SUMMARY REPORT

of the Conference on Conjugated Linoleic Acid and Human Health:

Research-in-Progress

November 21-22, 2003

Toronto, Ontario, Canada

DIABETES RESEARCH & TREATMENT CENTRE
DRTC/CRTD
CENTRE DE RECHERCHE ET TRAITEMENT DE DIABÈTE
WINNIPEG

Summary Report of the
Conference on Conjugated Linoleic Acid and Human Health: Research-in-Progress

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FORWARD

In March 2003, a workshop on "The Role of CLA in Human Health" was organized in Winnipeg to explore the extraordinary potential of conjugated linoleic acid as a functional food. Senior Canadian and international scientists were brought together to discuss promising leads and definitive directions for future research that would provide convincing evidence of the efficacy of CLA in human health. Since that successful gathering, more research has been published demonstrating CLA's multipotent effects in modifying organ physiology and disease processes related to obesity, inflammation, immune responses, bone formation, cancer biology, adipogenesis, and insulin resistance. In view of the momentum and growing interest in finding CLA's place as a nutraceutical in human health, we felt that our nascent network should reconvene to discuss recent advances and promote collaborations nationally.

The conference program described in this document featured contemporary research from leading Canadian laboratories and demonstrated continuity with the previous workshop and priority areas for future enquiry.

The conference and these proceedings were made possible by the generous and unrestricted support of sponsors listed on page 4 of this document. We are grateful to these organizations and institutions for their encouragement and for providing the resources to assure a successful event.

A. Angel, MD, FRCPC
Professor of Medicine & Physiology, University of Manitoba
President, Diabetes Research & Treatment Centre
Chair, CLA Conference Organizing Committee

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DIABETES RESEARCH AND TREATMENT CENTRE (DRTC)

The Diabetes Research and Treatment Centre is an inter-disciplinary centre dedicated to promoting research, education and treatment of diabetes mellitus. The Centre is based at the University of Manitoba, Departments of Internal Medicine and Physiology and the Health Sciences Centre in Winnipeg and has devoted much of its effort towards educating the public and the health professions in advances in diabetes care and education. The principal goals of DRTC include: to facilitate diabetes research; to improve treatment and care of people with diabetes; to strengthen the training programs for health and allied professionals involved in the management of persons with diabetes; and to promote awareness of diabetes in the medical, academic and lay community. In recent years, DRTC has sponsored a number of international and national conferences on diabetes and cardiovascular disease, and the role of conjugated linoleic acid (CLA) in human health.

NOTE: This report is based on data provided by conference participants in presentations, abstracts and discussions.

PROGRAM of the Conference on Conjugated Linoleic Acid and Human Health: Research-in-Progress

FRIDAY, NOVEMBER 21, 2003

Opening Remarks: Dr. Aubie Angel

KEYNOTE SPEAKER:

Dr. Sebastiano Banni

Università degli Studi di Cagliari, Dipartimento di Biologia Sperimentale, Sezione di Patolgoia Sperimentale, Cittadella Universitaria Cagliari, Italy

Title: CLA and Human Health - An Overview

SATURDAY, NOVEMBER 22, 2003

Research Presentations

Research-in-Progress

CHAIR – Helen Bishop-McDonald

Recent developments in Canadian functional foods and natural health products regulations

Kelley C. Fitzpatrick
Marketing and Research Development Manager
Richardson Centre for Functional Foods & Nutraceuticals
University of Manitoba, Winnipeg, MB

Conjugated linoleic acids alter the metabolism of fatty acids and triacylglycerol in rat hepatoma (McA-RH-7777) cells

Roger S. McLeod, Andrea M. LeBlanc and Deborah L. Currie
Department of Biochemistry and Molecular Biology
Dalhousie University, Halifax, NS

Conjugated linoleic acid (CLA) isomers and the fa/fa Zucker rat model of obesity, insulin resistance and altered lipid metabolism

Carla Taylor, NR Ryz, D Defries, D Herchak, R Diakiw, J Zahradka, L Lee and P Zahradka
Department of Human Nutritional Sciences
University of Manitoba, Winnipeg, MB

Effect of media containing linoleic acid (LA), conjugated linoleic acid (CLA) *cis* (c) 9, *trans* (t) 11 and CLA t10,c12 on 3T3-L1 cells and bovine adipofibroblasts

Priya Mir, ML He, EK Okine and MV Dodson
Department of Agriculture, Agriculture and Agri-Food Canada
Lethbridge, AB

Adipogenic and antiadipogenic effects of CLA isomers

Aubie Angel, Patti Plett and Josh Manusow
Departments of Medicine and Physiology
University of Manitoba, Winnipeg, MB

Effect of the novel t8-c10 CLA isomer on lipoprotein profile and body composition in hamsters

Hélène Jacques, V Bissonauth, J Marin and Y Chouinard
Departement des sciences des aliments et de nutrition
Pavillon Paul-Comtois, Universite Laval, Quebec, QC

Research-in-Progress

CHAIR – Heather Loeppky

Conjugated linoleic acid (CLA) and human body weight and composition

Peter JH Jones, Yanwen Wang and Sudha Venkatramanan
School of Dietetics and Human Nutrition
McGill University at Macdonald Campus, Montreal, Quebec, QC

CLA in humans: Effects on body composition and adverse events during weight loss

Leah Whigham and Richard Atkinson
Department of Nutritional Sciences, University of Wisconsin, Madison, WI

The effects of Safflorin™-CLA on human immune response to respiratory viral infections

Marianne O'Shea and Inge Mohede
Loders Croklaan, Channahon, IL

CLA and immune function: An update
Catherine J. Field

Department of Agricultural, Food and Nutritional Science
University of Alberta, Edmonton, AB

Research-in-Progress
CHAIR – Michael Archer

CLA and musculoskeletal biology

Bruce Watkins
Purdue University Department of Food Science, Lipid Chemistry and Molecular Biology Laboratory, West Lafayette, IN

Effects of conjugated linoleic acid on human osteoblast-like cells

Ahmed El-Sohemy
Department of Nutritional Sciences
University of Toronto, Toronto, ON

The effects of CLA on bone mass in rodents

Hope Weiler
Department of Human Nutritional Sciences
University of Manitoba, Winnipeg, MB

Does conjugated linoleic acid (CLA) as a dietary supplement attenuate alterations in growth, fat/lean mass and bone mass observed secondary to steroid drug treatment in infant pigs?

Dawei Wang, Mehul Patel, Simon Berge and Stephanie Atkinson
Department of Pediatrics
McMaster University, Hamilton, ON

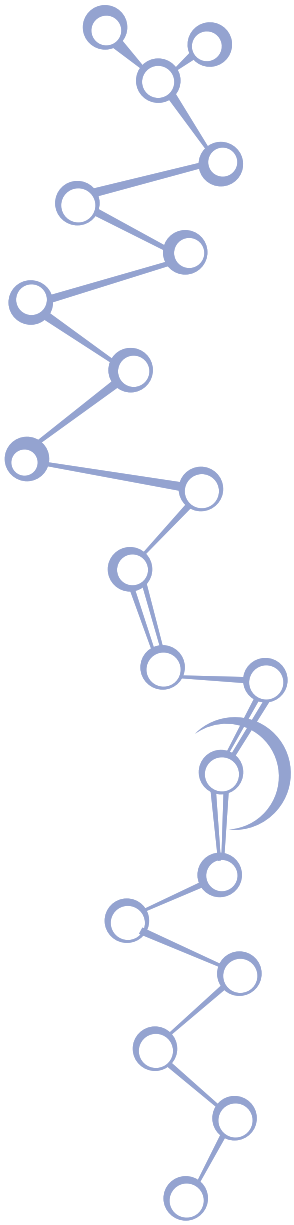
THE FUTURE:

1) Canadian/International CLA Network

2) CLA Workshop II, October '04

Summary Report of the
Conference on Conjugated Linoleic Acid and Human Health: Research-in-Progress

INTRODUCTION TO THE CONFERENCE AND KEYNOTE ADDRESS



INTRODUCTION to the Conference

Dr. Aubie Angel, chair of the CLA National Organizing Committee, opened the Toronto conference on CLA in human health on November 21, 2003. He provided an update on the outcomes of a previous national workshop on the role of CLA in human health, which was held in Winnipeg in March 2003. The main outcomes of the March workshop included the preparation and distribution of the workshop proceedings to the sponsors, organizers and attendees; securing an agreement with the *American Journal of Clinical Nutrition* to publish manuscripts based on workshop presentations as a supplement to the journal (published June 2004); identifying key challenges for CLA researchers; developing an action plan for CLA research; and planning a follow-up conference, the summary of which is the focus of this document.

This conference had two objectives: 1) to promote collaborations among Canadian scientists involved in CLA research and 2) to discuss recent advances in CLA research. This report draws on data from conference presentations, abstracts and discussions and includes Dr. Sebastiano Banni's invited manuscript.

