugated linoleic acid (CLA) is affected by the amount and composition of dietary fat consumed by the host. Because the anticancer agent of interest is a fatty acid, this approach may provide some insight into its mechanism of action, depending on the outcome of these fat feeding experiments. For the fat level experiment, a custom formulated fat blend was used that simulates the fatty acid composition of the US diet. This fat blend was present at 10, 13.3, 16.7 or 20% by weight in the diet. For the fat type experiment, a 20% (w/w) fat diet containing either corn oil (exclusively) or lard (predominantly) was used. Mammary cancer prevention by CLA was evaluated using the rat dimethylbenz[a]anthracene model. The results indicated that the magnitude of tumor inhibition by 1% CLA was not influenced by the level or type of fat in the diet. It should be noted that these fat diets varied markedly in their content of linoleate. Fatty acid analysis showed that CLA was incorporated predominantly in mammary tissue neutral lipids, while the increase in CLA in mammary tissue phospholipids was minimal. Furthermore, there was no evidence that CLA supplementation perturbed the distribution of linoleate or other fatty acids in the phospholipid fraction. Collectively these carcinogenesis and biochemical data suggest that the cancer preventive activity of CLA is unlikely to be mediated by interference with the metabolic cascade involved in converting linoleic acid to eicosanoids. The hypothesis that CLA might act as an antioxidant was also examined. Treatment with CLA resulted in lower levels of mammary tissue malondialdehyde (an end product of lipid peroxidation), but failed to change the levels of 8-hydroxydeoxyguanosine (a marker of oxidatively damaged DNA). Thus while CLA may have some antioxidant function in vivo in suppressing lipid peroxidation, its anticarcinogenic activity cannot be accounted for by protecting the target cell DNA against oxidative damage. The finding that the inhibitory effect of CLA maximized at 1% (regardless of the availability of linoleate in the diet) could conceivably point to a limiting step in the capacity to metabolize CLA to some active product(s) which is essential for cancer prevention.

Effect of timing and duration of dietary conjugated linoleic acid on mammary cancer prevention.
Ip C, Scimeca JA, Thompson H Nutr Cancer 1995;24(3):241-7

Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA.

Conjugated linoleic acid (CLA) is a minor fatty acid found predominantly in the form of triglycerides in beef and dairy products. Previous work by Ip and co-workers showed that free fatty acid-CLA at < or = 1% in the diet is protective against mammary carcinogenesis in rats. The present study verified that the anticancer activities of free fatty acid-CLA and triglyceride-CLA are essentially identical. This is an important finding, because it rules out a nonspecific free fatty acid effect. In terms of practical implication, we can continue the in vivo research with the less-expensive free fatty acid-CLA without compromising the physiological relevance of the data. A primary objective of this report was to investigate how the timing and duration of CLA feeding might affect the development of mammary carcinogenesis in the methylnitrosourea (MNU) model. We found that exposure to 1% CLA during the early postweaning and pubertal period only (from 21 to 42 days of age) was sufficient to reduce subsequent tumorigenesis induced by a single dose of MNU given at 56 days of age. This period incidentally corresponds to a time of active morphological development of the mammary gland to the mature state. In contrast to the above observation, a continuous intake of CLA was required for maximal inhibition of tumorigenesis when CLA feeding was started after MNU administration, suggesting that some active metabolite(s) of CLA might be involved in suppressing the process of neoplastic promotion/progression.

Conjugated linoleic acid inhibits proliferation and induces apoptosis of normal rat mammary epithelial cells in primary culture.

Ip MM, Masso-Welch PA, Shoemaker SF, Shea-Eaton WK, Ip C *Exp Cell Res* 1999 Jul10;250(1):22-34

Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, New York, 14263, USA.

The trace fatty acid conjugated linoleic acid (CLA) inhibits rat mammary carcinogenesis when fed prior to carcinogen during pubertal mammary gland development or during the promotion phase of carcinogenesis. The following studies were done to investigate possible mechanisms of these effects. Using a physiological model for growth and differentiation of normal rat mammary epithelial cell organoids (MEO) in primary culture, we found that CLA, but not linoleic acid (LA), inhibited growth of MEO and that this growth inhibition was mediated both by a reduction in DNA synthesis and a stimulation of apoptosis. The effects of CLA did not appear to be mediated by changes in epithelial protein kinase C (PKC) since neither total activity nor expression nor localization of PKC isoenzymes alpha, betaII, delta, varepsilon, eta, or zeta were altered in the epithelium of CLA-fed rats. In contrast, PKCs delta, varepsilon, and eta were specifically upregulated and associated with a lipid-like, but acetone-insoluble, fibrillar material found exclusively in adipocytes from CLA-fed rats. Taken together, these observations demonstrate that CLA can act directly to inhibit growth and induce apoptosis of normal MEO and may thus prevent breast cancer by its ability to reduce mammary epithelial density and to inhibit the outgrowth of initiated MEO. Moreover, the changes in mammary adipocyte PKC expression and lipid composition suggest that the adipose stroma may play an important in vivo role in mediating the ability of CLA to inhibit mammary carcinogenesis.

Differential stimulatory and inhibitory responses of human MCF-7 breast cancer cells to linoleic acid and conjugated linoleic acid in culture.

Shultz TD, Chew BP, Seaman WR *Anticancer Res* 1992 Nov-Dec;12(6B):2143-5

Department of Food Science and Human Nutrition, Washington State University, Pullman 99164-6376.

Consumption of dietary fat has been linked to the high incidence of certain cancers. However, recent research has stimulated interest in conjugated linoleic acid (CLA), a newly recognized anticarcinogenic fatty acid. Human MCF-7 breast cancer cells were incubated for 12 d in culture medium supplemented with various concentrations ($1.78\text{--}7.14 \times 10^{-5}$ M) of linoleic acid (LA) or CLA. Linoleic acid initially stimulated MCF-7 cell growth with an optimal effect at concentrations of $3.57\text{--}7.14 \times 10^{-5}$ M, but was inhibitory at similar concentrations after 8 and 12 d of incubation. In contrast, CLA was inhibitory to cancer cell growth at all concentrations and times tested. Cell growth inhibition by CLA was dose- and time-dependent. Growth retardation at the prescribed LA and CLA concentrations ranged, respectively, from 4 to 33% and 54 to 100% following 8 to 12 d of treatment. At similar LA and CLA concentrations, cytostatic and cytotoxic effects of CLA were more pronounced (8-81%) than LA. These in vitro results suggest that CLA is cytotoxic to MCF-7 cells.

