HEPATIC GENE NETWORKS DUE TO THE TRANS-10, CIS-12-CONJUGATED LINOLEIC ACID SUPPLEMENTATION IN GROWING MALE MICE

BY

JING LI

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Nutritional Sciences
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2011

Urbana, Illinois

Master's Committee:

Professor James K. Drackley, Chair
Assistant Professor Juan J. Loor, Director of Research
Professor Walter L. Hurley
Assistant Professor Yuan-Xiang Pan

ABSTRACT

Trans-10, *cis*-12-conjugated linoleic acid (*trans*-10, *cis*-12-CLA) has been reported to reduce body fat mass but accompanied by increased liver mass. However, the cellular and molecular mechanisms have been unknown. Our study investigated the effects of *trans*-10, *cis*-12-CLA supplementation on hepatic gene expression in growing male mice by measuring mRNA abundance of thirty-eight genes encoding important enzymes, ligand-dependent nuclear receptors, transcription regulators and transporters in lipid metabolism. Feeding growing male mice diets supplemented with 0.3% *trans*-10, *cis*-12-CLA for 6 weeks significantly increased the expression of hepatic genes involved in fatty acid uptake (Cd36), TG synthesis (Acaca, Gpam and Scd), lipid droplet formation (Plin2), VLDL assembling/secretion (Mttp and Cideb), ketogenesis and utilization (Hmgcs2 and Bdh1), fatty acid oxidation (Acox1), glyceroneogenesis (Pck1 and Pdk4). The results suggest that the increased liver weight in response to *trans*-10, *cis*-12-CLA may be due to increased fatty acid uptake, stimulated TG synthesis, enhanced formation of lipid droplets as well as insufficiently induced VLDL assembly/secretion mechanism in liver. We provided new evidences to advance in the knowledge of the effect of this supplement in a relatively low amount on hepatic gene expression in growing male mice.

ACKNOWLEDGMENTS

I wish to express my gratitude to all the people who helped me in making this research project a reality. Thanks to my advisor, Assistant Professor Juan J. Loor, for his constant support and guidance through the highs and the lows of my development as a scientist, colleague and lifelong learner. Also thanks to my committee members, Professor James K. Drackley, Professor Walter L. Hurley and Assistant Professor Yuan-Xiang Pan, who offered valuable comments and trustworthy counsel.

TABLE OF CONTENTS

CHAPTER 1

Introduction

The World Health Organization (WHO) reports that the global epidemic of overweight and obesity – "globesity" – is rapidly becoming a major public health problem in many parts of the world (1). Over the past two decades, the incidence of overweight and obesity has rapidly increased in industrialized countries and currently affects about 72.3% of male and 64.1% of female adults in the U.S. (2). Meanwhile, the most recent National Health and Nutrition Examination Survey (NHANES) shows that 16.9% of children ages 2-19 are obese and 31.7% are overweight (3). Besides, the medical care costs ascribed to obesity and associated disorders accounted for around $168 billion (4) in the U.S., thus constituting a substantial economic burden. In the course of this alarming epidemic trend, so-called functional foods that match both the growing health awareness and need for convenience attract much attention (5), such as conjugated linoleic acid (CLA).

CLA refers to a group of conjugated octadecadienoic acid isomers derived from linoleic acid (LA), a fatty acid (FA) that contains 18 carbons and 2 double bonds (6). CLA isomers are typically found in the products of ruminant animals, such as meat and milk, which arises in their gastrointestinal tract where microbes convert LA into different isoforms of CLA through biohydrogenation (7). This process changes the position and configuration of the double bonds, resulting in a single bond between one or both of the two

double bonds, i.e., *trans*-10, *cis*-12-CLA or cis-9, *trans*-11-CLA.

Experiments conducted using pure synthetic *trans* -10, *cis*-12-CLA have shown that this isomer might have beneficial effects against obesity (8), diabetes (9), atherosclerosis (10), and cancer (11). The anti-lipogenic effects of dietary CLA mixtures of *trans*-10, *cis*-12-CLA and *cis*-9, *trans*-11-CLA with a ratio of 1:1, were initially observed in several lines of mice, and later work using purified isomer preparations clearly showed that *trans*-10, *cis*-12-CLA was responsible for delipidation (12-16). Supplementation with *trans*-10, *cis*-12-CLA decreases body fat mass in many animal studies and some human studies (41, 42). Furthermore, some previous reports suggest that dietary supplementation with CLA reduces body fat mass in overweight humans without disclosing any adverse effects (17, 18), indicating that *trans*-10, *cis*-12-CLA may be considered as a weight loss agent. Therefore, CLA supplements containing *trans*-10, *cis*-12-CLA (e.g., Your Life®, Natrol® and Nature's Way®, Clarinol™, Met-Rx, Dymatize®, EAS®) are readily available in Health Food Stores and stores where nutritional supplements are sold.

However, recent studies revealed unfavorable metabolic effects of *trans*-10, *cis*-12-CLA associated with increased oxidative stress and elevated inflammatory biomarkers (19, 20). They have found that *trans*-10, *cis*-12-CLA indeed could considerably reduced body fat mass but accompanied by hepatic steatosis (5, 12, 21-25) which is an excess accumulation of triacylglycerol (TG) in the liver (26) and caused by an imbalance

between lipid availability (from circulating lipid uptake or de novo lipogenesis) and lipid disposal (via fatty acid oxidation or TG-rich lipoprotein secretion) (27). As *trans*-10, *cis*-12-CLA supplementation reduces a amount of adipose tissue, several studies suggest that much of the increased liver mass may be explained by accumulation of lipids (28, 29) derived from mobilization of delipidated adipose tissue stores (30, 31).

The hepatic steatotic effect of *trans*-10, *cis*-12-CLA has been considered to be related to modulate the expressions of genes relevant to energy expenditure, apoptosis, fatty acid oxidation, lipolysis, differentiation and lipogenesis (6, 32). A study conducted by Clément et al. showed that mice fed a diet enriched in *trans*-10, *cis*-12-CLA (0.4% w/w) for 4 weeks developed lipoatrophy, hyperinsulinemia and fatty liver (33). In this study, they investigated the levels of expression of genes encoding transcription factors, lipid-binding proteins and enzymes known to play a significant role in lipid metabolism (33). Finally, the results suggest that liver steatosis might be triggered by *trans*-10, *cis*-12-CLA-induced hyperinsulinemia, by inducing both fatty acid uptake and lipogenesis as evidenced by the substantial up-regulation of peroxisome proliferator activated receptor γ (Pparγ), Cd36, fatty acid binding protein 4 (Fabp4) and sterol regulatory element binding transcription factor 1 (Srebf1) (33). However, the cellular and molecular mechanisms by which *trans*-10, *cis*-12-CLA results in hepatic steatosis in mice have been unclear.

To our knowledge, there are no published studies dealing with the genomic effects

of chronic dietary *trans*-10, *cis*-12-CLA supplementation on liver of growing animals that could serve as models for humans. In our previous study (24), relatively low amount of *trans*-10, *cis*-12-CLA (0.30%) was used compared to other studies (22, 23, 28, 32, 34-36). We observed that dietary *trans*-10, *cis*-12-CLA resulted in a linear increase in liver weight as well as a reduction in adipose tissue weights in both male and female mice over the 6-week feeding period, which substantiated some published findings obtained at higher amounts of CLA (i.e., 0.5-1% of the diet) (28, 37). The present study utilized growing male mice which are more susceptible than females (24) to investigate the effects of *trans*-10, *cis*-12-CLA on hepatic gene expression. Our hypothesis was that long-term increased liver mass in response to *trans*-10, *cis*-12-CLA would be associated with greater mRNA abundance of lipogenic gene networks (Figure 1.1). Specific objectives were to measure mRNA abundance of thirty-eight genes (Table 1.1) encoding proteins required for FA uptake, FA oxidation, de novo FA synthesis, lipolysis, lipogenesis, ketogenesis, carbohydrate metabolism, inflammation, stress response, growth factor signaling, transcriptional regulation of lipogenesis and lipid droplet formation.

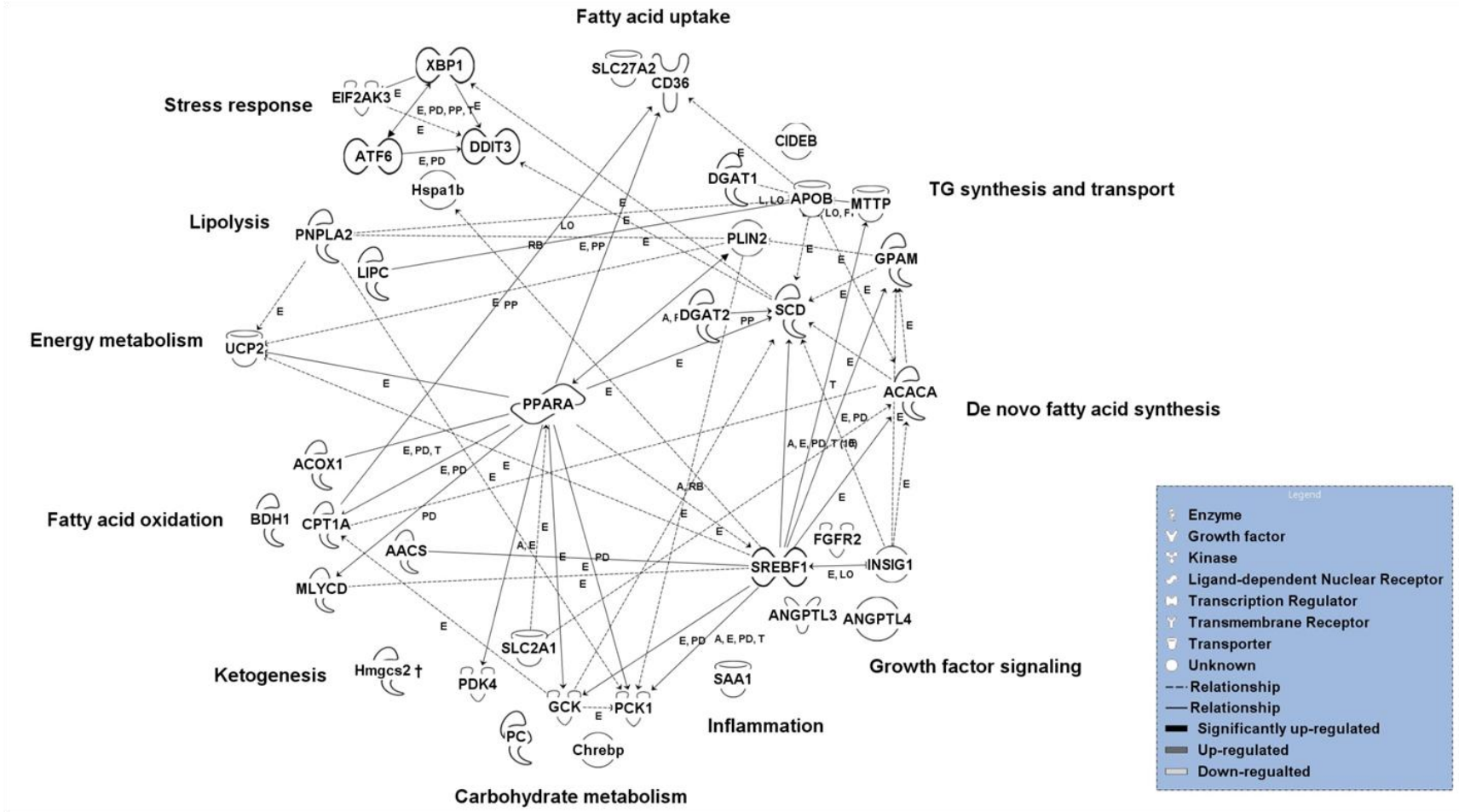

Figure 1.1 Currently known relationships among genes analyzed based on manually curated examination of the published literature within the Ingenuity Pathway Analysis (www.igenuity.com) knowledge base. Genes are grouped by the predominant process they play in lipid metabolism. Different shapes denote the type of protein encoded by the specific genes, including enzymes, ligand-dependent nuclear receptors, transcription regulators, and transporters. Letters along the edges denote effects on activity (A), expression (E), localization (LO), proteolysis (L), RNA binding (RB), protein-DNA binding (PD), and protein-protein binding (PP). Gene names are as in Table 1.1.

Table 1.1 Gene symbol, description, location, biological process and overall % mRNA abundance among genes measured

Gene symbol	**Description**	**Localization**	**Biological process[1]**	**% RNA[2]**
Aacs	Acetoacetyl-CoA synthetase	Cytoplasm	FA metabolism	0.57
Acaca	Acetyl-Coenzyme A carboxylase alpha	Cytoplasm	FA biosynthesis	0.52
Acox1	Acyl-Coenzyme A oxidase 1	Cytoplasm, peroxisome, mitochondrion	FA oxidation	6.83
Angptl3	Angiopoietin-like 3	Extracellular space	FA metabolism	1.47
Angptl4	Angiopoietin-like 4	Extracellular space	TG homeostasis	0.06
Apob	Apolipoprotein B	ER, microsome	Lipoprotein metabolism	9.14
Atf6	Activating transcription factor 6	Nucleus	Transcription regulation	0.02
Bdh1	3-hydroxybutyrate dehydrogenase 1	Mitochondrion	Oxidation-reduction process	3.35
Cd36	CD36 molecule (thrombospondin receptor)	Golgi, cell surface, mitochondrion	FA transport	0.00
Chrebp	MLX interacting protein-like	Cytoplasm, nucleus	Glucose homeostasis, transcription regulator activity	2.83
Cideb	Cell death-inducing DNA fragmentation factor alpha subunit-like effector B	Cytosol	Apoptosis	3.46
Cpt1a	Carnitine palmitoyltransferase 1a	Mitochondrion, microsome	FA metabolism	1.91
Ddit3	DNA-damage inducible transcript 3	Cytoplasm, nucleus	Cell cycle, transcription regulation	0.06
Dgat1	Diacylglycerol O-acyltransferase 1	ER, membrane	TG biosynthesis, VLDL assembly	0.15
Dgat2	Diacylglycerol O-acyltransferase 2	ER, membrane	TG biosynthesis, glycerol metabolism	4.93
Eif2ak3	Eukaryotic translation initiation factor 2 alpha kinase 3	Cytoplasm, ER	Srebp-mediated signaling pathway, apoptosis	0.03

Table 1.1 (cont.)

Gene symbol	Description	Localization	Biological process[1]	% RNA[2]
Fgfr2	Fibroblast growth factor receptor 2	Nucleus, cell surface	Cell-cell signaling	0.05
Gck	Glucokinase	Cytoplasm, mitochondrion, nucleus	Carbohydrate phosphorylation	0.82
Gpam	Glycerol-3-phosphate acyltransferase, mitochondrial	Mitochondrion, membrane	TG biosynthesis, FA hemeostasis	3.18
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	Mitochondrion	Ketogenesis	13.88
Hspa1b	Heat shock 70kDa protein 1B	Cytoplasm, mitochondrion, nucleus	DNA repair, anti-spoptosis	0.11
Insig1	Insulin induced gene 1	Endoplasmic reticulum (ER)	Cholesterol biosynthesis, TG metabolism	4.14
Lipc	Lipase, hepatic	Cell surface, extracellular space	FA metabolism, TG metabolism, LDL clearance, HDL remodeling	3.92
Mlycd	Malonyl-CoA decarboxylase	Cytoplasm, mitochondrion, peroxisome	FA biosynthesis & oxidation	0.50
Mttp	Microsomal triglyceride transfer protein	Golgi, ER	Lipoprotein transport, TG metabolism	1.24
Pcx	Pyruvate carboxylase	Mitochondrion	Gluconeogenesis, lipid biosynthesis	1.42
Pck1	Phosphoenolpyruvate carboxykinase 1	Cytoplasm, nucleus	Gluconeogenesis, lipid metabolism, glycerol biosynthesis	14.79
Pdk4	Pyruvate dehydrogenase kinase, isoenzyme 4	Mitochondrion	Glucose metabolism	0.13
Plin2	Perilipin 2	Cytoplasm, nucleus, plasma membrane	Lipid storage, long-chain FA transport	1.61

Table 1.1 (cont.)

Gene symbol	Description	Localization	Biological process[1]	% RNA[2]
Pnpla2	Patatin-like phospholipase domain containing 2	Cytosol, membrane	Lipid catabolic process	0.33
Ppara	Peroxisome proliferator activated receptor alpha	Nucleus	FA metabolism, glucose metabolism, transcription	1.28
Saa1	Serum amyloid A 1	Extracellular space	Cholesterol metabolism	0.54
Scd	Stearoyl-Coenzyme A desaturase 1	ER membrane	FA biosynthesis	2.53
Slc27a2	Solute carrier family 27 member 2	ER, microsome, peroxisome	FA transport, lipid metabolism	3.63
Slc2a1	Solute carrier family 2 member 1	Cytoplasm, plasma membrane	Glucose transport	0.07
Srebf1	Sterol regulatory element binding factor 1	Golgi, ER, nucleus	Transcription regulation	0.67
Ucp2	Uncoupling protein 2	Membrane, mitochondrion	Mitochondrion transport, response to superoxide	0.19
Xbp1	X-box binding protein 1	Nucleus	Transcription	9.67

[1] Entrez Gene, National Center for Biotechnology Information (NCBI).

[2] The % mRNA abundance is calculated by [((1/$E^{\Delta Ct}$) specific gene/sum (1/$E^{\Delta Ct}$) all genes) x 100]. See Experimental Methods for details

CHAPTER 2

Literature review

Currently, more than two-thirds of American adults and nearly one-third of children and teens are either overweight or obese (2). Since 1980, the number of obese adults has doubled (38). Since 1970, the number of obese children ages 6-11 has quadrupled, and the number of obese adolescents ages 12-19 has tripled (38, 39). Obesity-related medical costs are nearly 10 percent of all annual medical spending (40). The alarming increases in obesity rates over the past two decades has attracted much attention to the anti-lipogenic effects of the *trans*-10, *cis*-12-CLA. Supplementation with *trans*-10, *cis*-12-CLA has been reported to reduce body fat mass in many animal studies and some human studies (41, 42). Besides, CLA supplements containing *trans*-10, *cis*-12-CLA are readily available in Health Food Stores and stores where nutritional supplements are sold. Although *trans*-10, *cis*-12-CLA seems to be a promising substance as regards reduction of body fat, it is important to consider the possibility of adverse effects found in animal models (43, 44) and more recently in specific groups of humans (45-47). The most recent studies have stated that *trans*-10, *cis*-12-CLA could cause hepatic steatosis in mice (5, 12, 21-25, 33). However, fatty liver formation due to *trans*-10, *cis*-12-CLA has not been reported in human as seen in mice. As CLA has been approved by FDA in GRAS category, considering the possible adverse effects of long-term use of CLA, especially *trans*-10, *cis*-12-CLA

isomer, further and more studies in animals and humans are urgently needed before the *trans*-10, *cis*-12-CLA usages as a dietary supplement to reduce obesity in humans (48). This chapter will begin with the introduction of CLA, focusing on *trans*-10, *cis*-12-CLA isomer. Then it will discuss the physiological effects of *trans*-10, *cis*-12-CLA on adipose tissue and liver by reviewing *in vitro, in vivo* and clinical studies. Finally, it will summarize proposed underlying mechanisms through which *trans*-10, *cis*-12-CLA isomer exhibits its hepatic steatotic effect.

Definition of *trans*-10, *cis*-12-CLA

CLA is a fatty acid belonging to the group of polyunsaturated fatty acids (PUFAs) (49) as well as a generic term for the geometric and positional isomers of LA (*cis*-9, *cis*-12, octadecadienoic acid) (50) which contains 18 carbons and 2 double bonds. But, there is no methylene group separating the double bonds of CLA as there is in LA (51). Instead, the two double bonds are separated by one single bond (51) and are present predominantly in positions of carbons 9 and 11 (*cis*-9, *trans*-11-CLA), and 10 and 12 (*trans*-10, *cis*-12-CLA), which are two most studied CLA isomers (Figure 2.1).

CLA is formed in the gastrointestinal tract of ruminant animals where microbes convert LA into different isoforms of CLA through incomplete biohydrogenation (49), resulting in a single bond between one or both of the two double bonds (52). But, it has

been demonstrated that CLA can be also endogenously synthesized from vaccenic acid by Δ9-desaturase in rumens of lactating cows (53) and in lactating women (54). Therefore, natural forms of CLA can be found predominantly in ruminant food products, such as milk, cheese and beef (55-57), and present primarily (90%) as *cis*-9, *trans*-11-CLA (52, 53). According to Atkinson, the amount of CLA obtained by foods is 200 mg/day (58); Chin et al. estimated that the daily intake of CLA in humans is approximately 160 mg (59) and Ritzenthaler et al. suggested 212 mg in men and 151 mg in women (60). Hence, in order to obtain the beneficial effects of CLA on health, it is necessary to include an additional supplementation of CLA in the habitual diet in view of the amount and type of isomer consumed habitually (49).

Commercial preparations of CLA supplements are synthesized by alkaline isomerization of LA-enriched vegetable oils (e.g. safflower and sunflower oil) (61). This type of processing yields a CLA mixture containing approximately 40% of the *cis*-9, *trans*-11-CLA isomer and 44% of the *trans*-10, *cis*-12-CLA isomer (16). Commercial preparations also include about 4-10% *trans*-9, *trans*-11-CLA and *trans*-10, *trans*-12-CLA as well as trace amounts of other isomers (52). In 1997, only two companies (Natural ASA, Hovdebygda, Norway; PharmaNutrients Inc., Lake Bluff, IL) marketed CLA as a dietary supplement (62). Today numerous companies offer dietary supplements containing CLA; examples include Your Life®, Natrol® and Nature's Way® (63), ClarinolTM, Met-Rx,

Dymatize® and EAS®, claiming a variety of health benefits. Supplement manufacturers recommend doses of 3-3.4 g per day for weight loss purpose (Clarinol, Tonalin) (64).

Physiological effects of *trans*-10, *cis*-12-CLA

CLA was discovered quite accidentally by Pariza and hargraves when they were investigating the carcinogenic properties of grilled beef; and they surprisingly and unexpectedly found that the fatty acids present in grilled beef exhibited anticarcinogenic rather than procarcinogenic properties (65). Ever since then, a large number of studies have been done using a 1:1 ratio of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA both in vivo and in vitro (16), aiming to investigate CLA's several beneficial effects in health-related disorders. The results have shown that CLAs have anti-adipogenic (12, 13, 16), anti-carcinogenic (56, 66-72), anti-atherogenic (73-76), anti-diabetogenic (77, 78) and anti-inflammatory (79-82) properties. Among all the CLA isomers, the *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA isomers have various physiological functions and exhibit health benefits, individually or in association with each other (83, 84). Park et al. were the first to demonstrate that CLA mixture of 50% *cis*-9, *trans*-11-CLA and 50% *trans*-10, *cis*-12-CLA modulated body composition by decreasing body fat mass and increasing lean body mass (13). Experiments conducted later have confirmed that *trans*-10, *cis*-12-CLA is the isomer responsible for the changes in vivo and in vitro, particularly in body composition and lipid

metabolism (12, 14-16, 33, 85).

Trans-10, *cis*-12-CLA's effect on adipose tissue

In vitro studies

Brown and Mclnstosh found that *trans*-10, *cis*-12-CLA attenuated TG content and differentiation in primary cultures of human adipocyte (86). Similarly, a study demonstrated that *trans*-10, *cis*-12-CLA treatment at doses to those found in serum from rodents reduced adipogenesis and lipid droplet accumulation (87). In a study conducted by Park et al., *trans*-10, *cis*-12-CLA reduced TG content in the 3T3-L1 adipocytes (88).

In vivo studies

Dietary *trans*-10, *cis*-12-CLA supplementation has been demonstrated to be extremely effective at reducing the fat content (≥ 60%) in a number of rodent models and pigs (89). Navarro et al. investigated the effect of 6 weeks of supplementation of 0.5% linoleic acid, *cis*-9, *trans*-11-CLA or *trans*-10, *cis*-12-CLA in atherogenic diet-fed hamsters. Although there was no difference in body weight, fat mass decreased significantly in *trans*-10, *cis*-12-CLA-fed hamsters (90). Another study showed that intake of *trans*-10, *cis*-12-CLA isomer for 3 weeks in genetically obese mice decreased gain in body weight and white fat pad weight (91). In a study conducted by Kang et al. using wild-type and

stearoyl-CoA desturase 1 (Scd1) null mice, *trans*-10, *cis*-12-CLA decreased fat mass and enhanced mRNA expression of lipogenic enzymes, fatty acid synthase (Fasn) and uncoupling protein 2 (Ucp2), suggesting that antiobesity effects of *trans*-10, *cis*-12-CLA is independent of Scd1 gene expression and enzyme activity (92). House et al. conducted a study using a polygenic obese line of mice fed 1% *trans*-10, *cis*-12-CLA and showed that adipose mass was 30% less in the epididymal depot of CLA-fed mice, 27% less in the mesenteric depot and 58% less in brown adipose tissue after 14 days of CLA treatment (28). In contrast, a study in adult male Wistar rats fed with diets containing 1% *trans*-10, *cis*-12-CLA in combination with moderate physical activity for 6 weeks did not observe any effect on body composition or body weight (93). Based on the studies above, it is suggested that adult animals have not reproduced the dramatic results seen in growing animals (51). Additionally, mice are more sensitive to CLA than rats in loosing fat mass (51), as Kim et al. stated that CLA-induced reductions in body fat gain varied in different species as following: mice > hamster > rats (94).

Some of the mechanisms suggested to be involved in fat reduction with *trans*-10, *cis*-12-CLA intake are increased energy expenditure (95), increased fat oxidation (96), decreased adipocyte size (97), and inhibition of enzymes involved in fatty acid metabolism and lipogenesis (83, 98, 99).

Clinical studies

Although the body fat-lowering effect of *trans*-10, *cis*-12-CLA has also been reported in humans, most clinical studies have not reflected the dramatic findings obtained in vitro and in vivo studies (51). In a controlled, randomized, double-blind parallel group study, the participants were given 2.2-4.2 g/d of *trans*-10, *cis*-12-CLA, or *cis*-9, *trans*-11-CLA, or combined for 4-12 wk (100). The results showed a certain reduction of the proportion of body fat especially abdominal fat by *trans*-10, *cis*-12-CLA (100). It should be noted that the loss of body fat is 40-50% greater in mice than observed in humans (61); however, mice are generally fed approximately 5 times more CLA than humans (per kilogram body weight) (101). In another randomized, double-blind controlled trial, 60 abdominally obese men were treated with 3.4 g/day CLA mixture, purified *trans*-10, *cis*-12-CLA or placebo for 12 weeks (46). The study found that *trans*-10, *cis*-12-CLA decreased body fat, sagittal abdominal diameter and weight versus baseline, but the difference was not significantly different from placebo; besides, the CLA mixture did not change body composition or weight compared with placebo (46).

Malpuech-Brugere et al. examined the effects of *cis*-9, *trans*-10-CLA and *trans*-10, *cis*-12-CLA isomers at two different intakes on body composition in 81 middle-aged overweight healthy humans (102). This was a bicentric, placebo-controlled, double-blind, randomized study. After 6-week consuming daily a dairy product containing 3

g of high oleic acid sunflower oil, volunteers were randomized to receive daily either 3 g of high oleic acid sunflower oil, 1.5 g of *cis*-9, *trans*-11-CLA, 3 g of *cis*-9, *trans*-11-CLA, 1.5 g of *trans*-10, *cis*-12-CLA, or 3 g of *trans*-10, *cis*-12-CLA administrated as TG in a dairy product for 18 weeks. At the end of the 24 weeks, they did not find any significant differences in body composition and energetic uptake among the groups. Similarly, a study investigating the effects of 3 doses of highly enriched *cis*-9, *trans*-11 or *trans*-10, *cis*-12-CLA in healthy men did not demonstrate any significant alteration caused by any of those two isomers in the body composition of healthy adults (103).

In the vast majority of the studies with human volunteers, CLA mixtures rather than pure isomers have been used for supplementation (104). The effectiveness of CLA on humans is controversial. This may be partly because CLA dosage used in human studies is much lower than doses used in animal studies (51). For example, subjects in the human study received approximately 0.05 g/kg body weight CLA (105), the mice received 1.07 g/kg body weight CLA (106), which was 20 times the human dose based on body weight. Moreover, most animal studies have been in growing mice or rats, whereas studies in humans were mostly in mature volunteers whose changes in body composition in response to CLA supplementation have not been as dramatic as those seen in young animals (51).

