Food Research International 172 (2023) 113158

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Review

Conjugated linoleic acid (CLA) as a functional food: Is it beneficial or not?

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ARTICLE INFO

Keywords:
Conjugated linoleic acid
Functional food
Health benefits
CLA adverse effects
Toxicity

ABSTRACT

Conjugated linoleic acid (CLA) has attracted great attention in recent years as a popular class of functional food that is broadly used. It refers to a group of geometric and positional isomers of linoleic acid (LA) with a conjugated double bond. The main natural sources of CLA are dairy products, beef and lamb, whereas only trace amounts occur naturally in plant lipids. CLA has been shown to improve various health issues, having effects on obesity, inflammatory, anti-carcinogenicity, atherogenicity, immunomodulation, and osteosynthesis. Also, compared to studies on humans, many animal researches reveal more positive benefits on health. CLA represents a nutritional avenue to improve lifestyle diseases and metabolic syndrome. Most of these effects are attributed to the two major CLA isomers [conjugated linoleic acid *cis-9,trans-11* isomer (c9,t11), and conjugated linoleic acid *trans-10,cis-12* isomer (t10,c12)], and their mixture (CLA mix). In contrast, adverse effects of CLA have been also reported, such as glucose homeostasis, insulin resistance, hepatic steatosis and induction of colon carcinogenesis in humans, as well as milk fat inhibition in ruminants, lowering chicken productivity, influencing egg quality and

Abbreviations: ACC, acetyl-CoA carboxylase; ACO, acetyl-CoA oxidase; AD, atopic dermatitis; ALA, alpha-linolenic acid; AMPK, AMP-activated protein kinase; BHB, beta-hydroxybutyrate; Bglap2, bone gamma-carboxyglutamate protein 2; BMD, bone mineral density; BMI, body mass index; BW, body weight; BWG, body weight gain; CAT, catalase activity; Col1α1, collagen Iα1; COCOA, Cohort for Childhood Origin of Asthma and Allergic Disease; COPD, chronic obstructive pulmonary disease; COX, cyclooxygenase; c9, t11, cis-9,trans-11; CLA, conjugated linoleic acid; CPT1, carnitine palmitoyl *trans*ferase 1; CRP, c-reactive protein; CVD, cardiovascular disease; CYP17A1, Cytochrome P450 Family 17 Subfamily A Member 1; DAG, diacylglycerol; DILI, drug-induced liver injury; DSS, dextran sodium sulfate; EFSA, European Food Safety Authority; eNOS, endothelial nitric oxide synthase; ERK, extracellular signal-related kinase; ESI-MS, electrospray ionisation mass spectrometry; FAS, fatty acid synthetase; FDA, Food and Drug Administration; FM, fat mass; FMO, flavin-containing monooxygenase; GC, follicular granulosa cells; GLUT4, glucose *trans*porter-4; GPR120, G protein-coupled receptor 120; GPx, glutathione peroxidase; GRAS, generally recognized as safe; GST, glutathione S-*trans*ferase; DL, high-density lipoprotein; HMGR, hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase; HSL, hormone-sensitive lipase; IBD, inflammatory bowel disease; IBDV, immunosuppressive infectious bursal disease virus; IL1-β, interleukin 1β; LA, linoleic acid; LBM, lean body mass; Lb, Lactobacillus; Lc, Lactococcus; LDL, low density lipoprotein; LOX, lipoxygenase; LPL, lipoprotein lipase; MAD, mothers against decapentaplegic; MCP-1, monocyte chemoattractant protein 1; MDA, malondialdehyde; MUFA, monounsaturated fatty acid; NRF-1, nuclear respiratory factor 1; PATS, pressure-assisted thermal sterilization; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1-alpha; PPARs, peroxisome proliferator-activated receptors; PTH, parathyroid hormone; PUFA, polyunsaturated fatty acids; RAS, renin angiotensin system; RANKL, receptor activator of nuclear kappa-B ligand; RBP, retinol-binding protein; ROS, reactive oxygen species; SCD1, stearoyl-CoA desaturase; SFA, saturated fatty acid; SOD, superoxide dismutase activity; SMAD8, mothers against decapentaplegic related family of molecules 8; SREBF1, sterol regulatory element-binding *trans*cription factor1; Tfam, mitochondrial *trans*cription factor A; TFF3, intestinal trefoil factor; t10,c12, *trans*-10,*cis*-12; TNFα, tumor necrosis factor alpha; UCP-1, uncoupling protein 1; UHT, ultra-high temperatura; VCAM-1, vascular cell adhesion molecule-1; VO2 max, oxygen consumption; VMS, vitelline membrane strength; WAT, white adipose tissue; WC, waist circumference; WHO, World Health Organization.

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https://doi.org/10.1016/j.foodres.2023.113158
Received 30 November 2022; Received in revised form 15 June 2023; Accepted 16 June 2023
Available online 25 June 2023

altering growth performance in fish. This review article aims to discuss the health benefits of CLA as a nutraceutical supplement and highlight the possible mechanisms of action that may contribute to its outcome. It also outlines the feasible adverse effects of CLA besides summarizing the recent peer-reviewed publications on CLA to ensure its efficacy and safety for proper application in humans.

1. Introduction

Functional foods provide great health benefits for the consumer as they improve the general condition of the body and decrease the risk of some diseases (Anadón et al., 2021; Bigliardi & Galati, 2013). Conjugated linoleic acid (CLA) is considered a food supplement that is used to reduce body mass, muscle damage and inflammatory responses; thereby, it has drawn significant scientific attention in the past few decades. It was discovered for the first time in 1932. However, its physiological function has been emphasized since the 1980s when the antimutagenic effects of CLA in mice were firstly discovered (Koba & Yanagita, 2014; Macaluso et al., 2013; McCrorie, Keaveney, Wallace, Binns, & Livingstone, 2011). CLA is a family of polyunsaturated fatty acids (PUFAs) which refers to a mixture of positional and geometric isomers of linoleic acid (LA) with conjugated double bonds that can be located in any position of the carbon chain, commonly between 8 and 13, and in a *cis* or *trans* configuration. The most common isomers are *cis*-9,*trans*-11 (c9,t11) and *trans*-10,*cis*-12 (t10,c12) which occur naturally in dairy products (milk, cheese and yogurt) and ruminant meats (beef and lamb), synthesized from LA by the action of ruminal bacteria such as *Butyrivibrio fibrisolvens* (which has been used as a model for the production of c9,t11-CLA) in the process of biohydrogenation of LA into stearic acid. However, it occurs in very small amounts, in the range of 2–5 mg/g of total milk fat, and is dependent upon the ruminant ration, breed, age and feed additives, such as polyether ionophores. C9,t11 represents 80–90% of total CLA, while t10,c12 represents approximately 3–5% (Benjamin & Spener, 2009; Churruca, Fernández-Quintela, & Portillo, 2009; Koba & Yanagita, 2014; Kumari, Meng, & Ebrahimi, 2017; Macaluso et al., 2013; Philippaerts, Goossens, Jacobs, & Sels, 2011; Yang et al., 2015) (Fig. 1).

The European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition, and Allergies has recognized CLA combination (c9, t11 and t10,c12) as a safe new food ingredient in amounts up to 3.5 g/day (EFSA, 2010a). CLA 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. The optimal method of CLA intake for the human body can vary depending on individual factors and health goals. The United States Food and Drug Administration (FDA) does regulate CLA as a dietary supplement; however, it treats it like food rather than medication (Keservani, Kesharwani, Vyas, Jain, Raghuvanshi, & Sharma, 2010). Moreover, CLA mix or 50:50 has been approved for food as GRAS (generally recognized as safe) in the USA since 2008 (Kim, Kim, Kim, & Park, 2016). Currently, a semi-synthetic lipid CLA has been obtained by alkaline isomerization from high linoleic plant oils such as safflower oil (Saebo, 2003). Most LA is converted to CLA composed of approximately 50% c9,t11 and 50% t10,c12 isomers. Therefore, most CLA studies have been performed using a mixture of c9,t11- and t10,c12-CLA as the major compounds. However, as there is accumulating evidence that c9,t11 and t10,c12 isomers have various physiological functions, both individually and together, these isomers should be treated as different food factors (Fan, Fang, Ma, & Jiang, 2015; Yamasaki & Yanagita, 2013). The weight of the evidence strongly supports that of *cis*-9,*trans*-11 and *trans*-l0,*cis*-12 CLA isomers (50:50 mixture) is safe in the pivotal human studies from 3.4 − 6 g per day (FDA, 2007).