Reduction of murine mammary tumor metastasis by conjugated linoleic acid.

Hubbard NE, Lim D, Summers L, Erickson KL *Cancer Lett* 2000 Mar 13;150(1):93-100

Department of Cell Biology and Human Anatomy, University of California, School of Medicine, Davis 95616-8643, USA.

Recent studies have shown that conjugated linoleic acid (CLA) can inhibit the initiation and thus, incidence of mammary tumors in rodents. The concentration of CLA required for these effects was as low as 0.1% of the diet, with no increased effects above 1%. To date, there is little evidence that CLA has any effect on growth or metastasis of mammary tumors. In this report, we demonstrate that CLA, at the concentrations used in previous studies, had a significant effect on the latency, metastasis, and pulmonary tumor burden of transplantable murine mammary tumors grown in mice fed 20% fat diets. The latency of tumors from mice fed CLA was significantly increased when compared with the 0% CLA control diet. The volume of pulmonary tumor burden, as a result of spontaneous metastasis, decreased proportionately with increasing concentrations of dietary CLA. With 0.5 and 1% CLA, pulmonary tumor burden was significantly decreased compared to mice treated with the eicosanoid inhibitor, indomethacin and fed diets containing no CLA. Tumors of mice fed as little as 0.1% CLA and as much as 1% had significantly decreased numbers of pulmonary nodules when compared with diets containing no CLA. The decrease in the number of pulmonary nodules by CLA was nearly as effective as indomethacin, a known suppressor of tumor growth and metastasis in this malignant model. These data suggest that effects of CLA on mammary tumorigenesis may go beyond the reported alterations in tumor incidence and effect later stages, especially metastasis.

Conjugated linoleic acid and linoleic acid are distinctive modulators of mammary carcinogenesis.
Ip C, Scimeca JA *Nutr Cancer* 1997;27(2):131-5

Department of Surgical Oncology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA.

Previous work by Ip and co-workers showed that mammary cancer prevention by conjugated linoleic acid (CLA) is independent of the level of fat in the diet. Because CLA is an isomer of linoleic acid, there is the question regarding whether the effect of CLA is due to a displacement of linoleic acid in cells. To further evaluate whether there might be an interaction between linoleic acid and CLA, the present study was designed to examine the dose response to CLA (at 0.5%, 1%, 1.5%, and 2%) in rats fed a 2% or a 12% linoleate diet (both basal diets contained 20% total fat by weight). The end points of investigation included the bioassay of mammary tumorigenesis in the rat dimethylbenz[a]anthracene model as well as the incorporation of CLA, linoleic acid, and arachidonic acid in mammary glands. The mammary carcinogenesis results showed that the efficacy of tumor suppression by CLA was not affected by linoleate intake. With either linoleate diet, no further protection was evident with levels of CLA > 1%. Analysis of neutral lipids and phospholipids of the mammary tissue indicated that 1) the accumulation of CLA in mammary tissue was dose dependent from 0.5% to 2%, 2) CLA concentration was 10 times higher in neutral lipids than in phospholipids, 3) the incorporation of CLA in either fraction was not affected by the availability of linoleic acid, and 4) CLA did not appear to displace linoleic acid or arachidonic acid in the mammary tissue. The above findings suggest that there may be distinctive mechanisms in the modulation of tumor development by linoleic acid and CLA.

Conjugated linoleic acid suppresses the growth of human breast adenocarcinoma cells in SCID mice.

Visonneau S, Cesano A, Tepper SA, Scimeca JA, Santoli D, Kritchevsky D *Anticancer Res* 1997 Mar-Apr;17(2A):969-73

Wistar Institute, Philadelphia, Pennsylvania 1910, USA.

Conjugated linoleic acid (CLA), which is mainly derived from dairy products, has been shown both in vitro and in animal models to have strong anti-tumor activity. Particular effects were observed on the growth and metastatic spread of transplantable mammary tumors. In this study, we examined the effect of dietary CLA on the growth of human breast adenocarcinoma cells in severe combined immuno-deficient (SCID) mice. Mice were fed 1% CLA for two weeks prior to subcutaneous inoculation of 10(7) MDA-MB468 cells and throughout the study. Dietary CLA inhibited local tumor growth by 73% and 30% at 9 and 14 weeks post-inoculation, respectively. Moreover, CLA completely abrogated the spread of breast cancer cells to lungs, peripheral blood, and bone marrow. These results indicate the ability of dietary CLA to block both the local growth and systemic spread of human breast cancer via mechanisms independent of the host immune system.

Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats.

Ip C, Banni S, Angioni E, Carta G, McGinley J, Thompson HJ, Barbano D, Bauman D J *Nutr* 1999 Dec;129(12):2135-42

Department of Experimental Pathology, Roswell Park Cancer Institute, Buffalo, NY 14263, USA.

Conjugated linoleic acid (CLA) is a potent cancer preventive agent in animal models. To date, all of the in vivo work with CLA has been done with a commercial free fatty acid preparation containing a mixture of c9,t11-, t10,c12- and c11,t13-isomers, although CLA in food is predominantly (80-90%) the c9,t11-isomer present in triacylglycerols. The objective of this study was to determine whether a high CLA butter fat has biological activities similar to those of the mixture of free fatty acid CLA isomers. The following four different endpoints were evaluated in rat mammary gland: 1) digitized image analysis of epithelial mass in mammary whole mount; 2) terminal end bud (TEB) density; 3) proliferative activity of TEB cells as determined by proliferating cell nuclear antigen immunohistochemistry; and 4) mammary cancer prevention bioassay in the methylnitrosourea model. It should be noted that TEB cells are the target cells for mammary chemical carcinogenesis. Feeding butter fat CLA to rats during the time of pubescent mammary gland development reduced mammary epithelial mass by 22%, decreased the size of the TEB population by 30%, suppressed the proliferation of TEB cells by 30% and inhibited mammary tumor yield by 53% ($P < 0.05$). Furthermore, all of the above variables responded with the same magnitude of change to both butter fat CLA and the mixture of CLA isomers at the level of CLA (0.8%) present in the diet. Interestingly, there appeared to be some selectivity in the uptake or incorporation of c9,t11-CLA over t10,c12-CLA in the tissues of rats given the mixture of CLA isomers. Rats consuming the CLA-enriched butter fat also consistently accumulated more total CLA in the mammary gland and other tissues (four- to sixfold increases) compared with those consuming free fatty acid CLA (threefold increases) at the same dietary level of intake.