Trans-10, *cis*-12-CLA's effect on liver

Although CLA seems to be a promising substance as regards reduction of body fat, it is important to consider the possibility of adverse effects found in animal models and more recently in specific groups of humans (49). Liver has been reported to be an important target for the effects of CLA on lipid metabolism (36). Many recent studies revealed that CLA could considerably reduced body fat mass but accompanied by hepatic steatosis (5, 12, 21-25). They also verified that *trans*-10, *cis*-12-CLA is the isomer responsible for the presence of fatty liver in mice associated with the loss of adipose tissue (12, 25, 31, 33, 107). The studies discussed in this section were summarized in Table A.5-A.7.

In vitro studies

A study conducted by Eder et al. showed that treating HepG2 cells with *trans*-10, *cis*-12-CLA increases the concentration of saturated fatty acid (SFA) in various cell lipid fractions at the expense of monounsaturated fatty acid (MUFA) (108), which confirms the investigation conducted by Choi et al. that clearly demonstrated that *trans*-10, *cis*-12-CLA strongly suppresses Δ9-desaturase (109). Choi et al. found that treating HepG2 cells with *trans*-10, *cis*-12-CLA did not cause changes in the Scd gene transcription, mRNA and protein levels; however, this isomer decreased both the Scd activity as well as the levels of MUFAs, suggesting that in HepG2 cells the it regulates human Scd activity mainly by a

posttranslational mechanism (109). Since data from Belury and Kempa-Stedzko (110) suggested that liver microsomes may desaturate linoleate as well as CLA, Bretillon et al. (111) utilized male Wistar rat liver microsomes to investigate the effects of *trans*-10, *cis*-12-CLA on the desaturation of C18 fatty acids. They found that *trans*-10, *cis*-12-CLA significantly inhibited the conversion of stearic acid and the Δ6 desaturation of LA at the highest inhibitor/substrate (I/S) ratio. Another study demonstrated significant cytotoxic effects of 1 μM *trans*-10, *cis*-12-CLA on dRLh-84 rat hepatoma cells (50).

In vivo studies

Studies in *trans*-10, *cis*-12-CLA supplementation have shown inconsistent results when comparing its effects on liver metabolism in different animal species. Thus, as Moya-Camarena and Belury suggested, there was the existence of a species-specific response of liver to CLA (and *trans*-10, *cis*-12-CLA) feeding (112).

Numerous studies have shown that feeding *trans*-10, *cis*-12-CLA leads to an increase in liver weight in mice. As mentioned previously, the study observing a reduction in adipose tissue in response to 1% *trans*-10, *cis*-12-CLA supplementation for 14 days, found that livers of the CLA-fed mice accumulated more fat and mass than the livers from LA-fed mice (control group) (32). Clément et al. showed that C57B1/6J female mice fed a diet enriched in *trans*-10, *cis*-12-CLA (0.4% w/w) for 4 weeks resulted in a dramatic

decrease in the mass of peri-uteral white adipose tissue (WAT) but accompanied by triggering a massive enlargement of the liver (3.1-fold increase) with typical features of a fatty liver: pale color and accumulation of intracellular lipids (33). They also suggest that *trans*-10, *cis*-12-CLA-induced hyperinsulinemia may trigger liver steatosis by inducing both fatty acid uptake and lipogenesis (33). Another group using aging female C57B1/6J mice pointed out that feeding 0.5% *trans*-10, *cis*-12-CLA for 6 months reduced the fat mass and induced liver hypertrophy accompanied by the accumulation of lipids in liver (48) . In a study conducted by Degrace et al., C57BL/6j male mice were randomized to receive either 10g of oleic acid, *cis*-9, *trans*-11-CLA, or *trans*-10, *cis*-12-CLA for 4 weeks (30). The study observed a marked regression of the perieididymal adipose tissue (PAT) and a concomitant enlargement of liver associated with a sever fat accumulation (30). Their follow-up study confirmed their previous findings and found that the livers of *trans*-10, *cis*-12-CLA-fed mice had 170 μmol more TG and 1.1 μmol more total protein compared to those of controls (31). Due to the increased fatty acid oxidation capacities, they also suggested that hepatic steatosis was not caused by impaired fatty acid oxidation in C57BL/6J mice fed the *trans*-10, *cis*-12-CLA (31). A study using eighteen C57BL/6N female mice fed with either a control diet or diets supplemented with 0.5% *cis*-9, *trans*-11-CLA or 0.5% *trans*-10, *cis*-12-CLA for 8 weeks, found that the weights of the livers in the mice fed *trans*-10, *cis*-12-CLA was significantly ($p < 0.05$) greater than those in the control and *cis*-9, *trans*-11-CLA groups;

similarly, the weight of total liver lipids was approximately 4 times greater in the *trans*-10, *cis*-12-CLA than other groups, which was mainly due to an increase (5-fold) in the TG fractions, but cholesterol (3-fold), cholesterol esters (3-fold), and free fatty acids (2-fold) were also significantly increased (25). House et al. measured gene expression during *trans*-10, *cis*-12-CLA-induced fat reduction in polygenic obese line of mice. They observed an 33% increase ($p < 0.0001$) in liver weight by day 14 with 61% more fat present in the liver of *trans*-10, *cis*-12-CLA-fed mice compared with LA-fed mice ($p < 0.001$) (28). Similarly, Warren et al. also found that livers from the mice fed *trans*-10, *cis*-12-CLA contained 5 times more lipids than in the control group, with 30% lipids of the liver wet weights compared with 12% lipids of the liver wet weights in the control group (113). According to the studies (25, 30, 32, 33) that also measured the hepatic TG content, hepatic enlargement was associated with a 3- to 7-fold increase in TG content. Furthermore, our previous study suggested the lower threshold for these hepatic responses may be 0.15% *trans*-10, *cis*-12-CLA or less in a diet (24).

Several studies in hamsters have demonstrated different effects of *trans*-10, *cis*-12-CLA on liver compared with other rodent species, such as the mouse and the rat (114). Zabala et al. also observed a significantly greater liver weight ($p < 0.05$) produced by 5 g *trans*-10, *cis*-12-CLA, but with significantly lower TG content ($p < 0.01$) in 9-week-old male hamsters, indicating that the increased liver weight was not due to increased fat

accumulation (114). Moreover, they pointed out that the liver TG content in those mice was even significantly lower than that in the linoleic acid group; and, that reduced fat deposition in liver was not apparently due to changes in lipogenesis and should be ascribed to an increase in fatty acids oxidation (114). Another group showed that feeding male F1B hybrid hamsters with *trans*-10, *cis*-12-CLA (6.6 g/kg diet) for 8 weeks reduced weight gain as well as increased liver weight by 25% but not liver lipids (85).

In the study conducted by Gudbrandsen et al., feeding male Zucker fa/fa rats diets supplemented with 1% *trans*-10, *cis*-12-CLA for 10 days reduced the liver TG content without improving the overall adiposity or any change in liver weight (36). On contrary, another study observed a 33% increase in liver lipid content in the growing female Zucker fa/fa rats fed *trans*-10, *cis*-12-CLA (1.5 g CLA/kg BW) for 21 days (89).

Clinical studies

So far CLA supplement on the market has been a mixture of *trans*-10, *cis*-12-CLA and *cis*-9, *trans*-11-CLA at an equal proportion and supplement manufacturers recommend doses of 3-3.4 g per day for weight loss purpose (Clarinol, Tonalin) (64). Most clinical trials studying the safety of CLA in humans typically lasted between 4 and 13 weeks and dosages were 1.4-4.2 g, with a few exceptions that employed 6-6.8 g (115). A very recent study (64) determining the safety of a very high-dose (14.6 g *cis*-9, *trans*-11-CLA and 4.7 g *trans*-10,

cis-12-CLA per day) of CLA which was almost 3 times higher than the highest dose in previous studies on CLA (115), did not found any clinically relevant effects on liver function tests of the 19 participants after taking CLA supplement for 3 weeks. Thus, it ended up with the conclusion that doses of up to 3 g per day as commonly ingested from CLA supplements are unlikely to affect liver function at least over a period of weeks (64). However, the results of this study did not suggest that *trans*-10, *cis*-12-CLA would not cause any liver damage in such high dose. First, the content of *trans*-10, *cis*-12-CLA in CLA mixture they used was much lower than the other isomer. Because these two CLA isomers are responsible for different biological functions, so the high *cis*-9, *trans*-11-CLA content might affect or mask the influence of *trans*-10, *cis*-12-CLA on liver. Second, the subjects were mostly young women and only 19 participants were involved in this study; thereby, they were not very representative. Finally, the study only measured several biomarkers of liver function and those measurements could not indicate any change in liver weight.

A randomized, crossover, single-blind clinical trial (116) was carried out by Venkatramanan et al. in 11 moderately overweight, borderline hyperlipidemic individuals who consumed milk enriched with a 4.2% synthetic mixture of *trans*-10, *cis*-12-CLA and *cis*-9, *trans*-11-CLA isomers providing 1.3 g/d of CLA. The results indicated that supplementation with CLA-enriched milk did not significantly affect liver function or body weight (116). Since *trans*-10, *cis*-12-CLA is responsible for reducing fat mass and

increasing liver weight, the relatively low amount of this isomer used in the study may be at a level below the threshold necessary to elicit changes in body composition (116). However, this study utilized CLA in a food format with milk, which could possibly enhance the absorption and metabolism of the CLA supplement compared with those in a purified compound format. Similarly, neither Berven et al. (117) nor Blankson et al. (45) reported any clinically important changes in the blood levels of liver enzymes during the 12-week treatment with a mixture of *trans*-10, *cis*-12-CLA and *cis*-9, *trans*-11-CLA at an equal proportion. Lowery et al. also found no effect of the CLA mixture on the liver enzyme transferases in novice body builders supplemented with a two-isomer preparation (3.9 g/d) for 6 weeks (118).

In a study (119) trying to determine the safety of CLA in Japanese, 60 healthy overweight male were randomly assigned to three groups: 5.4 g CLA-triacyglycerol (3.4 g CLA), 10.8 g CLA- triacyglycerol (6.8 g CLA) and placebo daily for 12 weeks. The CLA-triacyglycerol contained *trans*-10, *cis*-12-CLA and *cis*-9, *trans*-11-CLA at an equal proportion (119). The study observed a slight increase in the level of liver enzymes (serum AST and ALT activities) in the 6.8 CLA group at 12 weeks; however, these changes were within the normal range (119). The results also indicate that CLA at a dose of 3.4 g/d is a safe dietary level in healthy Japanese populations based on no clinically significant changes in vital signs.

The long-term safety of CLA supplement was evaluated by a randomized, double-blind study consisting of 50 obese humans for one-year period (120). Subjects were randomized to receive either 6 g/day of CLA mixed isomers (as 7.5 g/day ClarinolTM) containing 37.3% *cis*-9, *trans*-11-CLA and 37.6% *trans*-10, *cis*-12-CLA, or placebo (high oleic sunflower oil) (120). The results showed that body composition did not differ between groups and liver function was not affected, in terms of ALT, AST and alkaline phosphatase in serum (120). The study concluded that CLA as ClarinolTM is safe for use in obese humans for at least one year (120).

So far there have been no clinical studies investigating the effects or safety of *trans*-10, *cis*-12-CLA supplementation by utilizing the purified isomer. Hence, we can get few hints from those studies using mixed isomers which may negate one another. Moreover, doses used in human trials were much lower than those used in animal studies (86). Therefore, in future, a large number of studies using different amounts of purified *trans*-10, *cis*-12-CLA supplement need to be carried out to investigate its effect on liver weight in humans. Furthermore, those studies need to be duplicated in other labs giving emphasis to men and women, age groups, ethnic background or food style (121). All in all, *trans*-10, *cis*-12-CLA-specific dose-titrated world-wide network clinical trials combined with mechanistic studies in cultures of primary cells should provide the much needed insight on potential human applications for this isomer (121). When extrapolating rodent data, such as

mouse, rat and hamster, to the human situation, the differences in species' sensitivities are of great importance (122, 123).

Hypothesized working mechanisms of *trans*-10, *cis*-12-CLA in liver

Increased hepatic lipogenesis

It was suggested that fatty liver could be a consequence of the increased lipogenesis in the liver in compensating for the reduction of fat deposition in the adipose tissue (124-126). A study found that increased fatty acid synthesis seemed to contribute to the *trans*-10, *cis*-12-CLA-induced fatty liver, evidenced by the increased expression of four lipogenic genes by more than 2-fold, including acetyl-CoA carboxylase (Acaca), fatty acid synthase (Fasn), malic enzyme (Me) and stearoyl CoA desaturase-1 (Scd1) (127). Indeed, the strong and specific induction of genes expressed at only very low levels in the normal liver, such as those encoding PPARγ, Fat/Cd36, and Albp, was observed in mice fed the *trans*-10, *cis*-12-CLA (33).These modifications may be accounted for by the concomitant induction of Srebf1 gene (33), which is known to be a major regulator of hepatic lipogenic program (128). Jaudszus et al. in a very recent study speculated that the reduction in the white adipose tissue in the *trans*-10, *cis*-12-CLA group was likely compensated by the hepatomegaly due to redistribution, altered oxidation and/or increased de novo synthesis of fatty acids (5). Therefore, the profound hepatic steatosis showed by animals consuming

trans-10, *cis*-12-CLA isomer has been attributed to increased hepatic TG, cholesterol, cholesterol esters and free fatty acids (FFAs) (25), reflecting increased hepatic fatty acids synthesis (124). These observations strongly indicated that an increase in lipogenesis is primarily responsible for the *trans*-10, *cis*-12-CLA-dependent accumulation of TG in the liver (129).

On contrary, a study (114) assessing the effects of *trans*-10, *cis*-12-CLA on mRNA levels and the activities of several lipogenic enzymes in hamsters, demonstrated that *trans*-10, *cis*-12-CLA isomer produced significantly greater liver weight but also significantly decreased liver fat accumulation. The study also observed no changes in mRNA levels or the activities of lipogenic enzymes (114). In terms of HepG2 cells, treatment of *trans*-10, *cis*-12-CLA did not cause changes in the Scd gene transcription, mRNA and protein levels (109).

Direct activation of PPARs

It was suggested that the observed *trans*-10, *cis*-12-CLA-mediated increased liver weight result from the direct activation of peroxisome proliferator-activated receptors (PPARs) (121, 130) and subsequent "switching on and/or off" of the target genes to elicit a host of biochemical pathways (131). According to the proposed CLA-mediated signal transduction by Benjamin et al. in Figure 2.2, during gene regulation, the well characterized

PPARs (α, β or γ subtypes) bind to the peroxisome proliferator responsive element (PPRE) on the nuclear DNA as heterodimers with the α, β or γ subtypes of the retinoic acid receptor (RXR), which in turn, needs to be activated by *cis*-9-retinoic acid to effect target gene transcription (132, 133).

As *trans*-10, *cis*-12-CLA has been reported to be a potent ligand for Pparα (134), the underlying regulations might imply the activation of Pparα, which was shown to repress apoptosis (135) and therefore to favor liver enlargement. Rasooly et al. observed a 65% reduction in Pparα gene expression in the liver of the mice fed *trans*-10, *cis*-12-CLA diet (127). The down-regulation of Pparα in the liver seems to be pivotal for the hepatic abnormalities (113). LaRosa et al. pointed out that suppression of Pparα is considered to be an integrated response to cellular stress, e.g. due to lipid peroxidation, leading to the activation of the nuclear factor of kappa light polypeptide gene enhancer in B-cells (NFκB) (136). Furthermore, down-regulation of Pparα as a central regulator of lipid homeostasis determines the inhibition of β oxidation and results, for example, in an over-expression of Srebf1 in the liver (137). Srebf1 controls a multitude of genes involved in hepatic de novo lipogenesis, such as Dgat (30) and Scd (5). See Figure 2.3.

Indirect mechanism: hyperinsulinemia

Several lines of evidence strongly suggest that the liver steatosis occurring in

trans-10, *cis*-12-CLA-fed mice is secondary to hyperinsulinemia, which causes high levels of fatty acid uptake and synthesis (33). First, because white adipose tissue plays a crucial role in metabolizing and converting glucose to fatty acid for storage purposes, the large decrease of fat pad mass in response to dietary *trans*-10, *cis*-12-CLA may result in a retardation of whole-body glucose metabolism (129). Therefore, Ide et al. speculated that there is a possibility that the counteraction of *trans*-10, *cis*-12-CLA-mediated induction of hepatic lipogenesis aggravates glucose intolerance and hyperinsulinemia, despite being potentially effective in preventing fatty liver (129). A large increase in lipogenesis and accumulation of TG in the liver after *trans*-10, *cis*-12-CLA treatment may represent the physiological response of the animal to metabolize excess glucose to fatty acid and store it as TG in the liver rather than in adipose tissue (129). Second, Clément et al. pointed out that fatty liver was not observed in *cis*-9, *trans*-11-CLA-fed mice, which remained normoinsulinemic (33). Third, hyperinsulinemia is associated with the induction of Pparγ gene expression in the liver and with liver steatosis in several mouse models (126). Fourth, insulin is known to upregulate Pparγ gene expression in adipocytes (138) and to induce Fas gene expression in the liver (139, 140). Finally, Clément et al. also demonstrated down-regulation of Pck1, strongly suggesting that the livers of *trans*-10, *cis*-12-CLA-fed mice remain sensitive to insulin (33).

Several in vivo studies have reported that dietary CLA caused marked glucose

intolerance and hyperinsulinemia (33, 141). Clément et al. showed that the mice fed a diet enriched in *trans*-10, *cis*-12-CLA (0.4% w/w) for 4 weeks developed lipoatrophy, hyperinsulinemia and fatty liver (33). In their study, a dramatic increase in non fasting plasma insulin levels was observed in mice fed the *trans*-10, *cis*-12-CLA-enriched diet, these mice displaying insulin concentrations 10 times higher than mice fed control and LA- or *cis*-9, *trans*-11-CLA-enriched diets; and, despite this marked hyperinsulinemia, plasma glucose concentration remained within the normal range in these mice (33). Another study found that *trans*-10, *cis*-12-CLA diet promoted insulin resistance and increased serum glucose ($p = 0.025$) and insulin ($p = 0.01$) concentrations in ob/ob C57BL-6 mice (141). Risérus et al. (142) conducted a clinical study including 57 non-diabetic abdominally obese men to randomly receive either 3.4 g *trans*-10, *cis*-12-CLA or control oil. After 12-week treatment, they demonstrated that *trans*-10, *cis*-12-CLA induced hyperproinsulinaemia that was related to impaired insulin sensitivity, independently of changes in insulin concentrations in obese men (142). Thus, they suspected the use of weight-loss supplements containing this CLA isomer (142).

However, the cause of the dramatic hyperinsulinemia triggered by the *trans*-10, *cis*-12-CLA remains to be determined.

Insufficient hepatic VLDL secretion in liver

In general, TG which is formed by esterification of fatty acid derived from plasma, is stored as droplets or secreted in the form of VLDL whose secretion depends on the synthesis of TG as well as apoB-containing lipoproteins which are major lipoproteins in transporting the hepatic lipids out of the liver (143). Thus, an alteration of VLDL secretion rates could also result in liver fat accumulation (30).

A study (144) aimed to investigate the effects of *trans*-10, *cis*-12-CLA on liver lipid metabolism in cultured HepG2 cells, which are human hepatoma cells and are known to exert a number of human liver functions, including fatty acid uptake, lipid synthesis, VLDL assembly and VLDL secretion (145). A novel finding in this study is that this CLA isomer suppressed TG secretion in spite of the fact that it increased 4-fold the cellular TG content (144). Since the secreted TG is mainly contained in VLDL, the decreased TG secretion by *trans*-10, *cis*-12-CLA reflects a diminished secretion of VLDL (144). Yotsumoto et al. found that apoB secretion by HepG2 cells treated with 10 μM *trans*-10, *cis*-12-CLA for 24 hours was decreased markedly when compared with the control group; and this CLA isomer also inhibited cellular TG and cholesteryl ester synthesis (146). Thus, this study suggests that *trans*-10, *cis*-12-CLA inhibited TG synthesis and reduced apoB secretion (146).

In terms of in vivo studies, Degrace et al. (30) demonstrated that the steatosis

accompanying the fat loss induced by *trans*-10, *cis*-12-CLA in C57BL/6j mice was not due to an alteration of the liver lipoprotein production that was even increased. The data obtained in that study showed that the ability of the total liver to secrete VLDL was practically two times greater in *trans*-10, *cis*-12-CLA-fed mice than in controls (30). Therefore, they indicated that the liver TG accumulation could not be attributed to a decreased VLDL secretion and that the VLDL overproduction from the enlarged liver was however insufficient to eliminate the flux of fatty acid entering the whole esterification and lipoprotein assembling/secretion pathway (30). According to the different results between HepG2 cells and mice, the explanations regarding the relations between the setup of the liver steatosis and VLDL secretion in mice fed diets containing purified *trans*-10, *cis*-12-CLA are very scarce and still confused.

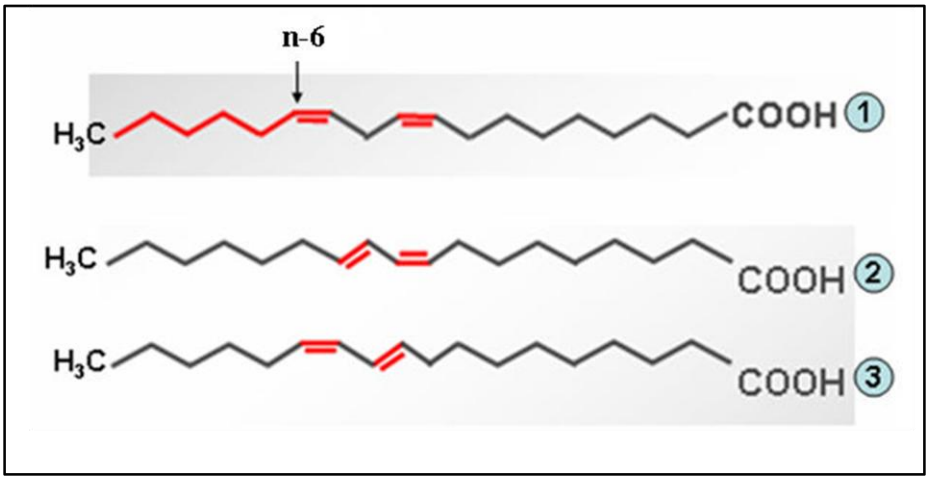

Figure 2.1 Structure of LA and its major CLA derivatives. 1. LA; 2. *cis*-9, *trans*-11-CLA; 3. *trans*-10, *cis*-12-CLA (121).

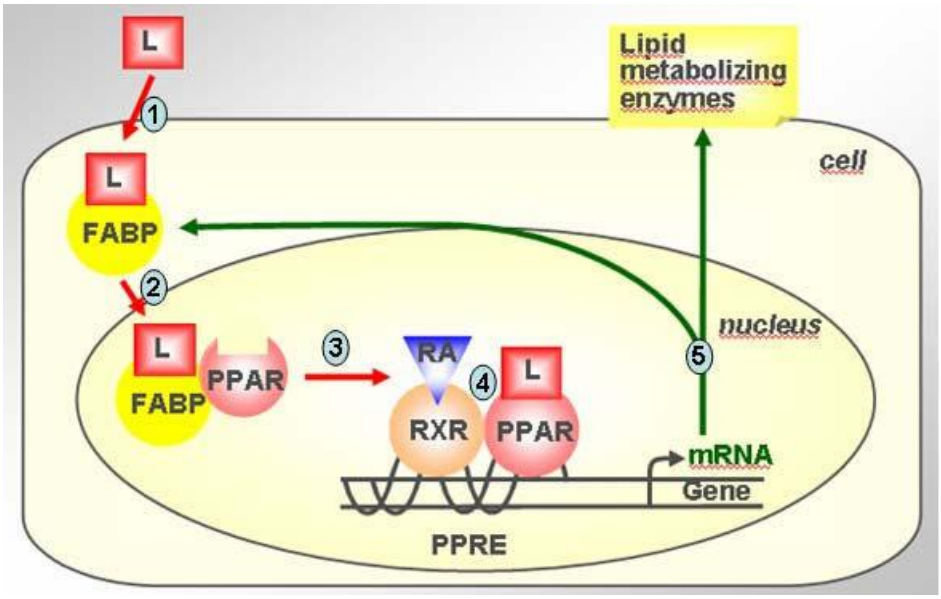

Figure 2.2 Proposed CLA-mediated signal transduction. The dietary lipid nutrient L (L = here CLA) crossing the cell membrane with the help of specific membrane-bound fatty acid transporters and binds to tissue-specific fatty acid binding protein (FABP) in the cytosol; 2. The L/FABP complex enters the nucleoplasm, where L is transferred to the specific peroxisome proliferators activated receptor (PPAR) subtype; 3. The L/PPAR complex heterodimerises with retinoic acid (RA)/retinoic acid receptor (RXR) subtype; 4. This heterodimer binds to the peroxisome proliferator responsive element (PPRE) on the target gene; and 5. Specific gene expression occurs, whose products act intra- or extracellularly to elicit a host of various biological functions. (121)

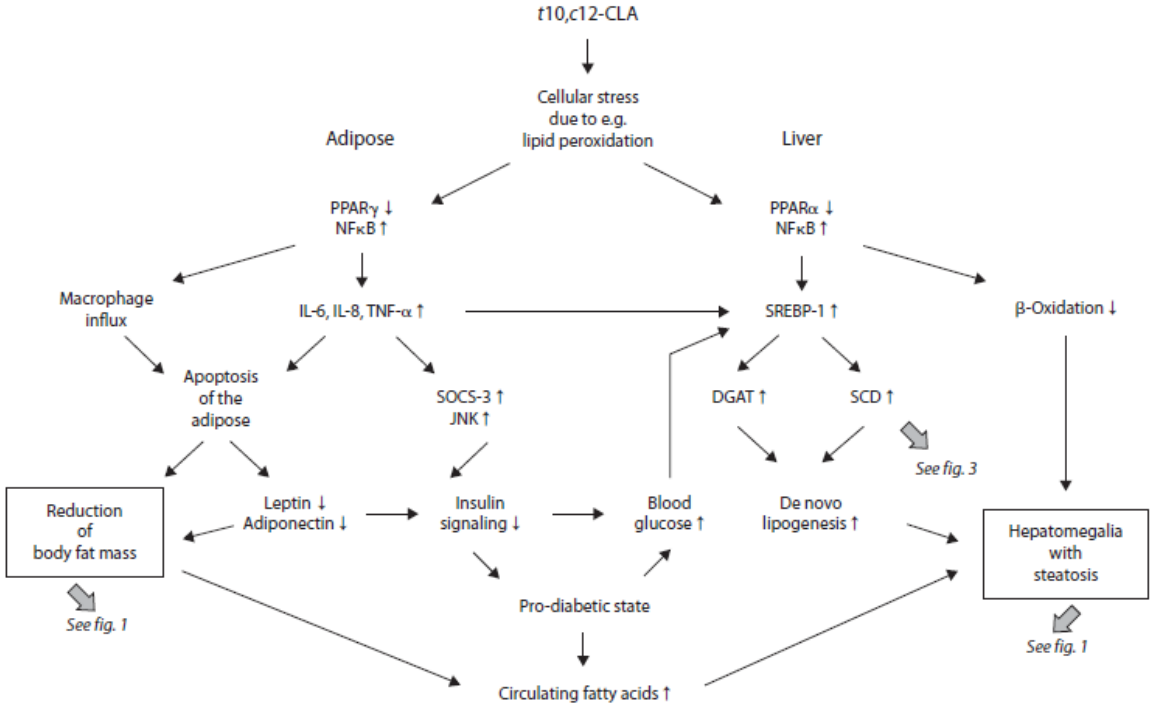

Figure 2.3 Observations from the present study in relation to previously suggested and approved mechanisms underlying the *trans*-10, *cis*-12-CLA-induced lipodystrophic alterations in rodents (5).