CLA exhibits numerous health benefits such as anti-obesity, anti-

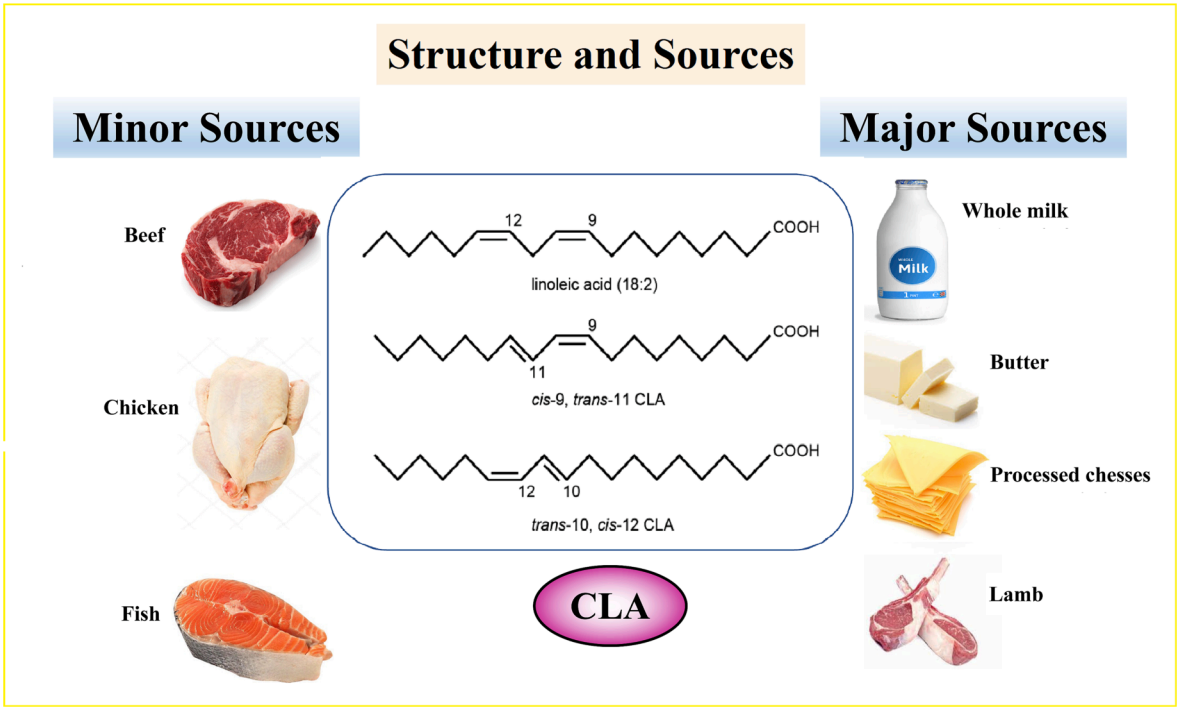

Fig. 1. CLA is an essential polyunsaturated fatty acid (PUFA) which refers to a mixture of positional and geometric isomers of linoleic acid (LA) with conjugated double bonds. The double bonds in CLA can be *cis* or *trans* configurations, giving rise to possible CLA isomers. The double bonds are usually located at positions 9 and 11 or 10 and 12. Therefore, the most common isomers are *cis*-9,*trans*-11 (c9,t11) and *trans*-10,*cis*-12 (t10,c12) which have proven to have health benefits. Meat and dairy products derived from ruminants (such as milk, butter, yogurt and cheese) are the main natural sources of CLA in the human diet.

carcinogenic, antihypertensive, anti-diabetogenic, immunomodulatory and osteosynthetic effects besides being used for the treatment of cardiovascular disease, metabolic syndrome and asthma. Most of these biological effects have been attributed to the c9,t11 and t10,c12 CLA isomers. Moreover, newer research studies indicate that both isomers have very different health effects: t10,c12-CLA is thought to be an anti-carcinogenic, anti-obesity and anti-diabetic agent, whereas c9,t11-CLA is mainly anti-inflammatory. It is also likely that some effects are induced by the synergistic action of these isomers. Thus, CLA could be favourable to improving human metabolic syndrome and represents a nutritional avenue to prevent lifestyle diseases or metabolic syndrome. However, both isomers seem to be responsible for insulin resistance, especially in humans. In addition, some other drawbacks of CLA have been reported, such as fatty liver (Benjamin & Spener, 2009; Koba & Yanagita, 2014; Kumari, Meng, & Ebrahimi, 2017; Philippaerts, Goossens, Jacobs, & Sels, 2011; Reynolds & Roche, 2010; Viladomiu, Hontecillas, & Bassaganya-Riera, 2016).

To date, several review articles have focused on CLA's beneficial effects such as anti-obesity, anti-carcinogenic, antihypertensive, anti-diabetogenic and immunomodulatory effects (den Hartigh, 2019; Kennedy, Martinez, Schmidt, Mandrup, LaPoint, & McIntosh, 2010; Kim, Kim, Good, & Park, 2016; Koba & Yanagita, 2014; Oleszczuk, Oleszczuk, Siwicki, & Skopińska-Skopińska, 2012; Viladomiu, Hontecillas, & Bassaganya-Riera, 2016; Yang et al., 2015). Although some hazardous effects of CLA have been denoted, the purpose of this review is to evaluate the health benefits of CLA as a functional food and highlight the possible biological mechanisms that contribute to its activity. In addition, the possible adverse effects of CLA such as hyperinsulinaemia, hepatic steatosis and induction of colon cancer are stated. It also summarizes the recent scientific publications on the effects of CLA, to ensure its safety and proper application before human usage.

2. CLA content in milk from different animal sources

The management system has a significant impact on the total CLA content in milk, and milk from the semi-intensive system has the highest levels. These results may be explained by the higher concentration of LA in the pasture, which are known to be the primary precursors of CLA (Chamekh et al., 2020; La Terra et al., 2013). The main factor influencing the content of CLA in milk is animal nutrition. Moreover, factors such as the period of lactation, seasonal variation, geographical area, and presence of mastitis can also affect CLA content (Mondragón, 2016). According to health claims, grass-fed beef has a 62% lower fat content than grain-fed beef, a 65% lower level of saturated fat, and higher levels of omega-3 fatty acids and CLA. Depending on the cows' diet, CLA concentration in milk typically ranges between 2 and 37 mg/g of fat (Gutiérrez, 2016), with the average CLA content being 4.3 mg/g of fatty acids in cow's milk (Kelsey, Corl, Collier, & Bauman, 2003). The total average CLA content of milk samples from milk, light milk, and fruit milk were determined to be 1.020%, 0.965%, and 0.961%, respectively (Guler, Cakmak, Zengin, Aktumsek, & Akyildiz, 2010). CLA content in food may vary widely. Representative concentration of CLA in a variety of food and dairy products is summarized in Table 1. Concentrations are highest in milk and dairy products (4–29 mg/g fat) (Chin, Liu, Storkson, Ha, & Pariza, 1992; Watkins, & Li, 2000, 2006).

The larger size of buffalo fat globules, 5 vs. 3.5 μm, is related to the higher amount of fat in buffalo milk: 73.4 ± 9.9 vs. 41.3 ± 3.7 g/kg for cow milk (Ménard, Ahmad, Rousseau, Briard-Bion, Gaucheron, & Lopez, 2010). Buffalo milk contains a significantly higher amount of CLA compared to cow milk. CLA levels in organic buffalo milk were found to be significantly greater than those in regular milk with 7.3 and 5.5 mg/g fat, respectively (Bergamo, Fedele, Iannibelli, & Marzillo, 2003).

Due to the semi-extensive nature of the system used to raise small ruminants, sheep and goat milk are typically higher in CLA than cow milk. Compared to cow milk fat, goat milk fat contains a greater 56.3% CLA (Barłowska, Szwajkowska, Litwińczuk, & Król, 2011). Goat and ewe milk contains approximately 3.25%-4.2% and 7.1% fat, respectively. According to research, sheep's milk fat contains as much as 2.2% more CLA than goat's milk. Sheep milk typically contains more CLA than goat milk when given the same dietary regimen, which can be explained by the variations in their mammary adipocytes' mRNA (Barłowska, Szwajkowska, Litwińczuk, & Król, 2011; Markiewicz-Kęszycka, Czyżak-Runowska, Lipińska, & Wójtowski, 2013; Nudda et al., 2020). The sheep milk fat content of CLA was 2.4% in May but fell to 1.3% in August and went up to 2.6% in September. The seasonal variations in the pasture are directly responsible for the content of CLA and indirectly responsible for the proportion of CLA in the milk fat (Meľuchová et al., 2008).

There is no enough information on CLA concentration in camel milk fat. According to Chamekh et al. (2020), camel milk is a rich source of CLA and second and third parties of camel milk and colostrum had the highest concentrations of CLA. Additionally, Abdelsalam, Ali, and Al-Sobayil (2017) reported that the stage of lactation had a significant influence on the CLA content of camel milk. However, it's important to note that the exact CLA content of camel milk can vary depending on other factors, such as the diet of the camels and the season in which the milk was produced.

Thus, it's important to note that these values vary depending on factors such as the animal's diet and breed, as well as the processing methods used to produce the milk. Additionally, the specific CLA isomers present in each type of milk may differ, which can affect their potential health benefits.