Proliferative responses of normal human mammary and MCF-7 breast cancer cells to linoleic acid, conjugated linoleic acid and eicosanoid synthesis inhibitors in culture.

Cunningham DC, Harrison LY, Shultz TD *Anticancer Res* 1997 Jan-Feb;17(1A):197-203

Department of Food Science and Human Nutrition, Washington State University, Pullman

Potential mechanisms for the stimulation or inhibition of cell growth by linoleic acid (LA) and conjugated linoleic acid (CLA) were investigated by using eicosanoid synthesis inhibitors. Normal human mammary epithelial cells (HMEC) and MCF-7 breast cancer cells were incubated in serum-free medium supplemented with LA or CLA and cyclo-oxygenase (indomethacin; INDO) or lipoxygenase (nordihydroguaiaretic acid; NDGA) inhibitors. Linoleic acid stimulated the growth and [3H]thymidine incorporation of normal HMEC and MCF-7 cancer cells, while CLA was inhibitory. Supplementation with LA increased intracellular lipid peroxide concentrations in normal HMEC and MCF-7 cancer cells, whereas CLA did not affect lipid peroxide formation. Normal HMEC and MCF-7 cells supplemented with LA and INDO and or NDGA resulted in growth inhibition. The treatment of normal HMEC with CLA INDO or NDGA, and MCF-7 cells with CLA and INDO stimulated cell growth. However, the addition of CLA and NDGA to MCF-7 cells resulted in synergistic growth suppression suggesting that CLA effects were mediated through lipoxygenase inhibition. Although NDGA was more inhibitory of cell growth in the presence of LA or CLA than INDO, growth was associated with both prostaglandin and leukotriene production. Additional studies are warranted to elucidate the mechanism(s) whereby LA or CLA affect breast cell growth.

SKIN

Conjugated linoleic acid modulation of phorbol ester-induced events in murine keratinocytes.

Liu KL, Belury MA *Lipids* 1997 Jul;32(7):725-30

Department of Foods and Nutrition, Purdue University, West Lafayette, Indiana, USA.

Recent work in our lab has shown that the chemoprotective fatty acid, conjugated linoleic acid (CLA), inhibits phorbol ester skin tumor promotion in mice. Because little is known about the deposition of CLA into tissues as well as its biological activity, this study compared the incorporation and biological activity of CLA to linoleic acid (LA; 18:2, c9,c12) and arachidonic acid (AA; 20:4 c5,c8,c11,c14) in cultured keratinocytes. When keratinocytes (HEL-30) were grown in media containing 14C-CLA for various periods, more than 50% of the 14C-CLA was incorporated into cellular lipids by 9 h. The distribution of CLA in phospholipid classes was similar to LA. Approximately 50% of 14C-LA and 14C-CLA were incorporated into phosphatidylcholine (PC), while the remainder was taken up by phosphatidyl-ethanolamine (PE) and phosphatidylserine/ phosphatidylinositol (PS/PI). In contrast, 14C-AA was more equitably distributed into PC, PE, or PS/PI (27, 30, or 38%, respectively). When keratinocytes were prelabeled with radiolabeled fatty acids, phorbol ester-induced release of 14C-CLA was 1.5 times higher than 14C-LA and 14C-AA. However, 14C-prostaglandin E (PGE) release in 14C-CLA prelabeled cultures was 6 and 13 times lower than cultures treated with 14C-LA and 14C-AA, respectively. Moreover, the ability of non-radiolabeled CLA to support ornithine decarboxylase activity, a hallmark event of tumor promotion, was significantly lower than in LA- and AA-treated cultures. These studies suggest that CLA inhibits skin tumor promotion, in part, through a PGE-dependent mechanism.

Dietary conjugated linoleic acid modulation of phorbol ester skin tumor promotion.

Belury MA, Nickel KP, Bird CE, Wu Y *Nutr Cancer* 1996;26(2):149-57

Department of Foods and Nutrition, Purdue University, West Lafayette, IN USA.

The fatty acid derivative conjugated dienoic linoleate (CLA) has been shown to inhibit initiation and postinitiation stages of carcinogenesis in several experimental animal models. The goal of the present study was to determine the role of increasing levels of dietary CLA in mouse skin tumor promotion elicited by 12-O-tetradecanoylphorbol-13-acetate (TPA). Mice were fed control (no CLA) diet during initiation, then switched to diets containing 0.0%, 0.5%, 1.0%, or 1.5% (wt/wt) CLA during skin tumor promotion by TPA. Body weights of mice fed 0.5%, 1.0%, or 1.5% CLA were similar to each other but were significantly lower ($p < 0.05$) than weights of mice fed no CLA (0.0%) throughout promotion. A reduction in papilloma incidence was observed in mice fed 1.5% CLA from Weeks 8 to 24 compared with mice fed diets containing 0.0-1.0% CLA ($p < 0.05$). Twenty-four weeks after tumor promotion was begun, diets containing 1.0% and 1.5% CLA inhibited tumor yield (4.94 and 4.35 tumors/mouse, respectively) compared with diets without CLA (0.0% CLA, 6.65 tumors/mouse, $p < 0.05$) or 0.5% CLA (5.92 tumors/mouse, $p < 0.05$). These data indicate that CLA inhibits tumor promotion in a manner that is independent of its anti-initiator activity. Further studies are warranted in identifying cellular mechanisms that are likely to be involved with the anti-promoter effects of CLA.

PROSTATE

Opposite effects of linoleic acid and conjugated linoleic acid on human prostatic cancer in SCID mice.

Cesano A, Visonneau S, Scimeca JA, Kritchevsky D, Santoli D *Anticancer Res* 1998 May-Jun;18(3A):1429-34

Wistar Institute, Philadelphia, PA 19104, USA.