CHAPTER 3

Methodology

Animals, diets, and sampling

Male CD-1 mice from Harlan (Madison, WI), aged 3- to 4-week-old, were divided into two experimental groups of ten mice each. The mice were housed individually with access to food and water at all times. A 12-h light/12-h dark cycle was maintained throughout the study. For 7 d before the start of the study, all mice were fed Harlan Teklad (Harlan, Madison, WI) Global Rodent Diet (2018) (18% protein, 5% fat) with 3% (w/w) high-oleic sunflower oil added. On day 1 of the study, mice were randomly assigned to receive diets containing 3.0% high-oleic sunflower (control) or 2.70% high-oleic sunflower oil + 0.30% *trans*-10, *cis*-12-CLA. The free FA form of *trans*-10, *cis*-12-CLA (>95% purity) was obtained from Natural Lipids (Hovdebygda, Norway). Mice were fed daily at 16.00 hours, and food refusals were weighed to estimate intake during the previous 24 h. Samples of each diet were obtained each week and stored at 4℃ prior to FA analysis. Table 3.1 gives an overview of the FA composition of the diets fed to growing male mice. Data on body weight, feed intake, and tissue fatty acid profiles have been published previously (24).

At the end of weeks 2 and 6, five mice per dietary treatment were anesthetized with Metofane ® (Pitman-Moore, Inc, Washington Crossing, NJ) prior to cervical dislocation. The liver was removed, rinsed with diethlpyrocarbonate (Sigma, St. Louis, MO)

in distilled water (1:1000, v/v), and weighed. A portion of liver was frozen in liquid nitrogen and stored at -80℃ prior to RNA extraction and gene expression analysis. The remainder of the liver was stored at -20℃ prior to FA analysis, which is reported in the previous study (24).

Extraction and analysis of RNA and semiquantification by RT-PCR

Liver RNA was extracted using TRI REAGENT® (MRC, Cincinnati, OH) and according to the manufacturer's instructions. Genomic DNA was removed from RNA with DNase (Qiagen) using RNeasy Mini Kit columns (Qiagen, Germany). RNA concentration was measured with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). The purity of RNA (A_{260}/A_{280}) for all samples was above 1.8. RNA quality was assessed using a 2100 Bioanalyzer (Agilent Technologies). A portion of assessed RNA was diluted to 100 ng/μl using DNase-RNase free water prior to reverse transcriptase.

Each cDNA was synthesized by RT-PCR using 100 ng RNA, 1 μl dT18 (Operon Biotechnologies, AL), 1 μl 10 mmol/L dNTP mix (Invitrogen Corp., CA), 1 μl random primers (Invitrogen Corp., CA), and 10 μl DNase/RNase free water. This mixture was incubated at 65℃ for 5 min and kept on ice for 3 min. A total of 6 μl of Master Mix composed of 4.5 μl 5X First-Strand Buffer, 1 μl 0.1 M DTT, 0.25 μl (50u) of SuperScript™ III RT (Invitrogen Corp., CA), and 0.25 μl of RNase Inhibitor (10U, Promega, WI) was

added. The reaction was performed in an Eppendorf Mastercycler® Gradient using the following temperature program: 25℃ for 5 min, 50℃ for 60 min, and 70℃ for 15 min. cDNA was the diluted 1:4 with DNase/RNase free water.

Quantitative PCR (qPCR) was performed using 4 μl of diluted cDNA combined with 6 μl of a mixture composed of 5 μl 1× SYBR Green master mix (Applied Biosystems, CA), 0.4 μl each of 10 μM forward and reverse primers, and 0.2 μl DNase/RNase free water in a MicroAmp™ Optical 384-Well Reaction Plate (Applied Biosystems, CA). Each sample was run in triplicate and a 5 point relative standard curve plus the non-template control (NTC) were used (User Bulletin #2, Applied Biosystems, CA). The reactions were performed in an ABI Prism 7900 HT SDS instrument (Applied Biosystems, CA) using the following conditions: 2 min at 50℃, 10 min at 95℃, 40 cycles of 15 s at 95℃ (denaturation), and 1 min at 60℃ (annealing + extension). The presence of a single PCR product was verified by the dissociation protocol using incremental temperatures to 95℃ for 15 s plus 65℃ for 15 s. Complete details regarding amplification of target genes can be found at http://docs.appliedbiosystems.com/pebiodocs/04364014.pdf.

Selection and evaluation of internal control genes (ICG)

Data were calculated with the 7900 HT Sequence Detection Systems Software (version 2.2.1, Applied Biosystems) using a five-point standard curve, as required for the

evaluation of gene stability. Stability (M = gene – stability measure) using geNorm (147) refers to the constancy of the expression ratio between two non-co-regulated genes among all samples tested. The more stable the expression ratio among two genes, the more likely that the genes are appropriate internal controls, i.e. two ideal control genes should have an identical expression ratio in all samples regardless of experimental conditions, cell, and/or tissue type. The lower the M value, the higher the stability. geNorm also performs an analysis to determine the utility of including more than 2 genes for normalization by calculating the pairwise variation (V) between the normalization factor (NF) obtained using n genes (best references) (NF_n) and the NF obtained using n+1 genes (addition of an extra less stable reference gene) (NF_{n+1}). A large decrease in the pairwise variation indicates that addition of the subsequent more stable gene (i.e. with lowest M value) has a significant effect and should be included for calculation of the NF (147). Once the most stable internal reference genes are selected, the NF is calculated using the geometrical average between them to normalize qPCR data.

At the beginning, a total of seven genes were selected as internal control genes, including β-actin, β_2 microglobulin (β-2M), Procollagen-lysine-2-oxoglutarate 5-dioxygenase 3 (PLOD3), neuroglobin (NGB), Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), sentrin specific peptidase 7 (SENP7), and syntaxin 8 (STX8). According to the results generated by geNorm, β-actin, β_2 microglobulin (β-2M),

Procollagen-lysine-2-oxoglutarate 5-dioxygenase 3 (PLOD3), and neuroglobin (NGB) expression data were the four most stable genes among the ones tested as internal controls.

Selection of genes and primer design

Thirty-eight genes encompassing networks with central biological functions in lipid metabolism were evaluated (Figure 1.1). Those genes encode proteins required for fatty acid uptake, fatty acid oxidation, de novo fatty acid synthesis, lipolysis, lipogenesis, ketogenesis, carbohydrate metabolism, apoptosis, inflammation, stress response, growth factor signaling, transcriptional regulation of lipogenesis and lipid droplet formation. Primers were designed with Primer Express software fixing the amplicon length to 100-120 bp with low specific binding at the 3'-end (limit 3'-G+C; Applied Biosystems). Primers for the thirty-eight selected genes and the four internal control genes are shown in Table S2. Primers were aligned against publicly available databases using BLAST software at the National Center of Biotechnology Information and also UCSC's Mouse Genome Browser Gateway (http://genome.ucsc.edu/cgi-bin/hgGateway?hgsid=183612843&clade=mammal&org=Mouse&db=0). Prior to qPCR, primers were tested by semiquantitative PCR using a total of 20 µl mixture composed of 8 µl pooled cDNA, 10 µl SYBR Green, 1µl each of forward and reverse primers. The PCR reaction was set to 2 min at 50℃, 10 min at 95℃, 40 cycles of 15

s at 95℃ (denaturation). PCR products were run in a 3% agarose gel (Invitrogen) stained with ethidium bromide. PCR products were sequenced at the Core DNA Sequencing Facility of the Roy J. Carver Biotechnology Center at the University of Illinois, Urbana-Champaign. Aliquots of all PCR products are stored to enable additional confirmatory sequencing of PCR products in the future if required.

Gene network analysis

Gene networks were evaluated using Ingenuity Pathway Analysis® (IPA; http://www.ingenuity.com), Redwood City, CA). This is a web-based application that enables the discovery, visualization, and exploration of interaction networks. The software relies on currently known relationships (i.e., published manuscripts) among human, mouse and rat genes/proteins.

Statistical analysis

Data were analyzed as a completely randomized design with a factorial arrangement of treatments using the MIXED procedure of SAS® (Windows version 9.1, Cary, NC). Interaction was dietary treatment × week. Leastsquare means ± SEM (combined observations for weeks 2 and 6) are presented in tables and figures.

Relative mRNA abundance among transcripts

Efficiency of PCR amplification for each gene was calculated using the standard curve method ($E = 10^{-1/\text{-log curve slope}}$). Relative mRNA abundance among measured genes was calculated as reported by Bionaz et al. (148), using the inverse of PCR efficiency raised to ΔCt (gene abundance = $1/E^{\Delta Ct}$, where ΔCt = Ct sample – geometric mean Ct of 4 internal control genes). Overall mRNA abundance for each gene among all samples measured was calculated using the median ΔCt. Use of this technique for estimating relative mRNA abundance among genes was necessary because relative mRNA quantification was performed using a standard curve (made from a mixture of RNA from several mouse tissues, which precluded a direct comparison among genes. Together, use of Ct values corrected for the efficiency of amplification plus internal control genes as baseline overcome this limitation. Description of genes measured and overall % relative mRNA abundance are reported in Table 1.1.

Table 3.1 Fatty Acid Composition of Diets Fed to Growing Male Mice for 6 Wk

Fatty Acid	***trans*-10, *cis*-12-CLA**	
	0%	**0.30%**
10:0	0.02	0.02
12:0	0.04	0.05
14:0	0.12	0.12
16:0	9.26	9.18
cis-9-16:1	0.18	0.19
18:0	2.49	2.52
trans-11-18:1	0.02	0.02
cis-9-18:1	34.41	31.77
18:2n-6	47.34	46.28
cis-9, *trans*-11-18:2	0.02	0.32
trans-10,*cis*-12-18:2	0.00	2.67
18:3n-3	3.72	3.77
20:0	0.39	0.38
20:4n-6	0.02	0.03
Total (μg/mg of diet)	44.07	46.14

CHAPTER 4

Results and Discussion

Data from this study published previously (24) observed a reduced deposition of lipids in peripheral tissues as well as a 15-18% increase in liver weight of male mice in response to relatively low amounts of *trans*-10, *cis*-12-CLA in a dose-dependent manner; and, the increased liver weight was accompanied by an increase in hepatic total fatty acid content. Apart from our previous study, many other studies have shown that *trans*-10, *cis*-12-CLA increases liver weight and reduces adipose mass; and, those studies that also measured the hepatic TG content, hepatic enlargement was associated with a 3- to 7-fold increase in TG content as summarized in Table 4.2. In the present study, we examined a total of thirty-eight genes related to lipid metabolism using real-time PCR in the livers of mice fed *trans*-10, *cis*-12-CLA, aiming to explore the underlying cellular and molecular mechanisms causing increased liver weight and potentially impaired function. The results of hepatic gene expression in response to *trans*-10, *cis*-12-CLA were listed in Table 4.3-4.5.

Trans-10, *cis*-12-CLA increased fatty acid uptake in the liver

Accumulating evidence suggests that an increase in lipogenesis and esterification due to dietary *trans*-10, *cis*-12-CLA is primarily responsible for liver triacylglycerol (TG) accumulation (33, 124, 129). Liver TG accumulation also could be a result of increased

hepatic uptake of circulating non-esterified fatty acids (NEFA) (149). In a study conducted by Gruffat et al., liver tissue samples of rats were incubated with 0.75 mM of fatty acid mixture (representative of circulating NEFA) and with 55 μM *trans*-10, *cis*-12-CLA or oleate. They found that the uptake of CLA by hepatocytes was three times higher ($p < 0.01$) than for oleate (150). Passive diffusion of fatty acid across membranes plays a minor role compared with protein-mediated fatty acid uptake and the flip-flop mechanism (151). The main proteins involved in fatty acid uptake in non-ruminant cells include fatty acid translocase Fat/Cd36 (Cd36) (152) and fatty acid transport proteins (Slc27a) (153). Slc27a2 is a member of the family of fatty acid transport proteins (FATPs) (151) and is predominantly expressed in liver and kidney (153).Serum NEFAs traverse into the hepatocytes mainly by interaction of the NEFAs directly with Slc27a2 or by a preceding binding to cell-surface proteins, such as Cd36, which subsequently hands the NEFAs to Slc27a2 (151).

In the present study, Cd36 mRNA abundance accounted for 0.0005% whereas Slc27a2 accounted for 3.63% of total genes measured. However, Cd36 mRNA expression (CLA = 1.11, control = -1.47; $p = 0.001$) was greater with *trans*-10, *cis*-12-CLA treatment and there was no change for Slc27a2 expression (CLA = -0.04, control = -0.23; $p = 0.42$). The increased Cd36 expression suggests that *trans*-10, *cis*-12-CLA caused more NEFAs uptake into hepatocytes, which was in agreement with a higher hepatic FFA content

(two-fold) (25) as well as a lower serum FFA concentration in mice fed *trans*-10, *cis*-12-CLA (31). Furthermore, a previous study showed not only a significant increase in hepatic Cd36 expression in mice fed *trans*-10, *cis*-12-CLA, but also significant association between Cd36 expression and the degree of hepatic steatosis (154). Therefore, upregulation of Cd36, although not the most abundant NEFA transporter, is part of the mechanism leading to increased hepatic fatty acid uptake and hepatic steatosis induced by *trans*-10, *cis*-12-CLA.

Trans-10, _cis_-12-CLA stimulates lipogenesis and TG synthesis in liver

Acaca and de novo fatty acid synthesis

Several authors suggested that de novo fatty acid synthesis may play a role in the onset of hepatic steatosis related to CLA supplementation (124, 129). Acetyl-CoA carboxylase (Acc) is one of the two enzymes required for long-chain fatty acid (LCFA) synthesis; the other is Fasn (155). ACC catalyzes the carboxylation of acetyl-CoA to generate malonyl-CoA, which is the committed step in this pathway (155). Two forms of Acc have now been identified: Acc-α (Acaca) and Acc-β (155). Acaca is involved in the synthesis of LCFAs, while Acc-β may be involved in the regulation of mitochondrial oxidation of fatty acids (156).

In our study, mRNA abundance of this gene accounted for 0.52% of total genes

measured. We observed that hepatic Acaca expression was significantly up-regulated (CLA = 0.72, control = 0.22; p = 0.0027) in response to *trans*-10, *cis*-12-CLA, suggesting that the liver was generating more malonyl-CoA which was then used for palmitic acid synthesis by the multifunctional enzyme Fasn (155). Results from Ashwell et al. (32) and Kelley et al. (25) support this hypothesis by observing more C16:0 in liver tissue of obese mice fed *trans*-10, *cis*-12-CLA than those fed a LA or corn oil diet.

However, there are some studies showing no significant changes in hepatic Acaca expression in mice fed *trans*-10, *cis*-12-CLA. In a study conducted by Ashwell et al., feeding mice 1% *trans*-10, *cis*-12-CLA for 14 days resulted in accumulation of 62.5% more fat in livers and a 33% increase in liver weight (32). They surprisingly found Acaca and other genes involved in fatty acid and glycerol lipid synthesis and/or degradation were not impacted by *trans*-10, *cis*-12-CLA (32). Zabala et al. also pointed out that *trans*-10, *cis*-12-CLA (5g/kg diet for 6 weeks) increased liver weight but reduced hepatic TG content without affecting mRNA levels and the activities of several lipogenic enzymes, including Acaca, in hamsters (114). In that study, the increased liver weight might be due to a significant increase in hepatocytes number (23% $p < 0.01$) instead of excessive fat accumulation (MT Macarulla, A Fernández-Quintela, A Zabala, V Navarro, E Echevarria, I Charruca, VM Rodríguez and MP Portillo, unpublished results). These findings suggest the existence of a species-specific response of liver to CLA feeding (112).

Moreover, although our study observed an upregulation of Acaca in mice fed *trans*-10, *cis*-12-CLA, increasing fate of fatty acid synthesis by stimulating Acaca may not guarantee that the fatty acids will be diverted towards TG biosynthesis (157), because the fatty acids may be used either for biosynthesis of TG or for degradation via β oxidation in the mitochondrial matrix (157).

Scd and TG synthesis

Stearoyl-CoA desaturase (Scd) is the rate-limiting enzyme involved in the synthesis of mono unsaturated fatty acids (16:1 or 18:1) from saturated fatty acids (16:0 or 18:0) (158). Although not in the G3P pathway of TG synthesis, Scd plays an important role in the synthesis of TG (158). A proper ratio of saturated to monounsaturated fatty acids contributes to membrane fluidity and alterations have been implicated in a variety of disease states (159). The expression of the mouse Scd gene is regulated by PUFAs and cholesterol at the levels of transcription and mRNA stability (160). Many studies have showed that Scd mRNA expression in adipose tissue decreased as the *trans*-10, *cis*-12-CLA content of the diet increased (5, 24).

We observed a significant upregulation of expression of Scd (CLA = 0.05, control = -0.92; p = 0.0007) in the liver of the mice fed *trans*-10, *cis*-12-CLA. mRNA abundance of Scd accounted for 2.53% of total genes measured. Guillen et al. supported our finding by

observing an increase in hepatic Scd expression in mice fed *trans*-10, *cis*-12-CLA (154). Moreover, they indicated that Scd had a significant association with the degree of hepatic steatosis (154). Because a large number of studies have shown that *trans*-10, *cis*-12-CLA reduces fat mass considerably, the upregulation of hepatic Scd mRNA due to feeding *trans*-10, *cis*-12-CLA may be a response to greater influx of 16:0 and 18:0 derived from adipose tissue. Moreover, the increased in de novo fatty acid synthesis via up-regulation of hepatic Acaca expression also may have contributed indirectly to the greater hepatic Scd expression. Together with the up-regulation of Gpam expression in the liver (discussed as below), *trans*-10, *cis*-12-CLA-induced hepatic steatosis may, at least in part, be attributed to stimulated lipogenesis and esterification. Our result was in agreement with the findings from previous studies (5, 32) which measured the ratio of saturated to monounsaturated fatty acids as an indicator of the enzymatic activity of Scd.

GPAM and TG synthesis

In the glycerol phosphate pathway to synthesize TG, acyl-chains from acyl-CoA are transferred consecutively to G3P produced either via glyceroneogenesis or through phosphorylation of glycerol released from adipose tissue during lipolysis (161). This reaction is catalyzed by G3P acyltransferase (Gpam) which resides in the outer mitochondrial membrane (162), and it is the first committed step in the synthesis of TG

(162, 163) via the glycerol phosphate pathway. Besides, Gpam is an enzyme that can switch the fate of fatty acids from β oxidation to glycerolipid synthesis (164, 165).

In our study, Gpam mRNA abundance accounted for 3.18% of total transcripts measured. Although a previous study found that *trans*-10, *cis*-12-CLA did not change statistically the hepatic expression of Gpam in mice (32), we observed a marked increase in hepatic Gpam expression (CLA = 0.44, control = -0.31; p = 0.003) in mice fed *trans*-10, *cis*-12-CLA, which was suggestive of stimulation of esterification in order to synthesize TG in liver. It could be possible that the concerted upregulation of both Acaca and Gpam was a mechanistic response to promote biosynthesis of TG, e.g. the up-regulation of Acaca stimulated de novo fatty acid synthesis, thus, generated more precursor for TG synthesis and resulting in increased Gpam expression.

Moreover, there is a potential that blood NEFAs derived and exported from adipose tissue went into liver and most of them would be esterified into TG via Gpam. From this point, synthesis of TG under catabolic conditions (i.e. as it would occur during lipolytic stimulation) is not a result of lipogenesis but esterification of blood derived NEFA and lower rates of oxidation of fatty acids to ketones and carbon dioxide. All in all, our results supported the hypothesis that *trans*-10, *cis*-12-CLA-induced lipogenesis and esterification are primarily responsible for liver TG accumulation (33, 124, 129).

Dgat1, Dgat2 and TG synthesis

There are two major biochemical pathways for TG synthesis: the monoacylglycerol (MAG) pathway and the G3P pathway (166). The former pathway plays a predominant role in the enterocytes (167) and adipose tissue (168), while the latter is a de novo pathway in most tissues. These two pathways share the final step in converting diacylglycerol (DG) to TG, a reaction catalyzed by diacylglycerol acyltransferase (Dgat) (158, 169). Yamazaki et al. found that Dgat1 is located in the lumen of the endoplasmic reticulum and promotes VLDL secretion, while Dgat2 has potent Dgat activity and plays a key role in cytosolic lipid accumulation; Dgat2 overexpressing mice had a 3.1-fold increase in hepatic fat content (170). In line with this observation, in high-fat fed obese mice liver-specific Dgat2 inhibition caused a marked reduction in hepatic TG content and plasma lipid levels (171).

In the present study, Dgat1 mRNA abundance accounted for 0.15%, whereas Dgat2 mRNA abundance accounted for 4.93% of total genes measured. We observed numerical increases in the hepatic expression of Dgat1 (CLA = 0.30, control = -0.02; p = 0.18) and Dagt2 (0.25, control = 0.03; p = 0.30) in the mice fed *trans*-10, *cis*-12-CLA. Although there was substantial variation in the expression of Dgat2 among animals, it could be speculated that this increase in Dgat2 mRNA expression corresponded to an actual metabolic requirement for stimulating TG synthesis. Such response has been clearly

observed in numerous studies with *trans*-10, *cis*-12-CLA that resulted in liver enlargement (25, 28-32, 113) (See Table 4.2).

Trans-10, *cis*-12-CLA stimulated glyceroneogenesis in liver

Pck1 and gluconeogenesis and glyceroneogenesis

Phosphoenolpyruvate carboxykinase 1 (Pck1) catalyzes the rate-limiting step in the gluconeogenesis pathway, converting oxaloacetate to phosphoenolpyruvate (172). In addition, Pck1 also is important for generation of G3P during the process of glyceroneogenesis which under most physiological circumstances is the predominant pathway to generate the glycerol backbone for TG synthesis (173). Pck1 mRNA abundance accounted for 14.79% of total genes measured. We observed a significant increase in hepatic Pck1 expression (CLA = -0.29, control = -1.74; p = 0.05) in mice fed *trans*-10, *cis*-12-CLA, which was indicative of increased glyceroneogenesis and/or gluconeogenesis in liver. Although we did not measure the plasma insulin level in mice, the *trans*-10, *cis*-12-CLA-induced hyperinsulinemia with a normal range of blood glucose level demonstrated by Clément et al. (33) would inhibit gluconeogenesis via Pck1. Furthermore, a greater degree of glyceroneogenesis would agree with the greater expression of Gpam. Therefore, this could contribute to enhanced TG synthesis and accumulation in liver due to *trans*-10, *cis*-12-CLA.

Pdk4 and glucose metabolism

The pyruvate dehydrogenase complex (PDC) catalyzes the irreversible oxidative decarboxylation of pyruvate into acetyl-CoA (174). Regulating PDC is an important step in fuel selection of energy utilization in animals during different nutritional and hormonal states as the modulation of PDC activity impacts fatty acid as well as pyruvate and glucose metabolism (175). Phosphorylation of PDC via pyruvate dehydrogenase kinase (Pdk) inhibits its activity (175, 176), thereby reducing the conversion of pyruvate to acetyl-CoA (177). Four isoenzymes of Pdk have been identified in mammalian tissues, including Pdk1, Pdk2, Pdk3 and Pdk4 (176). The abundance of the Pdk4 isoform, which is highly expressed in liver, heart and skeletal muscle, is transcriptionally controlled (178).

Pdk4 mRNA abundance accounted for < 1% of total genes measured. In our study, the group fed with *trans*-10, *cis*-12-CLA had significantly higher hepatic Pdk4 expression (CLA = 0.47, control = -1.85; p = 0.001) compared to the control mice, indicative of reduced glucose oxidation. Since expression of the Pdk4 gene is induced by high fat diets and long chain fatty acids (179), the results may suggest that increased Pdk4 expression could be the consequence of *trans*-10, *cis*-12-CLA intake.

Trans-10, *cis*-12-CLA enhanced lipid droplet formation in the liver

Plin and lipid droplet formation

Perilipin (Plin) proteins are vital for cytoplasmic lipid droplet (LD) formation and also lipolysis (180). In isolated hepatocytes, as well as livers from mice and humans, Plin2 levels are proportional to hepatic lipid content (181); however, it is unclear if increased Plin2 is a cause or consequence of fatty liver (182). Importantly, the Plin2 knockout mice display a 60% reduction in hepatic TG and are resistant to diet-induced fatty liver (183). To determine the mechanism for the reduced hepatic TG content, that study measured hepatic lipogenesis, VLDL secretion, and lipid uptake and utilization, all of which were shown to be similar between mutant and wild-type mice (183). The finding of similar VLDL output in the presence of a reduction in total TG in the Plin2 knockout liver was explained by retention of TG in the microsomes where VLDL is assembled. Given that LDs are thought to form from the outer leaflet of the microsomal membrane, the reduction of TG in the cytosol with concomitant accumulation of TG in the microsome of Plin2 knockout cells suggested that Plin2 facilitates the formation of new LDs (183).

In our study, expression of Plin2 mRNA accounted for 1.61%. We found that the hepatic expression of Plin2 in the mice fed *trans*-10, *cis*-12-CLA was significantly up-regulated (CLA = 0.40, control = -0.43; $p = 0.0017$). Since Plin2 may play a role in facilitating the formation of new LDs (183), the up-regulation of Plin2 was suggestive of

enhanced formation of hepatic LDs in mice fed *trans*-10, *cis*-12-CLA, thereby, at least in part, contributing to TG accumulation and hepatic steatosis. This may be explained by its specific role in the early stages of lipid droplet formation (182). Therefore, we suggest that enhanced LD formation via up-regulated expression of Plin2 could be a novel mechanism of *trans*-10, cis-12-CLA's hepatic steatotic effect on liver.

Trans-10, *cis*-12-CLA increased VLDL assembly and secretion

Apob, Mttp and VLDL assembly/secretion

In the liver, synthesized TG is either stored in cytoplasmic droplets or secreted as VLDL particles (170). Most de novo synthesized TG is stored in cytosolic TG pools and a smaller portion is secreted in the form of VLDL. Cytosolic TG pool size has been shown to correlate with VLDL secretion. Apo-protein B 100 (Apob) is the key component whose rate of synthesis in the rough endoplasmic reticulum controls the overall rate of VLDL production (184). Lipid components are added to apoprotein B (185) by microsomal TG transfer protein (Mttp) (186). Then the apoproteins are glycosylated in the Golgi apparatus (184) where secretory vesicles bud off, migrate to, and fuse with the membrane of the hepatocytes and release the VLDL into blood (184). Since Mttp catalyzes the transfer of lipids to the apoB molecule, Mttp may play a crucial role in the assembly and secretion of VLDL in the liver (187). Patients with abetalipoproteinemia have a defect in the Mttp gene

resulting in severely deficient lipoprotein secretion (188). Furthermore, Mttp inhibitors can reduce the production of apoB-lipoproteins in both hepatoma cell cultures (189-191) and *in vivo* in a rabbit model of human homozygous familial hypercholesterolemia (192). Minehira et al. showed that in mouse liver, mutant Mttp gene (homozygous knockout) increases hepatic steatosis in mouse (193).

Both Lin et al. and Yotsumoto et al. indicated that *trans*-10, *cis*-12-CLA reduced both apoB synthesis and TG secretion in HepG2 cells (144, 146). Several *in vivo* studies (31, 48, 113) observed reduced plasma TG levels in mice fed *trans*-10, *cis*-12-CLA, supporting the findings from *in vitro* studies. In contrast, when it comes to clinical studies, previous short-term studies in humans have revealed varying effects of CLA on blood lipids. Whigham et al. (120) found increased plasma TG levels in subjects receiving 6 g/d of CLA mixture (*trans*-10, *cis*-12-CLA: *cis*-9, *trans*-11-CLA = 1:1) after 28 and 52 weeks of feeding compared with controls. In contrast, Iwata et al. observed no significant changes in blood TG from Japanese male volunteers receiving low or high CLA mixtures or control (119). Another study found a reduction of VLDL-TG and TG in the blood in human subjects receiving 3 g/d of CLA mixture (*trans*-10, *cis*-12-CLA: *cis*-9, *trans*-11-CLA = 1:1) for 8 weeks (194).

In our study, Apob mRNA abundance accounted for 9.14% and Mttp mRNA abundance accounted for 1.24% of total genes measured; however, we observed a numerical

increase in Apob expression (CLA = -0.05, control = -0.33; p = 0.29) and a significant up-regulation of expression of Mttp (CLA = 0.14, control = -0.37; p = 0.04) in the mice fed *trans*-10, *cis*-12-CLA. Despite the apparent accumulation of TG in the liver (24) leading to greater weight (Table 4.1), our results suggest that the VLDL assembly/secretion mechanism in mice fed *trans*-10, *cis*-12-CLA was not impaired and may have actually been enhanced via the up-regulation of hepatic Mttp. It was reported previously that in *trans*-10, *cis*-12-CLA-fed mice the VLDL secretion rate was increased; whereas, the plasma TG concentration was decreased (30). Together with our results, studies suggest that TG-rich lipoproteins and NEFAs are taken up in the *trans*-10, *cis*-12-CLA-fed animals to such an extent that the VLDL secretion rate, even when increased, is insufficient to eliminate excess fatty acid (Table 4.1) entering the whole esterification and VLDL assembly/secretion pathways, which would account for part of the fat deposit within liver cells.