Table 1
Concentration of conjugated linolenic acid (CLA) in commercial and natural food products (Chin, Liu, Storkson, Ha, & Pariza,1992; Watkins & Li, 2000, 2006).

Products	CLA (mg/g fat)
Milk	
Human	1.7–36.4
Cow	0.7–10.1
Goat	6.1–10.35
Sheep	10.8–29.7
Dairy products	
Homogenized milk	5.5
Butter	4.7–8.11
Sour cream	4.14 – 7.49
Plain yogurt	4.8 – 9.01
Blue cheese	7.96
Cheddar cheese	5.86
Mozarella cheese	4.9
Cottage cheese	4.5 – 5.9
Meat	
Fresh ground beef	4.3
Beef round	2.9
Beef smoked sausage	3.8
Veal	2.7
Lamb	5.6
Pork	0.6
Poultry	
Chicken	0.9
Fresh ground turkey	2.5
Seafood	
Salmon	0.3
Lake trout	0.5
Shrimp	0.6
Vegetable oils	
Safflower	0.7
Sunflower	0.4
Canola	0.5
Corn	0.2

Note: Daily intake to get FDA recommended amount of CLA (3.4 - 6 g per day) (FDA, 2007).

3. Studies on absorption, distribution, metabolism and excretion

The metabolism of CLA has been extensively investigated and

follows known standard pathways for fatty acids consumed as triglycerides (EFSA, 2010a). LA (18:2v6; *cis*, *cis*-9,12-octadecadienoic acid) is the most highly consumed PUFA found in the human diet. Like the parent compound for the family of v6 PUFAs, LA can be elongated and desaturated to form other bioactive v6 PUFAs, such as γ-linolenic acid (18:3v6) and arachidonic acid (20:4v6). Subsequently, arachidonic acid can be converted to a myriad of bioactive compounds called eicosanoids, such as prostaglandins and leukotrienes. In tracer kinetic studies, fractional conversion of LA to arachidonic acid is believed to be between 0.3% and 0.6%, and this conversion appears to be offset by turnover. After consumption and absorption by enterocytes lining the small intestines, LA is packaged into chylomicrons as phospholipids, triacylglycerols, or cholesterol esters and enters the general circulation (subclavian vein) via the thoracic duct. LA is delivered to hepatic and extrahepatic tissues as chylomicrons that are delipidated route and cleared by the liver during their transition to much smaller remnant particles. After cellular uptake, the fate of LA is determined by the needs of the tissue, i.e., incorporation into membrane phospholipids, desaturation and elongation (Whelan & Fritsche, 2013).

4. Biosynthesize of CLA by microorganisms

The main dietary sources of CLA are dairy products, which contain CLA produced by the rumen microorganism *Butyrivibrio fibrisolvens* through the biotransformation of PUFAs derived from forage, in a process called biohydrogenation, and to the conversion of vaccenic acid. Following CLA formation in the rumen, absorption occurs via enterocytes lining the small intestines to general circulation and distribution, mainly in muscle tissues and in mammary glands. CLA also forms in small amounts in animal tissues and mammary glands from *trans*-vaccenic acid (Andrade et al., 2012) (Fig. 2).

Although natural sources of CLA have insufficient concentrations to provide therapeutic effects, various strains of food-grade microorganisms have been discovered as potential producers of CLA. These microorganisms include *Propionibacterium, Enterococcus, Clostridium, Lactobacillus* (Lb .*), Lactococcus (*Lc *)* and *Bifidobacterium. Bifidobacterium* is considered the most promising CLA producer due to its high bioconversion rate and enhanced production abilities. These microorganisms can produce CLA in commercial quantities through in situ fermentation or a biotechnological process (Mei et al., 2022; Salsinha, Pimentel, Fontes, Gomes, & Rodríguez-Alcalá, 2018; Yang et al., 2017; Zahed, Khosravi-Darani, Mortazavian, & Mohammadi, 2021). Therefore, *Bifidobacterium* strains have attracted significant attention in the production of food grade CLA as well as the rational design of health-promoting fermented foods or synbiotics.

The ability of different probiotic bacteria to optimize the production of CLA has been studied. *Lactobacillus acidophilus* LA-5 and *Bifdobacterium lactis* BB12 in cheese whey under six significant factors including initial pH, temperature, incubation time, free LA and yeast extract and types of cultivation media to co-produce CLA (Amiri, Mokarram, Khiabani, Bari, & Alizadeh, 2021; Amiri, Rezazadeh-Bari, Alizadeh-Khaledabad, Rezaei-Mokarram, & Sowti-Khiabani, 2021). *Bifidobacteria* are human probiotics in the intestinal tract with beneficial human health properties. Some strains of *Bifidobacterium* breve have been shown to produce significant amounts of CLA when cultured in lactobacilli deMan-Rogosa-Sharpe broth (Raimondi, Amaretti, Leonardi, Quartieri, Gozzoli, & Rossi, 2016).

Many researchers investigated the role of lactic acid bacteria in CLA production. Several studies found higher levels of CLA in fermented dairy products and cheese than in nonfermented milk. The acidification

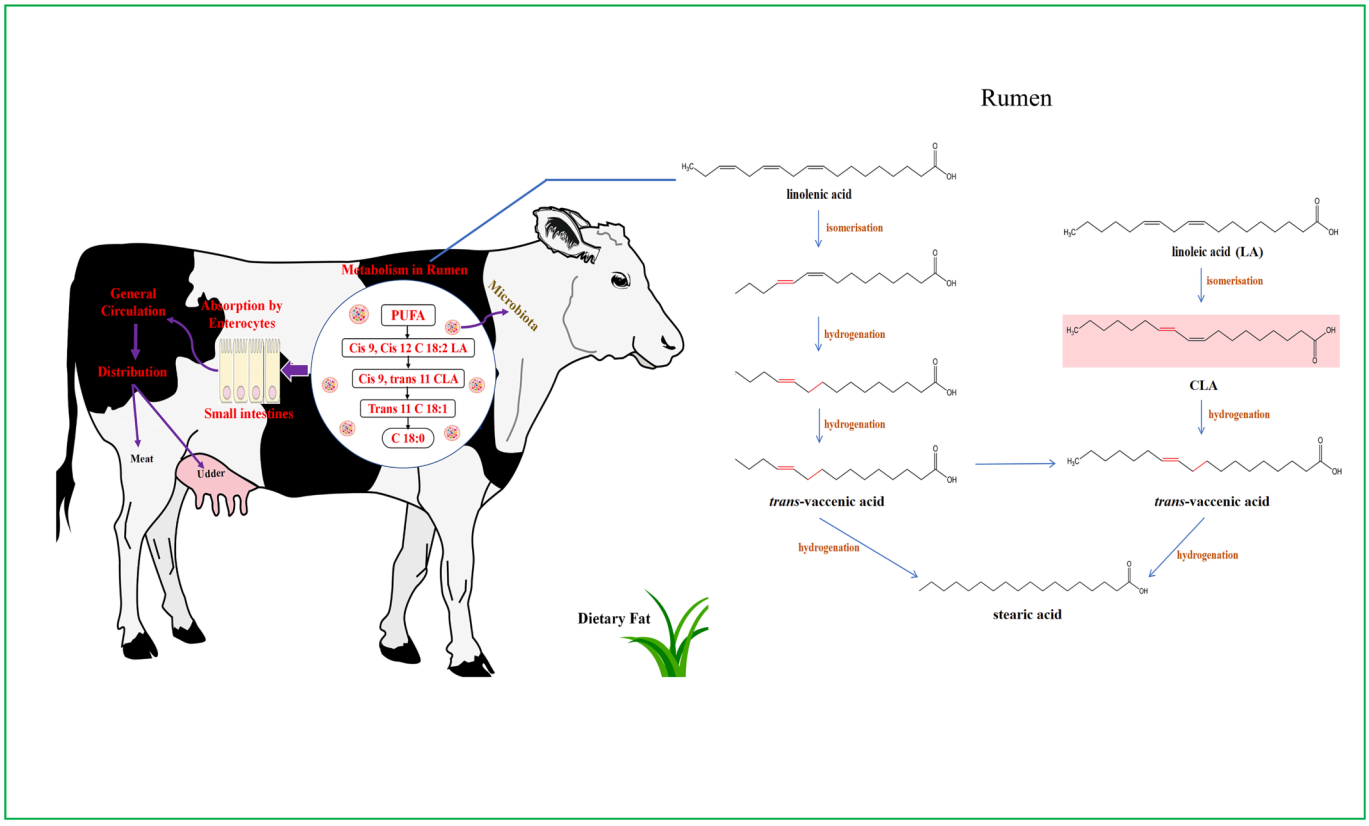

Fig. 2. CLA is mainly formed naturally in the rumen during ruminal biohydrogenation of polyunsaturated fatty acids (PUFAs). Dietary lipids, mainly linoleic acid (LA, an indirect precursor of the CLA isomers), undergoes lipolysis catalyzed by ruminal microbial lipase. Rumen bacteria play a major role in biohydrogenation. CLA is one of the intermediates of fatty acid biohydrogenation in the rumen to stearic acid. Biohydrogenation of LA begins with an isomerization reaction forming *cis*-9, *trans*-11-CLA which is the most prevalent isomer comprising 80–90% of the total CLA in food products from ruminants; then *cis*-9,*trans*-11-CLA is reduced to *trans*-11 C18:1 (*trans*-vaccenic acid) and finally to c18:0 (stearic acid).

process enhances CLA together with ALA decrease, at different levels in conventional and organic milk (Andrade et al., 2012). This result indicates that bacterial metabolism modified the relative fatty acid milk composition. The higher relative amounts of CLA in organic fermented milk and lower levels of SFA may be considered desirable from a nutritional perspective (Florence et al., 2012). The highest CLA content was observed in fermented milk containing only *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Manzo, Pizzolongo, Montefusco, Aponte, Blaiotta, & Romano, 2015).