The relationship between dietary fat intake (level and type) and cancer development is a matter of concern in Western society. The purpose of this study was to determine the effect of three different diets on the local growth and metastatic properties of DU-145 human prostatic carcinoma cells in severe combined immunodeficient (SCID) mice. Animals were fed a standard diet or diets supplemented with 1% LA or 1% CLA for 2 weeks prior to subcutaneous (s.c.) inoculation of DU-145 cells and throughout the study (total of 14 weeks). Mice receiving LA-supplemented diet displayed significantly higher body weight, lower food intake and increased local tumor load as compared to the other two groups of mice. Mice fed the CLA-supplemented diet displayed not only smaller local tumors than the regular diet-fed group, but also a drastic reduction in lung metastases. These results support the view that dietary polyunsaturated fatty acids may influence the prognosis of prostatic cancer patients, thus opening the possibility of new therapeutic options.

DIABETES

Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat.

Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, Portocarrero CP, Peck LW, Nickel KP, Belury MA *Biochem Biophys Res Commun* 1998 Mar 27;244(3):678-82 and 29;247(3):911

Department of Animal Sciences, Purdue University, West Lafayette, Indiana USA.

Conjugated linoleic acid (CLA) is a naturally occurring fatty acid which has anti-carcinogenic and anti-atherogenic properties. CLA activates PPAR alpha in liver, and shares functional similarities to ligands of PPAR gamma, the thiazolidinediones, which are potent insulin sensitizers. We provide the first evidence that CLA is able to normalize impaired glucose tolerance and improve hyperinsulinemia in the pre-diabetic ZDF rat. Additionally, dietary CLA increased steady state levels of aP2 mRNA in adipose tissue of fatty ZDF rats compared to controls, consistent with activation of PPAR gamma. The insulin sensitizing effects of CLA are due, at least in part, to activation of PPAR gamma since increasing levels of CLA induced a dose-dependent transactivation of PPAR gamma in CV-1 cells cotransfected with PPAR gamma and PPRE X 3-luciferase reporter construct. CLA effects on glucose tolerance and glucose homeostasis indicate that dietary CLA may prove to be an important therapy for the prevention and treatment of NIDDM.

IMMUNE FUNCTION

Immune modulation by altered nutrient metabolism: nutritional control of immune-induced growth depression.

Cook ME, Miller CC, Park Y, Pariza M *Poult Sci* 1993 Jul;72(7):1301-5

Department of Poultry Science, University of Wisconsin, Madison 53706.

The ability of conjugated isomers of linoleic acid (CLA) to prevent reduced growth rate following endotoxin (lipopolysaccharide, LPS) injection was studied in two chick trials and one rat trial. Chicks (10 per treatment) were fed a corn and soybean meal-based diet with or without .5% CLA. At 21 days of age, chicks were weighed and injected i.p. with 1 mg/kg BW *Escherichia coli* LPS and sterile PBS. Body weights were again determined 24 h later. Antibody responses to SRBC were also determined. Rats fed .5% stearic acid or CLA for 4 wk (seven per treatment) were also injected with LPS, and BW change over a 24-h postinjection period was determined. Antibody responses to BSA, phyto-hemagglutinin foot pad swelling, and phagocytosis of elicited peritoneal macrophages were also determined. The CLA had no adverse effects on any immune variables measured in the chicks and rats. The CLA enhanced the phytohemagglutinin response and macrophage phagocytosis in rats. Chicks fed CLA and injected with LPS continued to grow, whereas those not fed CLA either failed to grow or lost weight following LPS injection. Both control and CLA-fed rats lost weight over the 24-h period after LPS injection; however, the loss of weight in rats fed CLA was only half of the weight loss of the control rats. Thus, CLA is effective in preventing the catabolic effects of immune stimulation.

Dietary conjugated linoleic acid influences the immune response of young and old C57BL/6NCrIBR mice.

Hayek MG, Han SN, Wu D, Watkins BA, Meydani M, Dorsey JL, Smith DE, Meydani SN *J Nutr* 1999 Jan;129(1):32-8

Nutritional Immunology Laboratory, Jean Mayer U.S. Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, USA.

Aging is associated with a decline in the immune response in mammals. Conjugated linoleic acid (CLA) has been suggested to have immunoenhancing properties. We examined the influence of dietary

CLA on the immune response of young and old mice. Forty young (4 mo) and 40 old (22 mo) mice consumed ad libitum diets containing 0 or 1 g CLA /100 g for 8 wk. Splenocytes from half of the mice were isolated to evaluate proliferation to concanavalin A (Con A) (0.5, 1.5, 5.0 mg/L) and phytohemagglutinin A (PHA) (5, 20, 40 mg/L) and lipopolysaccharide (LPS) (5, 15, 30 mg/L), natural killer cell (NK) activity and prostaglandin (PG)E₂ and interleukin (IL)-2 production. The remaining mice were used to evaluate in vivo delayed-type hypersensitivity (DTH) skin response. There was a significant decline due to age in response to all three mitogens tested ($P < 0.05$). CLA supplementation significantly increased all CLA isomers measured in hepatic neutral lipids and phospholipids ($P < 0.05$). Young mice fed 1% CLA had greater splenocyte proliferation in response to Con A (0.5 and 5.0 mg/L) and PHA (40 mg/L) ($P < 0.05$) than young mice fed control diet. Old mice fed 1 g CLA/100 had significantly higher proliferative response to optimal concentrations of Con A (1.5 mg/L) ($P < 0.001$) than the mice fed the control diet. Old mice fed the control diet had significantly lower splenocyte IL-2 production than the young mice ($P < 0.005$). CLA-supplemented young mice had significantly higher splenocyte IL-2 production than those fed the control diet ($P < 0.05$). CLA had no effect on NK cell activity, PGE₂ production or DTH in young or old mice. Further studies are needed to determine the mechanism of CLA-induced enhancement of IL-2 production and T cell proliferation.

Conjugated linoleic acid modulates tissue levels of chemical mediators and immunoglobulins in rats.

Sugano M, Tsujita A, Yamasaki M, Noguchi M, Yamada K *Lipids* 1998 May;33(5):521-7

Laboratory of Food Science, Kyushu University School of Agriculture, Fukuoka, Japan.

