

Mechanisms for conjugated linoleic acid-mediated reduction in fat deposition

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ABSTRACT: Potential mechanisms for the decreased fat deposition observed after oral administration of conjugated linoleic acids (CLA) to mice, rats, hamsters, humans, and pigs will be reviewed. Most mechanisms are based on experiments with rodents or rodent-derived cells. Administration of CLA results in an increased metabolic rate in intact mice, but not in rats or sows. There is a decreased respiratory quotient in mice and rats, suggesting increased fat oxidation. Bovine milk-fat synthesis is decreased. Rat adipocyte size is smaller, but cell number is unchanged. In mice, there is increased adipocyte apoptosis. In 3T3-L1 preadipocytes, a clonal cell line derived from rodents, CLA decreases proliferation. Human, but not porcine, preadipocyte proliferation was inhibited by CLA. Differentiation of 3T3-L1 preadipocytes was diminished by CLA in two laboratories, but increased in a third laboratory. In porcine and human preadipocytes, CLA acutely increased lipid deposition, but lipid content quickly reached a plateau. Peroxisome proliferator-activated receptor- γ (PPAR γ), a key transcription factor in adipo-

cyte differentiation, requires an activating ligand; CLA are ligands for PPAR γ . The concentration of PPAR γ mRNA increases during adipocyte differentiation. In CLA-treated differentiating preadipocytes in culture, the PPAR γ mRNA concentration was decreased, increased, or not significantly changed, providing little evidence for modulation of differentiation through this mechanism. However, CLA might act as an agonistic or antagonistic ligand for PPAR γ to control differentiation. The primary CLA isomer in ruminant tissues is *cis* 9, *trans* 11-CLA. Most synthetic CLA preparations contain a considerable amount of *trans* 10, *cis* 12-CLA, in addition to 9,11-CLA. The 10,12-CLA is responsible for the body composition changes in mice and for the decreased bovine milk-fat synthesis. The two CLA isomers equally reduced lipid deposition in porcine preadipocytes, whereas there is evidence for both a preferential effect of 10,12-CLA and no isomer distinction in human preadipocytes. Elucidation of the mechanism(s) for a CLA-mediated reduction in fat deposition remains elusive and may be species-specific.

Key Words: Adipocytes, Dienoic Fatty Acids, Differentiation, Energy Metabolism, Lipid Metabolism, Tissue Proliferation

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Introduction

Conjugated linoleic acids (CLA) are geometric and positional isomers of the 18-carbon fatty acid with two *cis* double bonds, linoleic acid (C18:2; *cis*9, *cis*12). Among the many CLA isomers, the two isomers most studied for their biological effects are *cis* 9, *trans* 11-CLA (9,11-CLA), the predominant isomer produced in ruminant mammals, and *trans* 10, *cis* 12-CLA (10,12-CLA), one of the two predominant isomers, along with 9,11-CLA in most synthetic CLA preparations. The CLA have many interesting and unique biological properties compared to the common unsaturated fatty acids. Among the biological properties of CLA is the reduction of fat deposition in various mammalian species and chickens. Chemical and biological properties of CLA have been reviewed (Pariza et al., 2000, 2001; Yurawecz et al., 1999).

Before exploring potential mechanisms that might contribute to the CLA-mediated decrease in fat deposition, it is important to understand that there are conflicting data regarding some of the variables measured. This is not unexpected, because studies both *in vitro* and *in vivo* have many subtle as well as and not-so-subtle differences. In animal studies, the species varies, and, even within species, the breed or strain varies. The age, sex, and husbandry conditions (cage/building type and size, temperature, lighting regime, etc.) may all be quite variable. The composition of the feed is variable, even within species. Preparations diverge in percentage of CLA, CLA isomer composition and concentration, and the concentrations of other fatty acids. In cell culture, the cell type, medium composition, exogenous additions, and timing of various additions to the culture are highly variable. Finally, the choice of measurement times (blood sampling, tissue harvest, cell harvest from cultures, etc.) is extremely variable. For measurement of some variables, the timing may be critical (e.g., the changes in some mRNA and some enzymes are transient).

