PART I

Lipid based ingredients
CHAPTER 1

Conjugated Linoleic Acid

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Abstract

As consumers are becoming more educated about the importance of improving body composition not just weight loss, that is, preserving and increasing lean muscle mass while reducing body fat at the same time, public interest for functional ingredients, such as conjugated linoleic acids (CLAs), that can deliver such benefits is also growing. Research findings indicate that CLA may be useful in improving human health in controlling body fat gain and enhancing lean body mass. Functional foods that are developed with such ingredients could encourage healthier consumer attitudes toward body image and weight loss. The chapter reviews current research on mechanism, safety and effectiveness, regulatory details, and product development issues of CLA. These details provide food developers, marketers, academic researchers, and health professionals an overview of CLA and its application in functional foods.

Introduction

The current focus of the weight management category has steered away from typical stimulants-based concepts, which have been the basis for the products of the past. New trends include insulin management, appetite control, and optimal body composition (reduce body fat reduction and increase lean muscle mass). Consumers are becoming more educated about overall body composition, not just weight loss; they are learning about the importance of preserving and increasing lean muscle mass while reducing body fat at the same time. Preventing fat regain is another area of focus
especially for yo-yo dieters. Functional foods that are developed to target fat and retain lean body mass could encourage healthier attitudes toward body image and weight loss.

A recently published meta-analysis study along with other studies put conjugated linoleic acid (CLA) to the spotlight as the candidate for a healthier weight management aid. In the meta-analysis, the authors concluded that CLA could enhance overall health by effectively reducing body fat, maintaining or increasing lean muscle mass, and potentially preventing weight and fat regain commonly experienced by adults, especially yo-yo dieters (Whigham et al., 2007). Although this effect is modest, it could be important if accumulated over time, especially in an environment where continuous weight gain is the norm in the adult population. The following paragraphs provide an overview of CLA and its application in functional foods.

**Background**

CLA is found primarily in dairy products and ruminant meat as a result of bacterial biohydrogenation of linoleic acid in the rumen. CLA is a collective term for many modified forms of linoleic acid that are resulted from double bonds occurring in different locations along the fat molecules. The cis-9,trans-11-CLA (c9,t11-CLA) and trans-10,cis-12-CLA (t10,c12-CLA) are the two most bioactive isomers (see Fig.1.1) (Pariza et al., 2001) whereas the cis-9,trans-11 isomer is also the predominant form in whole fat dairy products and ruminant meat accounting for more than 90% of CLA intake in the diet (Bhattacharya et al., 2006). The estimated human intake of CLA from the U.S. diet is approximately 200 mg/day (Ritzenthaler et al., 2001).
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In addition to the bacterial biodehydrogenation in ruminant animals, CLA can also be produced commercially from safflower oil, such as the Tonalin® CLA manufactured by Cognis GmbH (Monheim, Germany). The high-quality CLA consists of approximately equal amounts of the c9,t11 (40%) and t10,c12 isomers (40%) with less than 2% of other isomers. It is produced by chemical isomerization of safflower linoleic acid under alkaline conditions.

CLA appears to have effects on the function of body based on its two most bioactive isomers. The c9,t11- and t10,c12-CLA isomers either act alone or in concert to produce their effects. In vitro and animal studies indicated that the t10,c12 isomer is solely responsible for the reduction of body fat gain while c9,t11-CLA enhances growth and feed efficiency in young rodents (reviewed in Ritzenthaler et al., 2001). In other cases, the isomers act together to induce an effect (Ip et al., 2002). This may be due to the fact that the mixed isomers seem to involve additive biochemical pathways or multiple interactions with numerous metabolic signaling pathways, which results in the superiority of the isomer blend (Ritzenthaler et al., 2001).

Absorption and Metabolism

The general metabolic fate of CLA is considered to be similar to that of linoleic acid (Banni, 2002). Like most fatty acids, CLA is well absorbed across the gastrointestinal mucosa. It is also widely distributed throughout the body, metabolized via oxidation and desaturation and extensively excreted from the body in expired air, and lesser amounts in urine and feces (Sébédo et al., 2003). Both isomers may also be oxidized in the β-oxidation pathway.