The effects of conjugated linoleic acid (CLA) on the levels of chemical mediators in peritoneal exudate cells, spleen and lung, and the concentration of immunoglobulins in mesenteric lymph node and splenic lymphocytes and in serum were examined in rats. After feeding diets containing either 0 (control), 0.5 or 1.0% CLA for 3 wk, there was a trend toward a reduction in the release of leukotriene B₄ (LTB₄) from the exudate cells in response to the dietary CLA levels. However, CLA did not appear to affect the release of histamine. A similar dose-response pattern also was observed in splenic LTB₄, lung LTC₄ and serum prostaglandin E₂ levels, and the differences in these indices between the control and 1.0% CLA groups were all statistically significant. The reduction by CLA of the proportions of n-6 polyunsaturated fatty acids in peritoneal exudate cells and splenic lymphocyte total lipids seems to be responsible at least in part for the reduced eicosanoid levels. Splenic levels of immunoglobulin A (IgA), IgG, and IgM increased while those of IgE decreased significantly in animals fed the 1.0% CLA diet. This was reflected in the serum levels of immunoglobulins. The levels of IgA, IgG, and IgM in mesenteric lymph node lymphocytes increased in a dose-dependent manner, while IgE was reduced in those fed the higher CLA intake. However, no differences were seen in the proportion of T-lymphocyte subsets of mesenteric lymph node. These results support the view that CLA mitigates the food-induced allergic reaction.

Effects of conjugated dienoic derivatives of linoleic acid and beta-carotene in modulating lymphocyte and macrophage function.

Chew BP, Wong TS, Shultz TD, Magnuson NS *Anticancer Res* 1997 Mar-Apr;17(2A):1099-106

Department of Animal Sciences, Washington State University, Pullman USA.

The in vitro effects of conjugated dienoic derivatives of linoleic acid (CLA) in combination with beta-carotene on lymphocyte and macrophage function was studied. Porcine blood lymphocytes and murine peritoneal macrophages were incubated with 0 (control), 1.78×10^{-5} , 3.57×10^{-5} and 7.14×10^{-5} M CLA and 0 (control), 10^{-9} , 10^{-8} and 10^{-7} M beta-carotene. CLA alone stimulated mitogen-induced lymphocyte proliferation, lymphocyte cytotoxic activity and macrophage bactericidal activity. In contrast, CLA inhibited interleukin-2 production by lymphocytes and suppressed the phagocytic activity of macrophages. beta-Carotene alone stimulated the cytotoxicity of lymphocytes and increased superoxide production by peritoneal macrophages. When present together, CLA and beta-carotene interacted in an additive manner to further enhance lymphocyte cytotoxicity and spontaneous lymphocyte proliferation. In addition, beta-carotene was able to negate the inhibitory action of CLA on the phagocytic activity of macrophages. Also, CLA and beta-carotene together seemed to suppress mitogen-induced lymphocyte proliferation. Therefore, CLA and beta-carotene; alone and in concert, act to modulate different aspects of cellular host defense.

Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection.

Miller CC, Park Y, Pariza MW, Cook ME Biochem Biophys Res Commun 1994 Feb 15;198(3):1107-12

Poultry Science Dept., U.W. Madison.

The ability of conjugated linoleic acid to prevent endotoxin-induced growth suppression was examined. Mice fed a basal diet or diet with 0.5% fish oil lost twice as much body weight after endotoxin injection than mice fed conjugated linoleic acid. By 72 hours post injection, mice fed conjugated linoleic acid had body weights similar to vehicle injected controls; however, body weights of basal and fish oil fed mice injected with endotoxin were reduced. Conjugated linoleic acid prevented anorexia from endotoxin injection. Splenocyte blastogenesis was increased by conjugated linoleic acid.

ATHEROSCLEROSIS

Conjugated linoleic acid and atherosclerosis in rabbits.

Lee KN, Kritchevsky D, Pariza MW Atherosclerosis 1994 Jul;108(1):19-25

Department of Food Microbiology and Toxicology, University of Wisconsin-Madison 53706.

Conjugated linoleic acid (CLA) consists of a series of positional and geometric dienoic isomers of linoleic acid that occur naturally in foods. CLA exhibits antioxidant activity in vitro and in vivo. To assess the effect of CLA on atherosclerosis, 12 rabbits were fed a semi-synthetic diet containing 14% fat and 0.1% cholesterol for 22 weeks. For 6 of these rabbits, the diet was augmented with CLA (0.5 g CLA/rabbit per day). Blood samples were taken monthly for lipid analysis. By 12 weeks total and LDL cholesterol and triglycerides were markedly lower in the CLA-fed group. Interestingly, the LDL cholesterol to HDL cholesterol ratio and total cholesterol to HDL cholesterol ratio were significantly reduced in CLA-fed rabbits. Examination of the aortas of CLA-fed rabbits showed less atherosclerosis.

Dietary conjugated linoleic acid reciprocally modifies ketogenesis and lipid secretion by the rat liver.

Sakono M, Miyanaga F, Kawahara S, Yamauchi K, Fukuda N, Watanabe K, Iwata T, Sugano M *Lipids* 1999 Sep;34(9):997-1000

Department of Biological Resource Sciences, Faculty of Agriculture, Miyazaki University, Japan.

The effects of dietary conjugated linoleic acid (CLA) and linoleic acid (LA) on ketone body production and lipid secretion were compared in isolated perfused rat liver. After feeding the 1% CLA diet for 2 wk, the concentration of post-perfused liver cholesterol was significantly reduced by CLA feeding, whereas that of triacylglycerol remained unchanged. Livers from CLA-fed rats produced significantly more ketone bodies; and the ratio of beta-hydroxybutyrate to acetoacetate, an index of mitochondrial redox potential, tended to be consistently higher in the liver perfusate. Conversely, cumulative secretions of triacylglycerol and cholesterol were consistently lower in the livers of rats fed CLA, and the reduction in the latter was statistically significant. Thus dietary CLA appeared to exert its hypolipidemic effect at least in part through an enhanced beta-oxidation of fatty acids at the expense of esterification of fatty acid in the liver.