KEYNOTE ADDRESS

Dr. Sebastiano Banni of the Università degli Studi di Cagliari, Italy, provided an overview of the role of CLA in human health. According to Dr. Banni, CLA research has increased exponentially over the past decade. Early work (1987-1991) determined that CLA has antimutagenic and anticancer activity in mice and rats. During the 1990's, research showed that CLA has antiatherogenic activity, modulates immune function, reduces body fat and exhibits antidiabetic activity in laboratory animals. Dr. Banni prepared the following paper of the possible mechanisms of action of CLA.

CLA AND HUMAN HEALTH—HYPOTHESES FOR MECHANISM OF ACTION BY WHICH CLA MAY EXERT ITS BIOLOGICAL ACTIVITIES IN HUMANS

Dr. Sebastiano Banni

Abstract

The discovery of the mechanism(s) of action by which conjugated linoleic acid (CLA) exerts its beneficial effects should give some clues regarding its actions in humans. One mechanism may be related in part to its metabolic fate and consequent perturbation of fatty acid metabolism. Even though it is a polyunsaturated fatty acid, CLA is mainly incorporated into neutral lipids, and it is desaturated and elongated in a manner similar to linoleic acid while also keeping its conjugated diene structure. As a consequence, CLA seems to inhibit linoleic acid metabolite accumulation in those tissues rich in neutral lipids, such as adipose and mammary tissues. Furthermore, other conjugated dienes with 16 carbon atoms, derived most probably from peroxisomal beta-oxidation of CLA and its metabolites, have been detected. This suggests an efficient metabolism of CLA and its metabolites in peroxisomes, lending support to the observation that CLA is a high-affinity ligand for peroxisome proliferator-activated receptors (PPARs), a family of transcription factors known to affect gene expression and, hence, glucose and lipid metabolism. In addition, CLA increases free retinol in different tissues; free retinol may regulate cell proliferation and the inflammatory process. The increase of retinol by CLA feeding may also be related to the activation of PPARs. We may conclude that CLA, when present in the diet, is able to perturb linoleic acid metabolism, may affect eicosanoid formation and, because it is efficiently metabolized in the peroxisomes and is a good ligand for PPARs, may influence fatty acid metabolism and cell trafficking of lipid-soluble molecules such as retinol. These factors may explain the pleiotropic effects of CLA in influencing inflammation, cell proliferation, glucose tolerance and immune functions. In humans, CLA is metabolized in a similar way as in experimental animals, suggesting that CLA may exhibit similar activities in humans. Identifying those conditions in which CLA exerts a biological effect will help determine whether CLA has a significant impact on human health.

Introduction

There is a general consensus that a causal link exists between food and nutrition and chronic diseases such as cancer, atherosclerosis and diabetes. Polyunsaturated fatty acids (PUFAs) may modulate the pathogenesis of these diseases. In fact, fatty acids belonging to the n-3, n-6 and n-9 PUFA families, obtained mainly from vegetable or marine oils, are able to drive lipid metabolism by affecting gene expression, and they appear to modulate several pathological states, most probably by interfering with eicosanoid production.

Conjugated linoleic acid (CLA) has received much attention for its varied biological activities in experimental animals. CLA is naturally present in milk, dairy products and the meat of ruminants. The formation of CLA is part of an overall process called biohydrogenation that takes place in the rumen and converts linoleic acid—or, less efficiently, other 18-carbon PUFAs with double bonds in the 9 and 12 positions—to CLA via an enzymatic isomerase reaction (1). Stearic acid is the ultimate end-product of linoleic acid biohydrogenation (2). The main CLA isomer formed is *cis*-9, *trans*-11-CLA (c9,t11-CLA), and manipulating cow's dietary PUFA intake modulates milk CLA content (3, 4). Therefore, ruminants are the major reservoir for this fatty acid. During this process, vaccenic acid (t11 18:1) is also formed. Vaccenic acid may be converted to CLA in all organisms that possess delta-9-desaturase (5-8). Therefore, vaccenic acid may serve as a precursor of CLA.

Even though CLA in milk was identified in the 1930s, it was not until 60 years later that its biological activities were discovered. Since the first report of Pariza's research group of CLA antimutagenic activity (9), the scope of CLA research has increased with the further discovery of several other biological activities in different experimental models, including protection against cancer (10-14) and atherosclerosis (15-20), stimulation of certain immune functions (21-29), reduction in body fat (30-40) and normalization of impaired glucose tolerance in type 2 diabetes (41, 42).

Most of the early data were obtained using a mixture of CLA isomers. In fact, in order to have sufficient material to perform the experiments, CLA was synthesized by alkali isomerization of linoleic acid from sunflower oil. The isomeric composition consisted of at least 12 isomers, as a combination of four positional isomers (8,10; 9,11; 10,12; 11,13) and three geometrical (t,t; c,t or t,c; c,c) isomers. Among them the most concentrated isomers were c9,t11-CLA and t10,c12-CLA. For this reason, recent studies have focused mainly on these two isomers.

The data emerging from the literature show that the two most thoroughly studied isomers seem to have different activities. It is not clear if the differences between them are due to the single isomer by itself or to a competition between the two isomers. In fact, it is quite difficult to obtain a pure isomer for *in vivo* studies. The t10,c12-CLA isomer seems to be active in decreasing fat mass (35) and also in inducing fatty liver and insulin resistance in animal models (43) and humans (44). The anticarcinogenic (7, 45-48) and antiatherogenic (unpublished data) activities seem to be exerted by both isomers. In order to answer the most important question—i.e., whether CLA may also be active in humans—attention should be focused on CLA's mechanism(s) of action in order to understand at which level CLA acts and if it interferes in those pathways that humans and experimental animals have in common.

Interference of CLA in PUFA Metabolism and Eicosanoid Formation

One proposed mechanism of action of CLA is that it interferes with eicosanoid production by decreasing the supply of arachidonic acid (20:4n-6) as a substrate for the lipoxygenase (LOX) and cyclooxygenase (COX) pathways (49) (Figure 1). In fact, naturally occurring CLA is well metabolized to conjugated dienes (CDs) of 18:3, 20:3 and 20:4 fatty acids by delta-6-desaturase, elongase and delta-5-desaturase, respectively (50). We have shown that the distribution of CLA and its metabolites in lipid classes in the liver was different from that of fatty acids derived from linoleic acid. In fact, while 18:2, 18:3 and 20:4 were principally incorporated into phospholipids (PL), CLA, CD 18:3 and CD 20:3 were mainly incorporated into neutral lipids (NL). CD 20:4 was the only metabolite that was preferentially incorporated into PL (51). Nevertheless, incorporation of CD 20:4 into PL, mainly in phosphatidylserine (PS) and phosphatidylinositol (PI), did not affect 20:4 levels in liver PL fractions (51).

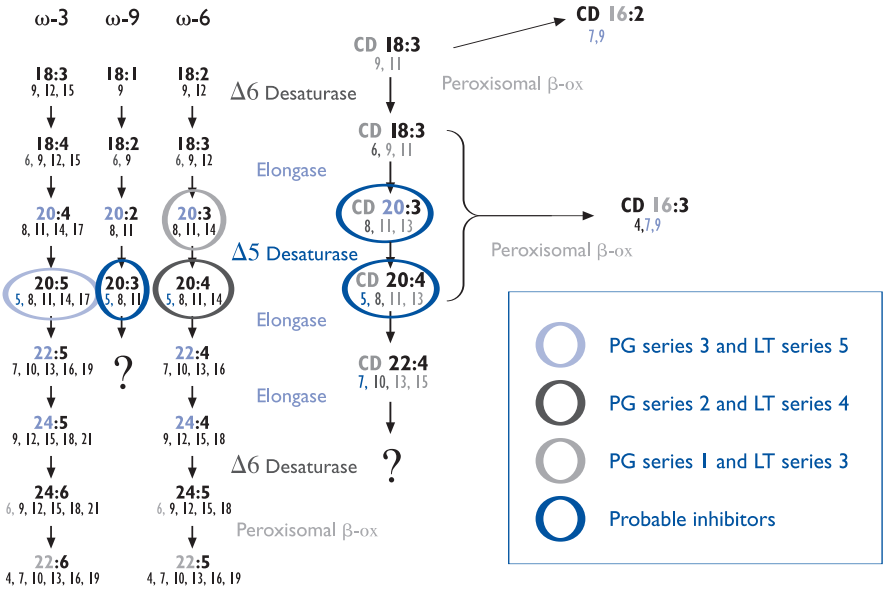


Figure 1.

Common pathway of the metabolism of n-3, n-9, n-6 and conjugated diene fatty acids.

Abbreviations:

β-ox, *β-oxidation;*
CD, *conjugated diene;*
LT, *leukotriene;*
PG, *prostaglandin.*

By comparison, the mammary gland, which is the target organ for CLA anticarcinogenic activity, differs from liver in terms of the NL/PL ratio. Whereas liver total lipids contain about 80% PL and 20% NL, NL in mammary gland accounts for more than 90% of total lipids. As a result, in the mammary gland, 20:4 is relatively low and CLA, being mainly incorporated into NL, is relatively high. Thus, the effect of CLA metabolism on 20:4 formation/incorporation in the mammary gland is more pronounced than in the liver. Indeed, we demonstrated that feeding a mixture of synthetic isomers of CLA reduced the incorporation of 18:2 metabolites, including 20:4, in the mammary gland but not in the liver (49). However, since it is known that PL 20:4 plays a major role as a substrate for the COX and LOX pathways for eicosanoid biosynthesis, we recently analyzed mammary gland phospholipids (Banni et al., manuscript in preparation). It resulted that 20:4 decreased significantly in PI, which is the major reservoir for eicosanoid synthesis.