Manipulating the animals' diet and using microorganisms with linoleate isomerase activity can increase the CLA content in milk and fermented milk products. However, several factors can affect the CLA concentration in milk, and the mechanisms governing CLA biosynthesis during milk fermentation are still unknown. The type of strain used is a significant factor, and various food-grade microorganisms such as *Bifidobacterium, Lactobacillus*, and *Propionibacterium* have demonstrated the others are still unclear (Gutiérrez, 2016).

The production of CLA by microorganisms is a promising approach for obtaining natural sources of CLA, as it is a sustainable and cost-effective method as shown in Table 2.

5. Synthetically produced CLA

Vegetable oils with high CLA content possess high nutritional value. LA is one of the most abundant fatty acids naturally found in vegetable oils. As a result, vegetable oils with a high LA concentration such as safflower, sunflower, corn, and soybean oils have a high affinity for producing CLA via carbon–carbon bond conjugation taking advantage of the alkaline-catalyzed reaction that converts linoleic acid into CLA (Quirino, 2014). Such a synthetic preparation renders a different proportion of the most common CLA isomers, yielding ~ 40%–45% 9,11 CLA, and ~ 40%–45% 10,12 CLA, with the remainder comprised of small amounts of other CLA isomers. The synthetic derivation of CLA is often termed "mixed" CLA because of the approximately 1:1 ratio of 9,11 and 10,12 CLA (den Hartigh, 2019).

Table 2
The production of conjugated linoleic acid (CLA) by different strains of bacteria.

Bacteria	Types of CLA	Product	References
Bifidobacterium spp	*cis*-9,*trans*-11-CLA and *trans*-10,*cis*-12-CLA	Fermentation of dairy products	(Mei et al., 2022; Salsinha, Pimentel, Fontes, Gomes, & Rodríguez-Alcalá, 2018; Yang et al., 2017)
Lactobacillus acidophilus LA-5	*cis*-9,*trans*-11-CLA and *trans*-10,*cis*-12-CLA	Cheese whey	(Amiri, Mokarram, Khiabani, Bari, & Alizadeh, 2021)
Bifidobacterium lactis BB12	*cis*-9,*trans*-11-CLA and *trans*-10,*cis*-12-CLA	Cheese whey	(Amiri, Rezazadeh-Bari, Alizadeh-Khaledabad, Rezaei-Mokarran & Sowti-Khiabani, 2021)
Propionibacterium freudenreichii	*cis*-9,*trans*-11-CLA and *trans*-10,*cis*-12-CLA	Yogurt	(Zahed, Khosravi-Darani, Mortazavian, & Mohammadi, 2021)
Lactic acid bacteria *Lactococcus lactis*	*cis*-9,*trans*-11-CLA and *trans*-10,*cis*-12-CLA	Fermented milk	(Kuhl & De Dea Lindner, 2016)
Streptococcus thermophilus and *Lactobacillus bulgaricus*	*cis*-9,*trans*-11-CLA	Cow milk and fermented products	(Manzo, Pizzolongo, Montefusco, Aponte, Blaiotta, & Romano, 2015)

6. Differences between synthetically and naturally produced CLA

Ruminant meat is a significant source of CLA isomers in the human diet (Troy & Kerry, 2010). The largest concentrations of CLA are often found in bovine products, while vegetable products and some seafood contain only trace amounts (Polidori, Vincenzetti, Pucciarelli, & Polzonetti, 2018). The quantity of the isomer *cis*-9,*trans*-11 varies about 75–90 % of the CLA in animal foods and less than 50 % in vegetable oils (Mondragón, 2016). The most environmentally friendly methods are based on microbial biosynthesis, but the isomerization yield is very low, the best yield obtained is 6 mg CLA per minute in a liter of reaction media. Due to this reason, microbial CLA synthesis is not competitive with the classical alkaline isomerization process (Salamon, Varga-Visi, András, Csapó Kiss, & Csapó, 2012). CLA is synthesized for mass production from a high linoleic type of sunflower oil via alkaline isomerization (Koba & Yanagita, 2014). Differences between synthetically produced CLA and naturally produced CLA are represented in Table 3.

7. Enhancement of CLA

Overall, obtaining CLA from natural food sources is generally recommended as part of a balanced diet. However, CLA levels in these foods are frequently low, so synthetic CLA production has been developed to provide a more sustainable source for fortification strategies. Lots of methods were developed for CLA synthesis. One of the primary methods for increasing CLA production is the selection of appropriate bacterial strains. The bacteria used for CLA production should be carefully selected to ensure high levels of production and minimize by-products. Several LAB strains (*Lactobacillus* and *Streptococcus), Propionibacterium*, and *Bifidobacterium* are effective in producing CLA (Kuhl & De Dea Lindner, 2016; Mei, Chen, Yang, Zhao, Zhang, & Chen, 2022).

Another important factor that reflects CLA synthesis and influences LAB growth is fermentation conditions, including temperature, pH, and time (Dahiya & Puniya, 2018). Pure *Lactobacillus* and isolated *L. reuteri* strains from camel, bovine, goat and sheep significantly raised CLA contents in eggs of 0.2–1.2 mg/g fat and 0.3–1.88 mg/g fat in broiler chicken tissue of leg, thigh and breast. These findings show that animal-derived lactic acid bacteria (*L. reuteri*) significantly increased CLA production in both eggs and broiler meat (Herzallah, 2013).

Table 3
The differences between two types of conjugated linoleic acid (synthetically and naturally produced CLA).

Property	Synthetically Produced CLA	Rumen Microflora-Produced CLA	References
Oxidative Stability	High oxidative stability.	Low oxidative stability due to its conjugated double bond.	(Katsouli & Tzia, 2019; Gammill, Proctor, & Jain., 2010)
Chemistry	1:1 ratio of c9,t11 and t10,c12	c9,t11 represents 80–90% while t10, c12 3–5% of total CLA.	(Kumari, Meng, & Ebrahimi, 2017; Koba & Yanagita, 2014)
Functionality	Often used as a dietary supplement or ingredient in functional foods.	Naturally present in dairy products and ruminant meats.	(Kuhl & De Dea Lindner, 2016; Koba & Yanagita, 2014)
Supplementary reaction step	Not need	Needed	(Salamon, Vargáné-Visi, András, Csapó Kiss, & Csapó, 2012)
Isomerization yield	Very low, about 6 mg CLA per minute in a liter of reaction media.	Much higher (approximately 80%) yields were obtained.	(Salamon, Vargáné-Visi, András, Csapó Kiss, & Csapó, 2012)

A recent study also investigates the pivotal effect of fermentation conditions on c9,t11-CLA synthesized by *Lactobacillus casei* and the variation of physicochemical characteristics, including pH, viable cell number, syneresis and texture profile of fermented soy milk (FSM) (Wang, Li, Meng, Tong, & Liu, 2022). The results concluded that optimizing these conditions can increase the yield and purity of the final CLA product.

The utilization of inexpensive, sustainable raw materials can reduce the cost of CLA production while also making it more environmentally friendly. Sunflower oil, for example, contains LA, which is turned into CLA by bacterial fermentation. When high concentrations of the substrate were added, the amount of CLA generated in sunflower oil increased (Li, Liu, Bao, Liu, & Zhang, 2012; Wang, Li, Meng, Tong, & Liu, 2022). Although CLA has numerous physiological activities and is becoming a popular food supplement, its water insolubility and oxidative instability limit its use in food systems. CLA degradation is reduced in nanoemulsions with an 8% weight lipid phase, according to research, since chemical stability is strongly dependent on surface contacts between oil droplets and the aqueous phase. Nanoemulsions have been found to be an excellent delivery system for CLA, resulting in good long-term physical and chemical stability even after 60 days of storage at 4 °C and 25 °C (Katsouli & Tzia, 2019).