Antiplatelet effects of conjugated linoleic acid isomers.

Truitt A, McNeill G, Vanderhoek JY *Biochim Biophys Acta* 1999 May 18;1438(2):239-46

Department of Biochemistry and Molecular Biology, The George Washington University, 2300 Eye Street, N.W., Washington, DC

Conjugated diene isomers of linoleic acid (CLA) are normal constituents of certain foods and exhibit anticarcinogenic and antiatherogenic properties. In the present study, the effects of several CLA isomers on human platelet aggregation and arachidonic acid metabolism were examined. It was found that 9c,11t-CLA, 10t, 12c-CLA and 13-hydroxy-9c,11t-octadecadienoic acid (13-HODE) inhibited arachidonic acid- and collagen-induced platelet aggregation with IC_{50} s in the 5-7 μ M range. The nonconjugated 9c, 12c-LA was about 300% and 50%, respectively, less potent an inhibitor with these aggregating agents. Using either thrombin or the calcium ionophore A23187 as aggregating agents, a CLA isomer mix was also found to be more inhibitory than 9c,12c-LA. The 9c,11t- and 10t,12c-CLA isomers as well as the CLA isomer mix inhibited formation of the proaggregatory cyclooxygenase-catalyzed product TXA₂, as measured by decreased production of its inactive metabolite [¹⁴C]TXB₂ from exogenously added [¹⁴C]arachidonic acid (IC_{50} s=9-16 μ M). None of the CLA isomers tested inhibited production of the platelet lipoxygenase metabolite [¹⁴C]12-HETE. The additional presence of a hydroxyl group gave opposite results: 13-HODE (IC_{50} =3 μ M) was about 4-fold more potent a cyclooxygenase inhibitor than the 9c,11t-CLA isomer but 9-HODE was 2- to 3-fold less effective an inhibitor (IC_{50} =34 μ M) of [¹⁴C]TXB₂ formation than the corresponding 10t,12c-CLA. In both the aggregation and arachidonic acid metabolism experiments, the inhibitory effects of CLA on platelets were reversible and dependent on the time of addition of either the aggregating agent or the [¹⁴C]arachidonic acid substrate. These studies suggest that CLA isomers may also possess antithrombotic properties.

Atherosclerosis and conjugated linoleic acid.

Rudel LL *Br J Nutr* 1999 Mar;81(3):177-9

Wake Forest University School of Medicine, Winston-Salem, NC 27157-1040, USA.

Species differences in the metabolism and regulation of gene expression by conjugated linoleic acid.

Moya-Camarena SY, Belury MA

Centro de Investigacion en Alimentacion y Desarrollo, Sonora, Mexico.

Conjugated linoleic acid (CLA) inhibits carcinogenesis and atherosclerotic plaque formation and delays the onset of diabetes in experimental animals. Whereas a plethora of data has demonstrated beneficial effects in rodent models, little work has been done to determine the role of dietary CLA in human health. The ability of CLA to modulate lipid metabolism appears to be a pivotal mechanism of CLA's beneficial effects in mice and rats. In particular, dietary CLA induces the expression of genes dependent in part on the transcription factor, peroxisome proliferator-activated receptor (PPAR). Furthermore, several CLA isomers are high-affinity ligands and activators for PPAR alpha. Within various rodent species and strains, dietary CLA exerts varying potencies; therefore, the differences in species' sensitivities are of great importance when trying to extrapolate the rodent data to be relevant in humans. This review presents the latest findings of the ability of CLA to alter lipid metabolism and gene expression in several different strains of mice and rats and speculates on the implications of these findings for human health.

OBESITY/BODY COMPOSITION

Conjugated linoleic acid and the control of cancer and obesity.

Pariza MW, Park Y, Cook ME Toxicol Sci 1999 Dec;52(2 Suppl):107-10

Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin-Madison, 53706-1187, USA.

The effects of conjugated linoleic acid (CLA) in animals are reviewed. In most of the CLA preparations that have been investigated to date for biological activity, two CLA isomers are present in about equal concentrations: cis-9,trans-11 CLA, and trans-10,cis-12 CLA. The occurrence of these isomers in foods and their production by rumen microorganisms are discussed. Potential mechanisms of action as regards the effects of CLA on cancer and body composition are reviewed, including recent evidence that body composition changes are produced by the trans-10,cis-12 CLA isomer. Evidence is presented indicating that CLA may modulate cellular response to tumor necrosis factor-alpha (TNF-alpha). The mechanistic implications of this finding are considered.

Dietary effect of conjugated linoleic acid on lipid levels in white adipose tissue of Sprague-Dawley rats.

Yamasaki M, Mansho K, Mishima H, Kasai M, Sugano M, Tachibana H, Yamada K Biosci Biotechnol Biochem 1999 Jun;63(6):1104-6

Department of Food Science and Technology, Faculty of Agriculture, Kyushu University, Fukuoka, Japan.

We examined the effect of dietary conjugated linoleic acid (CLA) on lipid parameters in the liver, white adipose tissue (WAT) and brown adipose tissue (BAT) of Sprague-Dawley rats and found that it reduced the levels of triglycerides and non-esterified fatty acid in the liver and WAT without significant change in the BAT lipid levels. These results suggest that CLA has an obesity-preventing action.

Effect of conjugated linoleic acid on body composition in mice.

Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW *Lipids* 1997 Aug;32(8):853-8

Department of Food Microbiology and Toxicology, University of Wisconsin-Madison

The effects of conjugated linoleic acid (CLA) on body composition were investigated. ICR mice were fed a control diet containing 5.5% corn oil or a CLA-supplemented diet (5.0% corn oil plus 0.5% CLA). Mice fed CLA-supplemented diet exhibited 57% and 60% lower body fat and 5% and 14% increased lean body mass relative to controls ($P < 0.05$). Total carnitine palmitoyltransferase activity was increased by dietary CLA supplementation in both fat pad and skeletal muscle; the differences were significant for fat pad of fed mice and skeletal muscle of fasted mice. In cultured 3T3-L1 adipocytes CLA treatment (1×10^{-4} M) significantly reduced heparin-releasable lipoprotein lipase activity (-66%) and the intracellular concentrations of triacylglyceride (-8%) and glycerol (-15%), but significantly increased free glycerol in the culture medium (+22%) compared to control ($P < 0.05$). The effects of CLA on body composition appear to be due in part to reduced fat deposition and increased lipolysis in adipocytes, possibly coupled with enhanced fatty acid oxidation in both muscle cells and adipocytes.