It may be postulated that CLA induces a mild essential fatty acid deficiency, regulating 18:2 metabolism and incorporation of its metabolites into various lipid species. This effect seems more marked in those tissues where CLA is better incorporated than 18:2—i.e., in those tissues rich in NL. As a matter of fact, there is a clear link between the tissue CLA concentration and events involved with eicosanoid production, which CLA has been shown to influence [e.g., modulation of immune functions (26), atherogenesis (20), carcinogenesis (49) and tumour promoter-mediated biochemical changes in keratinocytes (52)].

CLA may interfere with eicosanoid formation either by a direct inhibition of LOX and COX, as has previously been demonstrated (53-55), or by modulating the supply of 20:4 (56-58) (Figure 1). Both mechanisms are influenced by the experimental conditions and, in a particular way, by the dietary fatty acid composition, which might explain the apparent contrasting results found in the literature on modulation of eicosanoid synthesis by dietary CLA. Some studies, for example, show a significant decrease in eicosanoid synthesis following CLA supplementation (24, 52, 55-66), while others report no effect of dietary CLA on eicosanoid synthesis (22, 67). The individualization of tissue and/or specific cell targets for CLA activity should permit verification of the capacity of CLA to modulate eicosanoid production in different experimental conditions and also in humans. The possibility of modulating the 20:4 concentration for eicosanoid production by dietary means opens a range of applications for CLA in those pathological states where eicosanoid production is involved.

CLA as a Substrate of Peroxisomal beta-Oxidation and PPAR-alpha Ligand

Very recently, we detected CLA metabolites with 16 carbon atoms, CD 16:2 and CD 16:3, derived most probably from peroxisomal beta-oxidation of CLA and its metabolites, respectively (68) (Figure 1). This suggests an efficient metabolism of CLA and its metabolites in peroxisomes, an observation that may have important implications, given that good substrates for peroxisomal beta-oxidation may also be good ligands for peroxisome proliferator-activated receptors (PPARs) (69). These receptors belong to a family of transcription factors known to affect gene expression and, hence, glucose and lipid metabolism (69). Molecular assays indicate that CLA is a high-affinity ligand for PPARs.

While CLA and synthetic PPAR-alpha ligands have some common biological activities (Figure 2) (Banni et al., submitted for publication), the PPAR-alpha ligands show other quite different activities such as induction of peroxisome and cell proliferation and liver cancer in rodents (70). These differences may be ascribed to the fact that PPAR-alpha ligands, some of which (such as fibrates) are widely used as hypolipidemic agents in humans, are not efficiently catabolized. Accordingly, fibrates may activate PPARs to a greater extent than other compounds such as PUFAs (including CLA) that, being regularly metabolized and incorporated into lipid fractions, are less available as PPAR ligands. Thus, synthetic and natural ligands possess some important differences, and CLA is incorporated into lipid

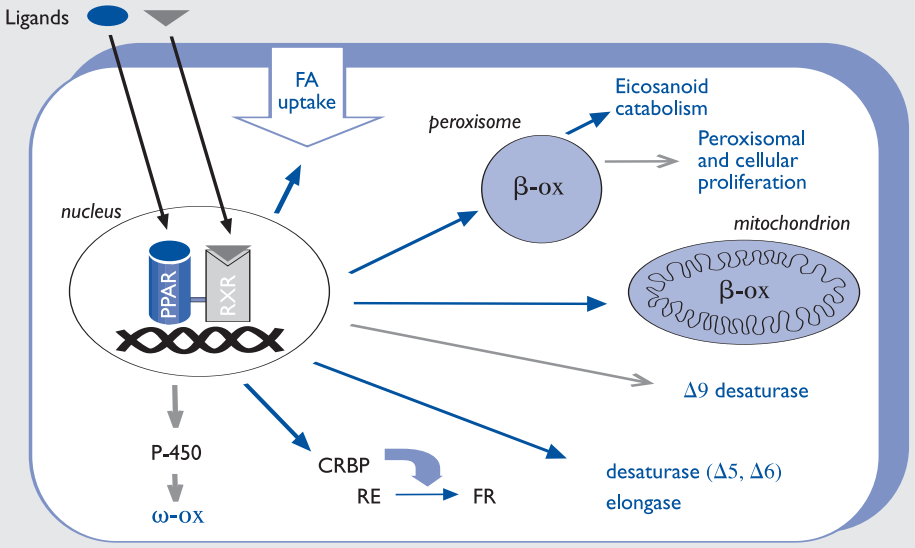


Figure 2.

Activities on fatty acid metabolism of synthetic PPAR-alpha agonists and CLA. In blue, the common; in grey, the opposite activities exerted by synthetic PPAR-alpha agonists and CLA.

Abbreviations:

β-ox, *β-oxidation;* CRBP, *cellular retinol-binding protein;* FA, *fatty acid;* FR, *free retinol;* PPAR, *peroxisome proliferator-activated receptor;* RE, *retinyl ester;* RXR, *retinoid X receptor.*

fractions and metabolized as a natural ligand.

Another effect that may be related to PPAR-alpha activation is the increase in free retinol concentration in different tissues of rats fed CLA (71). The increase of retinol in the free form suggested a dose response, with a 4-fold increase in liver free retinol at a dietary CLA level of 2% compared with mammary tissue, where free retinol increased 2-fold and reached a plateau at 0.5% dietary CLA (71).

More recent data show that CLA formed endogenously by delta-9-desaturation of vaccenic acid increased liver free retinol concentrations (Banni et al., unpublished data). This increase was strongly linked to CLA tissue concentration. A possible mechanism of action might be related to the ability of CLA to increase cellular retinol binding protein (72) by PPAR-alpha activation. The increased retinol level was closely related to CLA anticarcinogenic activity in all experimental models tested. As a matter of fact, dietary retinyl esters and CLA share similar biological activities such as suppression of mammary gland development and inhibition of terminal duct and alveolar cell

proliferation (73), which may explain in part their protection against mammary tumours.

CLA in Humans: Where Are We Now?

Since the discovery of the beneficial effects of CLA in experimental animals, several studies have been made of its occurrence, absorption, deposition and metabolism in humans. Actually, the first reports of its occurrence in blood plasma and other body fluids appeared in the 1980's in a series of papers by Dormandy (74, 75), who subsequently observed the presence of CLA in the PL of all fractions of serum lipoproteins (76). Dormandy and colleagues attributed such a presence to free-radical attacks on the lipids (77, 78), but there is no evidence, to date, that oxidative stress can generate CLA (79, 80). On the other hand, dietary CLA is absorbed and assimilated by humans (81, 82), and in samples of human adipose tissue there is a correlation between the levels of CLA and that of *trans* fatty acids, particularly vaccenic acid (83). An identical correlation was found in a variety of dairy products, the main dietary sources of CLA. It is highly probable, therefore, that most of the CLA detectable in human tissues is of dietary origin, even though there can be endogenous sources in humans.

Mean daily intake of c9,t11-CLA has been estimated to be between 246 mg/day and 323 mg/day in the American population (84). This dietary level of c9,t11-CLA, to which should be added vaccenic acid as a potential precursor with a conversion rate of about 20% (8), results in a plasma concentration of about 1 ug c9,t11-CLA/mg of lipids. However, it should be taken into account that high levels of c9,t11-CLA have been observed in patients with certain pathological states (77, 85-88). Lucchi et al. detected a high level of CD fatty acids in plasma and adipose tissue lipids of patients with chronic renal failure (89). Analyses of CLA and its metabolites showed that the CD fatty acids were CLA and revealed decreased levels of CD 18:3 and CD 20:3 in red blood cells (89). These data suggest that the increased levels of CD fatty acids observed in these patients may result from an impaired metabolism of CLA, rather than from endogenous synthesis or increased dietary intake. It should be kept in mind that the other active isomer t10,c12-CLA is virtually not present in human tissues if not voluntarily administrated, because if present in foods is there in trace amounts.

In physiological conditions, graded concentrations of dietary CLA correspond to increasing concentrations of CLA and its metabolites in plasma (Banni et al., unpublished data). The higher dietary levels of a 6 g/day mixture of t10,c12-CLA and c9,t11-CLA 50:50 that we tested in humans yielded a plasma concentration of about 7 ug of CLA mixture/mg of lipids, which corresponds with the level in plasma of rats fed about 0.4% of CLA in the diet (Figure 3). Comparison of plasma CLA levels between humans and rats is not quite scientifically sound, but it is apparently a better approximation than to extrapolate dietary CLA by comparing rat and human body weights (90).