In summary, to improve the synthetic synthesis of CLA for food fortification programs, its essential to optimize fermentation conditions, use sustainable raw materials, and develop novel delivery mechanisms. These strategies can make CLA fortification more practical and affordable, resulting in improved food nutrition.

8. Effect of food processing on CLA

The effect of food processing on CLA content can vary depending on the type of processing used. Some processing methods can increase or decrease the CLA content of a food product. Since milk and dairy products make up the majority of a person's daily CLA intake, numerous studies have evaluated the factors influencing the CLA content in these foods as reported in Table 4 (Gorissen, Leroy, De Vuyst, De Smet, & Raes, 2015). Levels of CLA ranged between 3.75 and 20.45 mg/g fat, corresponding to 2% milk and cooked ground beef. There was no direct relationship between CLA content, and the amount of fat provided per serving size in dairy foods (Herman-Lara, Santos-Blanco, Vivar-Vera, García, Ochoa-Martínez, & Martínez-Sánchez, 2012). Furthermore, cooked beef has a higher total CLA content compared to raw samples, mainly due to moisture loss during cooking. Cooking techniques with higher internal temperatures produce beef with the highest CLA concentrations, likely due to increased cooking losses. While CLA is susceptible to oxidation and isomerization, studies have reported minimal changes in the CLA isomeric profile of beef during cooking, with no significant variation in the relative proportions of the bioactive c9,t11 and t10,c12 isomers (Alfaia et al., 2010; Alfaia, Lopes, & Prates, 2013). CLA isomers show high stability during thermal processing (Sobral et al., 2018).

Table 4
The effect of different food processing methods on CLA content in natural products.

Food processing method	Effect on CLA content	References
High-temperature pasteurization	Increase	(Ioannidou, Maggira, & Samouris, 2022)
Freezing (of meat)	No or little effect	(Mungure et al., 2017)
Ultra-high-temperature treatment	Decrease	(Martínez-Monteagudo, Leal-Dávila, Curtis, & Saldaña, 2015; Martínez-Monteagudo, Saldaña, Torres, & Kennelly, 2012)
Fermentation (*Lactobacillus acidophilus, Streptococcus thermophilus and Lactobacillus bulgaricus*)	Increase	(Andrade et al., 2012; Manzo, Pizzolongo, Montefusco, Aponte, Blaiotta, & Romano, 2015)
Cooking	Increase	(Alfaia et al., 2010; Alfaia, Lopes, & Prates, 2013)
Refrigerating	Decrease	(Serafeimidou, Zlatanos, Kritikos, & Tourianis, 2013)

Neither the quantity nor the quality of fatty acids in milk is significantly changed by pasteurization. However, significant differences can be observed when raw milk passes through successive heat treatments of pasteurization and commercial sterilization including the main form of milk CLA (Costa et al., 2011). The effect of milk pasteurization on the progress of the physicochemical properties, fatty acids profile and lipid oxidation has been studied. CLA increased significantly from day 90 to day 120 of ripening and had an average content of 0.52% for raw cheese and 0.56% for the pasteurized one in the final stage of ripening (Ioannidou, Maggira, & Samouris, 2022).

The influence of PATS (pressure-assisted thermal sterilization) on CLA content in CLA-enriched milk has been studied. After 14 min of treatment at 100 MPa, regardless of temperature, at least 80% of the CLA was retained. Under the same PATS circumstances, CLA was not stable for 14 min at 600 MPa and 120 °C, with a retention value of about 3% (Martínez-Monteagudo, Saldaña, Torres, & Kennelly, 2012). The remaining CLA content decreased with an increase of temperature and more CLA was retained with an increase of pressure. According to Martínez-Monteagudo and Saldaña (2014), commercial sterilization utilizing high pressure sterilization (120 °C and 600 MPa with a 3 min holding duration) can retain more than 80% of CLA.

Milk naturally enriched in CLA was ultra-high temperature (UHT) treated at 125–145 °C for 2–20 s and stored at 4 and 25 °C for up to 120 d. After UHT treatment, more than 78% of CLA remained. After 15 d of storage at 25 °C, the CLA was relatively stable with a value in the range of 63–73% (Martínez-Monteagudo, Leal-Dávila, Curtis, & Saldaña, 2015).

The final quality of a CLA-enriched product is diminished by the oxidation of CLA and other unsaturated fatty acids. CLA was extremely unstable in air, and *cis*-CLA isomers are most vulnerable to oxidative degradation, while *trans*-CLA isomers are most stable in the air, indicating that the CLA must be protected from oxidation. Moreover, CLA can be oxidized faster than LA, suggesting that a conjugated double bond is more susceptible to auto-oxidation than a non-conjugated double bond (Moon, Lee, Chung, Choi, & Cho, 2008). The oxidative status of CLA remains unclear. Until now, there has been little research on CLA oxidation, and the results are sometimes contradictory. Knowing of CLA secondary products is critical for identifying the characteristic oxidation components of CLA products. However, there has been little investigation into the conversion of CLA to potentially hazardous chemicals during processing techniques.

Refrigerated storage resulted in a significant decrease of CLA in cow milk yogurts and a significant increase in sheep milk yogurts, CLA content of yogurts from sheep milk is higher than that of yogurts from cow's milk (4.7–7.6 vs.2.4–4.5 mg/g fat), indicating that the kind of milk used plays an essential role to the change of CLA during refrigerated storage (Serafeimidou, Zlatanos, Kritikos, & Tourianis, 2013).

A study investigated the effects of extended post-aging storage on dry-aged *versus* wet-aged, frozen-thawed beef. Striploins were dry or wet aged for 21 days, vacuum-packed, and stored at −20 °C for 24 months. The study analyzed tenderness, lipid oxidation, and the stability of bioactive CLA using 1 HNMR spectroscopy. The results showed that dry aging did not negatively affect CLA concentration or tenderness compared to wet aging. Therefore, dry-aged beef can be stored frozen for up to 24 months without affecting the concentration of CLA. This study provides valuable information for the meat industry on the storage of dry-aged beef and the preservation of its beneficial bioactive compounds (Mungure et al., 2017).

9. Techniques for the measurement of CLA

CLA analysis typically requires their conversion to derivatives that can be separated from other fatty acids in the sample using either gas chromatography (GC) or high-performance liquid chromatography (HPLC) (Bauman, Lock, Conboy Stephenson, Linehan, Ross, & Stanton, 2020). CLA can be measured using a variety of techniques, the choice of technique depends on the purpose of the analysis, the type of sample being analyzed, and the sensitivity required as in Table 5.

10. Beneficial effects of CLA

Recently, it has been well-documented that CLA has several health beneficial effects in animals and humans, as shown in Fig. 3.

10.1. Anti-obesity effect

There has been a marked elevation in overweight and obesity rates during the past 35 years and approximately more than one-third of the world's population is now classified as overweight or obese (Chooi, Ding, & Magkos, 2019). The World Health Organization (WHO) estimated that 38.2 million children under 5 years old were overweight or obese in 2019 (Yanes Cardozo & Romero, 2021) and by 2025, the prevalence of obesity will reach 18% and 21% in men and women, respectively (NCD, 2016). Taking into consideration the significant public health hazard due to its adverse effect on all physiological body functions, these results increase the risk for developing multiple disease conditions, such as diabetes mellitus, cardiovascular disease, musculoskeletal disorders and several types of cancers, all of which have negative effects on the quality of life, work productivity and healthcare costs (Chooi, Ding, & Magkos, 2019). To control obesity, several healthy strategies have been introduced but failed due to either lack of efficiency or high treatment cost and related adverse effects of long-term usage (Dahiya et al., 2017). Hence, there is a greater demand among consumers and nutritionists for using natural dietary supplements such as CLA for weight loss and the prevention of weight regain (Barrea et al., 2019). In this aspect, the anti-obesity effect of CLA as a nutritional supplement has been the main focus of interest. However, the exact mechanism of this effect is not clear, especially in humans (Churruca, Fernández-Quintela, & Portillo, 2009; Furlan, Marques, Marineli, & Maróstica, 2013; Macaluso et al., 2013). The majority of human intervention studies have used synthetic CLA supplements; the only data evidence that is broadly consistent is an effect on body fat and weight reduction (McCrorie, Keaveney, Wallace, Binns, & Livingstone, 2011). There are positive correlations between CLA dietary supplementation and a reduction in both body weight (BW) and fat mass (FM), as well improvement in lean body mass (LBM), body mass index (BMI) and waist measurement (Dilzer & Park, 2012; Polidori, Vincenzetti, Pucciarelli, & Polzonetti, 2018). CLA led to a lower total and lower body FM in healthy postmenopausal women (Raff et al., 2009). CLA supplementation (3.4 g/day) for 12 weeks reduces the obesity indices without obvious adverse effects in healthy overweight and obese people (Chen, Lin, Huang, Hsu, Houng, & Huang, 2012; Mądry et al., 2020). Also, a CLA mixture (3 g) for 24 weeks led to a decrease in BW and total FM in healthy, overweight individuals who maintained habitual diets and exercise patterns with a lack of adverse effects (López-Plaza et al., 2013). Thereby, CLA has been used recently in gyms/fitness centers as a supplement, to obtain a good shape quickly via reducing body FM (Della

Table 5
Different techniques used for measurement of CLA content.

