

Potential Mechanisms for CLA-Mediated Alteration of Body Composition and Lipid Metabolism

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For almost all proposed mechanisms there is supportive data, but also data that does not support the mechanism.

The multiple conclusions suggests the observed effects of CLA are contingent on the exact experimental design and conditions.

Models

Experiments that utilize a dose x response design provide stronger evidence for an effect than designs using a single concentration of the test compound.

The design is further improved if it includes a minimal dose below which there is no response and a clear maximal response.

The use of animal, cellular, subcellular, and molecular models is absolutely necessary to untangle mechanisms for the activity of an exogenous material administered to humans/animals.

The task is to distinguish between “apples and oranges” and to be very cautious about extrapolation from “apples to oranges”

Variation in Animal Studies

Species/Breed

Sex

Age

Diet

Husbandry

Timing of measurements

Laboratory Procedures

Variation in Cell and Subcellular Studies

Species/Breed

Cell Type

Culture Conditions

Timing of Measurements

Laboratory Procedures

Variation in Protein/Nucleic Acids Studies

Species

Complete or partial sequence

Details of experimental procedure

Effects of Dietary CLA

Weight: In most cases weight is not affected. However, in some cases it is decreased (particularly at the higher doses), or even increased. These changes must be factored into the interpretation of body composition changes, but seldom are great enough to totally account for the CLA effects.

Feed consumption: In most cases it is not affected. However, when it is decreased it must be considered as a cause for reduced fat deposition. Decreased feed intake does not totally account for the decrease in fat deposition.

Fat deposition. Major decrease in fat deposition in growing mammals:

mice (Park; West/DeLany; Miner, Tsuboyama-Kasaoka); rats (Yamasaki; Azain; Rahman; Sisk; Stangl; Miner); hamsters (deDeckere); chickens (Simon; Szymczyk); pigs (Dugan; Ostowska; O'Quinn; Parrish).

However, several pig studies indicate no effect or very minor changes (Azain; Odle; Parrish).

Minor or no effect in humans = low CLA dose or non-growing animal? However, mature mice have major decrease in fat (Pariza)

Mechanisms

Energy Metabolism

Increased in mice fed either high-fat or low-fat diets
(West/DeLany; Ohnuki);

No change in mice (Tsuboyama-Kasaoka: Miner);
rats (Azain); pigs (Muller)

UCP2 increased in BAT, but increased or decreased
in WAT (Tsuboyama-Kasaoka: West)

Does increased energy metabolism explain CLA-induced modification of fat deposition or is it an effect observed only in select species and perhaps under specific conditions?

Fatty Acid Oxidation

Increased in mice (West/DeLany), 3T3-L1 cells (McIntosh).

No change in pigs and sows (Muller)

Carnitine palmitoyltransferase activity increased in WAT, BAT, and muscle of mice and rats (Park; Rahman)

Is increased fatty acid oxidation a rodent-specific mechanism?

Cell Proliferation

Decreased cell number &/or DNA synthesis in 3T3-L1 cells (Smith; Hu; McIntosh), human preadipocytes (Mersmann), preadipocytes from pigs fed CLA (Smith).

No decrease in cell number in human preadipocytes treated with 10 μ M CLA (McIntosh), porcine preadipocytes (Mersmann), rat WAT after feeding CLA (Azain).

Proliferation is a continual process, but the ultimate question is, does hyperplasia make a measurable contribution to adipose tissue mass after the perinatal and early growing period – probably not except in states of prolonged excessive caloric intake.

Preadipocyte Differentiation

Decreased in 3T3-L1 cells (Hu; McIntosh), and human preadipocytes at 10 μM , but not at 100 μM CLA (McIntosh).

No effect on porcine preadipocytes #35 μM CLA (Hu).

Increased acutely in human and porcine preadipocytes (Mersmann), and chronically in 3T3-L1 cells (Smith).

Transcripts Associated with Differentiation

Decreased PPAR γ , C/EBP α , aP2 in 3T3-L1 cells (Hu), decreased FAS, AcCC, GPAT, LPL, FABP in bovine mammary gland (Baumgard/Bauman).

No change or increased PPAR γ , C/EBP α , LPL, aP2 in 3T3-L1 cells (McIntosh) and porcine and human preadipocytes (Mersmann).

Although a continual process, adipocyte differentiation probably does not make a measurable contribution to the mass of adipose tissue after weaning.

However, maintenance of the tissue probably depends on the continual presence of PPAR γ and C/EBP α .

Adipocyte Hypertrophy

Probably the major factor contributing to the increase in adipose tissue mass after weaning, except during extended excessive caloric intake.

Adipocyte size decreased by CLA: mice (Tsuboyama-Kasaoka), rats (Azain), and pigs (Smith).

Adipose Tissue Lipid Synthesis

Milk fat synthesis is decreased in cattle

(Bauman/Baumgard: Loo & Herbein) and pigs (Azain).

Decreased fat synthesis in porcine adipose tissue, but not in very lean pigs (Donkin/Mills).

Decreased triacylglycerol &/or glycerol 3-phosphate dehydrogenase activity in 3T3-L1 cells (Hu: McIntosh); human preadipocytes (McIntosh).

Decreased mRNA for:

phosphatidate phosphohydrolase in rat WAT.

fatty acid synthase in rat WAT, mouse WAT, porcine WAT, and bovine mammary gland.

acetyl CoA carboxylase in mouse WAT and bovine mammary gland.

glycerophosphate acyltransferase and acylglycerol phosphate acyltransferase in bovine mammary gland.

(Rahman: Tsuboyama-Kasaoka: Donkin/Mills:
Baumgard/Bauman)

Lipoprotein Lipase

Decreased activity in mouse WAT and 3T3-L1 cells
(Park; Lin)

Plasma triacylglycerol increased in pigs, suggesting
less uptake of FA & decreased LPL activity
(Ostrowska)

Negative Data

No decrease in WAT lipid synthesis in mice in vivo
(West/DeLany).

No decrease in lipid synthesis in WAT from treated pigs
(Bee; Smith).

Increased lipid synthesis in differentiating 3T3-L1 cells
(Smith; McIntosh).

Increased lipid deposition in differentiating porcine and
human preadipocytes (Mersmann); 9,11-CLA increased
(but 10,12-CLA decreased) in human preadipocytes
(McIntosh).

Increased LPL activity in 3T3-L1 cells at 10 μ M CLA (Lin).

Lipolysis

Lipolytic rate of 3T3-L1 cells modestly increased
(Park; McIntosh).

Plasma nonesterified fatty acids increased in pigs
(Ostowska).

However, no increase in human preadipocytes
treated with CLA (McIntosh).

StearoylCoA Desaturase

Inhibition of this enzyme is strongly implied from decreased monounsaturated and increased saturated fatty acids:

mice (Lee); 3T3-L1 cells (Smith); rats (Yamasaki; Azain); pigs (O'Quinn; Odle; Bee; Smith); milk (bovine = Baumgard/Bauman; Loor & Herbein: porcine = Bee).

Decreased mRNA:

mice(Lee); pigs, but not extremely lean (Donkin/Mills); bovine mammary gland (Baumgard/Bauman).

Decreased enzyme activity:

pigs (Smith).

Does decreased monounsaturated fatty acid concentration influence triacylglycerol (& phospholipid) synthesis?

Is inhibition of stearoylCoA desaturase a primary event in the mechanism for CLA modulation of fat deposition?

Does decreased monounsaturated (or increased saturated) fatty acid cause a decrease in triacylglycerol synthesis?

If so, inhibition of the desaturase should lead to decreased fat deposition. In cattle fed sterculic acid (a desaturase inhibitor), there is less intramuscular fat and in cattle with a great amount of desaturase (Japanese Black), there is more intramuscular fat (Smith). However, in young pigs with decreased oleic acid and desaturase activity, there is no decrease in fat (Smith).

CLA as a PPAR α Ligand

CLA binds to and activates PPAR α and PPAR γ
(VandenHeuvel/Belury).

Activated PPAR α -RXR α increases peroxisomal acylCoA oxidase and mitochondrial carnitine palmitoylCoA transferase = increased fatty acid oxidation.

May extend beyond the liver, depending on species-specific tissue distribution of PPAR α , e.g., high concentration in porcine adipose tissue (Mersmann).

However, PPAR α -null mice still respond to CLA
(Peters/Pariza)

Effects of CLA on ADD1

Adipocyte determination and differentiation-dependent factor1 (ADD1 or SREBP 1c) increases lipid synthesis enzymes (e.g., FAS and GPAT).

CLA acutely decreases ADD1 in differentiating porcine preadipocytes (Mersmann).

This effect is expected to decrease fatty acid and triacylglycerol synthesis in tissues that have ADD1 and the capacity to synthesize these lipids.

CLA as a PPAR γ Ligand

CLA binds to PPAR γ (VandenHeuvel/Belury; Mersmann).

CLA activates PPAR γ (Houseknecht).

Activation of PPAR γ should increase adipocyte differentiation as do TZD compounds: 3T3-L1 cells (VandenHeuvel/Belury; Smith); porcine and human preadipocytes (Mersmann).

How can CLA activate PPAR γ and decrease fat deposition?

1. Affinity for PPAR α > PPAR γ (VandenHeuvel).
2. The PPAR α and ADD1 mechanisms may predominate, especially in already differentiated adipocytes.
3. Although CLA is a ligand for PPAR γ and can stimulate differentiation, it may act as a competitive inhibitor if the CLA-PPAR γ -RXR α complex is not as potent or effective as other ligand-PPAR γ -RXR α complexes to bind to and activate specific response elements.

Other Potential Mechanisms

There is some evidence for these mechanisms:

Eicosanoid derivatives of CLA.

Decreased plasma leptin in mice (DeLany), rats (Rahman), and humans (Medina). Is this a cause or effect?

Increased apoptosis in mice (Tsuboyama-Kasaoka; Miner) and 3T3-L1 cells (McIntosh).

Isomers and Metabolites

Isomers: The modulation of body composition and of lipid metabolism are caused by 10,12-CLA compared to 9,11-CLA. In some cases this may not be an all or none response, but dependent on the concentration. Other isomers have not been extensively tested.

Metabolites: It is expected that at least some conjugated fatty acids can be elongated and desaturated by mammalian enzymes. The functional ligand may not be the parent molecule.

Other conjugated fatty acids: It is expected that other conjugated fatty acids would have the same effects as CLAs.

Recommended Research

Establish the mediator for 10,12-CLA to modify fat deposition and lipid metabolism. (The mediator may be a different molecule for distinct CLA effects and 9,11-CLA may modify effects of 10,12-CLA in some cases.)

Is the parent compound the active form or is it a metabolite, i.e., is it elongated &/or desaturated or is it converted to an eicosanoid-type derivative?

If it is an eicosanoid-type molecule, what are the mechanisms, i.e., are the effects as a fatty acid analog or through specific eicosanoid pathways?

Does 10,12-CLA act as a polyunsaturated fatty acid or metabolite?

as a PPAR α ligand = increased fatty acid oxidation
to decrease ADD1 = decreased fatty acid synthesis
as a PPAR γ ligand = increased fat synthesis?

What does PPAR α -null mice result mean?

How can activation of both PPAR α & PPAR γ be reconciled?

What is role of PPAR δ ?

How does PPAR γ activation lead to decreased fat?

How important is role of decreased ADD1?

What is contribution of PPAR α function in adipose tissue (at least in some species)?

Are other transcription factors modulated by CLA to mediate the observed effects?

What is the role of co-activators and repressors in the mechanism(s) for CLA effects on fat deposition and lipid metabolism?