Unfortunately, most intervention trials have been performed using dietary levels less than 6 g CLA/day—a level lower than that found effective for CLA to exert its biological activities in experimental animals, which is in the range of 0.5-1% of the dietary intake. It is therefore not surprising that these studies have been somewhat

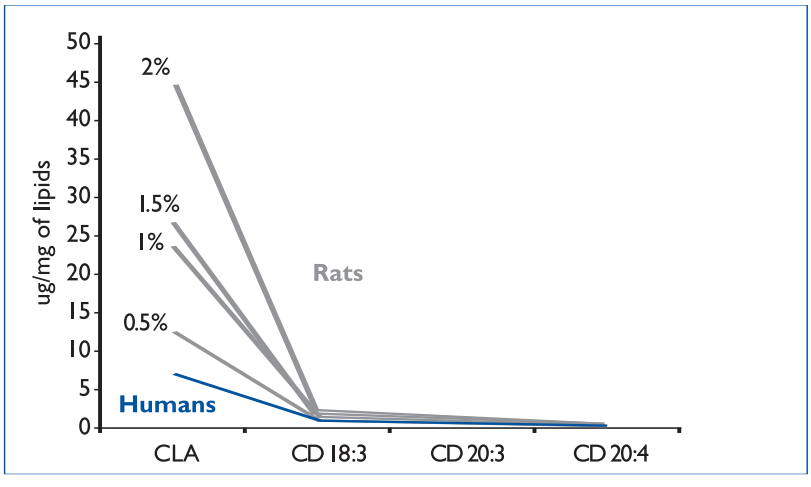


Figure 3.

Comparison between the plasma levels of CLA and its metabolites in rats fed different % of dietary CLA and in humans with a daily intake of 6 g.

Abbreviations:
CD, *conjugated diene;*
CLA, *conjugated linoleic acid.*

disappointing or marginally significant in terms of biological activities.

In terms of safety, CLA has been shown to be safe in the general population (91); while in patients with metabolic syndrome, some detrimental effects have been detected (44). Furthermore, this effect seems to be strictly linked to one isomer, t10,c12-CLA. The same isomer has been shown to depress milk fat in women (92). Therefore, it would be prudent that lactating women not consume commercially available CLA supplements containing t10,c12-CLA. By comparison, the natural CLA isomer c9,t11-CLA has not shown any adverse effect either in experimental animals or humans.

Conclusions

The discovery of the mechanism(s) of action of CLA in animals may provide some clues as to whether it may be active in humans. In humans, CLA appears to be metabolized in a similar way as in experimental animals, suggesting that CLA may exert similar activities in humans.

CLA, when present in the diet, perturbs linoleic acid metabolism and thus affects eicosanoid formation. Furthermore, because it is efficiently metabolized in the peroxisomes and is a good ligand for PPARs, CLA may influence fatty acid metabolism and cell trafficking of lipid-soluble molecules such as retinol. These factors may explain the pleiotropic effects of CLA in influencing inflammation, cell proliferation, glucose tolerance and immune functions—all aspects of the pathological states where CLA has been shown to be protective.

In most experimental conditions, CLA exerted its beneficial effects in a dose range between 0.5-1%. Our preliminary results show that for humans to reach the plasma levels seen in rats fed within that range, their daily intake would have to be higher than 6 grams a day—an intake level that exceeds the dose of 2-4 g CLA/day used in most intervention trials published to date. Furthermore, a typical diet for experimental animals includes 5% corn oil to provide an optimal level of the essential fatty acid, linoleic acid, but it doesn't mimic the human diet in terms of dietary fat (the experimental diet is low in omega-3 fatty acids), nor does it provide the optimal balance of dietary PUFAs.

Unfortunately, no data on fatty acid dietary intake are given in the studies present in the literature. It is likely that some of the ongoing human studies will address these important issues.

We may conclude that more nutritional studies on CLA are needed, and several factors should be taken into consideration for future research in humans, including: 1) length of feeding, 2) ways of CLA administration, 3) amount and type of fat present in the diet, 4) dose response with different isomers, 5) competition among CLA isomers, 6) competition with other PUFAs, and 7) different pathways of fatty acid oxidation in different tissues. Identifying those conditions in which CLA exerts its biological effects will help determine whether CLA has a significant impact on human health.

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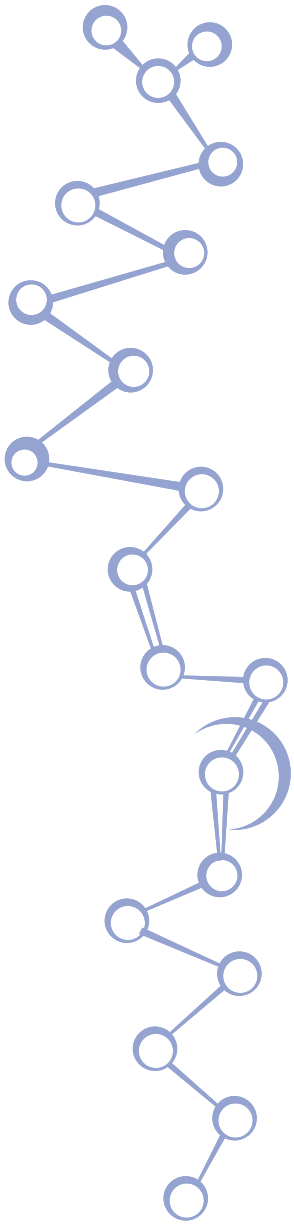
SCIENTIFIC SESSION—Summary of Speakers' Presentations



CELL CULTURE AND ANIMAL STUDIES

Helen Bishop-McDonald chaired the conference's session on regulatory issues related to CLA and on the findings of cell culture and animal studies.

Regulatory Developments

Kelley Fitzpatrick of the Richardson Centre for Functional Foods & Nutraceuticals summarized the Canadian regulations related to health claims. She indicated that health claims for CLA posted on U.S.-based Internet web sites—e.g., "promotes a decrease in body fat," "enhances immune function"—are structure/function claims that are not allowed in Canada. Five health claims are allowed in Canada and several claims are being reviewed, but none of them apply to CLA-containing foods or dietary supplements. Product-specific claims for foods will be allowed in Canada, but such claims apply only to one specific product and cannot be generalized to other products.

CLA Effects on Fatty Acid and Triacylglycerol Metabolism in Rat Hepatoma Cells

Dr. Roger McLeod of Dalhousie University described his interest in the mechanisms and regulation of the hepatic assembly of very-low-density lipoprotein (VLDL), a process that requires two steps, the first being co-translational and the second, post-translational. **Dr. McLeod** posed the question of whether CLA can affect the second, post-translational step of VLDL assembly in a rat hepatoma model. He described the findings of a study designed to assess the effects of CLA on fatty acid and triacylglycerol (TAG) metabolism in the rat hepatoma cell line McA-RH7777. He reported that compared with adding linoleic acid (LA) (the control fatty acid) to the cell medium, both CLA isomers (c9,t11-CLA, t10,c12-CLA) promoted myristic acid esterification into phospholipids and cholesteryl esters (CE). The c9,t11-CLA increased myristic acid esterification into TAGs to a greater extent than the t10,c12-CLA isomer. **Dr. McLeod** concluded that the two CLA isomers differed in their effects on cellular TAG synthesis and secretion in this model.

Dr. McLeod summarized the findings of this and other studies by indicating that both CLA isomers were incorporated into secreted VLDL and both decreased TAG mass accumulation and TAG synthesis from glycerol, but t10,c12-CLA had a greater effect on TAG synthesis than c9,t11-CLA. One other difference was that c9,t11-CLA increased CE mass, whereas t10,c12-CLA had no effect on CE mass. Neither isomer affected the esterification of oleic acid or fatty acid β-oxidation. The most consistent effect of the CLA isomers was to decrease the secretion of TAGs, suggesting that CLA may act at the level of cellular lipoprotein assembly. Future studies should assess the chronic effects of CLA on VLDL assembly and secretion in rat hepatoma cells and in primary hamster hepatocytes, the latter being an appropriate model for the study of human lipoprotein metabolism. The role of stearoyl-CoA desaturase (SCD) in fatty acid deposition and the effects of CLA on gene expression should also be determined.

CLA Effects in the fa/fa Zucker Rat

Dr. Carla Taylor of the University of Manitoba described findings of a study designed to determine the effects of CLA isomers, singly and in combination, on obesity, oral glucose tolerance and lipid metabolism in fa/fa and lean Zucker rats after eight weeks of dietary treatment. The researchers hypothesized that CLA activates peroxisomal proliferator-activated receptors (PPARs) and thus affects insulin sensitivity and glucose and lipid metabolism. Preliminary findings indicated that CLA did not alter body weight, but there was a genotype difference, with fa/fa

rats weighing more than lean rats. A genotype effect was also seen in fat pad weights—fatty rats had higher epididymal and peri-renal fat pad weights than lean rats. CLA intervention affected the fat pad weights differently, with the epididymal fat pad weight/body weight ratios in lean animals varying among the CLA groups, whereas the peri-renal fat pad weight/body weight ratios in lean animals did not differ among the CLA groups. A key finding was that the t10,c12-CLA isomer fed alone or in mixtures increased body fat (measured in epididymal and peri-renal fat pads) compared with the control group or the c9,t11-CLA isomer group. No data related to PPAR activity were presented.