Evidence that the trans-10,cis-12 isomer of conjugated linoleic acid induces body composition changes in mice.

Park Y, Storkson JM, Albright KJ, Liu W, Pariza MW *Lipids* 1999 Mar;34(3):235-41

Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin-Madison 53706, USA.

We investigated the effects of conjugated linoleic acid (CLA) preparations, which were enriched for the cis-9,trans-11 CLA isomer or the trans-10,cis-12 CLA isomer, on body composition in mice. Body composition changes (reduced body fat, enhanced body water, enhanced body protein, and enhanced body ash) were associated with feeding the trans-10,cis-12 CLA isomer. In cultured 3T3-L1 adipocytes, the trans-10,cis-12 isomer reduced lipoprotein lipase activity, intracellular triacylglycerol and glycerol, and enhanced glycerol release into the medium. By contrast, the cis-9,trans-11 and trans-9,trans-11 CLA isomers did not affect these biochemical activities. We conclude that CLA-associated body composition change results from feeding the trans-10,cis-12 isomer.

Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs.

Ostrowska E, Muralitharan M, Cross RF, Bauman DE, Dunshea FR *J Nutr* 1999 Nov;129(11):2037-42

Agriculture Victoria, Victorian Institute of Animal Science, Werribee, VIC 3030, Australia.

Conjugated linoleic acids (CLA) decrease the body fat content of rodents; the aim of this study was to determine whether dietary CLA altered carcass composition of pigs. Female Large White x Landrace pigs (n = 66) were used in this study. To obtain initial body composition, six pigs were slaughtered at 57 kg live weight, whereas the remaining pigs were allocated to one of six dietary treatments (0, 1.25, 2.5, 5.0, 7.5 and 10.0 g/kg CLA, containing 55% of CLA isomers). The diets, containing 14.3 MJ digestible energy (DE) and 9.3g available lysine per kg, were fed ad libitum for 8 wk. Dietary CLA had no significant effect on average daily gain (861 vs. 911 g/d for pigs fed diets with and without CLA, $P = 0.15$) or feed intake (2.83 vs. 2.80 kg/d, $P = 0.74$). The gain to feed ratio was increased by dietary CLA by 6.3% (0.328 vs. 0.348, $P = 0.009$). Fat deposition decreased linearly (-8.2 ± 2.09 g/d for each gram per kilogram increase in CLA concentration; $P < 0.001$) with increasing inclusion of CLA. At the highest level of CLA inclusion, fat deposition was decreased by 88 g/d (-31%). Similarly, the ratio of fat to lean tissue deposition decreased linearly (-0.093 ± 0.0216 for each gram per kilogram increase in CLA concentration; $P < 0.001$) with increasing dietary CLA. The carcass lean tissue deposition response to dietary CLA was quadratic in nature and was maximized (+25%) at 5.0 g/kg dietary CLA. Overall, dietary CLA increased the gain to feed ratio and lean tissue deposition and decreased fat deposition in finisher pigs.

Conjugated linoleic acid rapidly reduces body fat content in mice without affecting energy intake.

DeLany JP, Blohm F, Truett AA, Scimeca JA, West DB *Am J Physiol* 1999 Apr;276(4 Pt 2):R1172-9

Pennington Biomedical Research Center, Louisiana State University, Baton Rouge 70808, Louisiana, USA.

Recent reports have demonstrated that conjugated linoleic acid (CLA) has effects on body fat accumulation. In our previous work, CLA reduced body fat accumulation in mice fed either a high-fat or low-fat diet. Although CLA feeding reduced energy intake, the results suggested that some of the metabolic effects were not a consequence of the reduced food intake. We therefore undertook a study to determine a dose of CLA that would have effects on body composition without affecting energy intake. Five doses of CLA (0.0, 0.25, 0.50, 0.75, and 1.0% by weight) were studied in AKR/J male mice (n = 12/group; age, 39 days) maintained on a high-fat diet (%fat 45 kcal). Energy intake was not suppressed by any CLA dose. Body fat was significantly lower in the 0.50, 0.75, and 1.0% CLA groups compared with controls. The retroperitoneal depot was most sensitive to the effects of CLA, whereas the epididymal depot was relatively resistant. Higher doses of CLA also significantly increased carcass protein content. A time-course study of the effects of 1% CLA on body composition showed reductions in fat pad weights within 2 wk and continued throughout 12 wk of CLA feeding. In conclusion, CLA feeding produces a rapid, marked decrease in fat accumulation, and an increase in protein accumulation, at relatively low doses without any major effects on food intake.

DIETARY SOURCES

Relation between the intake of milk fat and the occurrence of conjugated linoleic acid in human adipose tissue.

Jiang J, Wolk A, Vessby B *Am J Clin Nutr* 1999 Jul;70(1):21-7

Department of Food Science, Swedish University of Agricultural Sciences, Uppsala, Sweden.

Conjugated linoleic acid (CLA) is a group of naturally occurring fatty acids mainly present in fats from ruminants. CLA has been shown to be a potential anticarcinogen. **OBJECTIVE:** In this study, the relation between bovine milk fat intake and the occurrence of CLA in human adipose tissue was investigated. One hundred twenty-three men weighed and recorded the foods they consumed for 1 wk. Afterward, recall interviews were conducted by telephone monthly for 7 consecutive months to inquire about food consumption during the previous 24 h. The entire dietary recording procedure was repeated once. The fatty acid composition of adipose tissue and serum was analyzed. **RESULTS:** The average amount of one isomer of CLA--9-cis,11-trans-octadecadienoic acid (9c,11t-18:2)--as a percentage of total fatty acids was found to be 0.50% in adipose tissue and 0.25% in serum. The amount of 9c,11t-18:2 in adipose tissue was significantly correlated with milk fat intake ($r = 0.42$). The percentage of 9c,11t-18:2 in both adipose tissue and in serum was strongly correlated with myristoleic acid (14:1). The amount of 9c,11t-18:2 in human adipose tissue was significantly related to milk fat intake.