Mechanism for Decreasing Body Fat Mass

The exact mechanism through which CLA is able to decrease body fat mass is yet not clear. However, it does appear that CLA has two main sites of action: the adipocytes that are the principal site of fat storage, and the skeletal muscle cells that are the principal site of fat burning (reviewed in Ritzenthaler et al., 2001 and Pariza et al., 2001). Studies have shown that CLA inhibits the activity of lipoprotein lipase (LPL) and stearoyl-CoA desaturase, and stimulates the breakdown of stored fat in the adipocytes (lipolysis) (Choi et al., 2000; Gavino et al., 2000; Park et al., 1997). LPL is the enzyme that transfers dietary fat after a meal into
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the adipocytes for storage. By inhibiting the LPL activities, CLA could reduce lipid uptake into adipocytes (Park et al., 1997, 1999). The \( t10,c12 \)-CLA isomer may also affect the number of newly formed adipocytes by reducing preadipocyte differentiation (Brown et al., 2003; Kang et al., 2003), or existing number of adipocytes by increasing adipocyte apoptosis or programmed cell death (Evans et al., 2000; Fischer-Posovszky et al., 2007; Hargrave et al., 2002; LaRosa et al., 2006; Tsuboyama-Kasaoka et al., 2000). Other evidence demonstrated that carnitine palmitoyltransferase (CPT) activity is increased with CLA. The increased CPT activity in skeletal muscle cells enhances the rate of fatty acid transport into the mitochondria and results in an increased \( \beta \)-oxidation (Park et al., 1997).

This may explain the enhancement of oxygen consumption and energy expenditure reported in CLA-fed OLETF rats (Nagao et al., 2003) and more recent in human studies (Close et al., 2007).

In summary, it is likely that CLA decreases body fat mass through four possible actions:

1. Decreasing the amount of fat that is stored after eating (decreases LPL).
2. Increasing the rate of fat breakdown in fat cells (lipolysis) and the rate of fat burning in the mitochondria (CPT and \( \beta \)-oxidation).
3. Reducing proliferation and differentiation of preadipocytes to mature adipocytes.
4. Decreasing the total number of fat cells (apoptosis).

The mechanisms are supported mostly by data from animal studies and in vitro studies using cultured mouse adiposities, human adipocytes, and modified markers of differentiation as well as cultured human preadipocytes (reviewed in Ritzenthaler et al., 2001). CLA may also decrease muscle protein breakdown, reduce the release of proinflammatory cytokines, and improve insulin-stimulated glucose transport (energy supply) into muscle that results in maintained or even increased muscle mass (Henriksen et al., 2003).

Body Fat Reduction by CLA

Introduction

CLA had been shown to reduce body fat and increase lean body mass in several species of animals, including chicken, mice, rats, and pigs (Ritzenthaler et al., 2001). In addition to extensive animal data, over 30
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clinical studies investigating doses from 0.7 to 6.8 g/day of the 50:50 mixture in over 1,700 human subjects for periods of 12 weeks to 2 years have been published. Many clinical studies have shown that CLA improves body composition with or without combination with exercise and in lean, overweight, and even obese subjects. For example, a long-term study demonstrated a 9% decrease in body fat in overweight subjects following 1-year supplementation with CLA without additional exercise (Gaullier et al., 2004, 2005). A recent study demonstrated its effect in overweight subjects during holiday period (Watras et al., 2006). Another recent study further demonstrated that the body fat reduction mainly occurs in waist and leg area suggesting a body-shaping benefit (Gaullier et al., 2007) and is effective for both men and women subjects.

Although a few studies used up to 6.8 g of CLA mixture/day (Kreider et al., 2002; Steck et al., 2007), the most common dosage is about 3.2 g of active CLA-mixed isomers per day either as free fatty acids or as triglycerides. While several studies showed significant reductions in body fat and/or other parameters of body composition, there are others that did not find such benefits.