CLA Effects on Proliferation of Bovine Adipofibroblasts

Dr. Priya Mir of Agriculture and Agri-Food Canada described the findings of an *in vitro* experiment designed to compare the independent effects of c9,t11-CLA and t10,c12-CLA at two concentrations (10 vs 70 mg/L) with those of linoleic acid (LA) on the proliferation, differentiation and lipid accumulation of bovine visceral or perimuscular adipofibroblasts. The restriction of adipocyte proliferation was greatest for the c9,t11-CLA isomer, although proliferation of bovine perimuscular cells was decreased by c9,t11-CLA only when added to the medium at the higher concentration (70 mg/L). Accumulation of C16:0, an index of general fatty acid synthesis, was greatest for c9,t11-CLA in 3T3-L1 cells, an observation that may be related to the fewer cells in the media. The t10,c12-CLA isomer decreased the number of fat bodies in cells to a greater extent than c9,t11-CLA and was effective in arresting lipid accumulation. **Dr. Mir** concluded that the isomers have different activities at different stages of adipocyte development. The findings suggest that in designing experiments to develop strategies for controlling lipid accumulation in adipocytes, the appropriate isomer must be provided at the appropriate stage of adipocyte development.

CLA Effects on Lipoprotein Profile and Body Composition in Hamsters

Dr. Hélène Jacques of Laval University described the findings of a previous study showing that when CLA was added to the diets of hamsters fed an atherogenic diet for 11 weeks, blood total cholesterol, VLDL, LDL and triacylglycerol (TAG) concentrations decreased significantly. **Dr. Jacques** described the protocol of a pilot study designed to evaluate the effects of CLA isomers on plasma lipids and lipoproteins, hepatic lipids, body composition, body weight and fecal fat content in Syrian Golden hamsters. The isomers being tested include purified c9,t11-CLA, purified t10,c12-CLA, a mixture of c9,t11-CLA and t10,c12-CLA (about 50/50), and a mixture of c9,t11-CLA and t8,c10-CLA (roughly 50/50). All isomers are being fed at a level providing 2% CLA in the diet for 28 days. Lard serves as the background fat because it meets the growth needs of hamsters. According to **Dr. Jacques**, several biologic materials (i.e., carcasses, organs, plasma samples) are being kept for further analysis.

CLA Adipogenic and Antiadipogenic Effects

Dr. Aubie Angel of the University of Manitoba reported findings of a study to determine the effects of dietary safflower oil [rich in linoleic acid (LA)], flax oil [rich in alpha-linolenic acid (ALA)] and standard chow on growth, adipose tissue mass and plasma leptin in mice and to assess the independent effects of c9,t11-CLA and t10,c12-CLA isomers on triacylglycerol (TAG) accumulation and leptin production in differentiating 3T3-L1 cells. The *in vivo* study showed that dietary safflower oil increased global fat mass and abdominal mass compared with dietary flax oil and chow in mice, suggesting that LA favours adipogenesis compared with ALA. The *in vitro* study showed that the c9,t11-CLA isomer increased, while the t10,c12-CLA isomer decreased, adipose tissue lipid and leptin secretion in dose response experiments. These findings suggest that the common naturally occurring c9,t11-CLA isomer favours lipogenesis and adipose cell growth, whereas the t10,c12-CLA isomer at higher concentrations is antiadipogenic. **Dr. Angel** concluded that common CLA isomers vary in their adipogenic and antiadipogenic effects, and these effects may be dose related. In addition, the findings suggest that c9,t11-CLA stimulates leptin secretion, while t10,c12-CLA inhibits leptin secretion, in a manner that may not be related to lipogenesis.

CLA RESEARCH-IN-PROGRESS—BODY WEIGHT, COMPOSITION AND IMMUNE FUNCTION

Heather Loeppky of Alberta Agriculture, Food and Rural Development chaired the session on CLA's effects on body weight, body composition and immune function in humans.

CLA and Human Body Weight and Composition

Dr. Yanwen Wang of McGill University indicated that with few exceptions, most clinical trials showed no effect of CLA on body weight when administered to volunteers at levels ranging from 0.7 g/day to 6.8 g/day for 6-12 weeks. Fat mass and blood lipids decreased in some, but not all, studies. Because the findings are inconsistent, a study designed to assess the effects of CLA obtained in regular milk (0.3% CLA), naturally enriched milk (4.2% c9,t11-CLA) or artificially enriched milk (4.2% c9,t11-CLA + t10,c12-CLA) on body weight and composition, lipid profiles, and other clinical outcomes in overweight, hyperlipidemic men is underway in **Dr. Peter Jones's** laboratory.

CLA's Effects on Body Composition and Adverse Events During Weight Loss

Dr. Leah Whigham of the University of Wisconsin described the findings of two clinical studies carried out among overweight and obese subjects. The hypothesis of Study 1 was that CLA consumed by obese adults participating in a weight loss program will facilitate body fat and body weight loss and preserve lean body mass compared with a placebo. The main study finding was that although there were no differences between the CLA and placebo groups related to changes in body weight, blood pressure, blood lipids, serum glucose, serum insulin and body composition, subjects who consumed CLA reported a better quality of life and fewer adverse events than control subjects. In particular, subjects in the CLA group reported fewer GI, negative mood and cognitive/CNS symptoms compared with those in the placebo group.

Study 2 had three hypotheses: 1) CLA will prevent body fat and body weight regain after weight loss compared with a placebo; 2) CLA will preserve lean body mass after weight loss compared with a placebo; and 3) Subjects in the CLA group will experience a decrease in the number of adverse health events during and after weight loss compared with subjects taking a placebo. In this study, consumption of a CLA supplement had no effect on body weight, lean body mass or fat mass. However, obese subjects who ate diets supplemented with CLA reported better quality of life (e.g., improved cognitive/CNS scores) and fewer adverse events (e.g., infections, skin rashes, depression, irritability and anger) compared with subjects in the placebo group. In addition, an analysis of safety data showed no differences in blood pressure, pulse, liver and renal function, cardiac arrhythmias, electrolytes, hemoglobin and hematocrit between the CLA and control groups, suggesting that long-term (12 months) consumption of 6 g CLA/day is safe.

Dr. Whigham concluded that CLA did not enhance weight loss or prevent weight gain in these subjects, but it appeared to have small but significant beneficial effects related to quality of life. She indicated that it is premature to conclude that CLA is ineffective in weight management in humans. Because most studies of body weight and body composition to date have been carried out in growing animals, there is a need to conduct trials in children and adolescents who are overweight or at risk of obesity.

Safflorin™-CLA and Human Immune Response to Respiratory Viral Infections

Dr. Marianne O'Shea of Loders Croklaan described the findings of one animal and two human studies designed to test the effects of Safflorin™ on the immune response to respiratory viral infections. Safflorin™ is a safflower oil supplement enriched with t10,c12-CLA. **Dr. O'Shea** reported that animals in the Safflorin™ group exhibited positive responses to active influenza infection, including increased spleen and lung weights and a reduction in the number of virus particles in the lungs. In addition, none of the animals in the Safflorin™ group died, whereas the expected number (21%) of the animals in the control group died.

In one human study, elderly adults who took the Safflorin™ supplement (2 g/d) for 7 wk had a higher antibody response after vaccination with the influenza vaccine than a control group, indicating their greater ability to resist infection. A second human study had three objectives: 1) to determine the effects of CLA treatment on the frequency and severity of colds; 2) to measure virology markers (e.g., virus shedding) in nasal lavage samples of subjects infected with a rhinovirus; and 3) to measure biochemical markers (e.g., cytokines) in nasal lavage samples of subjects infected with a rhinovirus. **Dr. O'Shea** reported that healthy adults who took Safflorin™ (2 g/d) for 5 wk and were then inoculated with the rhinovirus reported a decrease in the severity and frequency of symptoms (e.g., sore throat, cough, nasal obstruction) compared with control subjects. Safflorin™ may be an effective treatment in ameliorating the effects of infection with influenza and the common cold in adults, according to **Dr. O'Shea.**

CLA and Immune Function

Dr. Catherine Field of the University of Alberta described a preliminary study designed to examine the effects of CLA on immune function and incorporation into lipids in young rats fed diets with low (0.2) and high (1.0) ratios of polyunsaturated fatty acids to saturated fatty acids (P/S). CLA did not affect the proportion of immune cells in the spleen; the innate immune system (measured by natural killer cell function and nitric oxide production); or the production of interleukin-2 (IL-2), interferon-γ (IFN-γ) or tumor necrosis factor-α (TNF-α) after stimulation with concanavalin A (con A). However, the composition of the diet, specifically the P/S ratio, influenced the response of splenocytes to con A stimulation. **Dr. Field** concluded that an effect of CLA on one estimate of T cell function was seen in this study, but only when fed in a low P/S diet.