Bioactive lipids naturally occurring in bovine milk.

Molkentin J Nahrung 1999 Jun;43(3):185-9

Bundesanstalt für Milchforschung, Institut für Chemie und Physik, Kiel, Germany.

Bioactive properties of food components increasingly gain in importance in the modern diet. Bovine milk fat (BMF) exhibits bioactive substances mainly in the class of fatty acids. Currently, most interest is addressed to trans fatty acids (TFA) and particularly conjugated linoleic acids (CLA) with BMF being the main source of CLA in food. Whereas saturated fatty acids (C12-C16) and TFA are reported to be positively correlated (negatively for oleic acid) with atherosclerosis and coronary heart disease, CLA are regarded as potent anticarcinogens. Also butyric acid (C4) as well as some phospholipids and either lipids present in BMF are thought to have anticarcinogenic properties. Furthermore, BMF contains the essential fatty acids C18:2 n-6 and C18:3 n-3 that have many and diverse functions in human metabolism and, thus, control a variety of biochemical and physiological processes. Altogether, BMF contains approximately 75 wt% of bioactive substances.

Conjugated linoleic acid content of milk from cows fed different diets.

Dhiman TR, Anand GR, Satter LD, Pariza MW J Dairy Sci 1999 Oct;82(10):2146-56

US Dairy Forage Research Center USDA, Department of Dairy Science, University of Wisconsin, Madison 53706, USA.

Conjugated linoleic acid in milk was determined from cows fed different diets. In Experiment 1, cows were fed either normal or high oil corn and corn silage. Conjugated linoleic acid was 3.8 and 3.9 mg/g of milk fatty acids in normal and high oil treatments, respectively. In Experiment 2, cows consumed one-third, two-thirds, or their entire feed from a permanent pasture. Alfalfa hay and concentrates supplied the balance of feed for the one-third and two-third pasture treatments. Conjugated linoleic acid was 8.9, 14.3, and 22.1 mg/g of milk fatty acids in the one-third, two-third, and all pasture treatments, respectively. Cows grazing pasture and receiving no supplemental feed had 500% more conjugated linoleic acid in milk fat than cows fed typical dairy diets (Experiment 1). In Experiment 3,

cows were fed either a control diet containing 55% alfalfa silage and 45% grain, or similar diets supplemented with 3% fish meal, or 250 g of monensin/cow/per day, or fish meal and monensin together. Conjugated linoleic acid was 5.3, 8.6, 6.8, and 8.9 mg/g of milk fatty acids in the control, fish meal, monensin, and fish meal plus monensin treatments, respectively. In Experiment 4, cows were fed either finely chopped alfalfa hay (Treatment 1), or coarsely chopped alfalfa hay (Treatment 2) in a 50% forage and 50% grain diet, or 66.6% grass hay and 33.4% grain (Treatment 3), or 98.2% grass hay (Treatment 4). Conjugated linoleic acid was 7.3, 8.3, 9.0, and 7.9 mg/g of milk fatty acids in treatments 1 through 4, respectively.

Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds.

Dhiman TR, Helmink ED, McMahon DJ, Fife RL, Pariza MW J Dairy Sci 1999 Feb;82(2):412-9

Utah State University, Logan 84322-4815, USA.

Extruded oilseeds were fed to 24 dairy cows to study the influence on the conjugated linoleic acid content of milk and cheese. Cows were fed one of three diets that contained forage and grain in a ratio of 47:53. A control diet containing 13.5% soybean meal was compared with diets containing 12% full fat extruded soybeans or 12% full fat extruded cottonseed. The control, extruded soybean, and extruded cottonseed diets contained 2.73, 4.89, and 4.56% fatty acids, respectively. Measurements were made during the last 5 wk of the 8-wk experiment. The DM intakes and 3.5% fat-corrected milk yields were higher for cows fed the extruded soybean and extruded cottonseed diets than for cows fed the control diet. A tendency for lower fat and protein contents in the milk of cows fed the extruded soybean and extruded cottonseed diets was detected. Most of the C18 fatty acids were increased in the milk and cheese when extruded soybeans and cottonseeds were fed. The conjugated linoleic acid content in milk and cheese increased a mean of 109% when full fat extruded soybeans were fed and increased 77% when cottonseeds were fed compared with the conjugated linoleic acid content when the control diet was fed. Processing the milk into cheese did not alter the conjugated linoleic acid content. The conjugated linoleic acid content of milk and cheese can be increased by the inclusion of full fat extruded soybeans and full fat extruded cotton-seeds in the diets of dairy cows.

Effect of intake of pasture on concentrations of conjugated linoleic acid in milk of lactating cows.

Kelly ML, Kolver ES, Bauman DE, Van Amburgh ME, Muller LD J Dairy Sci 1998 Jun;81(6):1630-6

Department of Animal Science, Cornell University, Ithaca, NY 14853, USA.

We examined the effect of intake of fresh pasture on concentrations of conjugated linoleic acid in milk fat. Sixteen Holstein cows were paired and divided into either the control group or the grazing group. The study involved initial, transition, and final periods. During the initial period, all cows consumed a total mixed diet. Cows in the control group were fed the total mixed diet throughout the study, and cows in the grazing group were gradually adjusted to a diet consisting of intensively managed pasture. Performance of cows in the grazing group was significantly reduced from that of cows in the control group during the final period (dry matter intake, 19% less; milk yield, 29.6 vs. 44.1 kg/d; and live weight, 40 kg less). During the initial period, when both groups were consuming a total mixed diet, concentrations of conjugated linoleic acid in milk fat were similar ($X = 5.1$ mg/g of milk fat). As the grazing group was gradually adjusted to pasture, concentrations of conjugated linoleic acid in milk gradually increased. During the final period, when cows in the grazing group were consuming a diet

consisting of pasture only, conjugated linoleic acid concentrations in the milk fat were doubled (10.9 vs. 4.6 mg/g of milk fat). Furthermore, results showed the individual consistency of the milk fat content of conjugated linoleic acid over time but also demonstrated substantial variation among individual cows within treatment groups. Overall, this study indicated that the concentration of conjugated linoleic acid in milk fat is enhanced by dietary intake of fresh pasture.