Fat Deposition

It might be expected that the response to CLA would differ among species because the patterns of lipid metabolism are species-specific. This variability includes sites of *de novo* fatty acid synthesis, composition of lipids and lipoproteins, concentration and dynamics of individual lipoproteins, endocrine and genetic regulation of lipid metabolism, and depot sites for fat deposition. In spite of these qualifications, a CLA-mediated reduction in fat deposition has been observed in several species.

In mice fed CLA, there was reduced fat deposition at multiple sites. The effects have been observed in many laboratories, in several strains, and in both male and female mice.

The reduction in fat depot weight is approximately 50% after feeding 0.5 or 1.0% CLA for 4 wk (Park et al., 1997; West et al., 1998; Miner et al., 2001). In one study where mice were fed CLA for 5 mo, the fat weights in several depots were <5 to <30% of the fat weights in the control animals (Tsuboyama-Kasaoka et al., 2000).

Fat deposition decreased in rats fed CLA (Azain et al., 2000; Stangl, 2000; Yamasaki et al., 1999). In one study using both male and female rats, the decrease in fat deposition was restricted to female rats, with little or no response in male rats (M. J. Azain, personal communication). Reduced fat deposition has also been reported in hamsters (de Deckere et al., 1999) and chickens (Simon et al., 2000; Szymczyk et al., 2001).

In pigs, feeding of CLA caused a reduction in fat deposition (Dugan et al., 1997). Several additional studies, usually but not always conducted during the latter portion of growth (–50 kg to –100 kg), confirmed the decrease in fat deposition (Ostrowska et al., 1999b;

O'Quinn et al., 2000a,b) (F. R. Dunshea, personal communication). One study (Ostrowska et al., 1999b) clearly indicated that the decrease in fat deposition was related to the dose of CLA fed. Several recent pig feeding trials detected only a slight decrease or no decrease in fat deposition (M. J. Azain; L. A. Gatlin and J. Odle; R. L. Thiel-Cooper and F. C. Parrish; each by personal communication). No distinguishing feature in any of these trials suggests a reason for the lack of a substantial CLA effect. Perhaps in strains of pigs with reduced backfat thickness, further reduction does not occur to preserve the important metabolic functions of adipose tissue, or a reduction is not detectable. Regardless, a no-effect response is expected in a certain percentage of cases when an exogenous compound is administered. Such a lack of effects probably is the consequence of variables that are not controlled between trials, and may lead to less than the initial fervor for the positive responses. I would judge that "the jury is still out" regarding the pragmatic efficacy of CLA in pigs, because both positive and no-response results have been observed. Certainly, CLA is efficacious in pigs under some circumstances, as indicated by multiple positive results, and especially by the dose-related response.

In human adults fed CLA for periods up to 100+ d, the decrease in both weight and body fat was marginal or nonexistent (Berven et al., 2000; Blankson et al., 2000; Zambell et al., 2000) (M. W. Pariza, personal communication). Compared to animal studies in several different species, the dose of CLA was low in human trials, being 3 to 4 g/d in most trials and <7 g/d at the highest concentration administered. A 70-kg human with a CLA intake of 3 or 4 g/d can be compared to a 70-kg pig. The 70-kg pig is expected to eat 2.5+ kg/d, so that at 1% CLA in the diet, the CLA intake would be 25+ g/d. Even at 0.25% CLA, the lowest CLA dose used in experiments with multiple species, the pig would take in 6+ g CLA/d; the 0.25% CLA dose usually does not elicit a response in animal studies and is above the dose in most human trials. Animal trials for testing effects of CLA on fat deposition are in growing animals, whereas the human studies are in adults; the question in human studies is whether CLA administration will decrease existing fat depots, whereas the question in animal studies is whether CLA will decrease deposition of fat in growing animals. Of course, in growing humans, the question also is whether CLA will decrease fat deposition. I see no evidence of efficacy for CLA use in humans to lower fat stores, at least with the CLA doses used.