Dr. Field then described work deriving from her collaboration with **Dr. Carla Taylor** at the University of Manitoba. According to **Dr. Field,** there is little information about immune function in the fa/fa Zucker rat, an observation that led **Dr. Field** to ask two questions about this rat model: 1) Does immune function differ between fa/fa rats and lean controls? and 2) What are the effects of CLA isomers on immune function in this rat model? **Dr. Field** presented preliminary data from a study in progress designed to determine the effects of CLA isomers, singly and in combination, on immune function in insulin resistant/obese fa/fa and lean Zucker rats. She reported no effect of feeding c9,t11-CLA or t10,c12-CLA to fa/fa and lean Zucker rats on the concentrations of IFN-γ and TNF-α in splenocytes, although CLA reduced the splenocyte concentration of interleukin-4 after con A stimulation in both lean and obese rats. Production of the anti-inflammatory cytokine IL-10 in con A-stimulated splenocytes was increased in obese compared with lean rats only when the c9,t11-CLA isomer was fed. Future research should address the biological activity of individual isomers (c9,t11-CLA, t10,c12-CLA) when fed in the forms found in animal products (e.g., phospholipids, triacylglycerols) and when fed in diets similar in fat content and composition to that consumed by humans.

CLA RESEARCH-IN-PROGRESS – MUSCULOSKELETAL BIOLOGY

Dr. Michael Archer of the University of Toronto chaired this session on the effects of CLA on the musculoskeletal system.

CLA and Musculoskeletal Biology

Dr. Bruce Watkins of Purdue University reviewed the mechanostat theory of bone health, which can be defined as a balance of musculoskeletal biology needed to achieve the bone quality that will sustain an individual throughout life. The observation that disuse leads to weak muscles and muscle atrophy, which in turn reduces biomechanical strain and results in osteopenia, led **Dr. Watkins** to consider whether bone and muscle communicate and, if so, how. **Dr. Watkins** believes that polyunsaturated fatty acids may modify the communications between bone and muscle to minimize tissue loss associated with disuse. CLA may modulate the activity of cyclooxygenase (COX-2) in osteoblasts.

CLA and Human Osteoblast-like Cells

Dr. Ahmed El-Sohemy of the University of Toronto noted that CLA inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the rate-limiting enzyme of cholesterol biosynthesis, in mouse mammary gland and liver and postulated that CLA might act like statins to stimulate bone formation. He described a study designed to determine the effects of CLA on the number, size, differentiation and proliferation of mineralized bone nodules, HMG-CoA reductase activity and expression of peroxisome proliferator-activated receptor γ (PPARγ) and bone morphogenetic protein-2 (BMP-2) protein in human osteoblast-like cells (SaOS-2). Mixed CLA isomers at 100 μM increased the number and size of mineralized bone nodules and also increased osteocalcin production (by 40%) and BMP-2 protein expression, while decreasing proliferation, HMG-CoA reductase activity and PPARγ expression. **Dr. El-Sohemy** concluded that CLA increases bone nodule formation, possibly by increasing BMP-2 expression through the mevalonate pathway. He suggested that future directions for research assess the effects of individual CLA isomers, the functional significance of the decrease in HMG-CoA reductase activity and the effects of CLA on osteoclasts.

CLA Effects on Bone Mass in Rodents

Dr. Hope Weiler at the University of Manitoba reviewed the findings of nine studies related to CLA and bone mass or metabolism and reported a consensus that diets high in omega-6 fatty acids and CLA benefit bone, possibly by increasing bone formation, ash or bone weight. She had shown previously that CLA reduced parathyroid hormone concentrations by 60%. Using samples obtained from **Dr. Carla Taylor's** laboratory, **Dr. Weiler** sought to determine the effect of dietary CLA, combined with a moderate omega-6/omega-3 fatty acid ratio, on bone mass of fa/fa Zucker rats and lean controls and to determine the effect of single and mixed isomers from a variety of sources on bone mass. **Dr. Weiler** reported that eight weeks of feeding CLA to fa/fa and lean Zucker rats did not affect whole body bone mass, although it had a small effect on femur bone mass. **Dr. Weiler** concluded that the study findings may be due to the moderate omega-6/omega-3 fatty acid ratio in the basal diet and/or to the fact that bone mass is not reduced in rats until after six months of age.

CLA Effects on Growth and Bone Mass in Piglets

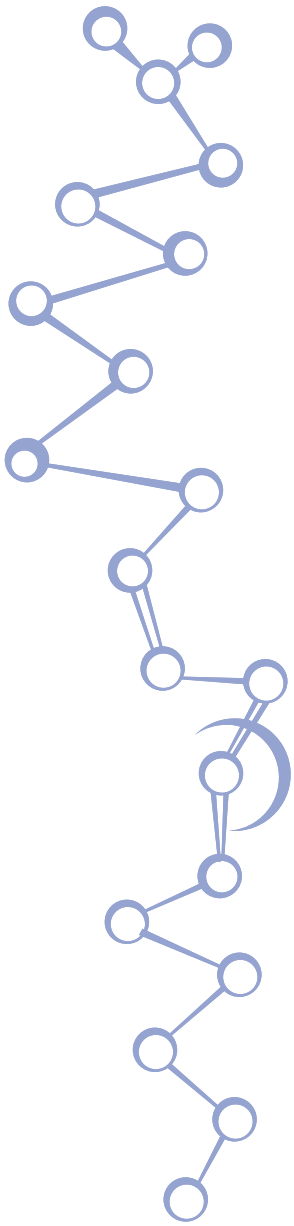
Dr. Dawei Wang of McMaster University designed a study to determine whether a CLA diet supplement attenuates the dexamethasone (DEX)-induced growth delay, altered fat/lean mass and bone abnormalities in piglets. The research was suggested by observations that human infants and piglets experience negative side effects such as decreased weight, lean body mass and bone mineral content and increased fat mass and mortality following DEX administration to improve lung function. In the piglet model, CLA demonstrated a trend to rescue the loss in lean mass and attenuate the loss in bone mineral content and bone mineral density associated with DEX administration. **Dr. Wang** concluded that CLA intervention may attenuate the altered bone formation associated with DEX in the short term but not in the long term. **Dr. Wang** noted that future research should consider a longer intervention period, larger sample size, other blood markers [e.g., insulin-like growth factor I (IGF-I), calcium] and bone analysis of prostaglandin E2 (PGE2).

Summary Report of the
Conference on Conjugated Linoleic Acid and Human Health: Research-in-Progress

FUTURE DIRECTIONS IN CLA RESEARCH

Abstracts of Presentations

at the Conference on Conjugated Linoleic Acid and Human Health: Research-in-Progress



FUTURE DIRECTIONS in CLA Research

In summarizing the conference proceedings, **Dr. Aubie Angel** noted that several emerging areas of CLA research, including bone biology, inflammation/infection and metabolic diseases, were identified. He observed that the researchers assembled in this conference are focused on the human health applications of CLA and called for greater uniformity of study design to allow for comparison among human studies.

Dr. Angel identified several strategies for maintaining the momentum generated by the Toronto conference:

- Build capacity for future collaborations by identifying other CLA researchers in Canada and sharing information about CLA-related meetings with current network members.
- Strengthen the current CLA network by developing a communications tool (e.g., a website or listserve).
- Develop an inventory of university-based functional food research groups such as the Functional Food Centers in the United States.
- Draw on the expertise and resources of the Richardson Centre for Functional Foods & Nutraceuticals at the University of Manitoba to enhance the CLA network of researchers.
- Build connections with European CLA networks and researchers.

Dr. Angel also informed conference participants of the plan to host a CLA workshop in October 2004. A planning meeting will be held early in 2004.

Abstract 1

Regulatory Issues in the Use of CLA in Functional Foods and Natural Health Products in Canada

K.C. Fitzpatrick. Richardson Centre for Functional Foods & Nutraceuticals, University of Manitoba, Winnipeg, MB.

The Canadian Food and Drugs Act and Regulations, through its definitions of "food" and "drug", currently restricts health-related claims for foods, food ingredients and natural health products (NHP). In 1998, Health Canada began to develop regulations related to the allowance of health claims for functional foods and NHP. Health Canada has three initiatives underway in the area of health claims for foods: 1) to adopt US generic health claims within a Canadian context; 2) to develop scientific standards of evidence and a guidance document for supporting the validity of product-specific claims; and 3) to develop an overall regulatory framework for functional foods. In 2000, Health Canada announced approval for the use of five generic diet-related health claims under its Mandatory Nutrition Labeling legislation. In October 2001, the agency released a proposed approach to regulating product-specific health claims for foods, in which each product with an intended claim would be evaluated on its own merit. Under a separate initiative, the Natural Health Products Directorate was established in March 1999. New regulations for NHPs were published in the Canada Gazette, Part II on June 18, 2003, to come into force on January 1, 2004. An update of these legislative initiatives will be described.

Abstract 2

Conjugated Linoleic Acids Alter the Metabolism of Fatty Acids and Triacylglycerol in Rat Hepatoma (McA-RH-7777) Cells

R.S. McLeod, A.M. LeBlanc and D.L. Currie. Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS.