To fully understand why there is inconsistency in CLA study outcomes, it is critical to take into consideration the key factors to the quality and outcome of CLA studies:

1. Quality of testing material
2. Suitable methods for measuring body composition
3. Study design includes random, double-blind design with adequate number of subjects. Other design factors include suitable dosage and study duration.

Quality of Testing Material

The quality of material is critical to clinical study outcomes. A CLA material containing high amount of less bioactive CLA isomers would certainly hamper the body fat reduction effect of the c9,t11- and r10,c12-CLA isomers (Gaullier et al., 2002). High-quality CLA-mixed isomers are usually manufactured under patented technology and process. In the case of Tonalin® CLA, manufactured by Cognis GmbH, the raw material used is food grade safflower oil rich in linoleic acid (C18:2 c9,c12) and containing very few other polyunsaturated fatty acids. The high linoleic acid then goes through isomerization to form CLA under a nitrogen atmosphere in order to limit oxidation. By carefully optimizing the reaction conditions, only the
c9,t11- and t10,c12-CLA isomers are formed in a 1:1 ratio. Conventional
deororation and/or bleaching stages in accordance with cGMP are per-
formed to further improve the quality of the final CLA product. The fin-
ished product contains >80% of c9,t11- and t10,c12-CLA isomers with
<2% of other isomers. The Tonalin CLA is produced according to FDA
GMP regulations and the production of GRAS substances.

A CLA product manufactured using nonpatented process and under
less-controlled condition would contain less c9,t11- and t10,c12-CLA isomers, but more other CLA isomers that may not be bioactive (Fig.1.2).

**Body Composition and Body Weight**

The effect of CLA is mainly on reducing body fat and improving body
composition that may not necessarily be accompanied by weight loss. An
optimal body composition is more critical to long-term health than just
weight loss. In fact, obesity is more a definition of having excessive body
fat rather than having excessive body weight. Although body weight is
an important health risk indicator, the amount of body fat, especially the
abdominal fat, is more directly related to diseases. This is because lipids
released from visceral fat could enter the portal vein and circulate to the
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Liver resulting in higher total cholesterol and LDL cholesterol. Maintaining a healthy body fat percentage, 14–20% for men and 17–24% for women, can reduce risk and help prevent the onset of these conditions.

Because body weight does not reflect percentage of body fat mass and lean mass, a muscular, lean person could have body mass index (BMI) over 30 (a criterion for being obese) even though he has high amount of muscle mass and low amount of body fat. Therefore, body composition is a much better indicator of overall health and fitness than body weight alone.

Comparing Methods for Measuring Body Fat

Body composition can be measured by several methods from simple often less accurate to more accurate but often more costly and complicated. The simple, less expensive, but less accurate methods include:

1. Calipers (anthropometry–skinfold measurements): The method is based on the assumption that the subcutaneous fat reflects a constant proportion of the total body fat.
2. Near-infrared interactance (NIR): This method is based on studies that show optical densities are linearly related to subcutaneous and total body fat.
3. Bioelectrical impedance (BIA): This method measures electrical signals as they pass through the fat, lean mass, and water in the body.

The more expensive and more accurate methods include:

1. Hydrodensitometry weighing (underwater weighing): This method measures whole-body density by determining body volume. Body fat percentage is calculated from body density using standard equations (either Siri or Brozek).
2. Dual energy X-ray absorptiometry (DEXA): A very accurate method, DEXA is based on a three-compartment model that divides the body into total body mineral, fat-free soft (lean) mass, and fat tissue mass. It measures the amount of photon energy absorbed by the bone, lean mass, and fat mass.
3. Total body electrical conductivity (TOBEC): This method is based on lean tissue being a better conductor of electricity than fat.
4. Air displacement plethysmography (Bod Pod): This method is based on the same principle as underwater weighing, but it measures how much air is displaced instead of water.
5. **Four-compartment model**: This is so far the most accurate body composition method. It incorporates measurements from DXA, underwa-ter weighing, and $^{18}$O stable isotope into Selinger’s four-compartment equation to calculate the criterion for body fat mass (Watras et al., 2006).