Furthermore, our findings were in agreement with recent kinetic studies. They have pointed out that the actual amount of hepatic TG-rich lipoprotein secretion rate in non-alcoholic fatty liver disease is actually increased, but is inadequate to match the increased TG synthesis in the liver (195). One of those studies found that subjects with NAFLD failed to further increase VLDL-TG secretion rate when hepatic TG infiltration exceeded 10% suggesting the liver of these subjects had a limited capacity to secrete VLDL particles (195).

Cideb and VLDL secretion

The cell death-inducing DFF45-like effector (Cide) family of proteins consists of three molecules, including Cidea, Cideb and Cidec/fat specific protein 27 (Fsp27) (196). Mouse Cideb is mainly expressed in liver and kidney (197). Recently, Li et al. observed that liver of Cideb null mice had higher levels of TG accompanied by lower levels of VLDL secretion (198). They also found that Cideb is localized to smooth ER and LDs and identified apoB as a Cideb-interacting protein (198). By infecting adenoviruses expressing various Cideb truncations into hepatocytes of Cideb-null mice, they found that Cideb requires both its apoB-binding and LD association domains to restore the secretion of TG-enriched VLDL particles (198). Their data suggest that Cideb promotes the formation of TG-enriched VLDL particles (198).

Cideb mRNA abundance accounted for 3.46% of total genes measured in the present study and its expression in mice fed *trans*-10, *cis*-12-CLA was significantly up-regulated (CLA = -0.04, control = -1.13; p = 0.05). The greater expression of Cideb in the liver suggested more effective TG secretion via VLDL and underscored that hepatic steatosis induced by *trans*-10, *cis*-12-CLA was not due to impaired VLDL secretion. However, given the increased liver weight, the increase in VLDL secretion might be insufficient to eliminate the fatty acid (Table 4.1) entering the whole esterification and VLDL assembly/secretion pathways.

Transcription regulation and lipogenesis and TG synthesis

Srebf1-related networks and TG synthesis

Sterol regulatory element binding transcription factor 1 (Srebf1), also known as sterol regulatory element-binding protein 1 (Srebp1), is a transcription factor involved in hepatic lipogenesis and also adipocyte differentiation (199, 200) . It is predominantly expressed in liver and adipose tissue (201-203). Srebf1 is synthesized as a precursor protein and is released from the ER membrane into the nucleus as a mature protein by a sequential cleavage process to bind sterol regulatory elements and then activate expression of the target genes (204, 205), such as Acaca (206) and Scd1 (207). Overall, Srebf1 plays a central role in energy homeostasis by promoting glycolysis, lipogenesis, and adipogenesis (208).

Srebf1 mRNA abundance accounted for 0.67% of total genes measured in our study. The hepatic Srebf1 expression in mice fed *trans*-10, *cis*-12-CLA was significantly up-regulated (CLA = 0.14, control = -0.22; p =0.02), which was in agreement with previous published reports indicating that a CLA-mix diet significantly induced the Srebf1 expression in the murine liver (33, 209). In freshly-isolated hepatocytes, Srebf1c mRNA was activated by insulin (210). Thus, the up-regulated Srebf1 gene in our study may have been a response to higher levels of insulin in mice fed *trans*-10, *cis*-12-CLA which has been previously reported by Clément et al, Halade et al. and Tsuboyama-Kasaoka et al (22, 33, 48). In our study, the expression of Srebf1's target genes, including Acaca, Scd, and Gpam

(206), were all significantly up-regulated in mice fed *trans*-10, *cis*-12-CLA, which suggests that the liver was generating more palmitic acid, oleic acid and TG (211). Collectively, these results indicate that *trans*-10, *cis*-12-CLA stimulates the lipogenic pathway by indirectly up-regulating Srebf1, leading to an overall increase in fat accumulation in liver.

Chrebp and lipid metabolism

Carbohydrate response element binding protein (Chrebp) is a transcription factor responsible for glucose-induced transcription of liver pyruvate kinase (LPK) gene (212). Some studies provided evidence for a direct and dominant role of Chrebp in the glucose regulation of two key liver lipogenic enzymes, Acaca and Fasn, therefore Chrebp is also known to be major regulator of lipogenic enzymes (157, 213). Chrebp is expressed constitutively in hepatocytes (214) and is known to recognize E box sequences in the promoters of target genes (215). The regulation of Chrebp involves phosphorylation-dependent mechanisms responsive to feeding (glucose and fatty acids) and fasting (glucagon) (216). Under low glucose conditions, Chrebp remains in the cytosol and unable to bind DNA as the result of phosphorylation at multiple sites (214). The dephosphorylation of Chrebp in response to high glucose does not require insulin (213).

In our study, Chrebp mRNA was numerically up-regulated (CLA = -0.17, control = -0.22; p = 0.62). Chrebp mRNA abundance accounted for 2.83% of total genes measured.

However, some of its target genes involved in lipid metabolism in the liver were significantly up-regulated, including Acaca and Gpam, suggesting that *trans*-10, *cis*-12-CLA indirectly stimulated lipogenic genes through Chrebp as well as Srebf1.

Pparα and lipid catabolism

The ligand-activated transcription factor, peroxisome proliferator-activated receptor α (Pparα), modulates lipid catabolism via specific Ppar responsive elements (PPREs) in the regulatory regions of genes such as lipoprotein lipase (134), Acox1 (217), Cpt1, thioesterases, fatty acid binding protein, peroxisomal and mitochondrial β oxidation enzymes, microsomal ω-oxidizing enzymes and apolipoproteins (131). Peroxisome proliferators (PPs) activate Pparα which then heterodimerises with RXR and binds to DNA at PPREs upstream of PP responsive genes (218). Activation of Pparα by ligands results in altered transcription of many of these genes which may stimulate fatty acid uptake and activation, mitochondrial oxidation, peroxisomal fatty acid oxidation, ketogenesis, and fatty acid elongation and desaturation (219).

Isomers of CLA have some structural features similar to PPs and importantly the physiological responses observed in mice (reduced body weights, hepatic lipid accumulation, hypolipidemia) are characteristic of this group of chemicals (134). As *trans*-10, *cis*-12-CLA has been reported to be a potent ligand of Pparα (134), the underlying

regulations might imply the activation of Pparα. In our study, the hepatic Pparα expression (CLA = -0.19, control = -0.33; p = 0.42) in mice fed *trans*-10, *cis*-12-CLA was numerically higher than in the control group. Pparα mRNA abundance accounted for 1.28% of total genes measured. Although no significantly up-regulation of Pparα was observed in our study, many of its target genes playing important roles in lipid metabolism (Cd36, Plin2, Scd, Pck1, Pdk4, Acox1, Ucp2 and Srebf1) were significantly up-regulated, providing indirect evidence that *trans*-10, *cis*-12-CLA might act through Pparα.

Despite dietary *trans*-10, *cis*-12-CLA induced expression of Pparα target genes, including fatty acid transporter, Srebp1 and Fasn genes, *in vitro* transactivation assays demonstrated that *trans*-10, *cis*-12-CLA and *cis*-9, *trans*-11-CLA were equally efficient at activating Pparα, thus the specific effect of *trans*-10, *cis*-12-CLA is unlikely to result from direct interaction with this nuclear receptor (33). In another study, wild-type or Pparα-null mice were fed a diet containing 0.5% CLA mixture (*trans*-10, *cis*-12-CLA represented 43.5% of CLA). After 4-week feeding period, while CLA feeding resulted in specific activation of Pparα target genes in liver that contribute to increased use of fatty acids as substrates and subsequent reductions in body weight/fat content, changes in those Pparα target gene expression were found in both wild-type and Pparα-null mice fed CLA (125) (125).

Apart from those, numerous lines of evidence indicate that it has a major role in

glucose metabolism (220). First, several studies (221-223) showed that fasting Pparα-null mice display marked hypoglycemia. Second, both Guerre-Millo et al. and Tordjman et al. found that high-fat feeding-induced insulin resistance in mice was mitigated in the absence of Pparα (224, 225). Third, a study found that induction of the gluconeogenic genes Pck1 and glucose 6-phosphatase by dexamethasone is Pparα dependent (226), although they are not direct target genes of Pparα (220). Fourth, Patsouris et al. showed that Pparα decreases plasma glycerol levels in mice and humans by directly upregulating the expression of genes involved in hepatic gluconeogenesis from glycerol, including cytosolic glycerol 3-phosphate dehydrogenase (cGpdh), mitochondrial glycerol 3-phosphate dehydrogenase (mGpdh), glycerol kinase, aquaporin 3 (AQP3), and aquaporin 9 (AQP9) (220). They also pointed out that the stimulatory effect of Pparα on gluconeogenic gene expression is associated with elevate hepatic glucose production during fasting (220). Above all, it can be concluded that Pparα has an important influence on glucose metabolism.

Trans-10, *cis*-12-CLA increased fatty acid oxidation in the liver

Since the decrease in fatty acid oxidation may cause fat accumulation in the liver (31), it is intuitive that the mRNA expression of the enzymes related to fatty acid oxidation may be down-regulated during liver lipidosis. However, recent investigations of the *trans*-10, *cis*-12-CLA's effects on fatty acid oxidation are controversial. Rasooly et al.

indicated that *trans*-10, *cis*-12-CLA supplementation decreased the expression of genes involved in fatty acid oxidation, including flavin monooxygenase-3 (FMO3) (95%, $p < 0.0001$), cytochrome P450 (cyt P450) (61%, $p = 0.002$), Cpt1a (77%, $p = 0.025$), acetyl CoA oxidase (50%, $p = 0.08$) and Pparα (65%, $p = 0.05$) (227). However, in the study of Gruffat at el., liver tissue samples of rats incubated with a fatty acid mixture (representative of circulating NEFA) and *trans*-10, *cis*-12-CLA or oleate they found that the rate of CLA isomer oxidation was two times higher than that of oleate ($p < 0.01$) (150). Furthermore, expression of mice hepatic fatty acid oxidation genes also increased in three studies with a mixture of CLA isomers (124, 129, 228) and in one study with *trans*-10, *cis*-12-CLA (31).

Cpt1 and fatty acid oxidation

Cpt1 is considered the rate-limiting enzyme for mitochondrial β oxidation (227). Degrace et al. found an increase in the activity of hepatic Cpt1 in mice supplemented with *trans*-10, *cis*-12-CLA accompanied by an increase in Acox1 activity and gene expression (31). Another study conducted by Viswanadha et al. noted that *trans*-10, *cis*-12-CLA increased the oxidation of fatty acids in murine hepatic cell lines, as indicated by increased expression of mRNA for Cpt1 (229). In our study, *trans*-10, *cis*-12-CLA supplementation the hepatic expression of Cpt1a (CLA = -0.32, control = -0.43; $p = 0.57$) was numerically greater with CLA. Cpt1a mRNA abundance accounted for 1.91% of total genes measured.

Moreover, malonyl-CoA plays a crucial role in the control of mitochondrial fatty acid oxidation through its inhibition of Cpt1 (230). As mentioned in the section discussing Acaca and de novo fatty acid synthesis, more malonyl-CoA was generated by up-regulated Acaca expression in liver in response to *trans*-10, *cis*-12-CLA. As a result, Cpt1 expression was supposed to down-regulated or inhibited. However, unexpectedly we observed a numerically upregulation of Cpt1 expression in liver.

Acox1 and fatty acid oxidation

Peroxisomes play an important role in lipid catabolism, since very long chain fatty acids (VLCFAs) are almost exclusively processed by the peroxisomal β oxidation system, whereas fatty acids up to 18 carbons in length are oxidized by the mitochondria (231). Acyl-CoA oxidase 1 (Acox1) catalyzes the first and rate-limiting enzyme of the peroxisomal fatty acid β oxidation pathway of VLCFAs (232). The reduction of the activity and/or mRNA of fatty acid oxidation enzymes in peroxisomes could contribute to the lipid accumulation in hepatocytes and to the severity of steatohepatitis. Fan et al. found that mice lacking the peroxisomal Acox1 gene developed severe steatohepatitis, lipogranulomas and hepatocellular carcinomas (233). Acox1 mRNA abundance accounted for 6.83% of total genes measured in our study and we found that mice fed *trans*-10, *cis*-12-CLA had greater Acox1 expression (CLA = 0.13, control = -0.22; p = 0.05), suggesting increased fatty acid β

oxidation in peroxisomes. The up-regulation of Acox1 might be due to the relatively lower amount of dietary *trans*-10, *cis*-12-CLA supplementation compared with previous studies (22, 23, 28, 32, 34-36).

A study conducted by Clément et al. showed that mice fed a diet enriched in 0.4% (w/w) *trans*-10, *cis*-12-CLA for 4 weeks developed fatty liver and observed a dramatic increase in non fasting plasma insulin levels which were 10 times higher in these mice than those fed control (33). Despite this marked hyperinsulinemia, they also pointed out that plasma glucose concentration remained within the normal range in these mice fed *trans*-10, *cis*-12-CLA (33). Thus, they suggest that the train of events leading to *trans*-10, *cis*-12-CLA-induced alterations in liver is probably indirect and that the liver steatosis occurring in *trans*-10, *cis*-12-CLA-fed mice is secondary to hyperinsulinemia which causes high levels of fatty acid uptake and synthesis (33).

In our study, the up-regulation of Acox1coupled with the lack of change in Cpt1 mRNA expression indicated that decreased fatty acid oxidation could not be the factor triggering hepatic steatosis (Table 4.1), but might be a consequence of it. Under hyperinsulinemia condition, the effect of elevated β oxidation due to *trans*10, *cis*-12-CLA was overwhelmed by an increase in uptake of fatty acids as well as greater lipogenesis in liver, thereby resulting in hepatic steatosis as observed previously (24).

Trans-10, *cis*-12-CLA stimulated ketogenesis in the liver

Hmgcs2 and ketogenesis

Under conditions of increased fatty acid uptake and low blood insulin to glucagon ratio, the liver often produces large amounts of the ketone bodies, acetoacetate and β–hydroxybutyrate, in a process known as ketogenesis (184). Moreover, ketogenesis is controlled indirectly by Cpt1 (234) and directly by the activity of the mitochondrial key regulatory enzyme 3-hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2) (235). Conversion of acetyl-CoA to ketone bodies, rather than complete oxidation in the TCA cycle, results in the formation of less ATP/mole of fatty acid oxidized, e.g. five time less in the case of palmitate (129 vs. 27 ATP/mole, TCA and oxidative phosphorylation in the electron transport chain vs. conversion of acetyl-CoA to ketone bodies) (184). Then why liver produces ketone bodies? Ketogenesis allows the liver to metabolize about five times more fatty acids (for the same ATP yield) and synthesize water-soluble fuels that other tissues can use when there is little glucose available (184).

In the present study, Hmgcs2 mRNA abundance accounted for 13.88% of total genes measured, which was the second highest gene expression among all the genes determined. Our results support previous findings of greater hepatic Hmgcs2 expression (CLA = 0.13, control = -0.16; p = 0.05) in mice fed *trans*-10, *cis*-12-CLA, suggesting that this CLA isomer induces more ketone body generation in liver, which was in agreement

with a previous study showing that the liver of rats fed CLA produced significantly more ketone bodies than those of rats fed a linoleic acid-rich diet (236).

Bdh1 and ketone body utilization

3-hydroxybutyrate dehydrogenase 1 (Bdh1) plays a key role in redox balance and energy metabolism since in the presence of NADH, the hepatic Bdh transforms acetoacetate into D-3-hydroxybutyrate, which is then transported through the blood stream to peripheral tissues, e.g., brain, heart, and kidney (237). Bdh1 mRNA abundance accounted for 3.35% of total genes measured. We observed that Bdh1 expression in mice fed *trans*-10, cis-1-CLA (CLA = 0.13, control = -0.20; p = 0.05) was significantly higher compared with the control. The increase in Bdh1 expression suggested that more 3-hydroxybutyrate was produced from acetoacetate, which was supported by previous findings showing that liver from CLA-fed rats produced significantly more ketone bodies and the ratio of 3-hydroxybutyrate to acetoacetate was consistently higher in the liver perfusate (236).

Together with data on Hmgcs2 this was indicative of stimulated ketone body production in liver. Indirectly, expression of Hmgcs2 and Bdh1 would support the notion that there likely was greater adipose tissue lipolysis in these mice, thus, providing ample NEFA for oxidation and ketogenesis. The results suggest that probably due to high rate of fatty acid oxidation in the liver, increased generation of acetyl CoA exceeded the capacity of

the TCA cycle; as a result, acetyl CoAs were used for ketogenesis and thereby leading to increased expression of Hmgcs2 and Bdh1 expression in liver.

***Trans*-10, *cis*-12-CLA changes energy metabolism in liver**

Ucp2 and energy metabolism

Uncoupling proteins (Ucps) belong to a family of mitochondrial anion carriers and are present in the mitochondrial inner membrane (238). Ucps dissipate the proton gradient by allowing the re-entry of protons into the mitochondrial matrix during oxidative ATP generation, resulting in the uncoupling of the respiratory chain and heat production (239). Ucp2 isoform shows the widest tissue expression of all Ucps including liver, skeletal muscle, heart and kidney (240). *In vivo* studies indicate that physiological and pathological elevation of blood LCFAs resulting from fasting (241-243) or high fat diet (244) induce upregulation of Ucp2. Peters et al. demonstrated that dietary CLA increases gene expression of Ucp2 in the liver of C57BL/6N mice (125). Ucp2 mRNA abundance accounted for 0.19% of total genes measured. Our study observed an upregulation of hepatic Ucp2 expression (CLA = 0.15, control = -0.31; p = 0.002) in mice fed *trans*-10, *cis*-12-CLA suggesting that the increased Ucp2 mRNA expression may serve as a counterregulatory mechanisms to lower cellular ATP levels and decrease metabolic efficiency, thereby reducing fat accumulation in the long-term (240).

***Trans*-10, *cis*-12-CLA and stress response**

According to the complex mechanisms proposed by Jaudszus et al. (5), the anti-adipogenic effect of *trans*-10, *cis*-12-CLA may be primarily the consequence of pro-apoptotic and pro-inflammatory responses in adipose tissue, including the nuclear factor κB (NFκB)-dependent production of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8) (21, 22, 245). These cytokines produced by adipose tissue induce the expression of the proteins suppressor of cytokine signaling-3 (SOCS-3) and JNK, which can inhibit insulin signal transduction leading to hyperinsulinemia and insulin resistance (21), thereby suppressing lipid synthesis and increasing lipolysis in adipocytes. The *trans*-10, *cis*-12-CLA-mediated suppression of the transcription factor PPARγ in the adipose tissue was suggested to be indirectly responsible for the pro-inflammatory conditions (21, 246).

In our study, the genes associated with inflammation or stress response as listed in Table 7 were all numerically up-regulated , a response likely related to the relatively low dosage of *trans*-10, *cis*-12-CLA that was fed.

Summary

In our study the excess fatty acid accumulation and greater liver mass in growing male mice consuming *trans*-10, *cis*-12-CLA seems to be attributed to increased fatty acid

uptake, stimulated TG synthesis, and enhanced LD formation in the liver. Guillén et al. observed a significant increase in hepatic Cd36 expression in mice fed *trans*-10, *cis*-12-CLA and demonstrated a significant association between Cd36 expression and the degree of hepatic steatosis. Our finding of up-regulation of Cd36 expression in the liver supported those earlier results. Hence, the increased hepatic fatty acid uptake could be one potential mechanism by which *trans*-10, *cis*-12-CLA causes hepatic steatosis, i.e. the peripheral action of *trans*-10, *cis*-12-CLA leading to adipose tissue catabolism provides NEFA which must be metabolized by tissues and primarily liver.

The greater up-regulation of Acaca, Gpam and Scd mRNAs indicates that increased TG synthesis in the liver contributes to hepatic steatosis in mice fed *trans*-10, *cis*-12-CLA. Furthermore, the up-regulation of Acaca indicated that the increased hepatic lipogenesis would be used for biosynthesis of TG via Gpam. Hepatic lipogenesis encompasses a complex series of reactions occurring in part in the cytosol and the mitochondrial matrix (247). Ferramosca et al. found that the activity of mitochondrial citrate carrier (Cic), considered as the bridge connecting these two different cellular compartments, was stimulated by a CLA mixture. The consequent increase in citrate efflux from mitochondria would supply the cytosol with the carbon source, in the form of acetyl-CoA for hepatic lipogenesis (247).

We also observed a potential for enhanced lipid droplet formation in the liver of

the mice fed *trans*-10, *cis*-12-CLA. The up-regulation of Plin2 which is vital for cytoplasmic lipid droplet formation, suggests that the steatotic effect of *trans*-10, *cis*-12-CLA may, in part, be achieved by increasing the lipid storage capacity of hepatocytes. Therefore, it is suggested that increased fatty acid uptake, stimulated TG synthesis, and enhanced lipid droplet formation might together contribute to the hepatic steatotic effect of *trans*-10, *cis*-12-CLA.

The mechanisms by which the liver becomes steatotic in response to *trans*-10, *cis*-12-CLA appear to be complex, since our study found that *trans*-10, *cis*-12-CLA induced β-oxidation in the peroxisome without changing it in the mitochondria as evidenced by the up-regulation of Acox1 and lack of effect on Cpt1. Induced β-oxidation would not be associated with lipid accumulation. There are two possibilities to explain these scenarios. First, induced β-oxidation in peroxisomes might be a consequence of excess fat accumulation in the liver and a favorable mechanism to help consume excess fatty acid influx into liver because expression of Pnpla2 (a TG lipase) was numerically greater with *trans*-10, *cis*-12-CLA. Second, the relatively low level of supplementation of *trans*-10, *cis*-12-CLA was not enough to impair hepatic β-oxidation which has been observed in the study (127) conducted by Rasooly et al.

Apart from β-oxidation, reduced VLDL assembling/secretion would not be a factor triggering hepatic steatosis in the mice fed *trans*-10, *cis*-12-CLA. In our study, the

expression of Mttp, which plays a crucial role in the assembly and secretion of VLDL in the liver, was up-regulated in the mice fed with *trans*-10, *cis*-12-CLA; and, hepatic CIDEB expression was significantly higher in the mice fed with *trans*-10, *cis*-12-CLA than that in control mice, indicating more effective TG secretion via VLDL. Thus, hepatic steatosis in the mice fed with *trans*-10, *cis*-12-CLA would not be due to impaired VLDL assembling/secretion.

The increased VLDL assembling/secretion could be a result of excess TG accumulation in liver and the subsequent lipolysis via Pnpla2. However, there is only one possibility that the increased VLDL assembling/secretion could be a triggering factor of fatty liver, when the VLDL overproduction from the enlarged liver was insufficient to eliminate the flux of fatty acids entering the whole esterification and lipoprotein assembling/secretion pathway. After being absorbed in hepatocytes, fatty acids must be therefore directed towards the esterification pathway. As a result, the increase in Dgat mRNA expression in the liver of *trans*-10, *cis*-12-CLA-fed mice corresponded to an actual metabolic requirement for meeting the excessive flux of fatty acids, which was in agreement with the findings from Degrace et al. They also indicated that the flux of lipids permanently cleared from blood and taken up more abundantly by a heavier liver was possibly the starting point of the observed inductions (30).

Table 4.1 Liver weight and fatty acid content for male mice fed trans-10, cis-12-CLA (24)

	Dietary *trans*-10, *cis*-12-CLA			
	0%	0.30%	SEM	P < *
Liver weight (g)	1.99	2.36	0.12	0.04
Adipose weight (g)	0.61	0.18	0.05	0.0001
Total fatty acids (́g/mg)	38.8	59.0	3.0	0.01

* Probability of a linear effect due to dietary trans-10, cis-12-CLA content.

Table 4.2 Summary of mouse studies including liver weight, liver TG, adipose tissue measurements in response to *trans*-10, *cis*-12-CLA

Reference	Dose	Duration	*trans*-10, *cis*-12-CLA*		
			Liver weight	Liver TG	Adipose tissue
Clément et al. (2002) (33)	0.4% (w/w)	4 wks	3.1-fold ↑		85% ↓
Degrace et al. (2003) (30)	10 g	4 wks	60% ↑	655% ↑	83% ↓
Warren et al. (2003) (113)	0.5%	8 wks	98% ↑	472% ↑	59% ↓
Degrace et al. (2004) (31)	1%	4 wks	80% ↑	646% ↑	85% ↓
Kelley et al. (2004) (25)	0.5%	8 wks		30% ↑	
House et al. (2005) (248)	1%	2 wks	33% ↑	61% ↑ (fat)	58% ↓
Viswanadha et al. (2006) (24)	0.30%	6 wks	17% ↑	52% ↑ (total fatty acids)	81% ↓
Halade et al. (2009) (48)	0.5%	0.5 y	50% ↑		73% ↓
Ashwell et al. (2010) (32)	1%	2 wks	33% ↑	62.5% ↑ (fat)	

* The increase or decrease in percentage was calculated by (CLA – Control) / Control.

Table 4.3 Hepatic expression of genes related to lipogenesis and carbohydrate metabolism following *trans* -10, *cis* -12-CLA consumption by growing male mice

	Treatments[1]		Time		
Gene	Control	CLA	Week 2	Week 6	SEM[2]
Carbohydrate Metabolism					
Gck	-0.45	-0.49	-0.31	-0.63	0.28
Pc	-0.31	-0.08	-0.08	-0.32	0.28
Pck1	-1.74 [a]	-0.29[b]	-0.80	-1.24	0.62
Slc2a1	-0.07	0.21	0.18	-0.04	0.14
Liver TG Synthesis & Transport					
Apob	-0.33	-0.05	-0.09	-0.29	0.19
Cideb	-1.13 [a]	-0.04 [b]	-0.35	-0.81	0.50
Dgat1	-0.02	0.30	0.23	0.049	0.17
Dgat2	0.03	0.25	0.26	0.02	0.15
Gpam	-0.31 [a]	0.44 [b]	0.13	0.004	0.17
Mttp	-0.37 [a]	0.14 [b]	0.002	-0.22	0.17
Plin2	-0.43 [a]	0.40 [b]	-0.01	-0.02	0.18
Scd	-0.92 [a]	0.05 [b]	-0.50	-0.37	0.19
De novo Fatty Acid Synthesis					
Acaca	0.22 [a]	0.72 [b]	0.42	0.51	0.11
Lipolysis					
Lipc	-0.95	-0.20	-0.15	-0.10	0.54
Pnpla2	-0.04	0.14	0.24	-0.13	0.24
Transcription Regualtion					
Chrebp	-0.22	-0.17	-0.19	-0.19	0.08
Srebf1	-0.22 [a]	0.14 [b]	0.04	-0.11	0.11

[1]Means with different superscripts differ ($P \leq 0.05$).

[2]SEM = the largest standard error of the mean is shown.

Table 4.4 Hepatic expression of genes related to lipid catabolism following *trans* -10, *cis* -12-CLA consumption by growing male mice

	Treatments[1]		Time		
Gene	Control	CLA	Week 2	Week 6	SEM[2]
Fatty Acid Oxidation & Energy Metabolism					
Aacs	-0.21	-0.29	-0.26	-0.25	0.31
Acox1	-0.22 [a]	0.13 [b]	-0.08	-0.0032	0.14
Bdh1	-0.20 [a]	0.13 [b]	0.06	-0.12	0.12
Cpt1a	-0.43	-0.32	-0.36	-0.39	0.14
Insig1	-0.79	0.35	-0.23	-0.20	0.45
Mlycd	0.00054	-0.17	-.0.07	-0.11	0.10
Pdk4	-1.85 [a]	0.47 [b]	-0.81	-0.57	0.50
Ucp2	-0.31 [a]	0.15 [b]	-0.04	-0.11	0.10
Ketogenesis					
Hmgcs2	-0.16[a]	0.13[b]	0.10[a]	-0.13[b]	0.10
Fatty Acid Uptake					
Cd36	-1.50 [a]	1.11 [b]	-0.01	-0.35	0.55
Slc27a2	-0.23	-0.04	-0.07	-0.20	0.18
Transcription Regulation					
Pparα	-0.33	-0.19	-0.23	-0.30	0.12

[1]Means with different superscripts differ ($P \leq 0.05$).

[2]SEM = the largest standard error of the mean is shown.

Table 4.5 Hepatic expression of genes related to stress following *trans* -10, *cis* -12-CLA consumption by growing male mice

	Treatments[1]		Time		
Gene	Control	CLA	Week 2	Week 6	SEM[2]
Inflammation					
Saa1	-0.50	-0.21	-0.17	-0.54	0.31
Stress Response					
Angptl3	-0.54	0.07	-0.26	-0.21	0.42
Angptl4	0.01	-0.29	-0.53	0.25	0.35
Atf6	-0.27	0.17	0.16	-0.26	0.46
Ddit3	-0.25	0.37	-0.11	0.24	0.30
Eif2ak3	-0.55	-0.10	-0.32	-0.32	0.46
Fgfr2	-0.09	0.19	-0.58	0.68	0.49
Hspa1b	-1.04	-0.54	-0.91	-0.67	0.62
Xbp1	-1.15	-0.55	-0.46	-1.23	0.63

[1]Means with different superscripts differ ($P \leq 0.05$).

[2]SEM = the largest standard error of the mean is shown.

Table 4.6 Summary of various hepatic measurements in response to *trans*-10, *cis*-12-CLA

Reference	Subject	Dosage*	Liver							
			Wt	TG	Protein	FFA	VLDL secretion	Ketone body production	BHBA/ AA	TG secretion
Degrace et al. (2003) (30)	Mice	10 g	60%↑	655% ↑			2-fold↑			
Kelley et al. (2004) (25)	Mice	0.5%		432%↑		↑				
Degrace et al. (2004) (31)	Mice	1%	80%↑	646%↑	19%↓	44%↓				
Sakono et al. (1999) (236)	Rat	1% CLA Mix	—	—				↑	↑	
Lin et al. (2001) (249)	HepG2 cell	1 mmol/l		4-fold↑						↓

* Without special indication (e.g. CLA Mix), dosage means the amount of *trans*-10, *cis*-12-CLA used.

Table 4.7 Summary of various serum measurements in response to *trans*-10, *cis*-12-CLA

Reference	Subject	Dosage*	Serum						
			Glucose	Insulin	Insulin resistance	CHL	TG	ApoB	NEFA
Clément et al. (2002) (33)	Mice	0.4% (w/w)	—	10-fold↑					
Halade et al. (2009) (48)	Mice	0.5%	31%↑	51%↑			20%↓		19%↑
Degrace et al. (2003) (30)	Mice	10 g					67%↓	53%↓	
Degrace et al. (2004) (31)	Mice	1%					64%↓		44%↓
Peters et al. (2001) (125)	Mice	0.5% CLA Mix	—			—	49%↓		
Tsuboyama-Kasaoka et al. (2000) (22)	Mice	1% (w/w)	—	↑	↑				

* Without special indication (e.g. CLA Mix), dosage means the amount of *trans*-10, *cis*-12-CLA used.

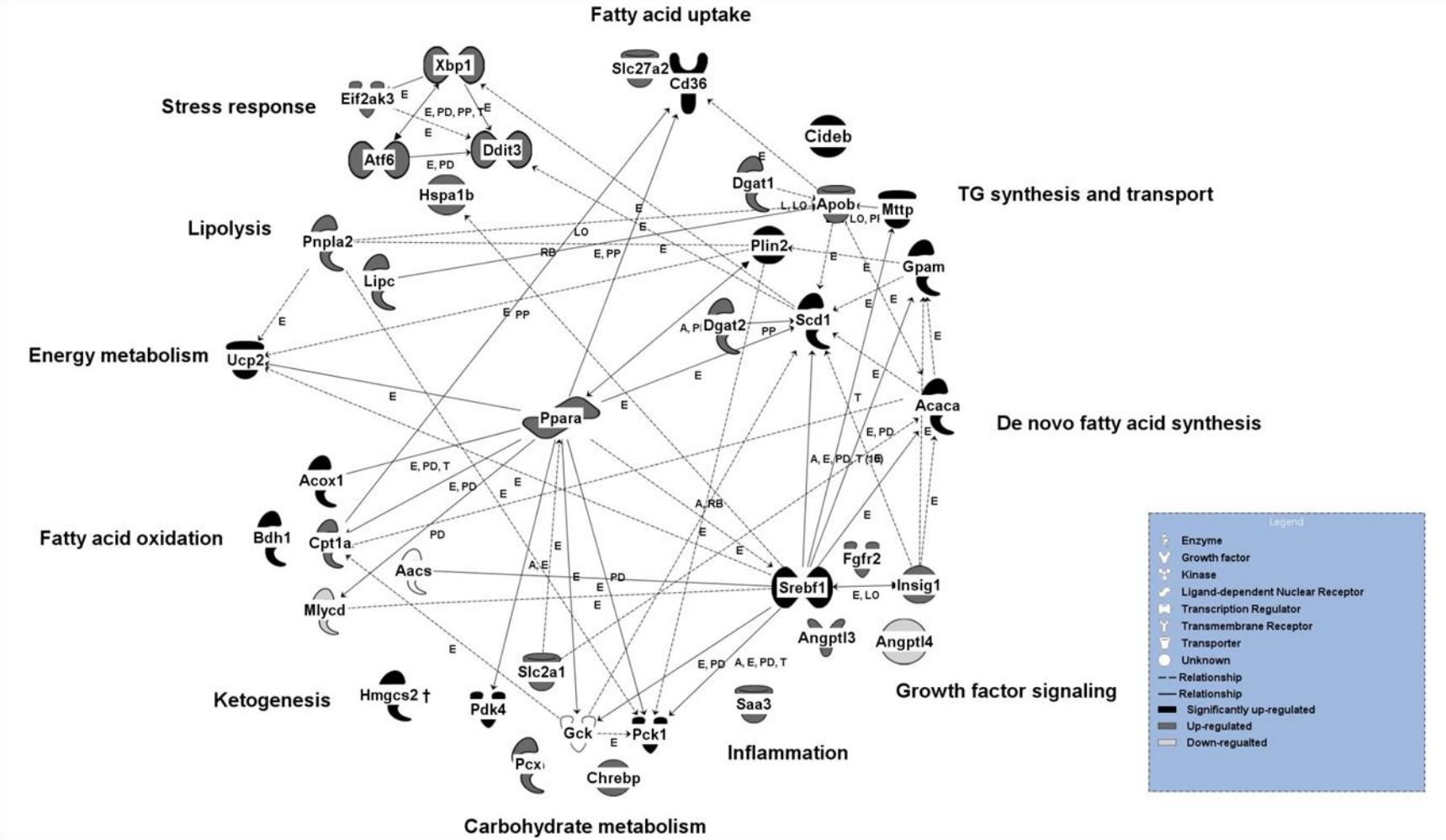

Figure 4.1 Currently known relationships among genes analyzed based on manually curated examination of the published literature within the Ingenuity Pathway Analysis (www.igenuity.com) knowledge base. Genes are grouped by the predominant process they play in lipid metabolism. Different shapes denote the type of protein encoded by the specific genes, including enzymes, ligand-dependent nuclear receptors, transcription regulators, and transporters. Letters along the edges denote effects on activity (A), expression (E), localization (LO), proteolysis (L), RNA binding (RB), protein-DNA binding (PD), and protein-protein binding (PP). Gene names are as in Table 1.1.

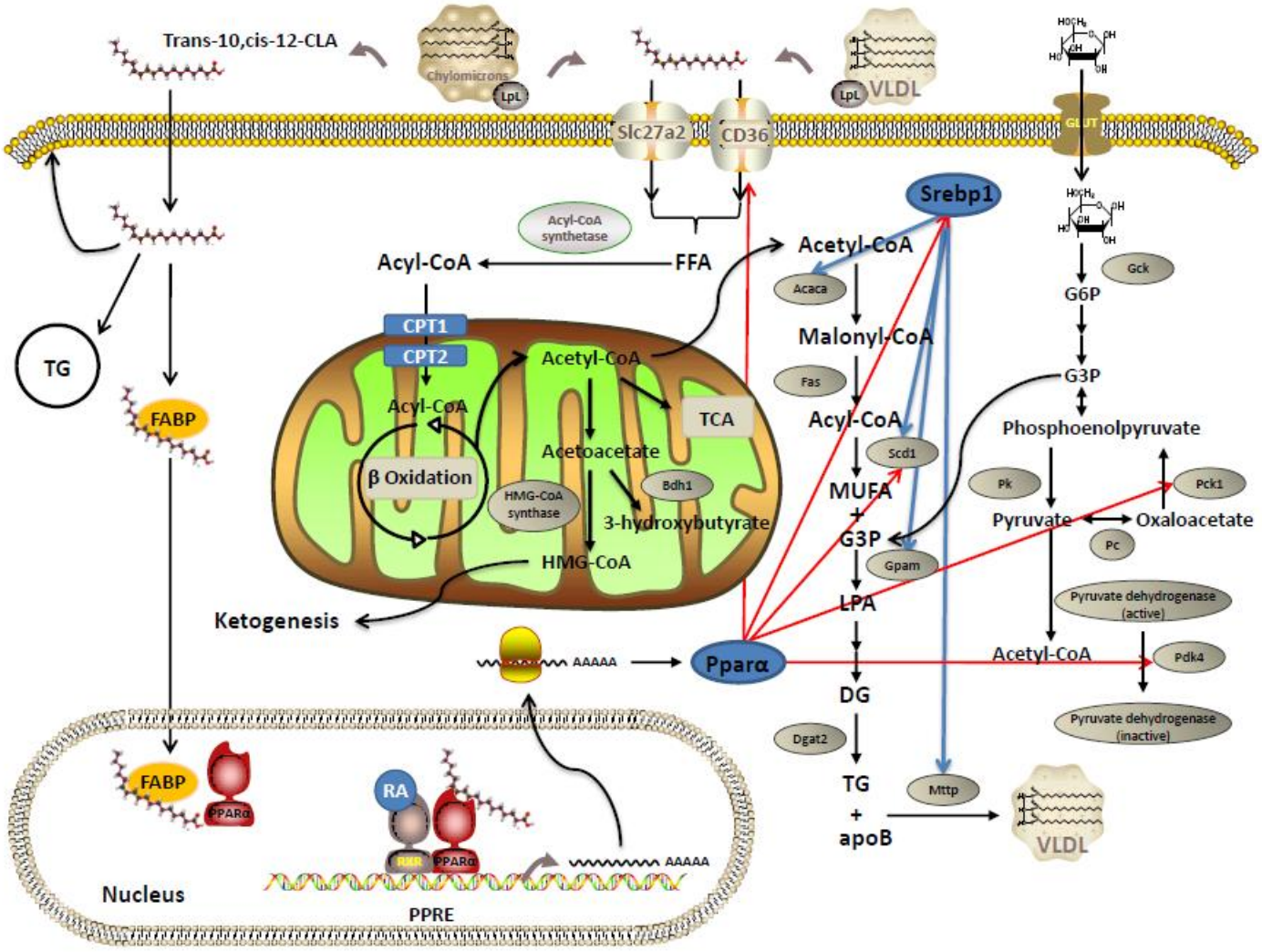

Figure 4.2 Interrelationships among cellular pathways regulating lipid and carbohydrate metabolism in the mouse liver.

CHAPTER 5

Conclusions

All in all, our study found that the hepatic steatotic effect of *trans*-10, *cis*-12-CLA might be due to increased fatty acid uptake, stimulated TG synthesis, enhanced storage capacity of lipid droplets as well as insufficiently induced VLDL assembly/secretion mechanism in the liver. Impaired β-oxidation would not be a factor contributing to hepatic steatosis induced by *trans*-10, *cis*-12-CLA. Our results provide new evidences to advance in the knowledge of the effect of this supplement on hepatic gene expression in a model of growing male mice. Furthermore, since the growing male mice was fed relatively low amount of *trans*-10, *cis*-12-CLA for 6 weeks, it seems to be a more reasonable model to speculate *trans*-10, *cis*-12-CLA's long-term effect on hepatic steatosis in humans. However, we did not capture the entire picture of the molecular mechanisms, since we did not measure gene product activity.

REFERENCES

1. Organization WHO. Global Database on Body Mass Index. 2006.
2. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. JAMA. 2010 Jan 20;303(3):235-41.
3. Ogden CL, Carroll MD, Curtin LR, Lamb MM, Flegal KM. Prevalence of high body mass index in US children and adolescents, 2007-2008. JAMA. Jan 20;303(3):242-9.
4. TODAY U. Obesity costs U.S. $168 billion, study finds. 2010.
5. Jaudszus A, Moeckel P, Hamelmann E, Jahreis G. Trans-10,cis-12-CLA-caused lipodystrophy is associated with profound changes of fatty acid profiles of liver, white adipose tissue and erythrocytes in mice: possible link to tissue-specific alterations of fatty acid desaturation. Ann Nutr Metab. 2010;57(2):103-11.
6. Kennedy A, Martinez K, Schmidt S, Mandrup S, LaPoint K, McIntosh M. Antiobesity mechanisms of action of conjugated linoleic acid. J Nutr Biochem. 2010 Mar;21(3):171-9.
7. Polan CE, McNeill JJ, Tove SB. Biohydrogenation of Unsaturated Fatty Acids by Rumen Bacteria. J Bacteriol. 1964 Oct;88:1056-64.
8. Wang Y, Jones PJ. Dietary conjugated linoleic acid and body composition. Am J Clin Nutr. 2004 Jun;79(6 Suppl):1153S-8S.
9. Taylor CG, Zahradka P. Dietary conjugated linoleic acid and insulin sensitivity and resistance in rodent models. Am J Clin Nutr. 2004 Jun;79(6 Suppl):1164S-8S.
10. McLeod RS, LeBlanc AM, Langille MA, Mitchell PL, Currie DL. Conjugated linoleic acids, atherosclerosis, and hepatic very-low-density lipoprotein metabolism. Am J Clin Nutr. 2004 Jun;79(6 Suppl):1169S-74S.
11. Belury MA. Dietary conjugated linoleic acid in health: physiological effects and mechanisms of action. Annu Rev Nutr. 2002;22:505-31.
12. Park Y, Storkson JM, Albright KJ, Liu W, Pariza MW. Evidence that the trans-10,cis-12 isomer of conjugated linoleic acid induces body composition changes in mice. Lipids. 1999 Mar;34(3):235-41.
13. Park Y, Albright KJ, Liu W, Storkson JM, Cook ME, Pariza MW. Effect of conjugated linoleic acid on body composition in mice. Lipids. 1997 Aug;32(8):853-8.
14. Evans M, Lin X, Odle J, McIntosh M. Trans-10, cis-12 conjugated linoleic acid increases fatty acid oxidation in 3T3-L1 preadipocytes. J Nutr. 2002 Mar;132(3):450-5.
15. Hargrave KM, Li C, Meyer BJ, Kachman SD, Hartzell DL, Della-Fera MA, et al. Adipose depletion and apoptosis induced by trans-10, cis-12 conjugated linoleic Acid in mice. Obes Res. 2002 Dec;10(12):1284-90.
16. Pariza MW, Park Y, Cook ME. The biologically active isomers of conjugated linoleic acid. Prog Lipid Res. 2001 Jul;40(4):283-98.
17. Watras AC, Buchholz AC, Close RN, Zhang Z, Schoeller DA. The role of conjugated linoleic acid in reducing body fat and preventing holiday weight gain. Int J Obes (Lond). 2007 Mar;31(3):481-7.
18. Gaullier JM, Halse J, Hoivik HO, Hoye K, Syvertsen C, Nurminiemi M, et al. Six months supplementation with conjugated linoleic acid induces regional-specific fat mass decreases in overweight and obese. Br J Nutr. 2007 Mar;97(3):550-60.
19. Riserus U, Basu S, Jovinge S, Fredrikson GN, Arnlov J, Vessby B. Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin resistance. Circulation. 2002 Oct 8;106(15):1925-9.

20. Tholstrup T, Raff M, Straarup EM, Lund P, Basu S, Bruun JM. An oil mixture with trans-10, cis-12 conjugated linoleic acid increases markers of inflammation and in vivo lipid peroxidation compared with cis-9, trans-11 conjugated linoleic acid in postmenopausal women. J Nutr. 2008 Aug;138(8):1445-51.
21. Poirier H, Shapiro JS, Kim RJ, Lazar MA. Nutritional supplementation with trans-10, cis-12-conjugated linoleic acid induces inflammation of white adipose tissue. Diabetes. [Research Support, N.I.H., Extramural]. 2006 Jun;55(6):1634-41.
22. Tsuboyama-Kasaoka N, Takahashi M, Tanemura K, Kim HJ, Tange T, Okuyama H, et al. Conjugated linoleic acid supplementation reduces adipose tissue by apoptosis and develops lipodystrophy in mice. Diabetes. 2000 Sep;49(9):1534-42.
23. Clement L, Poirier H, Niot I, Bocher V, Guerre-Millo M, Krief S, et al. Dietary trans-10,cis-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. J Lipid Res. 2002 Sep;43(9):1400-9.
24. Viswanadha S, McGilliard ML, Herbein JH. Desaturation Indices in Liver, Muscle, and Bone of Growing Male and Female Mice Fed trans-10,cis-12 Conjugated Linoleic Acid. Lipids. 2006;41:763-70.
25. Kelley DS, Bartolini GL, Warren JM, Simon VA, Mackey BE, Erickson KL. Contrasting effects of t10,c12- and c9,t11-conjugated linoleic acid isomers on the fatty acid profiles of mouse liver lipids. Lipids. 2004 Feb;39(2):135-41.
26. Machado M, Cortez-Pinto H. Non-alcoholic steatohepatitis and metabolic syndrome. Curr Opin Clin Nutr Metab Care. 2006 Sep;9(5):637-42.
27. Musso G, Gambino R, Cassader M. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). Prog Lipid Res. 2009 Jan;48(1):1-26.
28. House RL, Cassady JP, Eisen EJ. Functional genomic characterization of delipidation elicited by trans-10,cis-12-conjugated linoleic acid (t10c12-CLA) in a polygenic obese line of mice. Physiol Genomics. 2005;21:351-61.
29. Kelley D, Bartolini G, Newman J, Vemuri M, Mackey B. Fatty acid composition of liver, adipose tissue, spleen, and heart of mice fed diets containing t10, c12-, and c9, t11-conjugated linoleic acid. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2006;74(5):331-8.
30. Degrace P, Demizieux L, Gresti J, Chardigny JM, Sebedio JL, Clouet P. Association of liver steatosis with lipid oversecretion and hypotriglyceridaemia in C57BL/6j mice fed trans-10,cis-12-linoleic acid. FEBS Lett. 2003 Jul 10;546(2-3):335-9.
31. Degrace P, Demizieux L, Gresti J, Chardigny JM, Sebedio JL, Clouet P. Hepatic steatosis is not due to impaired fatty acid oxidation capacities in C57BL/6J mice fed the conjugated trans-10,cis-12-isomer of linoleic acid. J Nutr. 2004 Apr;134(4):861-7.
32. Ashwell MS, Ceddia RP, House RL, Cassady JP, Eisen EJ, Eling TE, et al. Trans-10, cis-12-conjugated linoleic acid alters hepatic gene expression in a polygenic obese line of mice displaying hepatic lipidosis☆. The Journal of Nutritional Biochemistry. 2010;21(9):848-55.
33. Clement L. Dietary trans-10,cis-12 conjugated linoleic acid induces hyperinsulinemia and fatty liver in the mouse. The Journal of Lipid Research. 2002;43(9):1400-9.
34. Takahashi Y, Kushiro M, Shinohara K, Ide T. Dietary conjugated linoleic acid reduces body fat mass and affects gene expression of proteins regulating energy metabolism in mice. Comp Biochem Physiol B Biochem Mol Biol. 2002 Nov;133(3):395-404.
35. Kelley DS, Bartolini GL, Newman JW, Vemuri M, Mackey BE. Fatty acid composition of liver, adipose tissue, spleen, and heart of mice fed diets containing t10, c12-, and c9, t11-conjugated linoleic acid. Prostaglandins Leukot Essent Fatty Acids. 2006 May;74(5):331-8.

36. Gudbrandsen OA, Rodriguez E, Wergedahl H, Mork S, Reseland JE, Skorve J, et al. Trans-10, cis-12-conjugated linoleic acid reduces the hepatic triacylglycerol content and the leptin mRNA level in adipose tissue in obese Zucker fa/fa rats. Br J Nutr. 2009 Sep;102(6):803-15.
37. LaRosa PC, Miner J, Xia Y, Zhou Y, Kachman S, Fromm ME. Trans-10, cis-12 conjugated linoleic acid causes inflammation and delipidation of white adipose tissue in mice: a microarray and histological analysis. Physiol Genomics. 2006 Nov 27;27(3):282-94.
38. E-Stats NCfHSH. Prevalence of overweight, obesity and extreme obesity among adults: United States, trends 1976-80 through 2005-2006. *NCHS E-Stats*. 2008.
39. Ogden CL, Carroll MD, Flegal KM. High body mass index for age among US children and adolescents, 2003-2006. JAMA. 2008 May 28;299(20):2401-5.
40. Finkelstein EA, Trogdon JG, Cohen JW, Dietz W. Annual medical spending attributable to obesity: payer-and service-specific estimates. Health Aff (Millwood). 2009 Sep-Oct;28(5):w822-31.
41. Whigham LD, Watras AC, Schoeller DA. Efficacy of conjugated linoleic acid for reducing fat mass: a meta-analysis in humans. Am J Clin Nutr. 2007 May;85(5):1203-11.
42. Wang YW, Jones PJ. Conjugated linoleic acid and obesity control: efficacy and mechanisms. Int J Obes Relat Metab Disord. 2004 Aug;28(8):941-55.
43. West DB, Blohm FY, Truett AA, DeLany JP. Conjugated linoleic acid persistently increases total energy expenditure in AKR/J mice without increasing uncoupling protein gene expression. J Nutr. 2000 Oct;130(10):2471-7.
44. Santomauro AT, Boden G, Silva ME, Rocha DM, Santos RF, Ursich MJ, et al. Overnight lowering of free fatty acids with Acipimox improves insulin resistance and glucose tolerance in obese diabetic and nondiabetic subjects. Diabetes. 1999 Sep;48(9):1836-41.
45. Blankson H, Stakkestad JA, Fagertun H, Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. J Nutr. 2000 Dec;130(12):2943-8.
46. Riserus U, Arner P, Brismar K, Vessby B. Treatment with dietary trans10cis12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. Diabetes Care. 2002 Sep;25(9):1516-21.
47. Mougios V, Matsakas A, Petridou A, Ring S, Sagredos A, Melissopoulou A, et al. Effect of supplementation with conjugated linoleic acid on human serum lipids and body fat. J Nutr Biochem. 2001 Oct;12(10):585-94.
48. Halade GV, Rahman MM, Fernandes G. Effect of CLA isomers and their mixture on aging C57Bl/6J mice. Eur J Nutr. 2009 Oct;48(7):409-18.
49. Baddini Feitoza A, Fernandes Pereira A, Ferreira da Costa N, Goncalves Ribeiro B. Conjugated linoleic acid (CLA): effect modulation of body composition and lipid profile. Nutr Hosp. 2009 Jul-Aug;24(4):422-8.
50. Yamasaki M, Miyamoto Y, Chujo H, Nishiyama K, Tachibana H, Yamada K. Trans10, cis12-conjugated linoleic acid induces mitochondria-related apoptosis and lysosomal destabilization in rat hepatoma cells. Biochim Biophys Acta. 2005 Aug 15;1735(3):176-84.
51. Bhattacharya A, Banu J, Rahman M, Causey J, Fernandes G. Biological effects of conjugated linoleic acids in health and disease. J Nutr Biochem. 2006 Dec;17(12):789-810.
52. Kennedy A, Martinez K, Schmidt S, Mandrup S, LaPoint K, McIntosh M. Antiobesity mechanisms of action of conjugated linoleic acid. The Journal of Nutritional Biochemistry. 2010;21(3):171-9.
53. Griinari JM, Corl BA, Lacy SH, Chouinard PY, Nurmela KV, Bauman DE. Conjugated linoleic acid is synthesized endogenously in lactating dairy cows by Delta(9)-desaturase. J Nutr. 2000 Sep;130(9):2285-91.

54. Mosley EE, McGuire MK, Williams JE, McGuire MA. Cis-9, trans-11 conjugated linoleic acid is synthesized from vaccenic acid in lactating women. J Nutr. 2006 Sep;136(9):2297-301.
55. Sehat N, Kramer JK, Mossoba MM, Yurawecz MP, Roach JA, Eulitz K, et al. Identification of conjugated linoleic acid isomers in cheese by gas chromatography, silver ion high performance liquid chromatography and mass spectral reconstructed ion profiles. Comparison of chromatographic elution sequences. Lipids. 1998 Oct;33(10):963-71.
56. Ha YL, Grimm NK, Pariza MW. Anticarcinogens from fried ground beef: heat-altered derivatives of linoleic acid. Carcinogenesis. 1987 Dec;8(12):1881-7.
57. Parodi PW. Cows' milk fat components as potential anticarcinogenic agents. J Nutr. 1997 Jun;127(6):1055-60.
58. RL. A. Conjugated linoleic acid for altering body composition and treating obesity. AOCS Press. 1999:328-53.
59. Chin SF LW, Storkson JM, Ha YL, Pariza MW. Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J Food Compos Anal. 1992;5:185-97.
60. Ritzenthaler KL, McGuire MK, Falen R, Shultz TD, Dasgupta N, McGuire MA. Estimation of conjugated linoleic acid intake by written dietary assessment methodologies underestimates actual intake evaluated by food duplicate methodology. J Nutr. 2001 May;131(5):1548-54.
61. Terpstra AH. Effect of conjugated linoleic acid on body composition and plasma lipids in humans: an overview of the literature. Am J Clin Nutr. 2004 Mar;79(3):352-61.
62. Gaullier JM, Berven G, Blankson H, Gudmundsen O. Clinical trial results support a preference for using CLA preparations enriched with two isomers rather than four isomers in human studies. Lipids. 2002 Nov;37(11):1019-25.
63. Larsen TM, Toubro S, Astrup A. Efficacy and safety of dietary supplements containing CLA for the treatment of obesity: evidence from animal and human studies. J Lipid Res. 2003 Dec;44(12):2234-41.
64. Wanders AJ, Leder L, Banga JD, Katan MB, Brouwer IA. A high intake of conjugated linoleic acid does not affect liver and kidney function tests in healthy human subjects. Food Chem Toxicol. Feb;48(2):587-90.
65. Pariza MW, Hargraves WA. A beef-derived mutagenesis modulator inhibits initiation of mouse epidermal tumors by 7,12-dimethylbenz[a]anthracene. Carcinogenesis. 1985 Apr;6(4):591-3.
66. Ha YL, Storkson J, Pariza MW. Inhibition of benzo(a)pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. Cancer Res. 1990 Feb 15;50(4):1097-101.
67. Ip C, Chin SF, Scimeca JA, Pariza MW. Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res. 1991 Nov 15;51(22):6118-24.
68. Liew C, Schut HA, Chin SF, Pariza MW, Dashwood RH. Protection of conjugated linoleic acids against 2-amino-3- methylimidazo[4,5-f]quinoline-induced colon carcinogenesis in the F344 rat: a study of inhibitory mechanisms. Carcinogenesis. 1995 Dec;16(12):3037-43.
69. Belury MA. Inhibition of carcinogenesis by conjugated linoleic acid: potential mechanisms of action. J Nutr. 2002 Oct;132(10):2995-8.
70. Belury MA, Nickel KP, Bird CE, Wu Y. Dietary conjugated linoleic acid modulation of phorbol ester skin tumor promotion. Nutr Cancer. 1996;26(2):149-57.
71. Ip MM, Masso-Welch PA, Shoemaker SF, Shea-Eaton WK, Ip C. Conjugated linoleic acid inhibits proliferation and induces apoptosis of normal rat mammary epithelial cells in primary culture. Exp Cell Res. 1999 Jul 10;250(1):22-34.

72. Ip C, Banni S, Angioni E, Carta G, McGinley J, Thompson HJ, et al. Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. J Nutr. 1999 Dec;129(12):2135-42.
73. Lee KN, Kritchevsky D, Pariza MW. Conjugated linoleic acid and atherosclerosis in rabbits. Atherosclerosis. 1994 Jul;108(1):19-25.
74. Nicolosi RJ, Rogers EJ, Kritchevsky D, Scimeca JA, Huth PJ. Dietary conjugated linoleic acid reduces plasma lipoproteins and early aortic atherosclerosis in hypercholesterolemic hamsters. Artery. 1997;22(5):266-77.
75. Kritchevsky D, Tepper SA, Wright S, Tso P, Czarnecki SK. Influence of conjugated linoleic acid (CLA) on establishment and progression of atherosclerosis in rabbits. J Am Coll Nutr. 2000 Aug;19(4):472S-7S.
76. Koba K, Akahoshi A, Yamasaki M, Tanaka K, Yamada K, Iwata T, et al. Dietary conjugated linolenic acid in relation to CLA differently modifies body fat mass and serum and liver lipid levels in rats. Lipids. 2002 Apr;37(4):343-50.
77. Ryder JW, Portocarrero CP, Song XM, Cui L, Yu M, Combatsiaris T, et al. Isomer-specific antidiabetic properties of conjugated linoleic acid. Improved glucose tolerance, skeletal muscle insulin action, and UCP-2 gene expression. Diabetes. 2001 May;50(5):1149-57.
78. Houseknecht KL, Vanden Heuvel JP, Moya-Camarena SY, Portocarrero CP, Peck LW, Nickel KP, et al. Dietary conjugated linoleic acid normalizes impaired glucose tolerance in the Zucker diabetic fatty fa/fa rat. Biochem Biophys Res Commun. 1998 Mar 27;244(3):678-82.
79. Yang M, Cook ME. Dietary conjugated linoleic acid decreased cachexia, macrophage tumor necrosis factor-alpha production, and modifies splenocyte cytokines production. Exp Biol Med (Maywood). 2003 Jan;228(1):51-8.
80. Yu Y, Correll PH, Vanden Heuvel JP. Conjugated linoleic acid decreases production of pro-inflammatory products in macrophages: evidence for a PPAR gamma-dependent mechanism. Biochim Biophys Acta. 2002 Apr 15;1581(3):89-99.
81. Iwakiri Y, Sampson DA, Allen KG. Suppression of cyclooxygenase-2 and inducible nitric oxide synthase expression by conjugated linoleic acid in murine macrophages. Prostaglandins Leukot Essent Fatty Acids. 2002 Dec;67(6):435-43.
82. Miller CC, Park Y, Pariza MW, Cook ME. Feeding conjugated linoleic acid to animals partially overcomes catabolic responses due to endotoxin injection. Biochem Biophys Res Commun. 1994 Feb 15;198(3):1107-12.
83. Choi Y, Kim YC, Han YB, Park Y, Pariza MW, Ntambi JM. The trans-10,cis-12 isomer of conjugated linoleic acid downregulates stearoyl-CoA desaturase 1 gene expression in 3T3-L1 adipocytes. J Nutr. 2000 Aug;130(8):1920-4.
84. Park Y, Storkson JM, Ntambi JM, Cook ME, Sih CJ, Pariza MW. Inhibition of hepatic stearoyl-CoA desaturase activity by trans-10, cis-12 conjugated linoleic acid and its derivatives. Biochim Biophys Acta. 2000 Jul 19;1486(2-3):285-92.
85. de Deckere EA, van Amelsvoort JM, McNeill GP, Jones P. Effects of conjugated linoleic acid (CLA) isomers on lipid levels and peroxisome proliferation in the hamster. Br J Nutr. 1999 Oct;82(4):309-17.
86. Brown JM, McIntosh MK. Conjugated linoleic acid in humans: regulation of adiposity and insulin sensitivity. J Nutr. 2003 Oct;133(10):3041-6.
87. Rodriguez E, Ribot J, Palou A. Trans-10, cis-12, but not cis-9, trans-11 CLA isomer, inhibits brown adipocyte thermogenic capacity. Am J Physiol Regul Integr Comp Physiol. 2002 Jun;282(6):R1789-97.

88. Park Y, Storkson JM, Liu W, Albright KJ, Cook ME, Pariza MW. Structure-activity relationship of conjugated linoleic acid and its cognates in inhibiting heparin-releasable lipoprotein lipase and glycerol release from fully differentiated 3T3-L1 adipocytes. J Nutr Biochem. 2004 Sep;15(9):561-8.
89. Sanders SR, Teachey MK, Ptock A, Kraemer K, Hasselwander O, Henriksen EJ, et al. Effects of specific conjugated linoleic acid isomers on growth characteristics in obese Zucker rats. Lipids. 2004 Jun;39(6):537-43.
90. Navarro V, Zabala A, Macarulla MT, Fernandez-Quintela A, Rodriguez VM, Simon E, et al. Effects of conjugated linoleic acid on body fat accumulation and serum lipids in hamsters fed an atherogenic diet. J Physiol Biochem. 2003 Sep;59(3):193-9.
91. Wargent E, Sennitt MV, Stocker C, Mayes AE, Brown L, O'Dowd J, et al. Prolonged treatment of genetically obese mice with conjugated linoleic acid improves glucose tolerance and lowers plasma insulin concentration: possible involvement of PPAR activation. Lipids Health Dis. 2005;4:3.
92. Kang K, Miyazaki M, Ntambi JM, Pariza MW. Evidence that the anti-obesity effect of conjugated linoleic acid is independent of effects on stearoyl-CoA desaturase1 expression and enzyme activity. Biochem Biophys Res Commun. 2004 Mar 12;315(3):532-7.
93. Mirand PP, Arnal-Bagnard MA, Mosoni L, Faulconnier Y, Chardigny JM, Chilliard Y. Cis-9, trans-11 and trans-10, cis-12 conjugated linoleic acid isomers do not modify body composition in adult sedentary or exercised rats. J Nutr. 2004 Sep;134(9):2263-9.
94. Kim MR, Park Y, Albright KJ, Pariza MW. Differential responses of hamsters and rats fed high-fat or low-fat diets supplemented with conjugated linoleic acid. Nutr Res. 2002;22:715-22.
95. Nagao K, Inoue N, Wang YM, Hirata J, Shimada Y, Nagao T, et al. The 10trans,12cis isomer of conjugated linoleic acid suppresses the development of hypertension in Otsuka Long-Evans Tokushima fatty rats. Biochem Biophys Res Commun. 2003 Jun 20;306(1):134-8.
96. Martin JC, Gregoire S, Siess MH, Genty M, Chardigny JM, Berdeaux O, et al. Effects of conjugated linoleic acid isomers on lipid-metabolizing enzymes in male rats. Lipids. 2000 Jan;35(1):91-8.
97. Evans M, Geigerman C, Cook J, Curtis L, Kuebler B, McIntosh M. Conjugated linoleic acid suppresses triglyceride accumulation and induces apoptosis in 3T3-L1 preadipocytes. Lipids. 2000 Aug;35(8):899-910.
98. Park Y, Pariza MW. Lipoxygenase inhibitors inhibit heparin-releasable lipoprotein lipase activity in 3T3-L1 adipocytes and enhance body fat reduction in mice by conjugated linoleic acid. Biochim Biophys Acta. 2001 Nov 30;1534(1):27-33.
99. Park Y, Pariza MW. The effects of dietary conjugated nonadecadienoic acid on body composition in mice. Biochim Biophys Acta. 2001 Oct 31;1533(3):171-4.
100. Riserus U, Smedman A, Basu S, Vessby B. Metabolic effects of conjugated linoleic acid in humans: the Swedish experience. Am J Clin Nutr. 2004 Jun;79(6 Suppl):1146S-8S.
101. House RL, Cassady JP, Eisen EJ, McIntosh MK, Odle J. Conjugated linoleic acid evokes de-lipidation through the regulation of genes controlling lipid metabolism in adipose and liver tissue. Obes Rev. 2005 Aug;6(3):247-58.
102. Malpuech-Brugere C, Verboeket-van de Venne WP, Mensink RP, Arnal MA, Morio B, Brandolini M, et al. Effects of two conjugated linoleic Acid isomers on body fat mass in overweight humans. Obes Res. 2004 Apr;12(4):591-8.
103. Tricon S, Burdge GC, Kew S, Banerjee T, Russell JJ, Jones EL, et al. Opposing effects of cis-9,trans-11 and trans-10,cis-12 conjugated linoleic acid on blood lipids in healthy humans. Am J Clin Nutr. 2004 Sep;80(3):614-20.

104. Churruca I, Fernandez-Quintela A, Portillo MP. Conjugated linoleic acid isomers: differences in metabolism and biological effects. BioFactors. 2009 Jan-Feb;35(1):105-11.
105. Nazare JA, de la Perriere AB, Bonnet F, Desage M, Peyrat J, Maitrepierre C, et al. Daily intake of conjugated linoleic acid-enriched yoghurts: effects on energy metabolism and adipose tissue gene expression in healthy subjects. Br J Nutr. 2007 Feb;97(2):273-80.
106. Purushotham A, Wendel AA, Liu LF, Belury MA. Maintenance of adiponectin attenuates insulin resistance induced by dietary conjugated linoleic acid in mice. J Lipid Res. 2007 Feb;48(2):444-52.
107. Arbones-Mainar JM, Navarro MA, Guzman MA, Arnal C, Surra JC, Acin S, et al. Selective effect of conjugated linoleic acid isomers on atherosclerotic lesion development in apolipoprotein E knockout mice. Atherosclerosis. 2006 Dec;189(2):318-27.
108. Eder K, Slomma N, Becker K. Trans-10,cis-12 conjugated linoleic acid suppresses the desaturation of linoleic and alpha-linolenic acids in HepG2 cells. J Nutr. 2002 Jun;132(6):1115-21.
109. Choi Y, Park Y, Pariza MW, Ntambi JM. Regulation of stearoyl-CoA desaturase activity by the trans-10,cis-12 isomer of conjugated linoleic acid in HepG2 cells. Biochem Biophys Res Commun. 2001 Jun 15;284(3):689-93.
110. Belury MA, Kempa-Steczko A. Conjugated linoleic acid modulates hepatic lipid composition in mice. Lipids. 1997 Feb;32(2):199-204.
111. Bretillon L, Chardigny JM, Gregoire S, Berdeaux O, Sebedio JL. Effects of conjugated linoleic acid isomers on the hepatic microsomal desaturation activities in vitro. Lipids. 1999 Sep;34(9):965-9.
112. Moya-Camarena SY, Belury MA. Species differences in the metabolism and regulation of gene expression by conjugated linoleic acid. Nutr Rev. 1999 Nov;57(11):336-40.
113. Warren JM, Simon VA, Bartolini G, Erickson KL, Mackey BE, Kelley DS. Trans-10,cis-12 CLA increases liver and decreases adipose tissue lipids in mice: possible roles of specific lipid metabolism genes. Lipids. 2003 May;38(5):497-504.
114. Zabala A, Churruca I, Macarulla MT, Rodriguez VM, Fernandez-Quintela A, Martinez JA, et al. The trans-10,cis-12 isomer of conjugated linoleic acid reduces hepatic triacylglycerol content without affecting lipogenic enzymes in hamsters. Br J Nutr. 2004 Sep;92(3):383-9.
115. Salas-Salvado J, Marquez-Sandoval F, Bullo M. Conjugated linoleic acid intake in humans: a systematic review focusing on its effect on body composition, glucose, and lipid metabolism. Crit Rev Food Sci Nutr. 2006;46(6):479-88.
116. Venkatramanan S, Joseph SV, Chouinard PY, Jacques H, Farnworth ER, Jones PJ. Milk enriched with conjugated linoleic acid fails to alter blood lipids or body composition in moderately overweight, borderline hyperlipidemic individuals. J Am Coll Nutr. Apr;29(2):152-9.
117. Berven G, Bye A, Hals O, Blankson H, Faggertun H, Thom E. Safety of conjugated linoleic acid in overweight or obese human volunteers. Eur J Lipid Sci Technol. 2000;102:455-62.
118. Lowery LM, Appicelli P, Lemon PWR. Conjugated linoleic acid enhances muscle size and strength gains in novice bodybuilders. Med Sci Sport Exerc. 1998;30:182.
119. Iwata T, Kamegai T, Yamauchi-Sato Y, Ogawa A, Kasai M, Aoyama T, et al. Safety of dietary conjugated linoleic acid (CLA) in a 12-weeks trial in healthy overweight Japanese male volunteers. J Oleo Sci. 2007;56(10):517-25.
120. Whigham LD, O'Shea M, Mohede IC, Walaski HP, Atkinson RL. Safety profile of conjugated linoleic acid in a 12-month trial in obese humans. Food Chem Toxicol. 2004 Oct;42(10):1701-9.

121. Benjamin S, Spener F. Conjugated linoleic acids as functional food: an insight into their health benefits. Nutr Metab (Lond). 2009;6:36.
122. Benjamin S, Hanhoff T, Borchers T, Spener F. An improved molecular test system for the screening of human PPAR transactivation by conjugated linoleic acid isomers and their precursor fatty acids. Eur J Lipid Sci Technol. 2005;107:706-15.
123. Belury MA, Moya-Camarena SY, Lu M, Shi LL, Leesnitzer LM, Blanchard SG. Conjugated linoleic acid is an activator and ligand for peroxisome proliferator-activated receptor-gamma (PPAR gamma). Nutr Res. 2002;22:817-24.
124. Takahashi Y, Kushiro M, Shinohara K, Ide T. Activity and mRNA levels of enzymes involved in hepatic fatty acid synthesis and oxidation in mice fed conjugated linoleic acid. Biochim Biophys Acta. 2003 Apr 8;1631(3):265-73.
125. Peters JM, Park Y, Gonzalez FJ, Pariza MW. Influence of conjugated linoleic acid on body composition and target gene expression in peroxisome proliferator-activated receptor alpha-null mice. Biochim Biophys Acta. 2001 Oct 31;1533(3):233-42.
126. Boelsterli UA, Bedoucha M. Toxicological consequences of altered peroxisome proliferator-activated receptor gamma (PPARgamma) expression in the liver: insights from models of obesity and type 2 diabetes. Biochem Pharmacol. 2002 Jan 1;63(1):1-10.
127. Rasooly R, Kelley DS, Greg J, Mackey BE. Dietary trans 10, cis 12-conjugated linoleic acid reduces the expression of fatty acid oxidation and drug detoxification enzymes in mouse liver. British Journal of Nutrition. 2007;97(01):58.
128. Shimano H, Horton JD, Hammer RE, Shimomura I, Brown MS, Goldstein JL. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. J Clin Invest. 1996 Oct 1;98(7):1575-84.
129. Ide T. Interaction of fish oil and conjugated linoleic acid in affecting hepatic activity of lipogenic enzymes and gene expression in liver and adipose tissue. Diabetes. 2005 Feb;54(2):412-23.
130. Moya-Camarena SY, Van den Heuvel JP, Belury MA. Conjugated linoleic acid activates peroxisome proliferator-activated receptor alpha and beta subtypes but does not induce hepatic peroxisome proliferation in Sprague-Dawley rats. Biochim Biophys Acta. 1999 Jan 4;1436(3):331-42.
131. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev. 1999 Oct;20(5):649-88.
132. Wolfrum C, Borrmann CM, Borchers T, Spener F. Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors alpha - and gamma-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus. Proc Natl Acad Sci U S A. 2001 Feb 27;98(5):2323-8.
133. Schachtrup C, Emmler T, Bleck B, Sandqvist A, Spener F. Functional analysis of peroxisome-proliferator-responsive element motifs in genes of fatty acid-binding proteins. Biochem J. 2004 Aug 15;382(Pt 1):239-45.
134. Moya-Camarena SY, Vanden Heuvel JP, Blanchard SG, Leesnitzer LA, Belury MA. Conjugated linoleic acid is a potent naturally occurring ligand and activator of PPARalpha. J Lipid Res. 1999 Aug;40(8):1426-33.
135. Roberts RA, Chevalier S, Hasmall SC, James NH, Cosulich SC, Macdonald N. PPAR alpha and the regulation of cell division and apoptosis. Toxicology. 2002 Dec 27;181-182:167-70.
136. LaRosa PC, Riethoven JJ, Chen H, Xia Y, Zhou Y, Chen M, et al. Trans-10, cis-12 conjugated linoleic acid activates the integrated stress response pathway in adipocytes. Physiol Genomics. 2007 Nov 14;31(3):544-53.

137. Yoshikawa T, Ide T, Shimano H, Yahagi N, Amemiya-Kudo M, Matsuzaka T, et al. Cross-talk between peroxisome proliferator-activated receptor (PPAR) alpha and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. I. PPARs suppress sterol regulatory element binding protein-1c promoter through inhibition of LXR signaling. Mol Endocrinol. 2003 Jul;17(7):1240-54.
138. Vidal-Puig AJ, Considine RV, Jimenez-Linan M, Werman A, Pories WJ, Caro JF, et al. Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. J Clin Invest. 1997 May 15;99(10):2416-22.
139. Tobin KA, Ulven SM, Schuster GU, Steineger HH, Andresen SM, Gustafsson JA, et al. Liver X receptors as insulin-mediating factors in fatty acid and cholesterol biosynthesis. J Biol Chem. 2002 Mar 22;277(12):10691-7.
140. Shimano H. Sterol regulatory element-binding proteins (SREBPs): transcriptional regulators of lipid synthetic genes. Prog Lipid Res. 2001 Nov;40(6):439-52.
141. Roche HM, Noone E, Sewter C, Mc Bennett S, Savage D, Gibney MJ, et al. Isomer-dependent metabolic effects of conjugated linoleic acid: insights from molecular markers sterol regulatory element-binding protein-1c and LXRalpha. Diabetes. 2002 Jul;51(7):2037-44.
142. Riserus U, Vessby B, Arner P, Zethelius B. Supplementation with trans10cis12-conjugated linoleic acid induces hyperproinsulinaemia in obese men: close association with impaired insulin sensitivity. Diabetologia. 2004 Jun;47(6):1016-9.
143. Nagayoshi A, Matsuki N, Saito H, Tsukamoto K, Wakashima M, Kinoshita M, et al. Deficiency of acyl CoA cholesterol acyl transferase activity in suncus liver. J Biochem. 1994 May;115(5):858-61.
144. Lin Y, Schuurbiers E, Van der Veen S, De Deckere EA. Conjugated linoleic acid isomers have differential effects on triglyceride secretion in Hep G2 cells. Biochim Biophys Acta. 2001 Aug 29;1533(1):38-46.
145. Lin Y, Smit MJ, Havinga R, Verkade HJ, Vonk RJ, Kuipers F. Differential effects of eicosapentaenoic acid on glycerolipid and apolipoprotein B metabolism in primary human hepatocytes compared to HepG2 cells and primary rat hepatocytes. Biochim Biophys Acta. 1995 Apr 28;1256(1):88-96.
146. Yotsumoto H, Hara E, Naka S, Adlof RO, Emken EA, Yanagita T. 10trans, 12cis-Linoleic acid reduces apolipoprotein B secretion in HepG2 cells. Food Research International. 1999;31(5):403-9.
147. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 2002 Jun 18;3(7):RESEARCH0034.
148. Bionaz M, Loor JJ. ACSL1, AGPAT6, FABP3, LPIN1, and SLC27A6 are the most abundant isoforms in bovine mammary tissue and their expression is affected by stage of lactation. J Nutr. 2008 Jun;138(6):1019-24.
149. Jourdan T, Djaouti L, Demizieux L, Gresti J, Verges B, Degrace P. Liver Carbohydrate and Lipid Metabolism of Insulin-Deficient Mice Is Altered by trans-10, cis-12 Conjugated Linoleic Acid. Journal of Nutrition. 2009;139(10):1901-7.
150. Gruffat D, De La Torre A, Chardigny JM, Durand D, Loreau O, Sebedio JL, et al. In vitro comparison of hepatic metabolism of 9cis-11 trans and 10trans-12cis isomers of CLA in the rat. Lipids. 2003 Feb;38(2):157-63.
151. Doege H, Stahl A. Protein-mediated fatty acid uptake: novel insights from in vivo models. Physiology (Bethesda). 2006 Aug;21:259-68.

152. Coburn CT, Hajri T, Ibrahimi A, Abumrad NA. Role of CD36 in membrane transport and utilization of long-chain fatty acids by different tissues. J Mol Neurosci. 2001 Apr-Jun;16(2-3):117-21; discussion 51-7.
153. Stahl A. A current review of fatty acid transport proteins (SLC27). Pflugers Arch. 2004 Feb;447(5):722-7.
154. Guillén N, Navarro MA, Arnal C, Noone E, Arbonés-Mainar JM, Acín S, et al. Microarray analysis of hepatic gene expression identifies new genes involved in steatotic liver. Physiological Genomics. 2009 May 2009;37(3):187-98.
155. Kim KH. Regulation of mammalian acetyl-coenzyme A carboxylase. Annu Rev Nutr. 1997;17:77-99.
156. Widmer J, Fassihi KS, Schlichter SC, Wheeler KS, Crute BE, King N, et al. Identification of a second human acetyl-CoA carboxylase gene. Biochem J. 1996 Jun 15;316 (Pt 3):915-22.
157. Guha P, Aneja KK, Shilpi RY, Haldar D. Transcriptional regulation of mitochondrial glycerophosphate acyltransferase is mediated by distal promoter via ChREBP and SREBP-1. Arch Biochem Biophys. 2009 Oct 15;490(2):85-95.
158. Coleman RA, Lee DP. Enzymes of triacylglycerol synthesis and their regulation. Progress in Lipid Research. 2004;43:134–76.
159. Ntambi JM. The regulation of stearoyl-CoA desaturase (SCD). Prog Lipid Res. 1995;34(2):139-50.
160. Ntambi JM. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. J Lipid Res. 1999 Sep;40(9):1549-58.
161. Reshef L, Olswang Y, Cassuto H, Blum B, Croniger CM, Kalhan SC, et al. Glyceroneogenesis and the triglyceride/fatty acid cycle. J Biol Chem. 2003 Aug 15;278(33):30413-6.
162. Gomez-Munoz A, Hamza EH, Brindley DN. Effects of sphingosine, albumin and unsaturated fatty acids on the activation and translocation of phosphatidate phosphohydrolases in rat hepatocytes. Biochim Biophys Acta. 1992 Jul 9;1127(1):49-56.
163. Weiss SB, Kennedy EP, Kiyasu JY. The enzymatic synthesis of triglycerides. J Biol Chem. 1960 Jan;235:40-4.
164. Haldar D, Kelker HC, Pullman ME. Properties of acyl coenzyme A: 1-acyl-sn-glycerol 3-phosphate acyltransferase from rat liver mitochondria and microsomes. Trans N Y Acad Sci. 1983;41:173-82.
165. Linden D, William-Olsson L, Rhedin M, Asztely AK, Clapham JC, Schreyer S. Overexpression of mitochondrial GPAT in rat hepatocytes leads to decreased fatty acid oxidation and increased glycerolipid biosynthesis. J Lipid Res. 2004 Jul;45(7):1279-88.
166. Shi Y, Cheng D. Beyond triglyceride synthesis: the dynamic functional roles of MGAT and DGAT enzymes in energy metabolism. Am J Physiol Endocrinol Metab. 2009 Jul;297(1):E10-8.
167. Phan CT, Tso P. Intestinal lipid absorption and transport. Front Biosci. 2001 Mar 1;6:D299-319.
168. Polheim D, David JS, Schultz FM, Wylie MB, Johnston JM. Regulation of triglyceride biosynthesis in adipose and intestinal tissue. J Lipid Res. 1973 Jul;14(4):415-21.
169. Cases S, Smith SJ, Zheng YW, Myers HM, Lear SR, Sande E, et al. Identification of a gene encoding an acyl CoA:diacylglycerol acyltransferase, a key enzyme in triacylglycerol synthesis. Proc Natl Acad Sci U S A. 1998 Oct 27;95(22):13018-23.
170. Yamazaki T, Sasaki E, Kakinuma C, Yano T, Miura S, Ezaki O. Increased very low density lipoprotein secretion and gonadal fat mass in mice overexpressing liver DGAT1. J Biol Chem. 2005 Jun 3;280(22):21506-14.

171. Chen HC, Smith SJ, Ladha Z, Jensen DR, Ferreira LD, Pulawa LK, et al. Increased insulin and leptin sensitivity in mice lacking acyl CoA:diacylglycerol acyltransferase 1. J Clin Invest. 2002 Apr;109(8):1049-55.
172. Yabaluri N, Bashyam MD. Hormonal regulation of gluconeogenic gene transcription in the liver. J Biosci. 2010 Sep;35(3):473-84.
173. Beale EG, Hammer RE, Antoine B, Forest C. Disregulated glyceroneogenesis: PCK1 as a candidate diabetes and obesity gene. Trends Endocrinol Metab. 2004 Apr;15(3):129-35.
174. Attia RR, Connnaughton S, Boone LR, Wang F, Elam MB, Ness GC, et al. Regulation of pyruvate dehydrogenase kinase 4 (PDK4) by thyroid hormone: role of the peroxisome proliferator-activated receptor gamma coactivator (PGC-1 alpha). J Biol Chem. 2010 Jan 22;285(4):2375-85.
175. Harris RA, Bowker-Kinley MM, Huang B, Wu P. Regulation of the activity of the pyruvate dehydrogenase complex. Adv Enzyme Regul. 2002;42:249-59.
176. Patel MS, Korotchkina LG. Regulation of mammalian pyruvate dehydrogenase complex by phosphorylation: complexity of multiple phosphorylation sites and kinases. Exp Mol Med. 2001 Dec 31;33(4):191-7.
177. Sugden MC, Holness MJ. Recent advances in mechanisms regulating glucose oxidation at the level of the pyruvate dehydrogenase complex by PDKs. Am J Physiol Endocrinol Metab. 2003 May;284(5):E855-62.
178. Kwon HS, Harris RA. Mechanisms responsible for regulation of pyruvate dehydrogenase kinase 4 gene expression. Adv Enzyme Regul. 2004;44:109-21.
179. Sugden MC, Bulmer K, Gibbons GF, Knight BL, Holness MJ. Peroxisome-proliferator-activated receptor-alpha (PPARalpha) deficiency leads to dysregulation of hepatic lipid and carbohydrate metabolism by fatty acids and insulin. Biochem J. 2002 Jun 1;364(Pt 2):361-8.
180. Bickel PE, Tansey JT, Welte MA. PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores. Biochim Biophys Acta. 2009 Jun;1791(6):419-40.
181. Motomura W, Inoue M, Ohtake T, Takahashi N, Nagamine M, Tanno S, et al. Up-regulation of ADRP in fatty liver in human and liver steatosis in mice fed with high fat diet. Biochem Biophys Res Commun. 2006 Feb 24;340(4):1111-8.
182. Stringer DM, Zahradka P, Declercq VC, Ryz NR, Diakiw R, Burr LL, et al. Modulation of lipid droplet size and lipid droplet proteins by trans-10,cis-12 conjugated linoleic acid parallels improvements in hepatic steatosis in obese, insulin-resistant rats. Biochim Biophys Acta. 2010 Dec;1801(12):1375-85.
183. Chang BH, Li L, Paul A, Taniguchi S, Nannegari V, Heird WC, et al. Protection against fatty liver but normal adipogenesis in mice lacking adipose differentiation-related protein. Molecular and Cellular Biology. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2006 Feb;26(3):1063-76.
184. Nguyen P, Leray V, Diez M, Serisier S, Le Bloc'h J, Siliart B, et al. Liver lipid metabolism. J Anim Physiol Anim Nutr (Berl). 2008 Jun;92(3):272-83.
185. White DA, Bennett AJ, Billett MA, Salter AM. The assembly of triacylglycerol-rich lipoproteins: an essential role for the microsomal triacylglycerol transfer protein. Br J Nutr. 1998 Sep;80(3):219-29.
186. Gordon DA, Jamil H, Sharp D, Mullaney D, Yao Z, Gregg RE, et al. Secretion of apolipoprotein B-containing lipoproteins from HeLa cells is dependent on expression of the microsomal triglyceride transfer protein and is regulated by lipid availability. Proc Natl Acad Sci U S A. 1994 Aug 2;91(16):7628-32.
187. Bremmer DR, Bertics SJ, Besong SA, Grummer RR. Changes in hepatic microsomal triglyceride transfer protein and triglyceride in periparturient dairy cattle. J Dairy Sci. 2000 Oct;83(10):2252-60.

188. Adeli K, Taghibiglou C, Van Iderstine SC, Lewis GF. Mechanisms of hepatic very low-density lipoprotein overproduction in insulin resistance. Trends Cardiovasc Med. 2001 Jul;11(5):170-6.
189. Benoist F, Nicodeme E, Grand-Perret T. Microsomal triacylglycerol transfer protein prevents presecretory degradation of apolipoprotein B-100. A dithiothreitol-sensitive protease is involved. Eur J Biochem. 1996 Sep 15;240(3):713-20.
190. Jamil H, Gordon DA, Eustice DC, Brooks CM, Dickson JK, Jr., Chen Y, et al. An inhibitor of the microsomal triglyceride transfer protein inhibits apoB secretion from HepG2 cells. Proc Natl Acad Sci U S A. 1996 Oct 15;93(21):11991-5.
191. Macri J, Kazemian P, Kulinski A, Rudy D, Aiton A, Thibert RJ, et al. Translocational status of ApoB in the presence of an inhibitor of microsomal triglyceride transfer protein. Biochem Biophys Res Commun. 2000 Oct 5;276(3):1035-47.
192. Wetterau JR, Gregg RE, Harrity TW, Arbeeny C, Cap M, Connolly F, et al. An MTP inhibitor that normalizes atherogenic lipoprotein levels in WHHL rabbits. Science. 1998 Oct 23;282(5389):751-4.
193. Minehira K, Young SG, Villanueva CJ, Yetukuri L, Oresic M, Hellerstein MK, et al. Blocking VLDL secretion causes hepatic steatosis but does not affect peripheral lipid stores or insulin sensitivity in mice. J Lipid Res. 2008 Sep;49(9):2038-44.
194. Noone EJ, Roche HM, Nugent AP, Gibney MJ. The effect of dietary supplementation using isomeric blends of conjugated linoleic acid on lipid metabolism in healthy human subjects. Br J Nutr. 2002 Sep;88(3):243-51.
195. Fabbrini E, Mohammed BS, Magkos F, Korenblat KM, Patterson BW, Klein S. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. Gastroenterology. 2008 Feb;134(2):424-31.
196. Inohara N, Koseki T, Chen S, Wu X, Nunez G. CIDE, a novel family of cell death activators with homology to the 45 kDa subunit of the DNA fragmentation factor. EMBO J. 1998 May 1;17(9):2526-33.
197. Chapman AB, Knight DM, Dieckmann BS, Ringold GM. Analysis of gene expression during differentiation of adipogenic cells in culture and hormonal control of the developmental program. J Biol Chem. 1984 Dec 25;259(24):15548-55.
198. Ye J, Li JZ, Liu Y, Li X, Yang T, Ma X, et al. Cideb, an ER- and lipid droplet-associated protein, mediates VLDL lipidation and maturation by interacting with apolipoprotein B. Cell Metab. 2009 Feb;9(2):177-90.
199. Briggs MR, Yokoyama C, Wang X, Brown MS, Goldstein JL. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. I. Identification of the protein and delineation of its target nucleotide sequence. J Biol Chem. 1993 Jul 5;268(19):14490-6.
200. Yokoyama C, Wang X, Briggs MR, Admon A, Wu J, Hua X, et al. SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell. 1993 Oct 8;75(1):187-97.
201. Shimomura I, Hammer RE, Richardson JA, Ikemoto S, Bashmakov Y, Goldstein JL, et al. Insulin resistance and diabetes mellitus in transgenic mice expressing nuclear SREBP-1c in adipose tissue: model for congenital generalized lipodystrophy. Genes Dev. 1998 Oct 15;12(20):3182-94.
202. Kim JB, Spiegelman BM. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev. 1996 May 1;10(9):1096-107.
203. Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS, Goldstein JL. Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. J Clin Invest. 1997 Mar 1;99(5):846-54.

204. Ferre P, Foufelle F. SREBP-1c transcription factor and lipid homeostasis: clinical perspective. Horm Res. 2007;68(2):72-82.
205. Shimano H. SREBPs: physiology and pathophysiology of the SREBP family. FEBS J. 2009 Feb;276(3):616-21.
206. Liang G, Yang J, Horton JD, Hammer RE, Goldstein JL, Brown MS. Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. J Biol Chem. 2002 Mar 15;277(11):9520-8.
207. Murase T, Misawa K, Minegishi Y, Aoki M, Ominami H, Suzuki Y, et al. Coffee polyphenols suppress diet-induced body fat accumulation by downregulating SREBP-1c and related molecules in C57BL/6J mice. Am J Physiol Endocrinol Metab. 2011 Jan;300(1):E122-33.
208. Chen J, Yang XJ, Xia D, Wegner J, Jiang Z, Zhao RQ. Sterol regulatory element binding transcription factor 1 expression and genetic polymorphism significantly affect intramuscular fat deposition in the longissimus muscle of Erhualian and Sutai pigs. J Anim Sci. 2008 Jan;86(1):57-63.
209. Takahashi Y, Kushiro M, Shinohara K, Ide T. Activity and mRNA levels of enzymes involved in hepatic fatty acid synthesis and oxidation in mice fed conjugated linoleic acid. Biochimica et biophysica acta. [Comparative Study]. 2003 Apr 8;1631(3):265-73.
210. Foretz M, Pacot C, Dugail I, Lemarchand P, Guichard C, Le Liepvre X, et al. ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. Molecular and Cellular Biology. [Research Support, Non-U.S. Gov't]. 1999 May;19(5):3760-8.
211. Thering BJ, Graugnard DE, Piantoni P, Loor JJ. Adipose tissue lipogenic gene networks due to lipid feeding and milk fat depression in lactating cows. J Dairy Sci. 2009 Sep;92(9):4290-300.
212. Yamashita H, Takenoshita M, Sakurai M, Bruick RK, Henzel WJ, Shillinglaw W, et al. A glucose-responsive transcription factor that regulates carbohydrate metabolism in the liver. Proc Natl Acad Sci U S A. 2001 Jul 31;98(16):9116-21.
213. Ishii S, Iizuka K, Miller BC, Uyeda K. Carbohydrate response element binding protein directly promotes lipogenic enzyme gene transcription. Proc Natl Acad Sci U S A. 2004 Nov 2;101(44):15597-602.
214. Kawaguchi T, Takenoshita M, Kabashima T, Uyeda K. Glucose and cAMP regulate the L-type pyruvate kinase gene by phosphorylation/dephosphorylation of the carbohydrate response element binding protein. Proc Natl Acad Sci U S A. 2001 Nov 20;98(24):13710-5.
215. Iizuka K, Bruick RK, Liang G, Horton JD, Uyeda K. Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. Proc Natl Acad Sci U S A. 2004 May 11;101(19):7281-6.
216. Menendez JA, Colomer R, Lupu R. Why does tumor-associated fatty acid synthase (oncogenic antigen-519) ignore dietary fatty acids? Med Hypotheses. 2005;64(2):342-9.
217. Tugwood JD, Issemann I, Anderson RG, Bundell KR, McPheat WL, Green S. The mouse peroxisome proliferator activated receptor recognizes a response element in the 5' flanking sequence of the rat acyl CoA oxidase gene. EMBO J. 1992 Feb;11(2):433-9.
218. Vanden Heuvel JP. Peroxisome proliferator-activated receptors (PPARS) and carcinogenesis. Toxicol Sci. 1999 Jan;47(1):1-8.
219. Rakhshandehroo M, Sanderson LM, Matilainen M, Stienstra R, Carlberg C, de Groot PJ, et al. Comprehensive analysis of PPARalpha-dependent regulation of hepatic lipid metabolism by expression profiling. PPAR Res. 2007;2007:26839.
220. Patsouris D, Mandard S, Voshol PJ, Escher P, Tan NS, Havekes LM, et al. PPARalpha governs glycerol metabolism. J Clin Invest. 2004 Jul;114(1):94-103.

221. Hashimoto T, Cook WS, Qi C, Yeldandi AV, Reddy JK, Rao MS. Defect in peroxisome proliferator-activated receptor alpha-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting. J Biol Chem. 2000 Sep 15;275(37):28918-28.
222. Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. J Clin Invest. 1999 Jun;103(11):1489-98.
223. Leone TC, Weinheimer CJ, Kelly DP. A critical role for the peroxisome proliferator-activated receptor alpha (PPARalpha) in the cellular fasting response: the PPARalpha-null mouse as a model of fatty acid oxidation disorders. Proc Natl Acad Sci U S A. 1999 Jun 22;96(13):7473-8.
224. Guerre-Millo M, Rouault C, Poulain P, Andre J, Poitout V, Peters JM, et al. PPAR-alpha-null mice are protected from high-fat diet-induced insulin resistance. Diabetes. 2001 Dec;50(12):2809-14.
225. Tordjman K, Bernal-Mizrachi C, Zemany L, Weng S, Feng C, Zhang F, et al. PPARalpha deficiency reduces insulin resistance and atherosclerosis in apoE-null mice. J Clin Invest. 2001 Apr;107(8):1025-34.
226. Bernal-Mizrachi C, Weng S, Feng C, Finck BN, Knutsen RH, Leone TC, et al. Dexamethasone induction of hypertension and diabetes is PPAR-alpha dependent in LDL receptor-null mice. Nat Med. 2003 Aug;9(8):1069-75.
227. Rasooly R, Kelley DS, Greg J, Mackey BE. Dietary trans 10, cis 12-conjugated linoleic acid reduces the expression of fatty acid oxidation and drug detoxification enzymes in mouse liver. Br J Nutr. 2007 Jan;97(1):58-66.
228. Javadi M, Beynen AC, Hovenier R, Lankhorst A, Lemmens AG, Terpstra AH, et al. Prolonged feeding of mice with conjugated linoleic acid increases hepatic fatty acid synthesis relative to oxidation. J Nutr Biochem. 2004 Nov;15(11):680-7.
229. Viswanadha S, McGilliard ML, Gandour RD, Herbein JH. Alterations in CPT-1 mRNA and fatty acid profile in hepatic cell lines in response to treatment with t10,c12- or c9,t11-conjugated linoleic acid. Eur J Lipid Sci Technol. 2007;110:16-22.
230. McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem. 1997 Feb 15;244(1):1-14.
231. Reddy JK, Mannaerts GP. Peroxisomal lipid metabolism. Annu Rev Nutr. 1994;14:343-70.
232. Fan CY, Pan J, Chu R, Lee D, Kluckman KD, Usuda N, et al. Hepatocellular and hepatic peroxisomal alterations in mice with a disrupted peroxisomal fatty acyl-coenzyme A oxidase gene. J Biol Chem. 1996 Oct 4;271(40):24698-710.
233. Fan CY, Pan J, Usuda N, Yeldandi AV, Rao MS, Reddy JK. Steatohepatitis, spontaneous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase. Implications for peroxisome proliferator-activated receptor alpha natural ligand metabolism. J Biol Chem. 1998 Jun 19;273(25):15639-45.
234. McGarry JD, Woeltje KF, Kuwajima M, Foster DW. Regulation of ketogenesis and the renaissance of carnitine palmitoyltransferase. Diabetes Metab Rev. 1989 May;5(3):271-84.
235. Hegardt FG. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase: a control enzyme in ketogenesis. Biochem J. 1999 Mar 15;338 (Pt 3):569-82.
236. Sakono M, Miyanaga F, Kawahara S, Yamauchi K, Fukuda N, Watanabe K, et al. Dietary conjugated linoleic acid reciprocally modifies ketogenesis and lipid secretion by the rat liver. Lipids. 1999 Sep;34(9):997-1000.
237. Williamson DH, Bates MW, Page MA, Krebs HA. Activities of enzymes involved in acetoacetate utilization in adult mammalian tissues. Biochem J. 1971 Jan;121(1):41-7.

238. Bouillaud F, Couplan E, Pecqueur C, Ricquier D. Homologues of the uncoupling protein from brown adipose tissue (UCP1): UCP2, UCP3, BMCP1 and UCP4. Biochim Biophys Acta. 2001 Mar 1;1504(1):107-19.
239. Aquila H, Link TA, Klingenberg M. The uncoupling protein from brown fat mitochondria is related to the mitochondrial ADP/ATP carrier. Analysis of sequence homologies and of folding of the protein in the membrane. EMBO J. 1985 Sep;4(9):2369-76.
240. Soh JR, Shin DH, Kwon DY, Cha YS. Effect of Cheonggukjang supplementation upon hepatic acyl-CoA synthase, carnitine palmitoyltransferase I, acyl-CoA oxidase and uncoupling protein 2 mRNA levels in C57BL/6J mice fed with high fat diet. Genes Nutr. 2008 Feb;2(4):365-9.
241. Gong DW, He Y, Karas M, Reitman M. Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. J Biol Chem. 1997 Sep 26;272(39):24129-32.
242. Weigle DS, Selfridge LE, Schwartz MW, Seeley RJ, Cummings DE, Havel PJ, et al. Elevated free fatty acids induce uncoupling protein 3 expression in muscle: a potential explanation for the effect of fasting. Diabetes. 1998 Feb;47(2):298-302.
243. Samec S, Seydoux J, Dulloo AG. Interorgan signaling between adipose tissue metabolism and skeletal muscle uncoupling protein homologs: is there a role for circulating free fatty acids? Diabetes. 1998 Nov;47(11):1693-8.
244. Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, et al. Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. Nat Genet. 1997 Mar;15(3):269-72.
245. Chung S, Brown JM, Provo JN, Hopkins R, McIntosh MK. Conjugated linoleic acid promotes human adipocyte insulin resistance through NFkappaB-dependent cytokine production. The Journal of biological chemistry. [Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't]. 2005 Nov 18;280(46):38445-56.
246. Evans M, Park Y, Pariza M, Curtis L, Kuebler B, McIntosh M. Trans-10,cis-12 conjugated linoleic acid reduces triglyceride content while differentially affecting peroxisome proliferator activated receptor gamma2 and aP2 expression in 3T3-L1 preadipocytes. Lipids. 2001 Nov;36(11):1223-32.
247. Ferramosca A. Conjugated linoleic acid and hepatic lipogenesis in mouse: role of the mitochondrial citrate carrier. The Journal of Lipid Research. 2006;47(9):1994-2003.
248. House RL, Cassady JP, Eisen EJ, Eling TE, Collins JB, Grissom SF, et al. Functional genomic characterization of delipidation elicited by trans-10, cis-12-conjugated linoleic acid (t10c12-CLA) in a polygenic obese line of mice. Physiol Genomics. 2005 May 11;21(3):351-61.
249. Lin Y, Schuurbiers E, Van der Veen S, De Deckere EA. Conjugated linoleic acid isomers have differential effects on triglyceride secretion in Hep G2 cells. Biochimica et biophysica acta. [Comparative Study]. 2001 Aug 29;1533(1):38-46.
250. Masson D, Staels B, Gautier T, Desrumaux C, Athias A, Le Guern N, et al. Cholesteryl ester transfer protein modulates the effect of liver X receptor agonists on cholesterol transport and excretion in the mouse. J Lipid Res. 2004 Mar;45(3):543-50.
251. Dutton S, Trayhurn P. Regulation of angiopoietin-like protein 4/fasting-induced adipose factor (Angptl4/FIAF) expression in mouse white adipose tissue and 3T3-L1 adipocytes. Br J Nutr. 2008 Jul;100(1):18-26.
252. Hirano K, Young SG, Farese RV, Jr., Ng J, Sande E, Warburton C, et al. Targeted disruption of the mouse apobec-1 gene abolishes apolipoprotein B mRNA editing and eliminates apolipoprotein B48. J Biol Chem. 1996 Apr 26;271(17):9887-90.

253. Mao C, Tai WC, Bai Y, Poizat C, Lee AS. In vivo regulation of Grp78/BiP transcription in the embryonic heart: role of the endoplasmic reticulum stress response element and GATA-4. J Biol Chem. 2006 Mar 31;281(13):8877-87.
254. H I, S T, A S, A S. Carbon tetrachloride affects inflammation-related biochemical networks in the mouse liver as identified by a customized cDNA microarray system. Environ Health Prev Med. 2009;15.
255. Kloting N, Wilke B, Kloting I. Phenotypic and genetic analyses of subcongenic BB.SHR rat lines shorten the region on chromosome 4 bearing gene(s) for underlying facets of metabolic syndrome. Physiol Genomics. 2004 Aug 11;18(3):325-30.
256. Coumoul X, Li W, Wang RH, Deng C. Inducible suppression of Fgfr2 and Survivin in ES cells using a combination of the RNA interference (RNAi) and the Cre-LoxP system. Nucleic Acids Res. 2004;32(10):e85.
257. Goldrath AW, Luckey CJ, Park R, Benoist C, Mathis D. The molecular program induced in T cells undergoing homeostatic proliferation. Proc Natl Acad Sci U S A. 2004 Nov 30;101(48):16885-90.
258. Kovacs WJ, Shackelford JE, Tape KN, Richards MJ, Faust PL, Fliesler SJ, et al. Disturbed cholesterol homeostasis in a peroxisome-deficient PEX2 knockout mouse model. Mol Cell Biol. 2004 Jan;24(1):1-13.
259. Oishi K, Uchida D, Ishida N. Circadian expression of FGF21 is induced by PPARalpha activation in the mouse liver. FEBS Lett. 2008 Oct 29;582(25-26):3639-42.
260. Son DS, Roby KF, Terranova PF. Tumor necrosis factor-alpha induces serum amyloid A3 in mouse granulosa cells. Endocrinology. 2004 May;145(5):2245-52.
261. Tsuchiya M, Tye CE, Sharma R, Smith CE, Bartlett JD. XBP1 may determine the size of the ameloblast endoplasmic reticulum. J Dent Res. 2008 Nov;87(11):1058-62.
262. Wei Y, Wang D, Pagliassotti MJ. Saturated fatty acid-mediated endoplasmic reticulum stress and apoptosis are augmented by trans-10, cis-12-conjugated linoleic acid in liver cells. Mol Cell Biochem. 2007 Sep;303(1-2):105-13.
263. Kamphuis MM, Lejeune MP, Saris WH, Westerterp-Plantenga MS. The effect of conjugated linoleic acid supplementation after weight loss on body weight regain, body composition, and resting metabolic rate in overweight subjects. Int J Obes Relat Metab Disord. 2003 Jul;27(7):840-7.
264. Gaullier JM, Halse J, Hoye K, Kristiansen K, Fagertun H, Vik H, et al. Supplementation with conjugated linoleic acid for 24 months is well tolerated by and reduces body fat mass in healthy, overweight humans. J Nutr. 2005 Apr;135(4):778-84.

APPENDIX A

Supplementary Tables

Table A.1 qPCR performance among the thirty-eight genes measured in liver.

Gene	Median Ct[1]	Median ΔCt[2]	Slope[3]	(R^2)[4]	Efficiency[5]
Aacs	24.6	3.0	-3.91	0.991	1.80
Acaca	24.4	2.8	-3.39	0.997	1.97
Acox1	20.6	-1.1	-3.54	0.996	1.92
Angptl3	22.7	1.0	-2.78	0.988	2.29
Angptl4	28.5	6.8	-3.82	0.948	1.83
Apob	19.9	-1.7	-4.01	0.976	1.78
Atf6	26.3	4.6	-2.07	0.971	3.05
Bdh1	21.7	0.0	-3.58	0.997	1.90
Cd36	26.5	4.9	-1.27	0.906	6.15
Chrebp	21.9	0.3	-3.72	0.995	1.86
Cideb	21.6	0.0	-2.95	0.999	2.18
Cpt1a	23.0	1.3	-5.37	0.971	1.54
Ddit3	26.6	5.0	-2.80	0.992	2.28
Dgat1	25.7	3.9	-2.93	0.992	2.19
Dgat2	21.1	-0.5	-3.20	0.993	2.05
Eif2ak3	26.2	4.5	-2.13	0.981	2.94
Fgfr2	26.7	5.2	-2.86	0.974	2.24
Gck	24.1	2.5	-4.03	0.990	1.77
Gpam	21.7	0.1	-3.63	0.997	1.89
Hngcs2	19.5	-2.1	-3.47	0.998	1.94
Hspa1b	25.6	3.9	-2.62	0.980	2.41
Insig1	21.4	-0.2	-2.55	0.997	2.47
Lipc	21.5	-0.2	-2.26	0.981	2.77
Mlycd	24.5	2.8	-3.44	0.998	1.95
Mttp	23.2	1.5	-3.46	0.999	1.95
Pc	23.1	1.4	-3.87	0.995	1.81
Pck1	20.0	-1.8	-2.86	0.999	2.24
Pdk4	26.2	4.5	-3.20	0.991	2.05
Plin2	22.7	1.1	-3.39	0.996	1.97
Pnpla2	25.1	3.2	-3.20	0.994	2.05
Ppara	23.3	1.6	-3.84	0.996	1.82

Table A.1 (cont.)

Gene	**Median Ct[1]**	**Median ΔCt[2]**	**Slope[3]**	**(R^2)[4]**	**Efficiency[5]**
Saa1	23.9	2.2	-2.79	0.997	2.28
Scd	22.0	0.4	-3.36	0.963	1.99
Slc27a2	21.5	-0.1	-3.99	0.997	1.78
Slc2a1	27.3	5.6	-3.33	0.987	2.00
Srebf1	23.9	2.2	-3.23	0.997	2.04
Ucp2	25.8	4.1	-3.27	0.993	2.02
Xbp1	21.0	-0.7	-1.57	0.959	4.34

[1] The median is calculated considering all time points and all steers.
[2] The median of ΔCt is calculated as [Ct gene – geometrical mean of Ct internal controls] for each time point and each steer.
[3] Slope of the standard curve.
[4] R2 stands for the coefficient of determination of the standard curve.
[5] Efficiency is calculated as [10(-1 / Slope)].

Table A.2 Accession number, sequence, amplicon size, reference and PCR efficiency of primers use to analyze gene expression by qPCR.

Accession #	Gene	Primer	Primers (5'-3')	Amplicon Size (bp)	Source
NM_030210.1	*Aacs*	F.230	TGGCGCTTGGGAATTACAAT	100	In this article
		R.329	CGTGAGTAGACGATTCCACTGAA		
NM_133360.2	*Acaca*	F.2003	CCTTCCTGCTCACACACTTCTG	85	
		R.2087	CTGCCGAGTCACCTTAAGTACATATT		
NM_015729	*Acox1*	F.436	GCCGTCGAGAAATCGAGAACT	102	
		R.537	TCTTAACAGCCACCTCGTAACG		
NM_013913.3	*Angptl3*		AGGGCTTTGGAGGAGCAGCTAACC		(250)
			GCAGTCGGCAGGAAGGTCATCTTG		
NM_020581.1	*Angptl4*		TTCCAACGCCACCCACTTACA		(251)
			ACCAAACCACCAGCCACCAGA		
NM_009693.2	*Apob*		ATCTGACTGGGAGAGACAAGTAG		(252)
			CGATCAATAATCTCAGCAATAGTTC		
NM_001081304.1	*Atf6*		TGCTAGGACTGGAGGCCAGGCTCAA		(253)
			CATGTCTATGAACCCAGCCTCGAAGT		
NM_175177.3	*Bdh1*	F.402	GTCAGGCAGATGCGGCTAGT	100	In this article
		R.201	AGGAAGCCTTTTGAGTGCAGAT		
NM_007643.3	*Cd36*	F.726	GAGGTCCTTACACATACAGAGTTCGTT	121	In this article
		R.846	ACAGACAGTGAAGGCTCAAAGATG		
NM_021455.3	*Chrebp*	F.733	GCTTCTAGACCTGGACTGCTTCTT	115	
		R.847	AGCATTGCCAACATAAGCATCTT		
NM_009894.2	*Cideb*		CCCCAAGAGTGGGATGTTGT	100	In this article
			GGGATTTTGCTTGTACACATCGA		
NM_013495.1	*Cpt1a*	F.1722	CCTGCATTCCTTCCCATTTG	117	
		R.1838	TTGCCCATGTCCTTGTAATGTG		
NM_007837.2	*Ddit3*		GCAAGGAAGAACTAGGAAACGGA		(254)
			CAATGTACCGTCTATGTGCAAGC		
NM_010046.2	*Dgat1*	F.804	ACAACCTGACCTACCGAGATCTCT	120	In this article
		R.923	TCTCAAGAACTCGTCGTAGCAGAA		

Table A.2 (cont.)

Accession #	Gene	Primer	Primers (5'-3')	Amplicon Size (bp)	Source
NM_026384.3	*Dgat2*	F.551	CCAAGAAAGGTGGCAGGAGAT	106	In this article
		R.656	GTCAGCAGGTTGTGTGTCTTCAC		
NM_010121.2	*Eif2ak3*		AACGGAAGGAGTCTGAAACTCAGT		(255)
			TTGGCTCAAAATCTGTTAGGTATCG		
NM_201601.2	*Fgfr2*		AAGGTTTACAGCGATGCCCA		(256)
			ACCACCATGCAGGCGATTAA		
NM_010292.4	*Gck*	F.389	TCTCTGACTTCCTGGACAAGCA	101	In this article
		R.489	GATGCCCTTGTCTATGTCTTCGT		
NM_008149.3	*Gpam*	F.725	TGGGTGTTACTAAAGCTCTTCAACA	100	In this article
		R.824	GCGGCAGGTTCGTCTCAGT		
NM_008256.3	*Hmgcs2*	F.515	CACTGACATCGAGGGCATAGATAC	107	In this article
		R.621	CATAGCGACCATCCCAGTAGCT		
NM_010478.2	*Hspa1b*		AATTTAACAGTCAACGCAATTACC		(257)
			AACAGACTCTTTGCACTTGATAGC		
NM_153526.3	*Insig1*		AGGACGACAGTTAGCTATGGGTG		(258)
			CCCCCTTACCCGACTCTCACATAC		
NM_008280.2	*Lipc*	F.722	TTCTCGGAGCAAAGTTCACCTAA	110	In this article
		R.831	CCAGCCCTGTGATTCTTCCA		
NM_019966.2	*Mlycd*	F.903	CGCTGCCATCTTCTACTCCAT	105	In this article
		R.1007	AATTCCTTCTGCAGCTCCTTGA		
NM_008642.1	*Mttp*	F.234	GACAGCGTGGGCTACAAAATC	101	In this article
		R.334	GCTGTTATCGTGACTTGGATCACTT		
NM_008797.2	*Pc*	F.686	CGCATGAGTTCTCCAACACCTA	114	In this article
		R.799	TGTAATTCTCTTCCAACTCCTCATAGC		

Table A.2 (cont.)

Accession #	Gene	Primer	Primers (5'-3')	Amplicon Size (bp)	Source
NM_011044.2	*Pck1*	F.1453	CTGAAGGTGTCCCCCTTGTC	124	In this article
		R.1576	GGGTCGTGCATGATGATCTTG		
NM_013743.2	*Pdk4*		CACATGCTCTTCGAACTCTTCAAG		(259)
			TGATTGTAAGGTCTTCTTTTCCCAAG		
NM_007408.3	*Plin2*	F.725	AGCTGGAGATGGAAGCAAAAAA	101	In this article
		R.825	CCGAGAGCAGAGCTTGGTAGA		
NM_025802.2	*Pnpla2*	F.875	TGGAACCAAAGGACCTGATGAC	121	In this article
		R.995	AACAAGCGGATGGTGAAGGA		
NM_011144.3	*Ppara*		ACTTCGCTATCCAGGCAGAA	117	
			CAGACCAACCAAGTGTTGTGA		
NM_009117.3	*Saa1*		GAAGGAAGCTAACTGGAAAAACTC		(260)
			CAGGCCCCCAGCACAACCTACT		
NM_009127.3	*Scd*		TCCAGTGAGGTGGTGTGAAA		
			TTATCTCTGGGGTGGGTTTG		
NM_011978.2	*Slc27a2*	F.1095	ACACCGCAGAAACCAAATGAC	100	In this article
		R.1194	CCCCAAATCTCTTGATGAACTCTCT		
NM_011400.2	*Slc2a1*	F.318	CCAGAAGGTTATTGAGGAGTTCTACAA	102	In this article
		R.419	GCCACGGAGAGAGACCAAAG		
NM_011480.2	*Srebf1*		GTGAGCCTGACAAGCAATCA		
			GGTGCCTACAGAGCAAGAGG		
NM_011671	*Ucp2*	F.881	GGCCTCTGGAAAGGGACTTC	103	
		R.983	TGGCTTTCAGGAGAGTATCTTTGA		
NM_013842.2	*Xbp1*		TCCGCAGCACTCAGACTATGT		(261)
			ATGCCCAAAAGGATATCAGACTC		

Table A.3 Sequencing results of PCR products from primers of genes designed for this experiment. Best hits using BLASTN (http://www.ncbi.nlm.nih.gov) are shown. Similar information for remaining genes was reported previously.

Gene	Sequence
Aacs	GGACTTTTGGGCTGAGTTCTGGAAGTTCAGTGGAATCGTCTACTCACG
Acaca	GTACATATCTTAGTAAGGAATCAAATATGTACTTAAGGTGACTCGGCAGA
Acox1	
Angptl3	CTCAGGAGCACCCAGAAGTAACATCACTCAAAAGTTTTGTAGAACAGCAAGACAACAGCATAAGAGAACTCCT CCAGAGTGTGGAAGAACAGTATAAACAATTAAGTCAACAGCACATGCAGATAAAAGAAATAGAAAAGCAGCTC AGAAAGACTGGTATTCAAGAACCCTCAGAAAATTCTCTTTCTTCTAAATCAAGAGCACCAAGAACTACTCCCCCT CTTCAACTGAACGAAACAGAAAATACAGAACAAGATGACCTTCCTGCCGACTGC
Angptl4	GGGAGCGGCACAGTGGACTTTTCCAGATCCAGCCTCTGGGGTCTCCACCATTTTTGGTCAACTGTGAGATGACTT CAGATGGAGGCTGGACAGTGATTCAGAGACGCCTGAACGGCTCTGTGGACTTCAACCAGTCCTGGGAAGCCTAC AAGGATGGCTTCGGAGATCCCCAAGGCGAGTTCTGGCTGGGCCTGGAAAAGATGCACAGCATCACAGGGAACC GAGGAAGCCAATTGGCTGTGCAGCTCCAGGACTGGGATGGCANTGCCNAATTGCTCCAATTTCCCATCCATTTG GGGGGTGAGGA
Apob	AAATTATAGAATTACAGATAATGATGTACTAATTGCCATAGATAGTGCCAAAATCAACTTCAATGAAAAACTCT CTCAACTTGAGACATACGCGATATAATTTGATCAGTATATTAAAGATAATTATGATCCACATGACTTAAAAAGA ACTATTGCTGAGATTATTGATCGAA
Atf6	AGGAGAATGGCTCCCTGAAGCGACAGCTGGACGAGGTGGTGTCAGAGAACCAGAGGCTCAAAGTCCCAAGTCC AAAGCGAAGAGCTGTCTGTGTGATGATAGTATTAGCATTTATAATGCTGAACTATGGGCCCATGAGCATGCTGG AGCAAGAATCCCGAAGAGTGAAACCTAGTGTGAGCCCTGCCAATCAGAGGAGGCATCTCTTGGAATTTTCAGCA AAAGAAGTTAAAGACACATCAGATGGTGACAACCAGAAAGACAGTTACAGCTATGATCACTCTGTGTCCAATGA CAAAGCTTTAATGGTGCTAAGTGAAGAGCCATTGCTTTATATGCCTCCACCTCCATGTCAACCCCTGATTAACAC AACAGAGTCTCTCAGGTTGAACCATGAACTTCGAGGCTGGGTTCATAGACATGA
Bdh1	GATTTGGGTTCTCACTGGCCAAGCATCTGCACTCAAAAGGCTTCCT

Table A.3 (cont.)

Gene	Sequence
Cd36	CCATCTTTGAGCCTTCACTGTCTGT
Chrebp	CTCTTCACCATGACACAGCCCAGTCCTTCGTCCCTGCAGCTGCCCCCAGAAGAATGCTTATGTTGGCAATGCTAA
Cideb	AGCAAGGACATCGCCCGCATCACCTTCGATGTGTACAAGCAAAATCCCC
Cpt1a	CTGATCAGAAGTGCCGGACGAGTCCCGATGCCTTCATCCAGCTGGCACTGCAGCTCGCACATTACAAGGACATGG GCAAA
Ddit3	CCGGCCTGGGAGCACGCATGAAGGAGAAGGAGCAGGAGAACGAGCGGAAAGTGGCACAGCTAGCTGAAGAGA ACGAGCGGCTCAAGCAGGAAATCGAGCGCCTGACCAGGGAGGTGGAGACCACACGGCGGGCTCTGATCGACCG CATGGTCAGCCTGCACCAAGCATGAACAGTGGGCATCACCTCCTGTCTGTCTCTCCGGAAGTGTACCCAGCACCA TCGCGCCAGCGCCAAGCATGTGACCCTGCACTGCACTGCACATGCTGAGGAGGGGACTGAGGGTAGACCAGGAG AGGGCTCGGCTTGCACATAGACGGTACATTG
Dgat1	TCAACTTTCCTCGGTCCCCCCGAATACGAAAGCGCTTTCTGCTACGACGAGTTCTTGAGA
Dgat2	GTGTGCGGCTACTTCCGAGACTACTTTCCCATCCAGCTGGTGAAGACACACAACCTGCTGAC
Eif2ak3	GTGCCGATGTCAGTGACAACAGCTGGAATGACATGAAGTACTCAGGATACGTATCCCGATACCTAACAGATTTTG AGCCAA
Fgfr2	ACGGCAGTAAATACGGGCCTGATGGGCTGCCCTACCTCAAGGTCCTGAAGCACTCGGGGATAAATAGCTCCAAT GCAGAAGTGCTGGCTCTGTTCAATGTGACGGAGATGGATGCTGGGGCAATATATATGTAAGGTCTCCAATTATAT AGGGCAGGCCAACCAGTCTGCCTGGCTCACTGTCCTGCCCAAACAGCAAGCGCCTGTGAGAGAGAAGGAGATCA CGGCTTCCCCAGATTATCTGGAGATAGCTATTTACTGCATAGGGGTCTTCTTAATCGCCTGCATGGTG
Gck	CCCCGTGGCTTCACCTTCTCCTTCCCGTTAAGGCACGAAGACATAGACAAGGGCATC
Gpam	AGGGTCAACTCGAGATGGTCAAGGCTGCAACTGAGACGAACCTGCCGC
Hmgcs2	GCGGCACAGCCTCCCTCTTCAATGCTGCCAACTGGATGGAGTCCAGCTACTGGGATGGTCGCTATG
Hspa1b	CCATGAAGAAGACTTTAAATAACCTTGACAGTAATCGGTGCCCAAGCAGCTATCAAGTGCAAAGAGTCTGTT

Table A.3 (cont.)

Gene	Sequence
Insig1	CACATGGTTCAGAGTGGAAGCAGGATGTAGAGACACTGGTCCTGGGTGTGATGAAGATGATCTTTTTCTCAATG TTCCATTTAGGCTGGGCTGACGATAAATGACTCCTAAAGACATGTTCACTTAGTCTAGACTAGCAAATGGAGGC AAGGACTGCTTACCTAAAAGTCTTACCTTGCTCCCCCACCCTCACACCTGTCTTCTTTGGAACATTCCATCCCAG GCTGTATGTGAGAGTCGGGGTAAGGGGG
Lipc	CGTCTCAGGGTTCGCAGGCAGCTCCATGGACGGGAAGAACAAGATTGGAAGAATCACAGGGC
Mlycd	GAGCTCGGGACCTTCCTCATAAAGAGAGTGGTCAAGGAGCTGCAGAAGGAATT
Mttp	GGAGGAATCCTGATGGTGATGATGATCAAGTGATCCAAGTCACGATAACAGC
Pc	GCCGCCTACGGAGGTGGGGGCCGCGGCATGCGGGTCGTGCATAGCTATGAGGAGTTGGAAGAGAATTACA
Pck1	GGAGCAGCCATGAGATCTGAGGCCACAGCTGCTGCAGAACACAAGGGCAAGATCATCATGCACGACCC
Pdk4	GAGCATCAAGAAAACCGTCCTTCCTTGACCCCAGTAGAGGCCACTGTCGTCTTGGGAAAAGAAGACCTTACAAT CAA
Plin2	AAGCCGAGCAACTATGAACGGCTGGAGTCCCTGTCTACCAAGCTCTGCTCTCGGG
Pnpla2	GCGCCTGGCAACGGCCATGATGGTGCCCTATACTCTGCCGCTGGAGAGTGCAGTGTCCTTCACCATCCGCTTGTT
Ppara	
Saa1	ATGCTGCTCAAAGGGGTCCCGGGGGAGTCTGGGCTGCTGAGAAAATCAGTGATGGAAGAGAGGCCTTTCAGGA ATTCTTCGGCAGAGGACATGAGGACACCATTGCTGACCAGGAAGCCAACAGACATGGCCGCAGTGGCAAAGAC CCCAATTACTACAGACCTCCTGGACTGCCTGACAAATACTGAGCGTCCTCCTATTAGCTCAGTAGGTTGTGCTGG GGGCCTGA
Scd	CTCAGCCAAGGTGCCTCTTAGCCACTGAATTGCTATGTTATCCTTTCTCTTGTAACAAACCCACCCCAGAGATAA A
Slc27a2	GGAAATGGCTTACGAGGAGATGTGTGGAGAGAGTTCATCAAGAGATTTGGGG
Slc2a1	GGAGAGCCCATCCCATCCACCACACTCACCACGCTTTGGTCTCTCTCCGTGGC
Srebf1	GGTCAAGAATCTGGAAATTGCAGAGGCTGCACTGGCCCGATGGCACCCTCTTGCTCTGTAGGCACCAAT
Ucp2	
Xbp1	TCCCCAGAACATCTTCCCATGGACTCTGACACTGTTGCCTCTTCAGATTCTGAGTCTGATATCCTTTTGGGCAT

Table A.4 Sequencing results of genes using BLASTN from NCBI against nucleotide collection (nr / nt) with total score.

Gene	Best hit in NCBI	Score
Aacs	Mus musculus acetoacetyl-CoA synthetase (Aacs), mRNA	80.1
Acaca	Mus musculus acetyl-Coenzyme A carboxylase alpha (Acaca), mRNA	89.1
Acox1	Mus musculus acyl-Coenzyme A oxidase 1, palmitoyl (Acox1), mRNA.	80.1
Angptl3	Mus musculus angiopoietin-like 3 (Angptl3), mRNA	89.1
Angptl4	Mus musculus angiopoietin-like 4 (Angptl4), mRNA	78.3
Apob	Mus musculus chromosome 12 genomic contig, strain C57BL/6J	89.1
Atf6	Mus musculus activating transcription factor 6 (Atf6), mRNA	94.5
Bdh1	Mus musculus 3-hydroxybutyrate dehydrogenase, type 1 (Bdh1),mRNA	78.3
Cd36	Mus musculus CD36 antigen (Cd36), mRNA	94.5
Chrebp	Mus musculus MLX interacting protein-like (Mlxipl), Mrna	87.3
Cideb	Mus musculus cell death-inducing DNA fragmentation factor, alpha subunit-like effector B (Cideb), mRNA	80.1
Cpt1a	Mus musculus carnitine palmitoyltransferase 1a, liver (Cpt1a),	78.3
Ddit3	Mus musculus DNA-damage inducible transcript 3 (Ddit3), mRNA	85.5
Dgat1	Mus musculus diacylglycerol O-acyltransferase 1 (Dgat1), mRNA	89.1
Dgat2	Mus musculus diacylglycerol O-acyltransferase 2 (Dgat2), mRNA	81.9
Eif2ak3	Mus musculus eukaryotic translation initiation factor 2 alpha kinase 3 (Eif2ak3), mRNA	81.9
Fgfr2	Mus musculus fibroblast growth factor receptor 2 (Fgfr2), transcript variant 2, mRNA	74.7
Gck	Mus musculus glucokinase (Gck), mRNA	83.7
Gpam	Mus musculus glycerol-3-phosphate acyltransferase, mitochondrial (Gpam), mRNA	81.9
Hmgcs2	Mus musculus 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2 (Hmgcs2), mRNA	85.5
Hspa1b	Mus musculus heat shock protein 1B (Hspa1b), mRNA	89.1
Insig1	Mus musculus insulin induced gene 1 (Insig1), mRNA	87.3

Table A.4 (cont.)

Gene	Best hit in NCBI	Score
Lipc	Mus musculus lipase, hepatic (Lipc), mRNA	80.1
Mlycd	Mus musculus malonyl-CoA decarboxylase (Mlycd), mRNA	80.1
Mttp	Mus musculus microsomal triglyceride transfer protein (Mttp), mRNA	83.7
Pc	Mus musculus pyruvate carboxylase (Pcx), mRNA	89.1
Pck1	Mus musculus phosphoenolpyruvate carboxykinase 1, cytosolic (Pck1), mRNA	76.5
Pdk4	Mus musculus pyruvate dehydrogenase kinase, isoenzyme 4 (Pdk4), mRNA	92.7
Plin2	Mus musculus adipose differentiation related protein (Adfp), mRNA	80.1
Pnpla2	Mus musculus patatin-like phospholipase domain containing 2 (Pnpla2) mRNA	78.3
Ppara	Mus musculus peroxisome proliferator activated receptor alpha (Ppara)	76.5
Saa1	Mus musculus serum amyloid A 1 (Saa1), mRNA	85.5
Scd	Mus musculus stearoyl-Coenzyme A desaturase 1 (Scd1), mRNA	74.7
Slc27a2	Mus musculus solute carrier family 27 (fatty acid transporter), member 2 (Slc27a2), mRNA	85.5
Slc2a1	Mus musculus solute carrier family 2 (facilitated glucose transporter),member 1 (Slc2a1), mRNA	87.3
Srebf1	Mus musculus sterol regulatory element binding factor 1 (Srebf1), mRNA	74.7
Ucp2	Mus musculus uncoupling protein 2 (mitochondrial, proton carrier)	81.7
Xbp1	Mus musculus X-box binding protein 1 (Xbp1), mRNA	81.9

Table A.5 *In vitro* studies of the *trans*-10, *cis*-12-CLA included in the literature review.

Reference	Subjects	Dose	Duration	Results
Bretillon et al. (1999) (111)	Rat liver microsomes	20 to 120 nmol/L	15 min	*Trans*-10, *cis*-12-CLA significantly inhibited the conversion of stearic aicd and the Δ6 desaturation of linoleic acid at the highest inhibitor/substrate ratio.
Yotsumotoet et al. (1999) (146)	HepG2 cells	10 μmol/L	24 hrs	*Trans*-10, *cis*-12-CLA inhibited cellular triglyceride synthesis and reduced apoB secretion.
Lin et al. (2001) (144)	HepG2 cells	1 mmol/L	5 hrs	*Trans*-10, *cis*-12-CLA suppressed TG secretion in spite of the fact that it increased 4-fold the cellular TG content.
Choi et al. (2001) (109)	HepG2 cells	45 μmol/L	48 hrs	Human SCD activity was ↓ by the *trans*-10, *cis*-12-CLA directly, without changes in SCD gene expression, suggesting that this isomer regulates human SCD activity mainly by a posttranslational mechanism.
Clément et al. (2002) (33)	COS-1 cells	10 to 200 μmol/L	36 hrs	*Trans*-10, *cis*-12-CLA and *cis*-9, *trans*-11-CLA were equally efficient at activating PPARα, β/σ and γ and inhibiting liver-X-receptor. Thus, the specific effect of *Trans*-10, *cis*-12-CLA is unlikely to result from direct interaction with these nuclear receptors.
Eder et al. (2002) (108)	HepG2 cells	1 to 100 μmol/L	0.5 or 24 hrs	Cells had a markedly lower ratio of MUFA to SFA in lipids than control cells, suggesting that *trans*-10, *cis*-12-CLA suppresses Δ9-, Δ6- and Δ5-desaturation and has significant effects on the metabolism of essential fatty acids in HepG2 cells.
Yamasaki et al. (2005) (50)	Rat hepatoma cells	0 to 10 μmol/L	72 hrs	1 μmol/L *Trans*-10, *cis*-12-CLA demonstrated significant cytotoxic effects on rat hepatoma cells. It also induced mitochondria-related apoptosis accompanied by lysosomal destabilization in rat hepatoma cells.
Wei et al. (2007) (262)	Rat hepatoma cells	80 μmol/L	3, 6 or 16 hrs	*Trans*-10, *cis*-12-CLA reduced SCD1 activity and increased the SFA composition of total lipids following incubations with palmitate. Co-supplementation of palmitate with *Trans*-10, *cis*-12-CLA resulted in a significant increase ER stress response genes and apoptosis.

Table A.6 *In vivo* studies of the *trans*-10, *cis*-12-CLA included in the literature review.

Reference	Subjects	Dose	Duration	Results
de Deckere et al. (1999) (85)	Male F_1B hybrid hamsters	6.6 g/kg diet	8 wks	*Trans*-10, *cis*-12-CLA and the CLA mix decreased fasting values of LDL- and HDL-cholesterol, increased VLDL-TG and decreased epididymal fat pad weights. *Trans*-10, *cis*-12-CLA increased liver weight but not liver lipids. Liver histology revealed that increased weight was due to hypertrophy. Data suggested that *Trans*-10, *cis*-12-CLA stimulated the oxidation of all C_{18} PUFAs.
Clément et al. (2002) (33)	Female C57B1/6J mice	0.4% (w/w)	4 wks	*Trans*-10, *cis*-12-CLA reduced adipose tissue and triggered a massive enlargement of the liver (3.1 fold increase), which displayed the typical features of a fatty liver: pale color and accumulation of intracellular lipids. *Trans*-10, *cis*-12-CLA-induced hyperinsulinemia may trigger liver steatosis by inducing both fatty acid uptake and lipogenesis.
Degrace et al. (2003) (30)	7-wk-old male C57BL/6j mice	10 g	4 wks	This study demonstrated that the steatosis accompanying the fat loss induced by *Trans*-10, *cis*-12-CLA. The 3-fold decrease in plasma TG contents and the induction of mRNA expression of LDL receptors concomitantly observed in *Trans*-10, *cis*-12-CLA-fed mice suggested an increase in the lipoprotein clearance at the level of the liver itself.
Warren et al. (2003) (113)	8-wk-old female mice	0.5%	8 wks	Body and retroperitoneal adipose tissue weights were significantly lower and liver weights were significantly greater (100%) in the *Trans*-10, *cis*-12-CLA group. Livers from animals in the *Trans*-10, *cis*-12-CLA group contained 5 times more lipids than in the control group. *Trans*-10, *cis*-12-CLA decreased PPARα mRNA, suggesting that PPARα did not mediate the effects of *Trans*-10, *cis*-12-CLA on body composition.

Table A.6 (cont.)

Reference	Subjects	Dose	Duration	Results
Kelley et al. (2004) (25)	8-wk-old femal C57BL/6N mice	0.5%	8 wks	Livers from animals fed *Trans*-10, *cis*-12-CLA diet contained 4 times more lipids than those of the control group; this was mainly due to an increase in the TG fractions. *Trans*-10, *cis*-12-CLA caused an increase in the weight percentage of 18:1n-9, possibly due to an increased activity of SCD.
Zabala et al. (2004) (114)	9-wk-old male hamsters	5 g	6 wks	*Trans*-10, *cis*-12-CLA produced significantly greater liver weight, but also significantly decreased liver fat accumulation. No changes in mRNA levels of Srebp-1a, Srebp-1c and lipogenic enzymes, or in the activities of these enzymes, were observed.
Sanders et al. (2004) (89)	Growing female obese Zucker rats	1.5 g/kg BW	3 wks	Average daily gain was reduced 44% by the *Trans*-10, *cis*-12-CLA. *Trans*-10, *cis*-12-CLA increased liver lipid content by 33% without effects on liver weight.
Degrace et al. (2004) (31)	7-wk-old male C57BL/6J mice	1%	4 wks	Mice fed the *Trans*-10, *cis*-12-CLA-fed had enlarged livers and hypotrophied periepididymal adipose tissues. CPT1 and carnitine-dependent palmitate oxidation activities were also significantly greater in *Trans*-10, *cis*-12-CLA-fed mice than in the two other groups, indicating that FA oxidation capacities were increased in mice fed *Trans*-10, *cis*-12-CLA.
House et al. (2005) (248)	Adult male mice	1%	2 wks	*Trans*-10, *cis*-12-CLA reduced weights of adipose tissues and increased liver weight by 34%.

Table A.6 (cont.)

Reference	Subjects	Dose	Duration	Results
Viswanadha et al. (2006) (24)	4-wk-old CD-1 mice	0.15% or 0.30%	6 wks	Liver weight of male and female mice increased in response to *Trans*-10, *cis*-12-CLA in a dose-dependent manner and the increase was accompanied by an increase in total FA content. Unlike adipose tissue, overall hepatic SCD mRNA expression was not affected by *Trans*-10, *cis*-12-CLA.
Gudbrandsen et al. (2009) (36)	5-wk-old male obese Zucker rats	1%	10 d	*Trans*-10, *cis*-12-CLA reduced the liver TG content without improving the overall adiposity, enhanced hepatic mitochondrial and peroxisomal β oxidation by stimulation of PPARα. The reduced hepatic TG content may be partly due to lower activity of SCD.
Halade et al. (2009) (48)	11-m-old female C57B1/6J mice	0.5%	0.5 y	*Trans*-10, *cis*-12-CLA was found to reduce the fat mass and to increase the lean mass but significantly contributed to increase insulin resistance and liver hypertrophy.
Ashwell et al. (2010) (32)	9-wk-old male mice	1%	2 wks	The mice fed *Trans*-10, *cis*-12-CLA had 62.5% more fat in the livers with a 33% increase in liver weight.

Table A.7 Clinical studies of the *trans*-10, *cis*-12-CLA included in the literature review.

Reference	Subjects	Dose	Duration	Results
Berven et al. (2000) (117)	55 overweight or obese > 18 years	3.4 g/d of CLA mixture	12 wks	A dose of 3.4 g of CLA daily for 12 weeks is well tolerated in the population studied. No clinically significant changes in blood parameters or vital signs occurred. The use of CLA does not impair liver functions in humans.
Blankson et al. (2000) (45)	47 overweight or obese > 18 years	1.7, 3.4, 5.1 or 6.8 g/d of CLA mixture (CLA1:CLA2 = 1:1)	12 wks	CLA may reduce body fat mass (BFM) in humans and no addition effect on BFM is achieved with doses > 3.4 g CLA/d.
Noone et al. (2002) (194)	51	3 g/d of CLA mixture (CLA1:CLA2 = 1:1)	8 wks	CLA significantly reduced fasting plasma TG concentrations. No significant effect on LDL-cholesterol, HDL-lipid-protein composition or reverse cholesterol transport occurred.
Kamphuis et al. (2003) (263)	54 overweight 20-50 years	1.8 or 3.6 g/d of CLA mixture (CLA1:CLA2 = 1:1)	13 wks	The regain of fat-free mass was favorably, dose-independently affected by a 13-week consumption of 1.8 or 3.6 g CLA/day and consequently increased the resting metabolic rate. However, it did not result in improved body weight maintenance after weight loss.
Malpuech-Brugere et al. (2004) (102)	81 overweight healthy middle-aged	1.5 or 3 g/d of CLA2	18 wks	A daily consumption of a drinkable dairy product containing up to 3 g of CLA isomers for 18 weeks had no statistically significant effect on body composition in overweight, middle-aged men and women.

Table A.7 (cont.)

Reference	Subjects	Dose	Duration	Results
Whigham et al. (2004) (120)	50 overweight or obese 18-50 years	6 g/d of CLA mixture (CLA1:CLA2 ≈ 1:1)	1 y	Body composition did not differ between groups. Laboratory tests showed no adverse effects of CLA. The study concluded that CLA as Clarinol^{TM} is safe for use in obese humans for at least one year.
Gaullier et al. (2005) (264)	125 Mean age: 45-48	3.4 g/d of CLA mixture (CLA1:CLA2 ≈ 1:1)	1 y	Aspartate amino transferase but not alanine amino transferase, increased significantly. Plasma total cholesterol and LDL cholesterol were reduced, whereas HDL cholesterol and TGs were unchanged. It confirms that CLA decreases BFM in overweight humans and may help maintain initial reductions in BFM and weight in the long term.
Iwata et al. (2007) (119)	60 healthy overweight 25-60 years	3.4 or 6.8 g/d of CLA mixture (CLA1:CLA2 = 1:1)	12 wks	There was a slight increase in the level of liver enzymes in 6.8 g/d CLA group at 12 weeks. But these changes were within the normal range. The study also indicated that the use of CLA does not impair liver function in overweight male humans whose blood parameters are within the normal range.
Wanders et al. (2010) (64)	20 healthy BMI < 30 kg/m^2 18-60 years	14.6 g CLA1 + 4.7 g CLA2	3 wks	A daily intake of 19.3 g CLA for 3 weeks does not produce clinically relevant effects on markers of liver and kidney function in healthy volunteers.
Venkatramanan et al. (2010) (116)	11 moderately overweight 30-60 years	Milk enriched with 1.3 g/d of CLA mixture	24 wks	Supplementation with CLA-enriched milk did not significantly affect liver function or body weight.

Curriculum Vitae

Jing (Amy) Li

Mobile: (781)708-3697; Email: lj870129@hotmail.com

Education

University of Illinois at Urbana-Champaign 08/2009-05/2011

M.S. of Nutritional Sciences, GPA: 3.83/4.0

Thesis: Hepatic gene networks due to the trans-10, cis-12-conjugated linoleic acid in growing male mice

The University of Hong Kong 09/2006-07/2009

B.S. of Food and Nutritional Sciences

Professional and Research Experience

Research Assistant, **University of Illinois at Urbana Champaign** 08/2009-05/2011

- Leading a project investigating the effects of t10, c12-CLA intake on hepatic gene expression in growing male mice
- Working independently with minimum supervision and successfully performed Primer Design & Testing, Internal Control Genes Selection, Gene Network Analysis, RT-PCR, Gel Electrophoresis, DNA/RNA Extraction & Purification, **Excellent Pipetting Skill**
- Strong ability to carry out scientific data analyses, interpretation and visualization in the form of graphs, charts and tables
- Have presented 5 most recently published and top-tier papers in one-hour seminars

Intern, **Product Development Department, Nestlé's R&D, Beijing** 07/2010-09/2010

Developing a signature taste for Nestlé dairy products, aiming to expand product line and improve quality/cost

- Screened and Evaluated a total of 40 flavors and more than 20 stabilizers and emulsifiers for dairy products
- Performed bench top and pilot plant experiments and Designed triangle tests/informal tasting test for innovative formula, quality improvement and ingredient substitution
- Collected data and Conducted analyses on industry/market, technology, competitors and

risks; Prepared reports, graphs and charts; Presented directly to Department Manager
- Successfully Offered a full-time position as Product Development Trainee for 2011 (the only intern to receive the honor)

Intern, **USDA Human Nutrition Research Center on Aging, Boston** 06/2008-08/2008
- Quickly mastered Western blotting, Cell Culture, Western Blot, BCA Protein Concentration Assay, Laser-Capture Microdissection in one week, generating reliable data for curcumin research
- Co-authored "Regulation of LPS-induced tissue factor expression in human monocytic THP-1 cells by curcumin"*
*http://www.fasebj.org/cgi/content/meeting_abstract/23/1_MeetingAbstracts/717.2

Research Student, **Genetics Lab, The University of Hong Kong** 09/2007-07/2008
- Undergraduate Research Student which was only offered to the students with excellent academic performance
- Successfully carried out a project on Campylobacter and Presented findings in a seminar to entire faculty of science
- Wrote a 50-page dissertation: **Surveying of Campylobacter jejuni in Local Poultry Farm with Phylogenetic Analysis on gyrB Gene**
- Learned and Performed DNA Extraction, qPCR, Gel Electrophoresis, Immunoassay Identification, Gram Stain Identification

Honors and Awards

2011 Nutrition Policy and Regulatory Affairs Summit, Washington, DC, Sponsored by Kraft Foods and University of Illinois Urbana Champaign 05/2011

The First Price Scholarship in the High School Entrance Examination 09/2002

The First Price of National English Proficiency Contest for Secondary Students 12/2001

Skills

Language: English, Chinese

Computer: MS Office, Advanced Excel, Adobe Suite, SAS, SigmaPlot,

DNASTARLasergene.7.1, Molecular Evolutionary Genetics Analysis, Primer Premier, Primer Express 3, Sequence Detection System Software, geNorm, Ingenuity Pathway Analysis

Projects

Graduate Study Proposal: Effect of High Pressure Heat Treatment and Polysaccharide Addition on Beta-Lactoglobulin (Allergy Project)

Individual Nutritional Topic: Bioactive Compounds Extracted From Juice-Making Waste Inhibit Human Pancreatic Cancer Cells

HACCP Project: Enjoy Sports, Drink O' Beer

Evidence Based Practice: Does Caffeine Increase the Risk for Osteoporosis in Postmenopausal Women?