Isomers of CLA

There is increasing evidence that the CLA isomer causing changes in fat accretion and lipid metabolism is 10,12-CLA and not 9,11-CLA (Martin et al., 2000; Park et al., 1999; K. Hargrave and J. L. Miner, personal communication). In addition, milk-fat in dairy

cows was decreased by 10,12-CLA rather than by 9,11-CLA (Baumgard et al., 2000). Lipoprotein lipase (**LPL**) activity in 3T3-L1 cells was decreased by treatment of the cells with 10,12-CLA but not by treatment with 9,11-CLA (Lin et al., 2001; Park et al., 1999). As additional enzymatic or receptor systems are studied, it might be expected that all will not exhibit specificity for a single CLA isomer. Furthermore, because of species variation in protein structure, the isotopic specificity of a particular enzyme or receptor may be species-specific.

Mechanisms

Preadipocyte Proliferation

One mechanism to increase fat deposition is to increase the number of adipocytes. Differentiated adipocytes do not proliferate, but preadipocytes proliferate and have the potential to differentiate and then fill with lipid to expand a fat depot. In rodent-derived clonal preadipocytes (3T3-L1 cells), cell proliferation, measured by cell counts and DNA synthesis, was decreased 10 to 50% by treatment with <25 to 100 μM CLA in a dose-related fashion. This has been observed in three different laboratories (Brodie et al., 1999; Satory and Smith, 1999; Evans et al., 2000). In human preadipocytes treated with 50 μM CLA in culture, there was a 25% decrease in cell proliferation (R. L. McNeel and H. J. Mersmann, unpublished data). The synthesis of DNA, during incubation in vitro of adipose tissue removed from pigs fed CLA, was decreased (V. L. Adams and S. B. Smith, personal communication). On the other hand, cell proliferation in human preadipocytes treated with 10 μM CLA was not decreased (J. M. Brown and M. McIntosh, personal communication). In cultured porcine preadipocytes treated with 50 μM CLA, there was no evidence of a reduction in cell proliferation (R. L. McNeel and H. J. Mersmann, unpublished data). Thus, preadipocyte proliferation is decreased by CLA in some circumstances. There is little evidence regarding this mechanism in vivo, although cell number in adipose depots was not decreased in rats fed CLA (Azain et al., 2000).

Preadipocyte Differentiation

Adipocyte differentiation has been reviewed multiple times (Brun et al., 1997; Mandrup and Lane, 1997; Fajas et al., 1998). The generally accepted model for adipocyte differentiation indicates that when preadipocytes are presented with the appropriate milieu of differentiation factors, i.e., growth factors and hormones, they begin to differentiate. Initially, the transcription factors, CCAAT/enhancer binding protein β (**C/EBP β**) and C/EBP δ , are increased. These factors stimulate an increase in another transcription factor, peroxisome proliferator-activated receptor γ (**PPAR γ**), which in turn causes an increase in C/EBP α . Genes

for several proteins characteristic of the adipocyte phenotype have response elements for either PPAR γ or C/EBP α so that transcription of these phenotypic genes is initiated to increase differentiation. After translation of genes for anabolic adipocyte lipid metabolism, the cells begin to fill with lipid.

The transcription factor, PPAR γ , does not function alone, but forms a heterodimer with another transcription factor, retinoid \times receptor α (**RXR α**). The PPAR γ -RXR α heterodimer must be activated. The nature of the physiological activator of PPAR γ is unclear, but fatty acids bind to PPAR γ and activate it (Tontonoz et al., 1994; Kliewer et al., 1997). Because CLA bind to PPAR γ and activate it (Houseknecht et al., 1998; Belury and Vanden Heuval, 1999), they may function, at least in part, as ligands for PPAR γ .

If fewer preadipocytes differentiate into mature adipocytes, there would be a decrease in the population of cells with the potential to fill with lipid and consequently expand the fat mass. Two laboratories observed a dose-related decrease in preadipocyte differentiation of CLA-treated rodent-derived 3T3-L1 clonal cells (Brodie et al., 1999; Evans et al., 2000; J. M. Brown and M. McIntosh, personal communication). Differentiation was measured by accumulation of oil red O-stained material (**OROSM**), glycerol-P dehydrogenase activity, cell size, or triacylglycerol concentration. A decrease in these differentiation end points could also represent decreased triacylglycerol synthesis in already differentiated cells. Human preadipocyte differentiation, using a thiazolidinedione compound as a PPAR γ ligand, was inhibited by treatment with 10 μM CLA, but treatment with 100 μM CLA did not inhibit differentiation (Brown et al., 2001b; J. M. Brown and M. McIntosh, personal communication). These latter data suggest the dose relationship for CLA effects on differentiation may not be linear or hyperbolic, but more complex in nature. Differentiation of porcine preadipocytes was not affected by addition of 5 to 35 μM CLA (C.-Y. Hu, personal communication).

The mRNA concentrations for the key transcription factors in adipocyte differentiation, PPAR γ and C/EBP α , were decreased in 3T3-L1 cells differentiating in the presence of CLA (Brodie et al., 1999), although researchers in another laboratory did not observe a decrease in PPAR γ mRNA concentration (Evans et al., 2001). The transcription factor, sterol regulatory element binding protein 1c, also known as adipocyte determination and differentiation-dependent factor 1 (**ADD1**), is also implicated in adipocyte differentiation. It increases PPAR γ and enzymes associated with fatty acid and triacylglycerol synthesis (e.g., fatty acid synthase and glycerol-phosphate dehydrogenase). An increase in fatty acids provides potential ligands for PPAR γ , as well as substrates for membrane and triacylglycerol biosynthesis (Kim and Spiegelman, 1996; Kim et al., 1998). Porcine preadipocytes treated with CLA had reduced ADD1 mRNA and protein concen-

trations (S. T. Ding and H. J. Mersmann, unpublished data).

One laboratory (Satory and Smith, 1999) indicated an increase in preadipocyte differentiation of 3T3-L1 cells measured by glucose incorporation into lipids and verified by OROSM. There is no obvious cause for the opposite effects in different laboratories using the same clonal cell line (i.e., CLA-induced inhibition vs stimulation of differentiation). The use of ethanol as a vehicle to add CLA to the culture medium by Satory and Smith (1999) was suggested as a cause for the stimulation of lipid synthesis in CLA-treated cells (Satory and Smith, 1999). I do not believe the presence of ethanol can explain this observation because the stimulation is related to the CLA concentration and ethanol was present at a low and constant concentration in all plates, including the controls. Furthermore, in porcine stromal-vascular cells (i.e., preadipocytes), addition of 50 μM CLA (without ethanol) plus differentiation factors caused a 5+ times increase in OROSM after 2 d, compared to the control cells without CLA (R. L. McNeel and H. J. Mersmann, unpublished data). The OROSM did not continue to increase at 5 or 7 d in the CLA-treated porcine cells as it did in the control cells. Human preadipocytes treated with differentiation medium + CLA also acutely accumulated OROSM (R. L. McNeel and H. J. Mersmann, unpublished data). In this case, the human control cells, i.e., without CLA, did not differentiate at all. In both porcine and human preadipocytes, there was a very slight numerical, but not statistical, increase in PPAR γ mRNA concentration at the early stages of differentiation when OROSM was dramatically increased. I propose that the stimulation of the porcine and human cell accumulation of OROSM results from the CLA acting as a ligand for PPAR γ at the early stages of differentiation when the capacity to synthesize PPAR γ ligands (perhaps fatty acids or fatty acid derivatives) is limited. This hypothesis is supported by the observations that CLA can activate PPAR (Houseknecht et al., 1998; Belury and Vanden Heuval, 1999); that the PPAR γ mRNA (Ding et al., 1999) and protein (Kim et al., 2000) are both present in porcine preadipocytes at initiation of differentiation; and that human preadipocytes, in specific culture conditions, do not differentiate unless an exogenous PPAR γ ligand is added (Zen-Bio Inc., Research Triangle Park, NC; verified by R. L. McNeel and H. J. Mersmann, unpublished data).

At this time, the possibility cannot be excluded that the observed increase in OROSM does not represent differentiation, but is the result of uptake of CLA by the porcine and human cells with subsequent incorporation into triacylglycerol, using existing enzymatic machinery and measured as OROSM. If this hypothesis is valid, the capacity to synthesize triacylglycerol must be considerable at the initiation of differentiation, because most cells have visible OROSM at 2 and 7 d for porcine and human cells, respectively. The

hypothesis is not supported by the low amount of glycerol-P dehydrogenase activity (the initiating enzyme for triacylglycerol synthesis) at the beginning of differentiation in porcine preadipocytes (Suryawan et al., 1997).

I also speculate that the reason CLA does not continue to stimulate differentiation in porcine preadipocytes is that as the cells differentiate, they have increased capacity to synthesize long-chain fatty acids that are more potent and (or) more efficacious ligands for PPAR γ . Under this circumstance, the CLA becomes an inhibitor through competitive binding to PPAR γ . The human preadipocytes have only limited or no capacity to synthesize fatty acids. However, the same type of competition with CLA is possible if the cells' capacity to take up fatty acids from the medium increases during differentiation, as expected by the increase in LPL during differentiation. In 3T3-L1 cells differentiated with a thiazolidinedione PPAR γ ligand, CLA inhibited the differentiation, and the CLA inhibition was reversed by addition of linoleic acid (Brown et al., 2001a; Evans et al., 2001). These observations are compatible with the concept of competition between CLA and other ligands, including fatty acids, for PPAR γ . Another possible mechanism for CLA-mediated inhibition of differentiation is a decrease in ADD1 as observed in differentiating porcine preadipocytes (S. T. Ding and H. J. Mersmann, unpublished data).

Overall, I conclude that CLA decreases preadipocyte differentiation, at least under some circumstances. Certainly there is evidence to the contrary and even evidence that CLA might stimulate differentiation under some circumstances. There is little or no evidence for CLA modulation of differentiation in vivo, but the observations of decreased fat deposition in vivo suggest that there is no evidence for continuous CLA-mediated stimulation of differentiation in vivo. Of course, the pig growth trials with no change in fat deposition in the presence of CLA suggest that if decreased differentiation is a mechanism in vivo, it may not be consistent, at least in pigs.

Energy Expenditure

A potential mechanism, operative at the whole-animal level, to decrease fat deposition would be an increase in energy expenditure. To be effective, this would have to occur without increased feed consumption. In mice fed both low-fat and high-fat diets, CLA treatment caused an increase in energy expenditure (West et al., 1998, 2000). Several other laboratories reported no change in energy expenditure in mice (Tsuboyama-Kasaoka et al., 2000; Miner et al., 2001), rats (Azain, et al., 2000), or pigs (Muller et al., 1999, 2000).

Although not clearly established, uncoupling protein 2 may function to disconnect energy conservation from substrate oxidation. Thus, heat production

would increase and energy conservation would decrease. This protein was increased in white and brown adipose tissue from mice fed CLA (Tsuboyama-Kasaoka et al., 2000); however, in another study, the protein was increased in brown, but not white adipose tissue (West et al., 2000).

Increased energy expenditure may be a mechanism to decrease fat deposition in some CLA-treated animals, but it does not appear to be a universal mechanism operative across species or even a consistent mechanism in mice.

Fatty Acid Oxidation

An increase in fatty acid oxidation might be expected to reduce the availability of fatty acids for synthesis of triacylglycerol, and consequently decrease fat deposition. A decreased respiratory quotient (**RQ**) represents increased fat oxidation. A decreased RQ was observed in mice fed a low-fat diet containing CLA, but not in mice fed a high-fat diet containing CLA (West et al., 1998). In pigs and sows, dietary CLA did not affect the RQ (Muller et al., 1999, 2000). Carnitine palmitoyltransferase is the enzyme responsible for initiation of the transport of fatty acids into the mitochondrion for oxidation. Activity of this enzyme was increased in white and brown adipose tissue of rats fed CLA (Rahman et al., 2001) and in adipose tissue and muscle of mice fed CLA (Park et al., 1997). As with many potential mechanisms, the evidence for increased fatty acid oxidation being a factor in the CLA-mediated reduction in fat deposition is restricted to individual studies.

Adipose Tissue Lipid Synthesis

Because much of the growth of adipose tissue, in many species, is by cell hypertrophy resulting from increased accretion of triacylglycerol in adipocytes, inhibition of adipocyte lipid synthesis would decrease fat deposition. There was decreased milk fat in dairy cows (Loor and Herbein, 1998; Chouinard et al., 1999; Baumgard et al., 2000) and sows (M. J. Azain, personal communication) fed CLA. There was decreased glucose incorporation into adipose tissue lipids in average-lean, but not high-lean pigs fed CLA (M. Heckart, S. Donkin, and S. Mills, personal communication). The activity of glycerol-P dehydrogenase and (or) the amount of triacylglycerol deposited was decreased in CLA-treated 3T3-L1 cells (Brodie et al., 1999; Evans et al., 2000; J. M. Brown and M. McIntosh, personal communication). The activity of phosphatidate phosphohydrolase, perhaps the rate-limiting enzyme in triacylglycerol synthesis, was decreased in adipose tissue of CLA-fed rats (Rahman et al., 2001). In support of these observations of decreased lipid synthesis, the transcript concentrations for fatty acid synthase and acetyl-CoA carboxylase were decreased in adipose tissue from mice fed CLA (Tsuboyama-Kasaoka et al.,

2000), and the fatty acid synthase mRNA was decreased in adipose tissue from average-lean, but not high-lean pigs fed CLA (M. Heckart, S. Donkin, and S. Mills, personal communication).

Lipoprotein lipase (LPL) is synthesized in the adipocyte and migrates to the endothelial cell surface, where it functions to cleave fatty acids from circulating lipoproteins. The fatty acids can then enter the adipocyte to be oxidized or to serve as building blocks for complex lipid synthesis, including the storage depot lipid, triacylglycerol. A decrease in LPL activity would decrease the fatty acid available for triacylglycerol synthesis and thus would decrease lipid deposition. Although very low concentrations of CLA cause an increase in LPL activity in 3T3-L1 cells, higher concentrations inhibit the enzyme activity (Park et al., 1997, 1999; Lin et al., 2001). Plasma triacylglycerol concentration was increased in pigs fed CLA, suggesting less uptake of lipids (Ostrowska et al., 1999a).

Mouse (Tsuboyama-Kasaoka et al., 2000), rat (Azain et al., 2000), and pig (V. L. Adams and S. B. Smith, personal communication) adipocyte cell size was decreased in animals fed CLA. A decrease in adipocyte size suggests a decrease in anabolic lipid metabolism in those cells.

There are data that do not support the concept of decreased adipose tissue lipid synthesis. There was no decrease in deuterated water incorporation into lipids *in vivo*, in mice fed CLA (West et al., 2000). Also, there was no decrease in adipose tissue malic enzyme or glucose-6P dehydrogenase activities (enzymes supporting *de novo* fatty acid biosynthesis) in rats fed CLA (Rahman et al., 2001). Although LPL activity decreased in 3T3-L1 cells treated with 100 μM CLA, it increased in cells treated with 10 μM CLA (Lin et al., 2001). Glucose incorporation into lipids increased in 3T3-L1 cells treated with CLA (Satory and Smith, 1999), and lipid deposition increased in porcine and human preadipocytes *in vitro* (R. L. McNeel and H. J. Mersmann, unpublished data).

Overall, there is a great deal of evidence that lipid synthetic processes are decreased in adipose tissue from CLA-treated animals or cells. This suggests that genetic or metabolic regulation of these synthetic pathways may provide a mechanism by which CLA decreases fat deposition, at least in many situations. It does not exclude the possibility that all of these effects are secondary responses and not the primary mechanism by which CLA lowers fat deposition.

Lipolysis

The sequential release of fatty acids from triacylglycerol, or lipolysis, is the highly regulated process of lipid degradation in the adipocyte. Increased lipolysis would be expected to lead to decreased fat deposition. The lipolytic rate of CLA-treated 3T3-L1 cells was almost double that of control cells (Park et al., 1997, 1999). Plasma nonesterified fatty acid concentration

was elevated in pigs fed CLA, suggesting an increased rate of lipolysis (Ostrowska et al., 1999a). Acute incubation of human preadipocytes with CLA did not increase the lipolytic rate, although isoproterenol stimulated lipolysis in these cells, indicating they had the capacity for lipolysis (J. M. Brown and M. McIntosh, personal communication). Increased lipid degradation may contribute to decreased fat deposition in CLA-treated cells or animals, but at this time, there are only a few studies.

Stearoyl-CoA Desaturase

This enzyme catalyzes the conversion of long-chain saturated fatty acids to monounsaturated fatty acids. The usual substrates are palmitic acid (C16:0) and stearic (C18:0) acids, which are converted to palmitoleic acid (C16:1) and oleic acid (C18:1), respectively. The mRNA concentration for stearoyl-CoA desaturase (SCD) was decreased in mice fed CLA (Lee et al., 1998). The mRNA concentration was also decreased in average-lean, but not in high-lean pigs fed CLA (M. Heckart, S. Donkin, and S. Mills, personal communication). Numerous studies demonstrated modified fatty acid composition in various tissues of animals fed CLA or cells treated with CLA. There was either increased C16:0 and/or C18:0 concentration coupled with decreased C16:1 and/or C18:1 concentration, or a decreased C16:1 to C16:0 and/or a decreased C18:1 to C18:0 ratio. This modification in fatty acid composition strongly suggests inhibition of SCD activity in 3T3-L1 cells (Satory and Smith, 1999), mice (Lee et al., 1998), rats (Yamasaki et al., 1999; Azain et al., 2000), pigs (O'Quinn et al., 2000a) (L. A. Gatlin and J. Odle; V. L. Adams and S. B. Smith, personal communications), and in dairy cow (Lor and Herbein, 1998; Chouinard et al., 1999; Baumgard et al., 2000) and sow (Bee, 2000) milk. The decrease in monounsaturated fatty acids coupled with an increase in saturated fatty acids is expected to decrease membrane fluidity, and could modify the function of multiple membrane bound structural and enzymatic proteins. Thus, inhibition of SCD may be a primary mechanism in CLA-fed animals, initiating many of the other metabolic changes observed in specific aspects of metabolism of the adipocyte or of other tissues.

There is evidence of inhibited SCD in both male and female rats fed CLA (M. J. Azain, personal communication). However, in these studies, only the female and not the male rats had decreased fat deposition after feeding CLA. This observation suggests that inhibition of SCD may not be pivotal to the mechanism for decreasing fat deposition in CLA-fed animals.

Eicosanoids

These molecules are powerful, positive and negative regulators of multiple aspects of metabolism. Linoleic acid (C18:2- *cis* 9, *cis* 12) is metabolized to arachidonic

acid (C20:4-*cis* 5, *cis* 8, *cis* 11, *cis* 14), a major precursor of eicosanoid molecules. One might postulate that CLAs (*cis* 9, *trans* 11-C18:2 or *trans* 10, *cis* 12-C18:2) would be metabolized in a fashion similar to linoleic acid (*cis* 9, *cis* 12-C18:2). For example, 10,12-CLA might be metabolized to the arachidonic acid analog, C20:4-*cis* 5, *cis* 8, *trans* 12, *cis* 14. Given this hypothesis, linoleic acid and CLA would compete for the same enzymes. In animals deficient for linoleic acid, it would be expected that more CLA would be converted to eicosanoid molecules. If eicosanoids are the mediators of CLA effects, the response to CLA would be expected to be greater in animals that are linoleic acid-deficient than in control animals with adequate linoleic acid. In mice deficient for linoleic acid, the CLA treatment led to a greater decrease in fat deposition than in control mice, supporting the hypothesis that eicosanoid molecules mediate at least some of the CLA effects (K. Hargrave and J. L. Miner, personal communication). At this time, there are too few data to firmly conclude that the mechanism for the CLA-mediated reduction in fat deposition is mediated through eicosanoids.

Leptin

This protein is produced by the adipocyte, is secreted into the plasma, and provides satiety signals to the hypothalamus. The amount of leptin produced is related to the size of the adipocyte, and the plasma concentration is related to the adipose tissue mass. Plasma leptin was reduced in mice (DeLany et al., 1999), rats (Rahman et al., 2001), and humans (Medina et al., 2000) treated with CLA. However, there is little evidence of increased feed consumption in CLA-treated mice or rats, as might be expected when the plasma leptin concentration is lowered. My interpretation is that the decreased leptin concentrations result from the decreased adipocyte size and/or decreased adipose tissue mass present in CLA-treated animals.

Apoptosis

Increased adipocyte apoptosis, the process of cellular degradation, could contribute or even be a primary cause for the decreased fat deposition in CLA-treated animals. Mice treated with CLA for 7 to 9 d had increased adipocyte DNA fragmentation, an evidence for increased apoptosis (Tsuboyama-Kasaoka et al., 2000; Miner et al., 2001). Confluent 3T3-L1 clonal preadipocytes treated with 100 μ M CLA had more cells with condensed nuclei, also an evidence for increased apoptosis (Evans et al., 2000). Increased apoptosis may be a fundamental mechanism to yield an apparent decrease in cell proliferation and differentiation, and ultimately to decrease fat deposition in CLA-treated animals or cells. A word of caution must be inserted, because CLA can be quite toxic to cells,

as observed by several investigators. Thus, at some concentrations, CLA may produce a toxic environment, so that increased apoptosis is a secondary mechanism or perhaps an artifact. The role of CLA toxicity to cells *in vivo* has not been investigated.

Tumor Necrosis Factor- α

Tumor necrosis factor- α (TNF α) is a major mediator of the inflammatory response, and has multiple biological effects. Among these effects are decreased adipocyte differentiation (Petuschke and Hauner, 1993), increased lipolysis, and decreased LPL activity (Hauner et al., 1995). The TNF α mRNA concentration was decreased in adipose tissue from mice fed CLA, and might be a driving force for the increase in apoptosis (Tsuboyama-Kasaoka et al., 2000). A decrease in TNF α would be expected to decrease lipolysis and increase LPL activity; potentially, both of these effects would produce more fat deposition, not less. Whether TNF α has a primary role in the CLA-mediated decrease in fat deposition awaits further investigation.

Conclusions

At this time, there is evidence for multiple mechanisms to execute the CLA-mediated reduction in fat deposition observed in numerous species and in cultured cells. The evidence, so far, does not indicate a primary mechanism to explain the effects of CLA on fat deposition. Some mechanisms are found only in a single species or even in a single laboratory, suggesting they probably are not universal mechanisms, but may be applicable only under specific conditions. Although a potential mechanism, such as decreased adipocyte lipid synthesis, that is, *de novo* fatty acid synthesis, triacylglycerol synthesis, lipoprotein lipase activity, is observed in a considerable number of studies across species, there are some negative results. Furthermore, a complex and multiple-step process such as lipid synthesis is probably not the focus for a primary mechanism in cells and animals treated with CLA. The CLA-mediated modulation of lipid synthesis probably is an effect rather than a cause. The body of evidence to support several possible mechanisms (decreased plasma leptin, increase adipocyte apoptosis, increased plasma TNF α) is very small at this time. The generalized mechanism for CLA-mediated effects on fat deposition may lie in a more fundamental step, such as modulation of the transcription factors, PPAR γ or ADD1, which in turn, could affect multiple metabolic pathways. The inhibition of SCD, perhaps controlled by PPAR γ , is an interesting possibility because it would alter membrane and complex lipid composition to potentially affect multiple metabolic processes. Species variation will have to be factored into the description of CLA-mediated decreased fat deposition. It is expected to complicate the picture

because of species-specific protein structure and function, and metabolic pathway divergence, including intra- and interorgan processing of lipids and lipoproteins.

Implications

Conjugated linoleic acid (CLA) decreases fat deposition when fed to several mammalian species and chickens. The practicality of feeding CLA to a meat-producing animal, such as the pig, is yet to be determined because there are both positive and negative data. Among the mechanisms proposed to explain the CLA-mediated decreased fat deposition are increased energy expenditure, fat oxidation, fat cell lipid degradation, or fat cell destruction. Also, decreased fat cell precursors, fat cell development, fat synthesis, or desaturation of fats could decrease fat deposition. Some mechanisms appear to be species-specific. Other mechanisms may be secondary rather than the primary cause. The primary mechanism probably resides in regulation of a few key genes that control a number of aspects of metabolism. The transcription factor, PPAR γ , is a candidate gene because it binds and is activated by CLA. However, the mechanism by which CLA reduces fat deposition remains unclear.

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