To determine the effects of conjugated linoleic acids (CLA) on liver fatty acid and triacylglycerol (TG) metabolism, we examined the changes in rat hepatoma (McA-RH-7777) cell metabolism when supplemented with purified c-9, t-11 or t-10, c-12 CLA isomer. When either CLA isomer was added as the sole source of medium fatty acid (0.4 mM), McA-RH-7777 cells accumulated TG and secreted very low density lipoprotein (VLDL). Linoleic acid (LA), c-9, t-11 CLA and t-10, c-12 CLA promoted similar levels of cellular TG mass accumulation, ~2.5-fold greater than in the absence of fatty acid. Conversely, while LA and t-10, c-12 CLA did not affect cellular cholesteryl ester (CE) levels, c-9, t-11 CLA increased CE storage approximately 3-fold. When cells were incubated with fatty acid and [^{3}H]glycerol, the initial rate of TG synthesis was slower in the presence of t-10, c-12 CLA than c-9, t-11 CLA and both were slower than in the presence of oleic acid (OA). Furthermore, t-10, c-12 CLA decreased [^{3}H]TG secretion by ~50% compared to OA, whereas TG secretion in the presence of c-9, t-11 CLA was similar to OA. When CLA was added as 10% total fatty acid with the balance as myristic acid (MA), the secreted VLDL were more TG-enriched than those secreted in with MA alone. To examine the effects of CLA isomers on the fate of hepatic fatty acids in the rat hepatoma cells, we measured esterification and oxidation of [^{3}H]fatty acids in cells treated with or without CLA. When compared to LA, t-10, c-12 CLA decreased the secretion of TG containing either MA or PA but had little effect on the secretion of TG synthesized from OA. t-10, c-12 CLA also reduced TG synthesis from all three fatty acids. The c-9, t-11 CLA isomer had similar effects although the magnitude of the changes was lower and neither CLA isomer had an appreciable effect on fatty acid β-oxidation. These results suggest that CLA may reduce McA-RH-7777 TG secretion by decreasing fatty acid esterification, although the fate of the fatty acids is not yet clear. (Supported by Dairy Farmers of Canada.)

Abstract 3

Conjugated Linoleic Acid (CLA) Isomers and the fa/fa Zucker Rat Model of Obesity, Insulin Resistance and Altered Lipid Metabolism

C. Taylor, N.R. Ryz, D. Defries, D. Herchak, R. Diakiw, J. Zahradka, L. Lee and P. Zahradka. Departments of Human Nutritional Sciences and *Physiology, University of Manitoba, Winnipeg, MB.*

Our previous study demonstrated that a CLA mixture improved oral glucose tolerance, and attenuated hyperleptinemia and adipose leptin mRNA levels in fa/fa Zucker rats, despite no changes in body weight. The objective of the current study is to determine the effects of CLA isomers (c9t11, t10c12), singly and in combination, on obesity, oral glucose tolerance, and lipid metabolism in fa/fa and lean Zucker rats after 8 weeks of dietary treatment. In collaboration with other research groups, the effects of the CLA isomers on kidney (H. Aukema), bone metabolism (H. Weiler) and immune function (C. Field) are being investigated. Initial analyses indicate that body weight was not different; however, adipose tissue to body weight ratios were elevated by t10c12 alone or isomer mixtures compared to c9t11 alone or the control group. Any positive effects of CLA treatment on insulin resistance may be related to changes in body fat. (Funding from Dairy Farmers of Canada and NSERC)

Abstract 4

Effect of Culture Media Containing Conjugated Linoleic Acid (CLA) *cis* (c) 9, *trans* (t) 11 and CLA t10,c12 or Linoleic Acid (LA) on Proliferation of Bovine Adipofibroblasts from Visceral and Perimuscular Adipose Tissues

M.L. He[1], P.S. Mir[1], Z. Mir[1], E.K. Okine[2] and M.V. Dodson[3]. [1]Agriculture and Agri-Food Canada, Lethbridge, AB. [2]Department of Agriculture, Food & Nutritional Sciences, University of Alberta, Edmonton, AB. [3]Department of Animal Sciences, Washington State University, Pullman, WA.*

In vitro experiments were conducted to determine the effect of CLA c9,t11, CLA t10,c12 or linoleic acid (LA) on proliferation of bovine visceral or perimuscular adipofibroblasts. Adipofibroblasts provided with 70 mg L^{-1} of CLA c9,t11 or CLA t10,c12 had the lowest ($P<0.05$) cell counts and absorbance for activity of mitochrondrial dehydrogenases. Proliferation restriction was greater for cells treated with CLA c9,t11 than the other two fatty acids. Provision of CLA c9,t11 at 70 mg L^{-1} inhibited proliferation of the bovine visceral and perimuscular adipofibroblasts, while CLA t10,c12 and LA suppressed cell proliferation moderately at similar concentrations.

Abstract 5

Adipogenic and Antiadipogenic Effects of Conjugated Linoleic Acid (CLA) Isomers

A. Angel, P. Plett and J. Manusow. Departments of Medicine and Physiology, University of Manitoba, Winnipeg, MB.

Linoleic acid supplements enhance lipogenesis and adipose tissue growth in intact animals and cultured adipocytes. In contrast, conjugated CLA is thought to reduce adipose mass but this effect appears to be variable. Earlier feeding experiments employed mixtures of CLA isomers. To clarify the response, we examined the independent effects of c-9, t-11 and t-10, c-12 isomers of CLA on triglyceride accumulation and leptin production in differentiating 3T3-L1 cells. In dose response experiments (0 – 100 μM) c-9, t-11 isomer stimulated and t-10, c-12 isomer reduced adipose lipid and leptin secretion. In time course experiments (0 – 11 days) a phasic profile was evident with a late fall in leptin secretion. These studies demonstrate that CLA isomers either stimulate or inhibit adipose lipid accumulation and leptin production depending on the isoform type and concentration. The results also suggest that leptin production is greatest early during lipid accumulation and decreases as lipid stores expand. Thus,

the common natural CLA (c-9, t-11) isomer, like linoleic acid, favours lipogenesis and adipose cell growth. The antiadipogenic properties of CLA mixtures appear to be due to the t-10, c-12 isomer.

Abstract 6

Effects of Various Isomers of Conjugated Linoleic Acid on Lipoprotein Profile and Body Composition in Hamsters

H. Jacques, V. Bissonauth, J. Marin and Y. Chouinard. Departement des sciences des aliments et de nutrition, Pavillon Paul-Comtois, Universite Laval, Quebec.

Few studies have reported the effects of CLA isomers on plasma lipoproteins and body composition. Among the predominant geometric isomers of CLA in foods are the c-9, t-11 CLA, the t-10, c-12 and the t-8, c-10 CLA isomers. The current problem under investigation is to examine whether consumption of c-9, t-11 CLA, t-10, c-12 CLA, a mixture of c-9, t-11 and t-10, c-12 CLA and a mixture of c-9, t-11 and the t-8, c-10 CLA isomer will result in overall weight loss, reduction in body fat and improved lipoprotein profile when compared with linoleic acid under dietary controlled conditions in hamsters. Thus, fifteen Golden Syrian hamsters per group are being fed with controlled purified diets (CLA isomers 2%) on an *ad libitum* basis for 28 days. At the same time, we are examining the effects of these CLA-isomers on hepatic triglyceride and cholesterol concentrations and fecal fat content. (Supported by Dairy Farmers of Canada)

Abstract 7

Conjugated Linoleic Acid (CLA) and Human Body Weight and Composition

P.J.H. Jones, Y. Wang and S. Venkatramanan. School of Dietetics and Human Nutrition, McGill University at Macdonald Campus, Montreal, QC.

A limited number of studies in humans suggest that CLA supplementation has marginal and equivocal effects on fat deposition, with no significant effects on body weight and insulin sensitivity observed. It is evident that effects of CLA on body weight and composition as well as the surrounding mechanisms in humans need to be clarified by conducting more experiments. The authors' laboratory is carrying on a human clinical trial to examine the effects of milk naturally enriched with 4.2% CLA isomer mixture or milk with 4.2% commercially prepared *trans*-10, *cis*-12 isomer of CLA, as compared with regular milk with 0.3% CLA, on body weight and composition, lipid profiles, cholesterol and triglyceride fatty acid synthesis in overweight and hyperlipidemic men. It is anticipated that results emerging from this study will assist in identification of mechanisms responsible for how CLA impacts on body weight regulation and fat deposition.

Abstract 8

Conjugated Linoleic Acid (CLA) in Humans: Effects on Body Composition and Adverse Events During Weight-loss

L.D. Whigham and R.L. Atkinson. Department of Nutritional Sciences, University of Wisconsin, Madison, WI.

Prior studies show modest effects of CLA on body composition (BC) or weight. We performed two randomized, double-blind, placebo-controlled human studies. Study 1 evaluated 80 obese subjects on 2.7 g/d of CLA vs placebo for 6 mo, with a 500 kcal/d energy deficit. Questionnaires evaluated adverse events at each visit; BC by underwater weight. Wt loss was 2.8 kg with no differences in BC. CLA group had fewer GI, negative mood, and cognitive/CNS complaints. In Study 2, 60 obese subjects received 6 g/d of CLA vs placebo for 28 wk. Hypothesis: CLA would prevent fat accumulation during regain after rapid weight loss. Diet from wk 1-12 was ~800 kcal/d, from wk 12-28 was maintenance (~20 kcal/kg). Changes in wt and BC were not different at any time. Several adverse events were lower ($p<.05$). Conclusions: CLA does not improve BC in obese people on diets, but improves tolerability of weight loss. (Funded by Natural and Loders-Croklaan)

Abstract 9

The Effects of Safflorin™-CLA on Human Immune Response to Respiratory Viral Infections

M. O'Shea[1] and I. Mohede[2]. [1]Loders Croklaan, Lipid Nutrition, Channahon, IL. [2]Loders Croklaan, Lipid Nutrition, Wormerveer, The Netherlands.

Safflorin™ CLA enhances innate and adaptive immune responses to respiratory viral infection in humans. In study I, the effect of Safflorin™ on the influenza specific serum antibody response of elderly adults to influenza vaccine was investigated. The volunteers (n=49) received 2 g Safflorin™/day or placebo for 7 weeks and were vaccinated with the influenza vaccine administered intramuscularly on day 28. The antibody levels were measured 3 weeks after vaccination. Safflorin™ in comparison to the control group clearly increased the antibody levels against each influenza component as used in the vaccine. In Study II, the effect of Safflorin™ supplementation on the frequency and severity of common colds in humans was investigated. The volunteers (n=45) received Safflorin™ (2 g/d) for 5 weeks. On week 5, they were inoculated (intranasally) with the rhinovirus (day 0) and remained in isolation for 5 days post inoculation as incidence and severity of symptoms were recorded. The sore throat was significantly inhibited by Safflorin™, and other symptoms like total obstruction (nose, throat), headache, malaise and chilliness showed clearly an inhibition (trend).

Abstract 10

CLA and Immune Function: An Update

C.J. Field. Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB.

A number of studies have reported immunological effects of mixtures of CLA isomers in poultry, rodents, guinea pigs, and pigs. There are reports of beneficial effects of feeding CLA to animals and rodents on inflammatory-induced growth suppression, endotoxin-induced anorexia, mucosal damage and growth failure in experimental colitis, and antigen-induced type 1 hypersensitivity response. The mechanism for these effects has not been clearly established. The aim of our research program has been to identify, using rodent models, the effect of feeding CLA in diets with similar fat content and composition to that of the human diet on lymphocyte function. A summary of our unpublished recent work on the effects of feeding CLA on lymphocyte function (proliferation and cytokine production) will be briefly presented.

Abstract 11

CLA and Musculoskeletal Biology

B. Watkins[1,2], Y. Li[1], M.F. Seifert[2] and K. Hannon[3] . [1]Department of Food Science, Center for Enhancing Foods to Protect Health, Purdue University, West Lafayette, IN. [2]Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, IN. [3]Basic Medical Sciences, Purdue University, West Lafayette, IN.

Musculoskeletal atrophy, the decrease in size and strength of muscle, bone and connective tissue, is a sequela of many different diseases and states (e.g., aging, cancer, heart disease, and stroke) as well as prolonged illness requiring bed rest. This atrophy progressively diminishes ambulatory function, thereby markedly diminishing the quality of life of millions of Americans each year. Age-associated musculoskeletal atrophy often necessitates costly nursing care, and disease-associated atrophy increases hospitalization time and extends requirements for regenerative therapy. Thus, in addition to quality of life issues, musculoskeletal atrophy is an important factor that drives up the cost of medical care. Our long-range goal is to discover potential treatments to attenuate musculoskeletal atrophy. One approach our group has taken to treat atrophy is by gene therapy, as we have found that electroporation and ectopic expression of anabolic growth factors such as IGF-I and Shh within the gastrocnemius muscle significantly attenuated the loss of muscle fiber area, muscle mass and muscle mass density that normally occurs during disuse muscle atrophy. In addition, we have found that ectopic expression of IGF-I and Shh within the gastrocnemius/soleus muscle inhibits parameters of osteopenia within the tibia and fibula associated with hindlimb unloading in the mouse. These results support the theory that skeletal muscle can regulate bone maintenance in an endocrine fashion and could offer potentially novel and efficient therapeutic options for attenuating muscle and bone atrophy during aging and illness. Another approach we have taken to control atrophy is through the use of nutrients such as polyunsaturated fatty acids (PUFA). We are testing mixtures of specific PUFA including CLA on muscle atrophy and osteopenia in rodent models of disuse. Some mixtures of PUFA are effective in attenuating tissue atrophy. This approach has immediate and significant practical implications in the fight against musculoskeletal atrophy.

Abstract 12

Effects of Conjugated Linoleic Acid (CLA) on Human Osteoblast-like Cells

I. Platt[1], L.G. Rao[2] and A. El-Sohemy[1]. [1]Department of Nutritional Sciences, University of Toronto, Toronto, ON; and [2]Calcium Research Laboratory, St. Michael's Hospital and Department of Medicine, University of Toronto, Toronto, ON.

Conjugated linoleic acid (CLA) has been shown to increase biochemical markers of bone formation in cultured mouse osteoblasts and in rat bone organ culture. However, the direct effects of CLA on bone formation using cells of human origin are not known. The purpose of this study was to determine the effects of CLA on the differentiation and mineralization of human osteoblast-like cells (SaOS-2). Cells were seeded in either 12 or 24 well plates at a density of 0.5-1 x 10^4 cells/well. On day 8, cells were treated with varying concentrations of CLA (25-100 μM, mixed isomers) or vehicle (0.1% ethanol). On day 17, mineralized bone nodules were stained using the Von Kossa technique and quantified using a FluorChem™ imaging system. Alkaline phosphatase (ALP) activity in cell lysates, and osteocalcin levels in the media were used as markers of early and late osteoblast differentiation, respectively. CLA at 100 μM significantly increased the number (6-fold; $P<0.001$), and area (15-fold; $P<0.01$) of mineralized bone nodules. CLA also increased ALP activity by ~3-fold after 4 days of treatment ($P<0.01$), and after 5 days increased osteocalcin production by ~40% ($P<0.01$). Western blot analyses were used to determine whether the effects of CLA on bone nodule formation might be mediated by a decrease in 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase. Drugs that inhibit this enzyme have been shown to stimulate bone formation by increasing bone morphogenetic protein-2 (BMP-2). After 24 hours of treatment, 100 μM CLA decreased HMG-CoA reductase and increased BMP-2 protein levels. These results demonstrate that CLA stimulates the mineralization of bone nodules from cells of human origin, possibly through changes in HMG-CoA reductase and BMP-2. (Supported by a grant from the Dairy Farmers of Canada and NSERC)

Abstract 13

The Effects of CLA on Bone Mass in Rodents

H. Weiler, H. Kovacs, R. Mollard and C. Taylor. Department of Human Nutritional Sciences, University of Manitoba, Winnipeg, MB.

Two studies were conducted to explore the effects of CLA on bone mass in health and disease. Both studies resulted from collaboration with C. Taylor where the lean (healthy) and fa/fa (hyperinsulinemic/hyperlipidemic) Zucker rat model of insulin resistance was fed CLA for 8 weeks. In the first study, bone mass of the femur was measured and in the second, whole body bone mass was measured using dual-energy X-ray absorptiometry (DXA). In both studies, CLA did not affect bone mass. These studies contradict existing literature where bone mass or ash weight was reported to be elevated with CLA.

Abstract 14

Does Conjugated Linoleic Acid (CLA) as a Dietary Supplement Attenuate Alterations in Growth, Fat/Lean Mass and Bone Mass Observed Secondary to Steroid Drug Treatment in Infant Pigs?

D. Wang, M. Patel, S. Berge and S. Atkinson. Department of Pediatrics, McMaster University, Hamilton, ON.

Aim: To determine if a dietary supplement containing conjugated linoleic acid (CLA) will attenuate dexamethasone (DEX)-induced growth delay, altered fat/lean mass and bone abnormalities. Methods: Infant piglets (n=16) were divided into three groups: placebo, DEX and DEX with CLA supplement (2% dietary fat) for 15 days. Growth change was recorded. Bone, lean and fat mass were measured by dual-energy X-ray absorptiometry (DXA). Serum osteocalcin (OC) and parathyroid hormone (PTH) were measured. Results: Compared to placebo, DEX treatment caused reduced growth, lean mass, bone mass and OC, while fat mass and PTH increased. Compared to DEX, CLA displayed a trend to rescue lean mass by 5% and BMC by 5%. The CLA group demonstrated a similar pattern in length (+13%), OC (-65%) and PTH (+112%) as the DEX group (+14%, -63% and +98%, respectively). Conclusion: CLA may attenuate the negative effect of DEX on growing animals. Further experiments will be conducted to confirm the results.

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