Technique	Principle	Advantages	Disadvantages	References
1H Nuclear magnetic resonance (NMR) spectroscopy	Magnetic properties of certain atomic nuclei, such as protons (i.e., hydrogen atoms).	Rapid, reliable non-destructive, and requires minimal sample preparation.	Requires a specialized instrument and expertise in NMR data acquisition and analysis.	(Manzano Maria, Colnago, Aparecida Forato, & Bouchard, 2010; Prema, Pilfold, Krauchi, Church, Donkor, & Cinel, 2013; Prema et al., 2015)
Near infrared reflectance spectroscopy (NIRS)	Based on the interaction of near-infrared radiation with samples containing CLA.	Fast, cost-effective and non-destructive method, neither requiring reagents nor producing waste.	Requires a well-developed calibration model, appropriate sample preparation, and maintenance of the NIRS instrument to ensure accurate and reliable results.	(Prieto, Dungan, López-Campos, McAllister, Aalhus, & Uttaro, 2012; Prieto, López-Campos, Aalhus, Dungan, Júarez, & Uttaro, 2014)
Attenuated total reflectance − Fourier transform infrared spectroscopy (ATR − FTIR)	Determination of total CLA, trans,trans CLA, *cis*-9, *trans*-11 CLA, *trans*-9,*cis*-11 CLA, *cis*-10,*trans*-12 CLA, and trans10, *cis*-12 CLA of the oil in potato chips prepared with CLA-rich oil, without oil extraction.	Rapid method for the measurement of CLA content in a food system and directly from the product without oil extraction or chemical modification. Minimizes the time, labor, and use of chemicals and costs.	Highly specific only for the matrix of potato chips fried in CLA rich soy oils and cannot be applied to other food systems.	(Kadamne, Castrodale, & Proctor, 2011; Kadamne, Jain, Saleh, & Proctor, 2009)
Fourier-transform infrared spectroscopy (FTIR)	Measurement of the vibrational spectra of molecules in the mid-infrared range.	A non-destructive and rapid technique. Differentiate CLA from other compounds in the sample and quantify the amount of CLA present.	Requires appropriate sample preparation and calibration to ensure accurate and reliable results.	(Najbjerg et al., 2011)
Gas chromatography (GC)	Separation of fatty acid methyl esters (FAMEs) on a polar capillary column based on their boiling points, polarities, and interactions with the stationary phase. and detection by flame ionization or mass spectrometry.	High specificity and sensitivity; quantification of individual CLA isomers.	Laborious due to required sample derivatization steps, which involve significant amounts of reagents and solvents. Not sufficient for direct quantitative analysis.	(Blaško, Kubenic, Ostrovský, Pavlíková, Krupčík, & Soják, 2009)
High-performance liquid chromatography (HPLC)	Based on the separation of the 9-anthrylmethyl ester derivatives of saturated and unsaturated (conjugated and non-conjugated) fatty acids by reversed-phase high-performance liquid chromatography with UV or fluorescence detection.	A simpler method and highly sensitive one offers high accuracy and precision for CLA determination.	A time-consuming sample preparation step and specialized equipment, as well as the cost of HPLC instrumentation and maintenance, can be relatively high compared to other analytical methods.	(Nishimura, Suzuki, Momchilova, Miyashita, Katsura, & Itabashi, 2005)

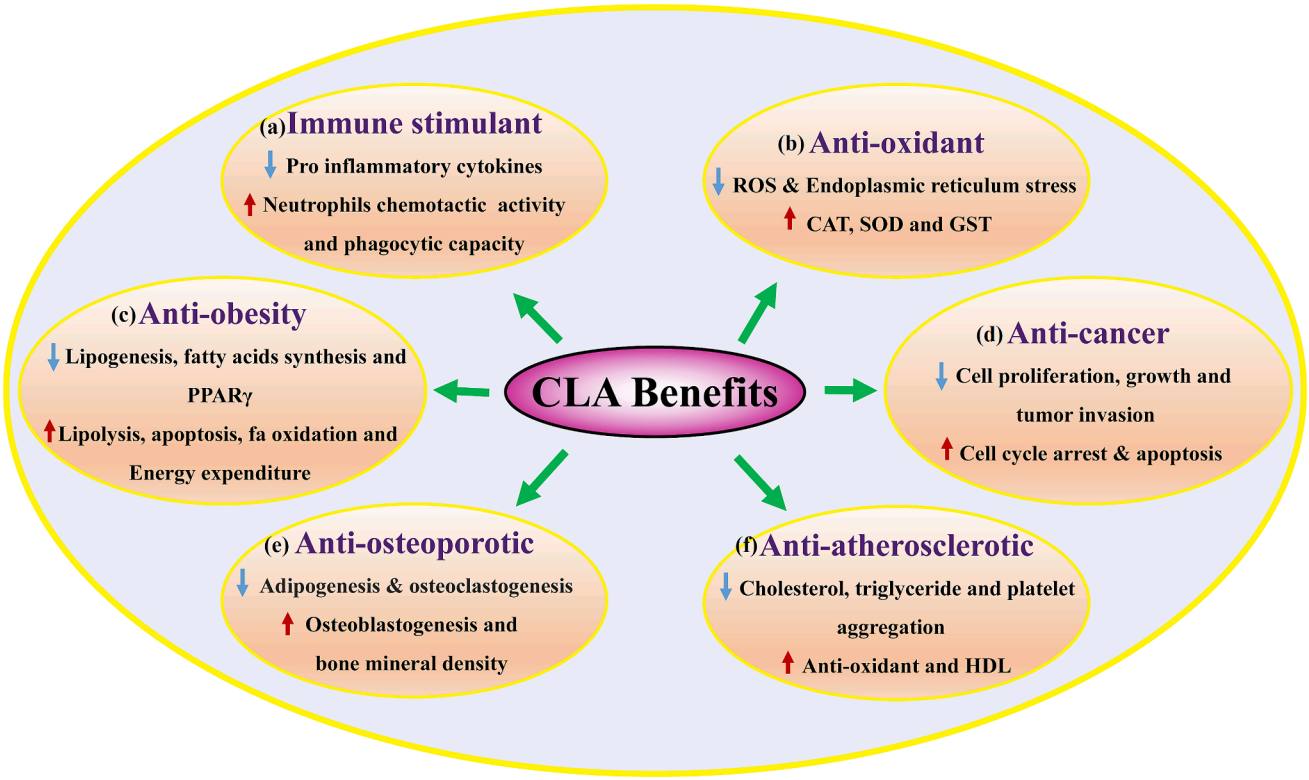

Fig. 3. CLA isomers, mainly c9,t11-CLA and t10,c12, have been shown to exert physiological properties such as (a) improved immunity by reducing the production of pro-inflammatory cytokines and increasing chemotactic activity and phagocytic capacity; (b) anti-oxidant activity, CLA acting as an efficient radical scavenger, improving the redox status and enhancing antioxidant enzymes; (c) anti-obesity activity due to a reduction in fat deposition and increase in lipolysis by enhancing fatty acid oxidation in both muscle cells and adipocytes, induction of apoptosis in adipocytes and increased oxygen consumption and energy expenditure; (d) anti-cancer activity through modulation of apoptosis and cell cycle control; (e) anti-osteoporotic effect, mainly by inhibiting excessive bone resorption and osteoclast activity; and (f) anti-atherosclerotic effect via decreasing plasma levels of atherogenic lipoproteins such as very low LDL cholesterol and increasing anti-atherogenic HDL cholesterol.

Casa, Rossi, Romanelli, Gibellini, & Iannone, 2016). CLA supplements have a beneficial effect on the whole lipid profile although the significant effect is only on low-density lipoprotein (LDL) cholesterol levels (Derakhshande-Rishehri, Mansourian, Kelishadi, & Heidari-Beni, 2015). Even so, its efficacy is still not clinically relevant and needs further investigation before being used in human diets to reduce body fat.

Several mechanisms have been used to illustrate the anti-obesity potential of CLA, including the promotion of fatty acid oxidation, increased lipolysis and energy expenditure, and modulating adipocyte metabolism, adipokines and cytokines independently of a reduction in food or energy intake (Fuke & Nornberg, 2017; Kennedy, Martinez, Schmidt, Mandrup, LaPoint, & McIntosh, 2010; Macaluso et al., 2013; Onakpoya, Posadzki, Watson, Davies, & Ernst, 2012). The gastrointestinal tract is the first site of action of the CLA isomers. That is why an alteration in gut microbial composition and function might contribute to the CLA mechanism of weight loss (den Hartigh et al., 2018; Marques et al., 2015). CLA induces the expression of genes encoding gastric proteins related to energy balance regulation and has a prebiotic effect on gut microflora, especially *Bacteroidetes/Prevotella* and *Akkermansia muciniphila*, which could improve the metabolic profile (Chaplin, Parra, Serra, & Palou, 2015). The potential mechanisms responsible for these anti-obesity properties of t10,c12-CLA include (a) decreasing energy intake by suppressing appetite; (b) increasing energy expenditure in white adipose tissue (WAT), muscle and liver tissue; (c) reducing lipogenesis or adipogenesis; (d) enhancing lipolysis or delipidation; and (e) apoptosis via adipocyte stress, inflammation and/or insulin resistance (Kennedy, Martinez, Schmidt, Mandrup, LaPoint, & McIntosh, 2010). Increased adipocyte plasma membrane glycerol fluxes may be part of the anti-adipogenic response to CLA treatments (Martins et al., 2015).

Another possible mechanism of CLA action is via the peroxisome proliferator-activated receptor (PPAR) family that contains three existing isoforms (PPAR-α, PPAR-β/δ and PPAR-γ) which control the expression of networks of genes involved in adipogenesis, lipid metabolism and maintenance of metabolic homeostasis besides inflammatory regulation (Wahli & Michalik, 2012; Wang et al., 2014; Yuan, Chen, & Li, 2015). PPAR-γ is expressed in adipose tissue, immune cells and the colon, being mainly responsible for regulating adipocyte differentiation and improving insulin resistance (Hong, Pan, Guo, Xu, & Zhai, 2019). The t10,c12-CLA isomer reduces adipogenesis and lipogenesis by regulating the expression of PPAR-γ target genes and modulating the transactivating activity of PPAR-γ by SIRT1 binding directly or indirectly to PPAR-γ in the adipocyte (Kumari, Meng, & Ebrahimi, 2017; Yuan, Chen, & Li, 2015). It also inhibits adipocyte differentiation by increasing β-catenin stability, resulting in binding to PPAR-γ and inhibiting the transcriptional activity of both PPAR-γ and β-catenin, leading to blockage of adipogenesis (Yeganeh, Taylor, Poole, Tworek, & Zahradka, 2016). CLA supplementation can increase lipolysis and reduce the accumulation of fatty acids in adipose tissue via reducing lipase lipoprotein activity, increasing carnitine palmitoyl transferase 1 (CPT1) activity, interacting with PPAR-γ, and raising the expression of uncoupling protein 1 (UCP1) (Lehnen, da Silva, Camacho, Marcadenti, & Lehnen, 2015).

In vitro, t10,c12-CLA-induced inflammatory gene expression and cytokine secretion, with suppression of lipogenic genes and markers of insulin signalling, suggests that the JNK signalling pathway and phospholipase C-dependent cell signalling play an important role in t10,c12-CLA-mediated regulation of inflammatory and lipogenic gene expression and insulin resistance in primary cultures of human adipocytes

(Martinez, Kennedy, & McIntosh, 2011; Shen, Martinez, Chuang, & McIntosh, 2013). Besides that, the suggestion of (Obsen et al., 2012) is that the anti-obesity mechanism of t10,c12-CLA in these cells is via decreasing *de novo* lipid synthesis due to the rapid repression of lipogenic transcription factors that regulate monounsaturated fatty acid (MUFA) synthesis. Moreover, in L6 myotubes, acute exposure to c9,t11- and t10,c12-CLA isomers mimic insulin action by stimulating glucose uptake and glucose transporter 4 (GLUT4) trafficking (Mohankumar, Taylor, Siemens, & Zahradka, 2012, 2013). Further, CLA influences various proteins which are particularly associated with reproduction, development, translation, metabolic processes, catabolism and proteolysis (Shen et al., 2018).

CLA's anti-obesity effect varies depending on the species, which could be due to three main reasons: (a) the type of CLA isomers used, (b) CLA dose and (c) individual variation including the age, BW, body fat, or metabolic status of the animals (Kennedy, Martinez, Schmidt, Mandrup, LaPoint, & McIntosh, 2010). Moderate doses of an equimolar CLA mix reduce body fat content, improve plasma lipid profile and maintain insulin sensitivity (despite a moderate degree of hyperinsulinaemia) without the promotion of inflammatory markers in the adipose tissue of male mice (C57BL/6J) fed a high-fat diet (Parra, Palou, & Serra, 2010; Parra, Serra, & Palou, 2010). CLA mixture leads to lower FM in hamsters without having an impact on the blood lipid profile and the liver (Joseph et al., 2010), and lower serum triglyceride (TG) beyond liver steatosis in Wistar rats (Kostogrys & Pisulewski, 2010). Moreover, CLA isomers reduce adiposity without causing steatosis or elevating serum free FA, TG, glucose, or insulin levels in mice (Shen et al., 2013). CLA has potent effects on lipid metabolism in ducks after 6 weeks via increasing liver mass, serum fatty acid synthase (FAS), acetyl-CoA carboxylase (ACC) and a lipid oxidation indicator (CPT1) and decreasing adipose tissue mass, but these effects differ depending on animal age (Fesler & Peterson, 2013). CLA supplementation reduces body weight gain (BWG), visceral adipose tissue and serum TG (Malinska, Hüttl, Oliyarnyk, Bratova, & Kazdova, 2015). The c9,t11-CLA-enriched butter significantly raises serum high-density lipoprotein (HDL) and cholesterol and prevents fasting hyperinsulinaemia in rats. Moreover, it increases serum TG levels (de Almeida et al., 2014). In comparison to CLA supplementation, foods high in CLA had a favourable effect on the entire lipid profile, however only the effect on LDL-cholesterol was statistically significant (Derakhshande-Rishehri, Mansourian, Kelishadi, & Heidari-Beni, 2015).

Other feed ingredients can improve CLA's anti-obesity effects: co-supplementation of CLA and fish oil reduces liver hypertrophy and improves insulin sensitivity, with remarkable attenuation of bone marrow adiposity, inflammation and oxidative stress in aging mice. Therefore, CLA in combination with fish oil might be a novel dietary supplement to reduce FM and improve bone mineral density (BMD) (Halade, Rahman, Williams, & Fernandes, 2011). Furthermore, the addition of calcium to CLA induces body fat loss through interference with the absorption and/or bioactivity of CLA; the mechanism underlying the anti-obesity effects of calcium supplementation is mediated mainly by changes in PUFAs. Besides that, it has a significant effect on energy metabolism, in particular, leptin and adiponectin tibia receptors which can be of relevance when using CLA against human obesity (Chaplin, Palou, & Serra, 2015; Laraichi, Parra, Zamanillo, El Amarti, Palou, & Serra, 2013).

10.2. Antioxidant effect

Redox homeostasis is the balance between oxidants and antioxidants, which ensures the proper cell response to endogenous and exogenous stimuli. However, an imbalance between reactive oxygen species (ROS) and antioxidants leads to the induction of oxidative stress, resulting in cell death and the development of many diseases (Bergamo, Cocca, Palumbo, Gogliettino, Rossi, & Palmieri, 2013; Tauler Riera, 2012; Trachootham, Lu, Ogasawara, Nilsa, & Huang, 2008). Ali, Kadir, Ahmad, Yaakub, Zakaria, and Abdullah (2012) demonstrated that both CLA isomers possess antioxidant capacity, and their free radical scavenging activity may contribute to their diverse biological activities. Moreover, CLA is reported as the most active antioxidant in milk fat, and its beneficial effect can be attributed to its synergistic interactions with other milk components (α-tocopherol, β-carotene, vitamin A and vitamin D3, phospholipids, short-chain saturated fatty acids, vaccenic acid, coenzyme Q10 and ether lipids) (Grażyna, Hanna, Adam, & Magdalena, 2017). Importantly, CLA induces differential regulation of redox status across all tissues and decreases hepatic and muscle endoplasmic reticulum stress. It also modulates mechanistic links between the actin cytoskeleton, insulin signalling pathway, glucose transport and inflammation in adipose tissue (Rungapamestry et al., 2012). The t10,c12-CLA isomer strongly improves the redox status of bovine mammary epithelial cells due to GSH synthesis without lipoperoxidation, with a reduction in intracellular ROS and thiobarbituric acid reactive substance levels (Basiricò et al., 2015), and shows a better antioxidant cellular response against oxidative damage induced by H_2O_2 compared with other fatty acids (Basiricò, Morera, Dipasquale, Tröscher, & Bernabucci, 2017). Furthermore, Qi et al. (2018) stated that t10,c12-CLA has a stronger antioxidant capacity than c9,t11-CLA in primary cultured laying hen hepatocytes via increasing the activity and mRNA expression of antioxidant enzymes by facilitating nuclear translocation of nuclear factor e2-related factor-2 (Nrf2). On the other hand, the pro-antioxidant activity of CLA in human endothelial cells is dose-dependent: a low dose 10 μmol/L seems to have pro-oxidant activity without inducing cytotoxicity while a dose of 100 μmol/L is cytotoxic (Nakamura, Dubick, & Omaye, 2012). CLA and oleic acid supplementation in mice increases body metabolism, inducing the activity of UCP2 (a protein-coding gene) and preserving the redox state in the liver. Thus, this co-administration may be a potential strategy for controlling obesity and oxidative stress (Baraldi, Dalalio, Teodoro, Prado, Curti, & Alberici, 2014). The addition of 10% CLA to commercial frying oil provides stability against oxidation for 5 days of frying, indicating that CLA can act as an antioxidant and a pro-oxidant at increased concentrations (Alavijeh, Goli, & Kadivar, 2015). c9,t11-CLA reduced oxidative stress and autophagy by enhancing milk fat synthesis through the Nrf2 signaling pathway in bovine mammary epithelial cells suggesting using c9,t11-CLA as a natural therapeutic strategy for mastitis alternative to antibiotics (Ma et al., 2022). With the increasing demand for natural antioxidants, CLA can be added to a food system to prevent oxidation.

10.3. Anti-inflammatory effect

The t10,c12-CLA isomer may attenuate lipopolysaccharide-induced production of tumor necrosis factor in bovine immune cells (Perdomo, Santos, & Badinga, 2011). CLA-enriched butter supplementation increases the serum levels of anti-inflammatory interleukin 10 (IL-10) but reduces levels of tumor necrosis factor alpha (TNF-α), IL-2, IL-8, matrix metalloproteinase-2 (MMP-2) and matrix metallopeptidase-9 (MMP-9), resulting in a reduction of systemic inflammatory mediators in healthy young adults with sub-clinical inflammation in overweight individuals (Penedo, Nunes, Gama, Leite, Quirico-Santos, & Torres, 2013). CLA supplementation for 2 weeks significantly decreases inflammatory factors such as MMP-2 and TNF-α following exhaustive exercise in young healthy males (Baghi, Mazani, Nemati, Amani, Alamolhoda, & Mogadam, 2016). CLA isomers inhibit monocyte migration and reduce the inflammatory output of macrophages in the human THP-1 monocyte cell line (McClelland et al., 2010). CLA can alter the differentiation of monocytes to macrophages which play a key role in the subsequent activation and differentiation of T and B cell subsets. Both c9,t11- and t10,c12-CLA isomers can reduce the production of pro-inflammatory cytokines such as TNF-α and modulate the environment that favours the differentiation of lymphocytes towards a more regulatory phenotype during the antigen presentation phase (Viladomiu, Hontecillas, & Bassaganya-Riera, 2016). CLA seems to ameliorate the inflammatory profile in adipose tissue, causing a reduction in the expression of monocyte chemoattractant protein 1 (MCP-1), the main macrophage

recruitment factor, and a decrease in the expression of the pro-inflammatory mediators iNOS and IL-6 in adipose tissue of male mice (C57BL/6J) fed a high-fat diet (Parra, Palou, & Serra, 2010). Also, t10, c12-CLA upregulation of inflammatory signalling pathways increases the secretion of cytokines and the activity of nuclear factor κB (NF-κB), AP-1 and MAPK (Yuan, Chen, & Li, 2015) and a significantly lower level of ROS production *in vitro* reduces the gene expression of pro-and anti-inflammatory cytokines by upregulating PPAR-γ mRNA expression (Dipasquale, Basiricò, Morera, Primi, Tröscher, & Bernabucci, 2018). A *meta*-analysis study revealed that CLA supplementation is associated with an increase in plasma c-reactive protein (CRP) concentrations and a reduction in serum adiponectin concentrations, which indicates CLA's pro-inflammatory effects (Mazidi, Karimi, Rezaie, & Ferns, 2017). Moreover, t10,c12-CLA may affect neuroinflammation by reducing the pro-inflammatory molecules in human cultured astrocytes, suggesting a potential role of CLA isomers in modulating the astrocyte inflammatory response (Saba et al., 2019). In a mouse model, dietary consumption of c9, t11-CLA will be useful in preventing Alzheimer's disease progression by lowering amyloid β-protein accumulation and increasing anti-inflammatory cytokines (Fujita et al., 2021). CLA prevents inflammation through controlling arachidonic acid transformations and promoting high levels of antioxidant activity which reduce the risk of atherosclerosis, cancer and neurological disorders (Grażyna, Hanna, Adam, & Magdalena, 2017). CLA has an anti-neuroinflammatory effect that is mediated by PPAR activation (Murru et al., 2021). However, only a few researches on the potential beneficial effects of CLA in brain function and neuroinflammation via PPAR activation are available.

PPAR's anti-inflammatory effect is mainly based on transrepression mechanisms in which protein–protein interactions are crucial and often include T cell, B cell, macrophage and dendritic cell functions (Wahli & Michalik, 2012). CLA is known as a potent agonist of PPAR-γ. Therefore, it can be employed as a complementary treatment for inflammatory bowel disease (IBD) (Yuan, Chen, & Li, 2015). Moreover, it improves colitis and suppresses inflammation-driven colonic carcinogenesis in mice, and significantly decreases the percentages of macrophages and increases regulatory T cell numbers in mesenteric lymph nodes (Bassaganya-Riera & Hontecillas, 2010; Evans, Misyak, Schmelz, Guri, Hontecillas, & Bassaganya-Riera, 2010). Furthermore, probiotic bacteria that favour the local production of CLA in the colon target myeloid cell PPAR-γ and suppress colitis in mice (Bassaganya-Riera et al., 2012b). CLA supplementation reduces mucosal damage and inflammatory infiltrate in dextran sodium sulphate (DSS)-induced colitis through PPAR-γ activation and the induction of the intestinal trefoil factor (TFF3) (Borniquel, Jädert, & Lundberg, 2012; Moreira et al., 2019). However, this effect appears to be dose-dependent in that CLA at doses of 40 and 20 mg/day regulates inflammatory cytokines (TNF-α, IL-10 and IL-6) which may be due to the activation of PPAR-γ and the inhibition of NF-κB. Besides the modulation of oxidative stress-related enzymes, CLA significantly protects against a reduction of goblet cells and destruction of the mucosal layer, maintaining the mucosal barrier and rebalancing the gut microbiota damaged by DSS. Thus, CLA not only indirectly improves the intestinal barrier and regulates inflammatory factors through the regulation of bacterial flora and oxidative stress but also can directly regulate mucin and TJ protein as well as inflammatory factors, which could directly improve related indices of colitis (Chen et al., 2019; Ren et al., 2020). In addition, CLA can directly reduce airway inflammation and hyper-reactivity in asthma via a PPAR-γ-dependent mechanism (MacRedmond & Dorscheid, 2011). Also, it significantly reduces serum levels of IL-1β and MMP-9, inhibiting the production of oxidative stress markers, regulating the appetite and improving nutritional status, which may be helpful for chronic obstructive pulmonary disease (COPD) patients (Ghobadi, Matin, Nemati, & Naghizadeh-Baghi, 2016; Matin, Nemati, Ghobadi, Alipanah-Moghadam, & Rezagholizadeh, 2018). The modulation of oxidative stress and the inflammatory state may be attributed to CLA's ability to activate specific receptors such as PPAR-γ and AMPK (Trinchese et al., 2020). Accordingly, the anti-inflammatory effects of CLA are partly mediated through its ability to activate PPAR-γ (Fig. 4).

10.4. Immunomodulatory effect

Numerous peer-reviewed publications have proven that CLA-induced immunoregulatory activity in both human and animal models is via regulation of lipid metabolism, cell survival and signal activation, besides the suppression of inflammatory cytokine production by peripheral blood T cells (Bassaganya-Riera et al., 2012a; Chen et al., 2019; Mohammadi, Mahdavi, Rabiee, Nasr Esfahani, & Ghaedi, 2020; Oleszczuk, Oleszczuk, Siwicki, & Skopińska-Skopińska, 2012). The t10, c12-CLA isomer exerts its immunostimulant effect through elevating phagocytic capacity, which is induced by TNF-α via a PPAR-γ-dependent pathway (Kang, Lee, Jeung, & Yang, 2007; Kim et al., 2011; Mohammadi, Mahdavi, Rabiee, Nasr Esfahani, & Ghaedi, 2020). Furthermore, it directly enhances chemotactic activity, potentially by increasing the F-actin polymerization of IL-8-polarized neutrophils (Paek, Kang, Kim, Son, Park, & Yang, 2010). Dietary CLA enhances immune function in chickens infected with the infectious bursal disease virus (IBDV) via suppressing the relative expression of IBDV-specific proinflammatory cytokine mRNA (Long, Guo, Wang, Liu, Zhang, & