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**Phytopharmaceuticals for Obesity Treatment: Pathophysiology
and Weight Management**

**Conjugated Linoleic Acid (CLA) and Weight Control: From the
Biomedical Immune Viewpoint**

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ABBREVIATIONS

BAT, brown adipose tissue; BMI, body mass index; CLA, Conjugated linoleic acid; c9t11-CLA, cis-9, trans-11 conjugated linoleic acid ; t10c12-CLA, trans-10, cis-12 conjugated linoleic acid ; CNF, ciliary neurotrophic factor; CRP, C reactive protein; CVD, cardiovascular disease; DM, diabetes mellitus; ELISA, enzyme-linked immunosorbent assay; FFA, free fatty acid; GADD45, Growth arrest and DNA damage-inducible gene; IL, interleukin; LA, Linoleic acid; LPS, lipopolysaccharide; MCP-1, monocyte chemotactic protein-1; MIF, migration

inhibitory factor; MTT, 3-(4,5-dimethylthiazol-2-yl) -2,5- diphenyltetrazolium bromide; MS, metabolic syndrome; NFAT, calcineurin/nuclear factor of activated T cells; NGF, nerve growth factor; PAI-1, plasminogen activator inhibitor 1; PPAR γ , Peroxisome proliferator activated receptor gamma; PPRE, Peroxisome proliferator responsive element; RA, Retinoic acid; RAR, Retinoic acid receptor; ROS, reactive oxygen species; RT-PCR, Reverse transcriptase polymerase chain reaction; RXR, Retinoid X receptor; TG, triglyceride; TNF α , tumor necrosis factor alpha; VEGF, vascular endothelial growth factor; WAT, white adipose tissue

I. Introduction

Obesity is the important medical problems worldwide today. It is associated with a number of acute and chronic medical complications, including cardiovascular disease, diabetes, arthritis, depression, respiratory and gastrointestinal problems. On the other hand, traditional weight loss treatments, usually involving reduction of fat intake, in fact, have generally had very limited success. In this article, from viewpoint of dietary supplement, we will report some our recent results and review the updated knowledge of the adipocytes regarding to conjugated linoleic acid (CLA) effects, focusing on gene expression and cytokines/adipokines in different model, including cultured cell, rodent, and human study. In 1987 Ha *et al.* [1] found that CLA present in fried ground beef, reduced tumor incidence in mice. CLA has also attracted a lot of attention over the past few years for antiobese issue. Earlier research has indicated that intake of CLA might reduce adiposity, providing antioxidant protection to treating diabetes and cardiovascular disease in humans and could have important other beneficial effects. A CLA-enriched diets lead to a rapid and marked decrease in fat stores in several species including pig, rat, hamster, chicken and mouse [2-6], suggesting that CLA might be useful as weight-loss agents. However unfortunately, several recent in human subjects data of CLA supplementation in contrast to animal studies there has been marked variation between reports on the health-related outcomes. Additionally, adverse side effects have been recently reported in mice fed a commercial CLA mixture. It indicated that the relation between CLA taken as supplements and antiobese could be more complex than initially general thought.

II. Chemical property, source, pharmacology and safety

Chemical property, origin and production

The CLA family consists of several different conjugated and many have currently been identified [7-9]. Natural forms of CLA can be found predominantly in ruminant products [10,11], more than 91% of the c9t11-CLA present in milk fat [12-14], also known as rumenic acid [15-19] see figure 1, and partially hydrogenated vegetable oils. Because the CLA content of dairy products is related to their fat content, CLA levels are greater in higher fat than in lower fat products. The two predominant isomers found in foods and commercial preparations are the c9t11-CLA and t10c12-CLA. Commercial dietary supplements, contain c9t11-CLA and t10c12-CLA isomers in approximately equal amounts. Measurements of c9t11-CLA in human adipose tissue have found that its presence is highly correlated with milk fat intake [20-24]. Anaerobic ruminant bacteria, such as *Butyrivibrio fibrisolvens* [25], produce predominantly c9t11-CLA through biohydrogenation of linoleic acid and α -linolenic acid obtained from plant material, and pathway of linoleic acid by c9t11-octadecadienoate reductase [26]. CLA also from fried ground beef, heat-altered derivatives of linoleic acid [1]. In the formation of CLA, hypothetical mechanism by which oxygen-derived free radicals might induce a double bond of linoleic acid to shift [27].

In addition to dietary sources, some CLA can be produced endogenously by humans [28]. Several methods are currently available to chemically synthesize CLA [29,30], either absorbed or further metabolized to vaccenic acid (trans-11-octadecenoic acid), a predominant trans fatty acid in milkfat, which can be converted to c9t11-CLA by the enzyme Δ^9 stearoyl-CoA desaturase, an alternative route in mammals, including in humans [31,32]. Blood levels of CLA in humans may reflect both dietary intake of CLA and endogenous synthesis from trans-vaccenic acid. Interestingly, in diabetes, the glycation and subsequent glycoxidation reactions are enhanced by elevated glucose concentrations. Ratios of CLA to LA significantly increased in diabetic erythrocytes compared with control erythrocytes. This indicate that glycation via chronic hyperglycemia links lipid peroxidation in the erythrocytes of both diabetic and healthy subjects [27].

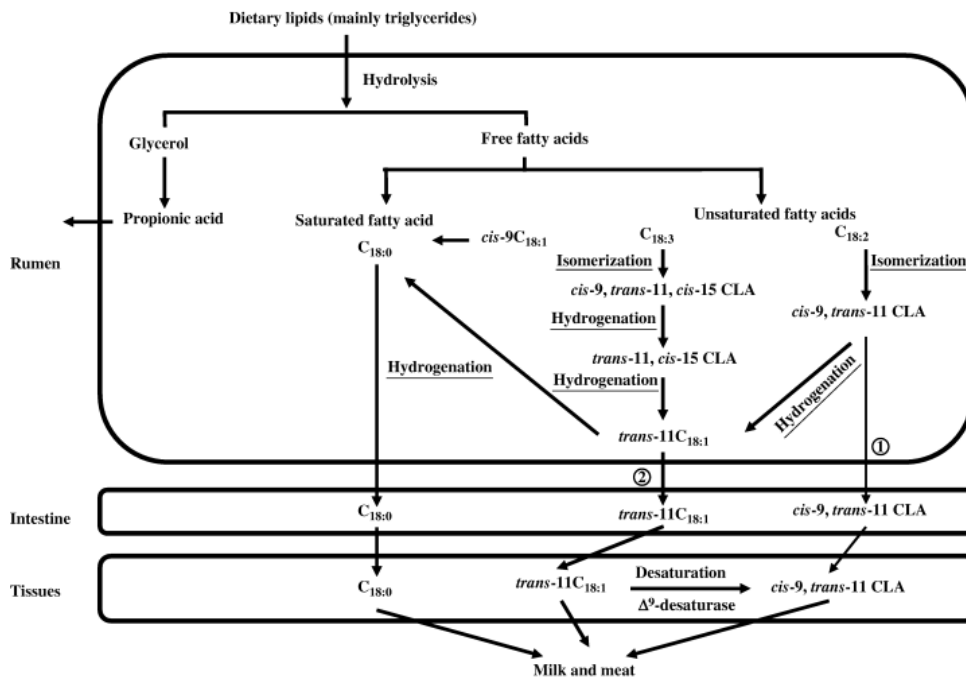


Figure 1. Biosynthesis of CLA in its incorporation into meat and milk ruminants [33]. (adapted from Tanaka, 2005 with permission) [34].

The conjugated trienoic fatty acids produced from α - and γ -linolenic acid were further saturated by *Lactobacillus plantarum* to trans-10, cis-15-18:2 and cis-6, trans-10-18:2 [35]. Recently, purified CLA isomers are commercially available, and is expected to facilitate the clarification of dietary function paradox and of each isomer's physiological activity.

Pharmacology and dosage

In vitro and experimental animal studies found a number of potential health benefits for CLA. CLA inhibits the proliferation of some cancer cells such as mammary, colorectal, prostate, and forestomach cancers [36]. Virtually most studies have used synthetic mixtures of CLA at dose from 10 to 25 μ M. In human serum, CLA has been reported to be around 7.1 μ M. One report indicates that potent cytotoxic effect on cancer cell line can be exerted at physiological concentration [37,38]. In study of body composition, intake of CLA reduces body fat and increases lean body mass in several species of growing animals [39], improves glucose utilization and reverses symptoms of diabetes in laboratory animals. CLA may lowers total and LDL cholesterol as well as TG levels and reduces the severity of atherosclerosis in experimental animals [40]. Recent reports also suggest that each CLA isomer has different functions, such as the t10c12-CLA has significantly anticarcinogenic, antiobese and antidiabetic effects, whereas the c9t11-CLA seem to exerts an

anticancer effect. In addition, CLA enhances select immune responses in experimental animals, as well as increases the rate of bone formation by influencing factors that regulate bone metabolism. Physiological difference between free and triglyceride-type CLA on the immune function of C57BL/6N mice also investigated [41].

The typical dosage of CLA ranges from 3 to 5 g daily as dietary supplement. CLA was found induces leptinaemia and adiponectinaemia, followed by hyperinsulinaemia, was determined in C57BL/6J female mice fed a 1% isomeric mixture of CLA for various periods of time ranging from 2 to 28 days. Additionally, due to still only few reports or weak evidence concerning the anticancer and antiobese effects of CLA in humans study. Therefore, more detailed evaluations of the physiological bioactivities, especially in double blind and placebo based research, using pure CLA isomers on lifestyle/aging-related diseases in humans and animals will be of great interest in future studies.

Dietary safety and adverse effects

The t10c12-CLA isomer is responsible for antiobese effect especially dramatic in the mouse. However, it is noteworthy that a significant impairment of insulin sensitivity has been reported in overweight subjects receiving the purified t10c12-CLA isomer, in which it is associated with severe hyperinsulinemia, insulin resistance and massive liver steatosis, also called the CLA-mediated lipotrophic syndrome [42,43]. This finding, raises the question of safety of dietary supplements containing CLA. In generally, the usual doses of CLA used in animal studies greatly exceed those used in human studies. This is reasonable to explain why animal studies come up with better results than human studies, and may also explain the adverse effects of CLA in rats. Collectively, evidences for a putative beneficial effect of a CLA supplementation in humans are still inconclusive, maybe the safety of dietary supplements containing CLA need more concerning, and more clearly further isomer-specific clinical trials are necessary.

III. CLA on obesity issue: weight control and body composition in cultured cell, animal and human studies

Disease related to obesity- role of inflammation and adipokine

Despite the enormous medical implications of obesity, effective prevention and treatment strategies are still lacking. It is important to distinguish the term obesity, used to describe excess body fat, from other forms of overweight. Obesity results from the hypertrophy, and hyperplasia of adipocytes within the organism later, is generally thought to be the result of both genetic and environmental influences. Being overweight or obese has become highly prevalent in Western countries and the population are rapidly upgrowing in the developing world [44]. Obesity-related disorders, such as insulin resistance, hypertension and diabetes, are also dramatic increasing. Obesity is also associated with endothelial dysfunction and arterial stiffness from as early as the first decade of life [45]. This probably mediated in part by low-grade inflammation associated with cytokine-like molecules, called adipokines. WAT is a major endocrine/secretory organ, which releases a wide range of adipokines. A number of adipokines, including IL-1 β , IL-6, MCP-1, MIF, TNF α , leptin, adiponectin, NGF, VEGF, PAI-1, are somehow linked to the inflammatory response. Recent research indicate those adipose tissue-derived factors influence metabolic and cardiovascular disease. Leptin is now considered to play a key role in in obese, hypertensive patients, and decreased secretion of adiponectin appears to be an important predictor of diabetes [46,47]. A high leptin concentration, in particular, is found in obese individuals and is strongly associated with vascular changes related to early atherosclerosis[48,49].

Metabolic syndrome: adipogenesis and neurotrophins

Obese adipose tissue origin from a long-term process of adipogenesis, is characterized by inflammation and progressive infiltration by macrophages as obesity develops [50], that link to metabolic pathways in metabolic disease and immune response, and the signaling pathways at the intersection contribute to diabetes [51]. The elevated production of inflammation-related adipokines is increasingly considered to be important in the development of diseases linked to obesity, particularly thought as the metabolic syndrome. [52]. Metabolic syndrome, such as diabetes, hypertension, dyslipidemia, , coronary artery disease, and obesity. also known as syndrome X, or the insulin resistance syndrome [53-56]. The global epidemic of obesity and DM has led to a marked increase in the number of persons with metabolic syndrome. Both type 2 DM and metabolic syndrome share common features, and patients.

An increasing number of researchers of the metabolic syndrome assume that many mechanisms are involved in the impact for complex pathophysiology of neurotrophins, such as disorders of the hypothalamo-pituitary-adrenal axis, an increased sympathetic activity, the chronic subclinical infections, proinflammatory cytokines, the effect of adipocytokines, and/or psychoemotional stress [57]. Scientific research in this field confirms the role of the neurotrophins and mastocytes in the pathogenesis of inflammatory and immune diseases [58]. CNF is a another neurocytokine expressed by glial cells in nervous system, generally recognized for its function in survival of non-neuronal and neuronal cell types. It was recently acknowledged for its potential role in the control of obesity [59].

Cell-cell interaction: integrin, matrix metalloproteinase

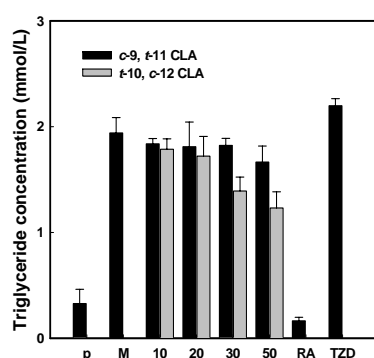
In obesity, changes in fat pad size lead to physical changes in the surrounding area and modifications of the paracrine function of the adipocyte. Such as adipocytes begin to secrete TNF α , which will stimulate preadipocytes to produce monocyte chemoattractant [60-64], and contribute to progressive inflammation occur later. Noteworthy, the processes of adipogenesis include a process of migration, adhesion, proliferation and differentiation of preadipocytes into mature adipocytes. Many of these biological functions are related to cell integrins, like the TG content and gene expression of PPAR γ and leptin also decreased in response to the treatment of disintegrin [65]. Moreover, *in vivo* model found that partial inhibition of gelatinolytic activity is associated with moderate effects on adipose tissue development. MMP inhibitor decreases adipose conversion of 3T3-L1, and enhancement of MMP expression counteracts the inhibitor in adipose tissue [66]. These support a role for the MMP system in the control of proteolytic processes and adipogenesis during obesity mediated fat mass development [67].

CLA on lipid metabolism: in cell, animal, and human study

Beneficial effect of CLA on body composition: Potential antiobesity mechanisms of CLA include decrease preadipocyte proliferation and differentiation into mature adipocytes, decrease fatty acid and TG accumulation, increase energy expenditure, lipolysis, and fatty acid oxidation. CLA intake has been demonstrated consistently to decrease in body fat accumulation and increase in lean body mass in several experimental animals including mice, rats, hamsters, and pigs. However, CLA's effect on overall body weight appears to be variable [68,69]. CLA was shown to accumulate

in the WAT much more than in the serum or liver, and found levels of triglycerides in the WAT and serum nonesterified fatty acid were reduced in a CLA dose-dependent manner [70]. In animal studies, the most dramatic and desirable effects of CLA on body composition are ascribed to the t10c12- isomer rather than the c9t11- isomer. Recent findings in mice and hamsters indicate that the t10c12-CLA isomer is largely responsible for CLA's effect on body composition, adipocyte morphology, and for many of the effects seen in diabetes and obesity [71,72]. As shown in figure 2, we also confirmed the similar result, t10c12-CLA prevent TG accumulation more remarkable. The t10c12-CLA has been reported to inhibit heparin-releasable lipoprotein lipase activity and leptin secretion from 3T3-L1 adipocytes, and to suppress delta-9 desaturase activity [73]. Although a lot of mechanisms are presumably involved, how CLA alters body composition is still unclear and remains to be determined.

Figure 2. Effects of c9t11- and t10c12-CLA on triglyceride accumulation in differentiated 3T3-L1



cells. (p: preadipocyte, M: MDI, RA: 5 μ M retinoic acid, TZD: 10 μ M thiazolidine.)

There is little direct evidence regarding CLA's effect on body composition in humans. Recent reports surprisingly suggest that supplementation with t10c12-CLA isomer or with the c9t11- isomer enriched foods may not contribute to the expected beneficial changes in the risk variables associated with MS. Especially, due to the effects of CLA on adiposity and lipid metabolism are dependent on its isomer specificity, the results of CLA on human are conflicting because these studies have used different mixtures and dosages of CLA isomers, and diverse subject populations [74]. Results of many published studies in human subjects is that CLA does not affect body weight, body composition or adipose tissue mass [75]. Such as, a randomized crossover study on plasma lipoproteins and body composition in men, found that the CLA-enriched butter induced no significant change in the CVD risk profile and had no effect on the distribution of body fat [76].

Collectively, CLA's effects from a fundamental viewpoint, an integral extrapolation of animal data to human seems unrealistic, CLA has only limited effects on immune functions in man. In fact however, dietary CLA is able to be incorporated and metabolized as linoleic acid, influence linoleic acid desaturation and elongation; and to be beta oxidized finally in peroxisomes, which via activation of PPARs increase free retinol levels, link to regulate gene expression [77]. In a study of 60 abdominally obese men with MS, who were randomly assigned to supplements containing either 3.4 g/d of a CLA isomer mixture, or the purified t10c12- isomer. Results found that after 12 wk of supplementation, the t10c12- isomer induced statistically significant deteriorations in insulin resistance, in glycemia as well as in plasma HDL-cholesterol concentrations compared with placebo [76]. CLA supplementation with purified t10c12-CLA isomer decreases fat mass and causes a significant impairment in insulin sensitivity in overweight humans [78].

IV. Mechanism of CLA effects: on differentiation, organelles, gene expression, putative receptor, and immune response

Adipocyte/preadipocyte differentiation, activation

Adopogenesis is a multistage process beginning with mesenchymal cells capable of forming muscle, bone or adipose tissue. Adipose tissue plays a key role in the pathogenesis of the obesity-related metabolic syndrome. Adipocyte serves as an important source of pro-inflammatory molecules, including leptin, TNF α , and IL-6, as well as anti-inflammatory molecules, such as adiponectin [79]. Most of these functions are carried out via adipocytokines capable of acting locally or at distant sites [80]. The recent study demonstrate that calcineurin is a critical effector of a calcium-dependent signaling pathway that acts to inhibit adipocyte differentiation [81]. Moreover, a constitutively active NFAT mutant preadipocytes inhibits their differentiation into mature adipocytes. Cell expressing NFAT lose contact-mediated growth inhibition, protected from apoptosis following growth factor deprivation.

The first, and best characterized, model of adipogenesis *in vitro* is the 3T3-L1 mouse fibroblast cell line [82,83]. When confluent/growth-arrested 3T3-L1 cells are subjected to the adipogenic hormones: 3-isobutylmethylxanthine (a phosphodiesterase inhibitor), dexamethasone, and insulin, collectively known as MDI, they undergo a defined genetic program of terminal differentiation, and giving rise to mature morphologically distinct adipocytes containing large cytoplasmic TG depots [84]. It is

dependent on the sequential activation of transcription factors including the C/EBP, PPAR, and SREBP, those leading to changes in gene expression [85]. Another cell lines, 3T3-F442A, at a later stage, requires only insulin to differentiate, as well as AP-18, a new non-embryo derived preadipocyte cell line established from an adult C3H/HeM mouse provides a useful model for investigating adipocyte differentiation and adipogenesis [86].

Organelle functions: mitochondria and endoplasmic reticulum

Increasing evidence show that mitochondrial dysfunction in the prediabetic/insulin-resistant state, contributes to a variety of human disorders, ranging from cardiac dysfunction, neurodegenerative diseases, obesity, insulin sensitivity and cancer, as well as their increasingly acknowledged key role during apoptosis [87-89]. Induction of mitochondrial uncoupling proteins, UCP1 in mouse or human white adipocytes promote fatty acid oxidation and resistance to obesity. UCP2 and UCP3 do not mediate adaptive thermogenesis physiologically, and do not seem to contribute to energy expenditure, but they may be significantly thermogenic under specific pharmacological conditions. Both UCP2 and UCP3 are should be considered as potential targets for treatment of aging, degenerative diseases, and perhaps obesity [90,91]. Noteworthlly, PPAR γ co-activator 1 α plays a key role in regulating mitochondrial biogenesis and fuel homeostasis, and overexpression favored a shift from incomplete to complete beta-oxidation enables muscle mitochondria to better cope with a high lipid load [92]. These possibly reflecting a fundamental metabolic benefit of exercise training, and up-regulation of PPAR γ co-activator 1 α may as a effective strategy for preventing or reversing insulin resistance and obesity [93]. On the other hand, obesity-induced endoplasmic reticulum stress recently has been demonstrated to underlie the initiation of inflammatory responses, and generation of peripheral insulin resistance [94]. It leads to suppression of insulin receptor signaling through hyperactivation of c-Jun N-terminal kinase and subsequent serine phosphorylation of insulin receptor substrate-1 [95].

The beneficial effects exerted by low amounts of CLA, and raise the question about their mitochondrial oxidizability. CLA appeared to be both poorly oxidizable and capable of interfering with the oxidation of usual FA at a step close to the beginning of the beta-oxidative cycle [96]. It was reported that, CLA more effective than vitamin A protecting mitochondria from peroxidative damage of 3T3-L1 cells [97]. Our research indicated that CLA induced apoptosis via mitochondrial pathway through PPAR γ signalling, reduce mitochondria transmembrane potential, increased

mRNA expression of apoptosis regulator Bax and Bcl-2 ratio, enhance cytochrome c release to cytoplasm and activation of caspase-3 (reversed by pan-caspase inhibitor). CLA reduced mRNA expression of cyclin D and increased mRNA expression of p53 and p21^{waf-1}, but increased mRNA expression of GADD45. In addition, research found that CLA inhibits the elongation and desaturation of 18:2n-6 into 20:4n-6. One might speculate that a diet enriched in CLA would be useful in preventing carcinoma [98].

CLA on gene expression: PPARs, signal transduction, and adipokines

Adipocytes act not only as a fuel storage depot but also as a critical endocrine organ that secretes a variety of signaling molecules into the circulation, they play a central role in the maintenance of energy homeostasis by regulating insulin secretion, glucose and lipid metabolism. These secretory factors include enzymes, growth factors, cytokines and hormones involved in fatty acid and glucose metabolism. For gene expression, the PPAR are ligand-activated transcription factors, family comprises three closely related gene products-PPAR α , PPAR γ , and PPAR δ/β -and is so named because PPAR α is activated that elicit increases in the number and size of peroxisomes. Fatty acids, eicosanoids, and some drugs are PPAR ligands, PPAR- α regulates fatty acid oxidation primarily in liver, PPAR γ serves as a key regulator of adipocyte differentiation and lipid storage, while PPAR δ is a positive factor for fat burning. PPARs function as important coregulators of energy homeostasis. Moreover, both PPAR γ -1 and PPAR γ -2 isoforms, generated by alternative splicing, PPAR γ 1 isoform is expressed in liver and other tissues, whereas PPAR γ -2 isoform is expressed exclusively in adipose tissue where it regulates adipogenesis and lipogenesis. Oxidized LDL regulates macrophage gene expression also through ligand activation of PPAR γ [99].

ICR and C57BL/6J mice were fed experimental diets containing CLA greatly decreased weights of white adipose tissue and interscapular brown adipose tissue in the two strains [100]. It is apparent that dietary CLA's function, accompanying changes in the gene expression of proteins regulating energy metabolism in white and brown adipose tissues, and skeletal muscle of mice. Inhibition of lipid accumulation induced by t10c12-CLA treatment during adipocyte differentiation is associated with a tight regulatory process between early (PPAR γ and C/EBP α) and late (LXR α , aP2 and CD36) adipogenic marker genes [101]. Furthermore, we also confirmed the effects of c9t11- and t10c12- isomers of CLA on the expression of adipogenic genes, results show in table 1. The t10c12-CLA at concentration 25 μ g/L significantly

decreased the mRNA expression of C/EBP α , PPAR γ and SREBP-1, and in a dose-dependent manner. However, c9t11- CLA presents no inhibitive function on the expression of SREBP-1. t10c12-CLA also decreases the triglyceride content of newly differentiated human adipocytes by inducing MEK/ERK signaling through the autocrine/paracrine actions of IL-6 and 8 [102]. Collectively, CLA may impart its effects by increasing expression of genes associated with apoptosis, fatty acid oxidation, lipolysis and inflammation, as well as decreasing stromal vascular cell differentiation, and lipogenesis [72].

Table 1. Inhibitory effect of c9t11- and t10c12-CLA in differentiated 3T3-L1 cells.

sample	Concentration (ppm)	Inhibitory activity (%)		
		SREBP-1	C/EBP α	PPAR γ
c9t11-CLA	10	4.14 \pm 4.56 a	6.28 \pm 1.53 a	0.48 \pm 4.28 a
	15	9.99 \pm 2.42 a	16.05 \pm 2.42 b	7.06 \pm 2.09 a
	20	7.09 \pm 3.09 a	18.29 \pm 1.94 b	7.27 \pm 5.67 a
	25	9.87 \pm 1.39 a	17.20 \pm 1.38 b	19.45 \pm 2.99 b
t10c12-CLA	10	2.02 \pm 2.15 a	12.46 \pm 1.38 a	5.82 \pm 2.58 a
	15	1.69 \pm 6.10 a	23.79 \pm 2.20 b	9.29 \pm 4.27 a
	20	13.86 \pm 2.46 a	34.09 \pm 1.19 c	32.46 \pm 3.88 c
	25	35.8 \pm 3.87 b	56.06 \pm 3.19 d	61.39 \pm 5.02 d

Effects of c9t11- CLA on C/EBP α mRNA expression in 3T3-L1 adipocytes. Two-day postconfluent 3T3-L1 were differentiated by MDI and treated with CLA. On day 8 after induction of differentiation, total RNA was extracted from 3T3-L1 cells and subjected to RT-PCR with primers specific for C/EBP α .

Serum leptin is correlated to body fat level, acts in the hypothalamus to regulate satiety [103]. Leptin, a cytokine, is the ob gene product from mature adipocytes, its expression is accelerated at obesity state that inhibits food intake and accelerates energy expenditure. Dietary CLA found acute reduction of serum leptin level in Sprague-Dawley rats [104].

Anti-adipogenesis of CLA involved to estrogen receptor ? RA receptor?

Estrogen receptor alpha plays an important role in mediating estrogen signaling is involved in osteoporosis and obesity [105,106]. Interestingly, obesity positively associated with breast cancer for postmenopausal women [107]. Estradiol affects the metabolic action of insulin in a concentration dependent manner, that high

concentrations of estradiol inhibit insulin signaling by modulating phosphorylation of IRS-1 via a JNK-dependent pathway [108]. In addition, estrogens and phytoestrogen genistein also found regulate adipogenesis and lipogenesis in males and females [109]. CLA compounds possess potent antiestrogenic properties that may partly account for their antitumor activity on breast cancer cells [110,111], caused by inducing the dephosphorylation of estrogen receptor alpha through stimulation of protein phosphatase 2A activity [112]. Nevertheless, at present no direct evidence to approve the antiestrogenic pathway of CLA contribute to its anti-obesity functions.

Retinoids modulate various biological functions, such as cell differentiation, proliferation and embryonic development, through specific nuclear receptors- RAR and RXR, and their endogenous ligands are all-trans-retinoic acid and 9-cis-retinoic acid, respectively [113-116]. We convinced that CLA induce apoptosis of 3T3-L1 preadipocyte, through reduced mRNA expression of cyclin D (see figure 3) and increased mRNA expression of p53, GADD45, and p21waf-1, but do not activate RAR in a reporter gene assay.

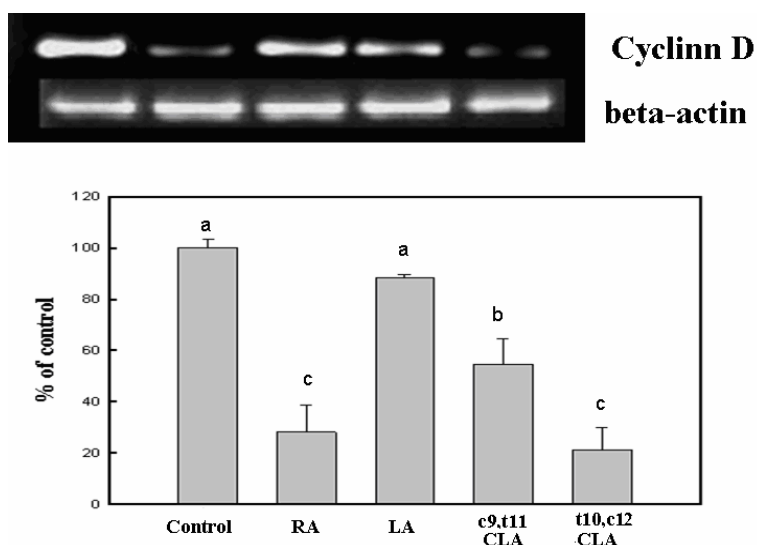


Figure 3. Effects of 10 μ M RA ,200 μ M LA , c9t11- CLA and t10c12- CLA on cyclin D mRNA expression. 3T3-L1 preadipocytes treated for 6hr. Results are expressed as mean \pm S.D. for n=3 each group ($P < 0.05$).

Immune viewpoint: CLA on inflammation and oxidative stress

Metabolic and immune responses are highly integrated and interdependent. In obesity and diabetes is now firmly links between inflammatory mediators. Peripheral blood mononuclear cells in obesity are in a proinflammatory state with an increase in

intranuclear NFκB binding [117]. NFκB plays a key role in inflammatory and immune responses [118]. TNFα and adiponectin are antagonistic in stimulating NFκB activation. In 3T3-L1 adipocytes model, NGF, a neurotrophin show as an important inflammatory response protein. NGF secrete in WAT, synthesis being influenced by TNFα [119]. In response to proinflammatory cytokines such as TNFα, the IκB kinase is activated, further stimulate the formation of additional inflammatory cytokines, along with adhesion molecules which promote endothelial dysfunction and downstream modulate specific metabolic syndrome. TNFα induces oxidative stress, which leading to oxidized low-density lipoprotein and dyslipidemia, insulin resistance, hypertension, endothelial dysfunction, and atherogenesis [120].

Oxidative stress levels are elevated in obesity correlated with fat accumulation in humans and mice [121], ROS increased selectively, augmented expression of NADPH oxidase and decreased expression of antioxidative enzymes, dysregulated production of adipocytokines, and those are important pathogenic mechanisms of obesity-associated metabolic syndrome [122]. Overall, serum concentrations of CRP, TNFα and IL-6 were significantly correlated with weight, BMI, and visceral adipose tissue [48,49,123,124]. Leptin expression is accelerated at obesity state, similar to other proinflammatory cytokines, it promotes Th1 cell differentiation and cytokine production [125]. Leukocyte populations within adipose tissue, which may be involved in the development of inflammation that is characteristic of obesity [126].

Both the innate and acquired immune responses are affected by dietary CLA supplementation. *In vitro* studies of the use of immune cells and animal models demonstrate that CLA modulates immune function, it enhance IL-2 production, lymphocyte proliferation, but decrease TNFα and IL-6 production [127]. We also confirmed the effect of CLA isoforms on TNFα production in human monocyte assay, and c9t11-CLA seems work more effectively, see figure 4. There is a slight but significant difference between the functionalities of triglyceride and free dietary CLA on modulate immunoglobulin and various cytokine productions [41]. A Double-blind, randomized, parallel, reference-controlled intervention study on human immune function, CLA may beneficially affect the initiation of a specific response to a hepatitis B vaccination [128].

Nitrolinoleic acid, LNO2 are formed via nitric oxide-dependent oxidative inflammatory reactions, is a potent endogenous ligand for PPARγ. NO-mediated cell signaling reactions can be transduced by FA nitration products and PPARs dependent gene expression [129]. CLA in endotoxin LPS-activated inflammatory events in macrophages (for enhance TNFα production), negatively regulating expression of inflammatory mediators [130], decrease both PGE2 and NO synthesis by suppressing transcription of COX-2 and iNOS [131]. Report reveal that CLA significantly (P<0.05) depressed rat PGF synthesis

in placenta, uterus and liver [132]. On the other hand, results also indicated that the t10c12- CLA supplementation led to a marked increase in human plasma CRP, a markers of inflammation and oxidative stress compared with the placebo [76].

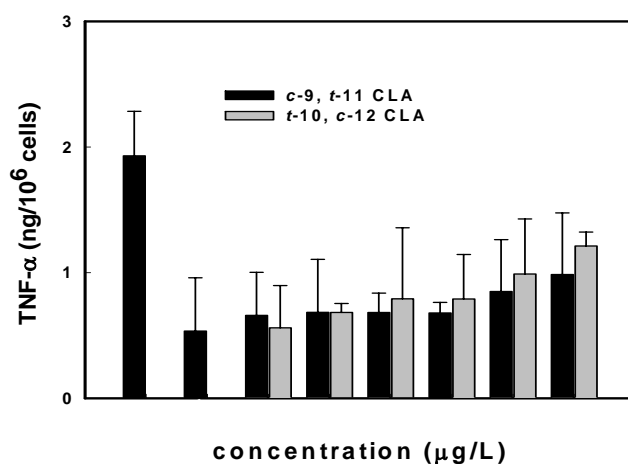


Figure 4. Production of TNF α by human monocytic THP-1 cells treated with CLA. The concentration of TNF α in the cell supernatant was measured by ELISA.

The adipokine resistin displays potent proinflammatory properties by strongly up-regulating IL-6 and TNF α , which were abrogated by NF κ B inhibitor[133]. The t10c12-CLA performed as an antioxidant, promotes at least in part, NF κ B activation and subsequent induction of IL-6, which are partly responsible for suppression of PPAR γ target gene expression and insulin sensitivity in mature human adipocytes [134]. On the other hand, c9t11-CLA possessed weak antioxidant activity, whereas at 200 μ M higher concentration, it act as a strong pro-oxidant [135]. Take together, it is likely that CLA modulate the accumulation of arachidonic acid in phospholipids, resulting in a reduced arachidonic acid pool and reduced production of downstream PGE₂, and following anti-inflammation [34].

V. Conclusion

Either ageing or hypertrophy is associated with increased body fat and insulin resistance, and with a higher risk for cardiovascular diseases. What happen in obese tissue? Here, we propose that obese tissue may activated via cell-cell interaction due to sustainable cell proliferation and size enlargement, which trigger progressive inflammation, related adipokine secreting from adipocyte, such as resistin displays potent proinflammatory properties, and induce mitochondrial dysfunction as well as

metabolic syndrome later. Those complicated mechanisms are involved to immune response, gene expression and intracellular signal transduction, which CLA or its specific isoform somehow, partly perform positive biological activity.

However, review updated research data, from *in vitro* to *in vivo* investigation, even in human clinic study, including functions, mechanisms, and safety of dietary CLA, we must conclude that it is still insufficient and ambiguous at present. Despite conflicting results, likely due to large variability in protocols used, the use of CLA for weight management in human should be under medical control. Meanwhile noteworthy, the question of the safety of high dosage dietary CLA supplements is raised. In addition, development of a commercial available functional food- CLA in soft gelatin capsules which is maybe not the best way to fit the consumer taste demand and to have the best bioavailability of the active form. Finally, a new approach to this problem may be a low glycemic index diet, refers to the effect of food on blood sugar and insulin level after a meal. It may be a practical and safe approach to the prevention and treatment of obesity and related complications.

VI. References

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