The methods to measure body composition could affect the CLA study outcome significantly. For example, in comparison to DXA, BIA is known (Newton et al., 2006) to underestimate body fat in obese subjects while showing a systematic overestimate of fat-free mass. In Berven et al. (2000), the Norwegian research group investigated the effect of 3-month supple-mentation with 3.4 g CLA/day in 60 overweight and obese subjects, but did not find significant differences between treatments, but only a trend (0.9 kg reduction of body fat). The body composition was measured using BIA. Two other studies using BIA methods investigated 3 g CLA supplementation in lean and overweight/obese subjects over 8 weeks and 12 months, respectively, also failed to detect any differences in body fat reduction (Noone et al., 2002; Whigham et al., 2004). Interestingly, when the same Norwegian group (Blankson et al., 2000) used DXA in a similar designed study, they were able to detect a significant body fat reduction by CLA supplementation. The superiority of the DXA method is further supported by later findings of the Norwegian group (Gaullier et al., 2004, 2005) in which after 12 months of supplementation with 3.4 g CLA/day, subjects experienced a significant reduction of body fat mass of 2.4 kg ($-9\%$) whereas subjects receiving the placebo did not show these changes.

Because methods involving DXA have been accepted as one of the most accurate methods to detect body fat reduction, the following review of CLA clinical studies focuses on studies conducted with DXA or equally accurate methods.

**Review of Clinical Studies of CLA on Body Composition**

**Long-Term Studies**

The longest study duration reported was a 24-month study in which a 12-month multicenter, double-blind, randomized, and placebo-controlled
study was followed by a 12-month open-label follow-up study. In the first study, 180 overweight (BMI 25–30) volunteers received 3.4 g CLA/day either as free fatty acid (Tonalin 80 FF, 4.5 g oil) or as triglycerides (Tonalin 80 TG, 4.5 g oil), or 4.5 g olive oil as the control group. Mean body fat mass in the CLA groups regardless of the forms was >8%, lower than that in the placebo group. This loss of body fat coincided with a slight increase of lean body mass (0.6 kg) and a reduction of body weight (−1.6 kg) whereas subjects receiving the placebo did not show these changes (Gaullier et al., 2004). In the follow-up study, 125 overweight (BMI 25–30) volunteers from both CLA group and the initial placebo group received 3.4 g CLA/day (given as Tonalin 80 TG). Subjects who lost body fat mass in year 1 did not lose further body fat, but were able to prevent fat regain. Subjects from the initial placebo group lost body fat mass significantly in the second year after they were given CLA supplement (Gaullier et al., 2005). The authors concluded that long-term supplementation with CLA reduced body fat mass (BFM) in healthy overweight adults and helped prevent fat regain.

A recent long-term study was conducted in 120 subjects who were put on energy restriction diet over a period of 8 weeks and investigated if CLA supplementation (3.4 g/day) for 1 year would prevent body weight and body fat regain. Although the differences on body fat regain did not reach statistical significance, the authors found that CLA group regained less body fat (2.1 kg with CLA vs. 2.7 kg with placebo) and more lean body mass (Larsen et al., 2006).

A recent randomized, double-blind CLA study using the four-compartment model method found that giving 40 healthy, overweight subjects 3.2 g CLA/day or placebo (safflower oil) for 6 months, the CLA supplementation significantly reduced body fat (−1.0 kg; \( p < 0.05 \)) and weight gain during a period that included the holiday season (November to January). The placebo group, however, gained weight and body fat during the same period (Watras et al., 2006). In a follow-up study, the research group discovered that fat oxidation was increased significantly during sleep (15%) compared to baseline in the CLA group, while it was decreased in the placebo group (−22%) (Close et al., 2007). Furthermore, the total energy expenditure during sleep was also increased in the CLA group. The results may explain partially the body fat reduction effect of CLA in the holiday study. The four-compartment model method is a combination of a few existing accurate methods, thus, making it the most accurate method so far to measure body composition. This method is able to detect even subtle change in body composition.
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In Exercise Subjects with BMI < 25

CLA not only has effect on body fat reduction in overweight or obese subjects, the same effect was demonstrated in lean subjects as well. An earlier study investigated the effect of CLA supplementation (1.8 g/day) upon body fat in healthy, lean humans over 12 weeks (Thom et al., 2001). In this placebo-controlled double-blind study, all 20 subjects (age 18–39 years) performed a standardized physical exercise for 90 minutes, three times per week. While the placebo group experienced no changes in body composition, the CLA-treated group reduced BFM from 22% to 17.5%. The number of subjects, however, was relatively small.

Another study evaluated whether CLA supplementation during resistance training affects body composition, strength, and/or general markers of catabolism and immunity (Kreider et al., 2002). In a double-blind and randomized study, 23 experienced, resistance-trained subjects were matched according to body mass and training volume and randomly assigned to supplement their diet with 6 g CLA/day versus placebo for 4 weeks. Although some statistical trends were observed with moderate to large effect sizes, CLA supplementation did not significantly affect (p > 0.05) changes in total body composition parameters, or general markers of catabolism and immunity during training. Unfortunately, the study duration was too short and the number of subjects was too small to observe any significant effects.

In a recent longer duration study, 76 subjects were randomized to receive 5 g CLA/day or placebo for 7 weeks while performing resistance training three times per week. The CLA group had greater increases in lean tissue mass and greater losses of fat mass by maintaining the resting metabolic rate (RMR) while RMR decreased in the placebo group. Additionally, supplementation with CLA lessened the catabolic effects of training (measured as myofibrillar protein degradation, 3-MH). This finding suggests that CLA might be most effective if combined with physical exercise (Pinkoski et al., 2006).

In Subjects with BMI > 25

Kamphuis et al. (2003aa, 2003bb) tested the hypothesis that CLA might reduce the regain of body fat and body weight in overweight adults who had undergone weight loss. All subjects received a very low calorie diet for 3 weeks followed by a 13-week intervention period during which they ate ad libitum but were given CLA (1.8 or 3.6 g) or placebo each day. The subjects consuming CLA (either dose level) significantly gained
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lean mass, but not fat mass comparing to the controls, and a significantly enhanced RMR. Interestingly, measures of appetite, feelings of fullness and satiety were also increased while feelings of hunger were decreased by CLA ingestion.

In another clinical study (Blankson et al., 2000) with overweight and obese subjects, BFM was reduced by 5.7% after 3 months of supplementation with CLA (3.4 g/day). The reduction in BFM was accompanied by a proportional increase in lean body mass and an improved overall feeling of well-being. Higher dosages of CLA were tested, but did not result in greater effects upon body composition.

In a recent study, 48 healthy obese subjects of both genders were randomized to receive placebo, 3.2 g CLA/day, or 6.4 g CLA/day for 12 weeks. Subjects were instructed to maintain their current diet and exercise routines throughout the study period. Although no significant difference on body fat was found between two groups, lean body mass was increased significantly by 0.64 kg in the 6.4 g of CLA group/day ($p < 0.05$) after 12 weeks of intervention (Steck et al., 2007).

Body Shaping and CLA

A new study recently demonstrated that CLA may have body-shaping effect by losing fat in certain areas of body. In a randomized, double-blind, placebo-controlled study (Gaullier et al., 2007), 83 overweight and obese subjects (BMI 28–32 kg/m\(^2\)) took 3.4 g of a 50:50 CLA mixture or placebo (olive oil) per day for 6 months; CLA significantly decreased BFM at month 3 ($-0.9\%$, $p < 0.05$) and at month 6 ($-3.4\%$, $p < 0.05$) compared to placebo. The reduction in fat mass was mostly in the legs ($-0.8$ kg, $p < 0.001$), and in women ($-1.3$ kg, $p \leq 0.05$) with BMI over 30 ($-1.9$ kg, $p < 0.05$), compared to placebo. Waist/hip ratio was also decreased significantly ($p < 0.05$) compared to placebo. Lean body mass was increased ($+0.5$ kg, $p < 0.05$) within the CLA group. All changes were independent of diet and/or physical exercise.

CLA Efficacy in Foods

The body fat reduction benefits of CLA began to draw mainstream food manufacturers to launch functional foods in an attempt to solve the widespread obesity problem. Since 2004, there are a few CLA-enriched functional foods that have been launched in Europe. One of the most successful examples is from a Spanish firm Asturiana. Asturiana has launched
a series of CLA-fortified dairy and fruit products in Spain since 2004 and has accomplished over 47 million Euros annual sales since then.

It is important to study the efficacy of CLA in food matrix because of any potential synergy or interaction between CLA and food components. A recent study confirmed that CLA in foods is as effective as it is in supplement forms (Laso et al., 2007). Sixty healthy subjects of both genders (BMI 25–35 kg/m²) were randomized to receive a placebo or 500 mL milk supplemented with 3 g CLA for 12 weeks. Total fat mass was decreased significantly among subjects with a BMI ≤ 30 kg/m² in the CLA-fortified milk group (~2%, p = 0.01) while no changes were detected in the placebo group. Trunk fat mass showed a trend toward reduction (~3%, p = 0.05). The authors concluded that supplementation of milk with 3 g CLA over 12 weeks results in a significant reduction of fat mass in overweight but not in obese subjects.

In a smaller study, 31 overweight subjects were given CLA-fortified milk (with or without 3 g CLA, respectively) in combination with programmed physical activity (60 minutes each time, four times a week) for a total duration of 4 months (Nazare et al., 2007). The group receiving 3 g CLA/day decreased BMI (p < 0.0001), and subcutaneous fat mass measured by anthropometry (p < 0.002) and the total fat mass measured by air displacement plethysmography (Bod Pod) (p < 0.0001) in comparison to the placebo group (the physical activity level was identical).

Another recent study using CLA-fortified yogurt, however, failed to find a significant body fat reduction effect in 44 healthy subjects even though the basal energy expenditure increased significantly in the CLA group (+5 kJ/kg fat-free mass/day on day 98 vs. day 0, p = 0.03) suggesting a potential fat burning effect of CLA (Villegas et al., 2007). The subjects were randomly assigned to consume daily either a 50:50 CLA-mixed isomer (3.76 g/day) supplemented yogurt or a placebo yogurt for 98 days.

Meta-Analysis and Summary

Despite some inconsistent results on body fat reduction, the totality of the evidence suggests a moderate body fat reduction by CLA mixture confirmed by a recent meta-analysis study (Whigham et al., 2007). The meta-analysis study collected and analyzed 18 eligible CLA studies that were longitudinal randomized, double-blind, placebo-controlled human clinical trials using validated body composition measurements. The researchers concluded that among participants given 3.2 g/day, CLA produces a significant but modest reduction of fat mass of 0.2 pounds a week or 0.8 pounds a month compared to participants in the placebo group.
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Although this effect is modest, it could be important if accumulated over time, gradual weight gain is the norm in the adult population.

In summary, when the high-quality CLA-mixed isomers were used in proper designed studies with adequate number of subjects, there was an indication of significant BFM reduction relative to placebo controls regardless of delivery vehicle. This effect was observed in both short-term and long-term studies and in various subject populations. More importantly, the fat reduction seems to occur in waist and leg areas where the accumulation of fat is the most detrimental to health. Although further evidence is needed, the potential of this benefit by CLA on long-term health is too important to be ignored. Furthermore, CLA may be most effective in reducing fat mass and increasing lean mass when combined with enhanced physical activity. CLA intake was not always associated with significant reductions in body weight, which is consistent with data from animal studies.

Safety Evaluation of CLA-Mixed Isomers

A large number of published studies—including traditional toxicology studies and extensive human trials—have assessed the safety of CLA (50:50 mixture). CLA is the subject of 32 clinical studies in which the 50:50 mixture of isomers was evaluated. A comprehensive review of the clinical data has demonstrated that consumption of 50:50 CLA isomers at levels up to 6 g/day for up to 1 year (Larsen et al., 2006; Whigham et al., 2004) and 3.4 g/day for up to 2 years (Gaullier et al., 2004, 2005, 2007) is safe and has no significant effects on cardiovascular parameters (lipid metabolism, markers of inflammation, and markers of oxidative stress), insulin sensitivity and glucose, and milk fat deposition (Cognis GmbH, 2007). Although most consumers for CLA products are expected to be adults, the safety evaluation has considered potential use among children and did not find any potential adverse effects among children at the recommended use level.

Pre-clinical data have demonstrated an absence of significant toxicological, mutagenic, or reproductive and developmental effects. Subchronic and chronic studies in rats demonstrate that CLA at 2,433 and 2,728 mg/kg body weight/day for males and females, respectively, for periods of 13 weeks to 18 months, produce no significant adverse effects. Reproductive and developmental toxicity studies in rats and pigs demonstrate a lack of adverse effects on maternal food consumption and body weight, litter size, or offspring growth and development following exposure to CLA.
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(0.25–2% in the diet, equivalent to 250–500 mg/kg body weight/day) throughout gestation, lactation, and/or during the postweaning period. In vitro assays demonstrate an absence of mutagenicity or genotoxicity (reviewed in Ritzenthaler et al., 2001, Cognis GmbH, 2007, and Campbell and Kreider, 2008).

Based on the clinical data, toxicological data, and the history of dietary consumption of CLA, an expert panel concluded in 2007 that estimated consumption of 50:50 CLA isomers is safe within the meaning of the FDA regulations for use as a food ingredient at levels of up to 1.5 g CLA per serving, with expected use of approximately 2 servings per day (Cognis GmbH, 2007). FDA agreed with the expert panel’s conclusion in July 2008 and issued no-question letter to the petitioners, one of which is Cognis GmbH (FDA, 2008); thus, Cognis’ CLA-mixed isomers achieved the so-called FDA-notified GRAS status.

Regulatory Status

Labeling

Commercial CLA can be labeled as CLA or conjugated linoleic acid. CLA is not considered as trans fat according to FDA rulings (68 Fed. Reg. 41433, 41462, 2003). Because commercial high-quality CLA oil, such as the Tonalin CLA TG80, is derived from highly refined natural safflower oil, it is also exempt from allergen labeling requirement.

CLA is fatty acid; thus, the calorie calculation for nutrition labeling is based on its fat content. In the case of Cognis’ Tonalin CLA, the TG 80 (100% fatty acids) = 9 kcal/g, 60 WDP (80% fatty acids) = 7 kcal/g, 35 WDP (67% fatty acids) = 6 kcal/g.

Claims

The claims on CLA’s effect on body composition were documented by several patents and licensed to a couple of CLA manufacturers, such as Cognis GmbH, the manufacturer of Tonalin CLA.

The following structure function claims can be used for either foods or dietary supplements:

Clinical and laboratory research indicates that Tonalin CLA

- helps reduce body fat,
- maintains lean body mass,
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- improves body composition,
- increases lean body mass,
- enhances lean body mass.

**Application of CLA in Functional Foods**

Cognis’ CLA is FDA GRAS. It is intended for use in specific foods within the following general food categories: beverages and beverage bases, grain and pasta products, milk and milk products, and processed fruits and fruit juices. Specifically, these food applications are as follows:

- Coffee creamers (only for Cognis’ Tonalin CLA)
- Chocolate (only for Cognis’ Tonalin CLA)
- Milk-based and fruit-based beverages
- Soy milk beverages
- Meal-replacement beverages and bars
- Milk and flavored milk products
- Yogurt products
- Fruit juice products

The level to be added into foods is at a level of up to 1.5 g CLA per labeled serving and up to 2 servings a day as a total recommended daily intake of 3 g CLA/day.

**CLA Forms**

There are a variety of CLA forms available from major suppliers for nearly any type of food applications. For instance, Cognis offers the Tonalin® range of CLA products in multiple concentrations and forms to meet a wide range of formulation requirements, such as 80% triglyceride or free fatty acids oil, 60% encapsulated water dispersible powder, and 35% acid-stable water dispersible powder. Additionally, a customized wet emulsion form is available for beverage applications. Table 1.1 lists the product forms available at Cognis GmbH.

When CLA oil used, it can replace existing fat or oil in food formula. If developers need to blend the CLA with other dry ingredients, a powder form is more appropriate. When CLA powder is used, food manufacturers must list in the ingredient statement any carriers contained in powders (i.e., gum arabic, maltodextrin).
### Table 1.1. The different forms of CLA product

<table>
<thead>
<tr>
<th>CLA Product Forms</th>
<th>Description</th>
<th>Food Applications</th>
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</thead>
<tbody>
<tr>
<td>Tonalin&lt;sup&gt;®&lt;/sup&gt; TG 80</td>
<td>78–84% as CLA triglyceride, transparent, colorless to slightly yellow liquid at room temperature with characteristic taste/odor</td>
<td>Oil-based food products, spreads, dairy, dressings</td>
</tr>
<tr>
<td>Tonalin&lt;sup&gt;®&lt;/sup&gt; 35 WDP</td>
<td>32–38% CLA cold water dispersible powder, fine, free flowing, cold water dispersible white powder with characteristic taste/odor, gum acacia as carrier</td>
<td>Cloudy beverages, baked goods, dairy, cereals, bars</td>
</tr>
<tr>
<td>Tonalin&lt;sup&gt;®&lt;/sup&gt; 40 WDP</td>
<td>37–42% CLA water dispersible powder, fine, free flowing, cold water dispersible white to off-white powder with characteristic taste/odor, modified food starch derived from waxy maize as carrier</td>
<td>Dry powder mixes, powdered drink mixes, formulas as chewable tablet and two piece capsules</td>
</tr>
<tr>
<td>Tonalin&lt;sup&gt;®&lt;/sup&gt; 60 WDP</td>
<td>53–62% CLA water dispersible powder, fine, free flowing, cold water dispersible, white to off-white powder with characteristic taste/odor, milk powder as carrier</td>
<td>Dairy beverages, yogurts, baked goods, cereals, bars</td>
</tr>
</tbody>
</table>

<sup>a</sup>Provided by Cognis GmbH.

### Stability

CLA, in general, is very stable and is not impacted by heat treatment and/or acid environments. The stability of CLA has been monitored by following the fatty acid profile, \(c9,r11\) and \(t10,c12\) isomer distribution, color (Hazen or Gardner), acid value, peroxide value, glyceride composition, and nonlipid components under a variety of storage conditions (e.g., plastic, steel, and glass containers). For storage of greater than 1 month, CLA should be stored in the dark in an air-free environment and not exposed to elevated temperatures (>25°C) or strong odors. Under the appropriate storage conditions, CLA triglycerides oil was completely stable in airtight steel drums over 24 months. In addition, CLA is an
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anhydrous system and will not support microbial growth. CLA is stable in various food matrices (orange juice, milk, yogurt, and nutrition bars) and following pasteurization and ultrahigh temperature (UHT) treatment. As far as interaction with other ingredients is concerned, the avoidance of pro-oxidants is recommended.

Sensory Impact

CLA is a bland tasting product with a slight characteristic flavor that can be masked by various flavoring agents. When used and handled in accordance with suppliers’ recommendations, the impact on sensory characteristics should be minimal.

References


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Gavino VC, Gavino G, Leblanc MJ, Tuchweber B. An isomeric mixture of conjugated linoleic acids but not pure cis-9, trans-11-octadecadienoic
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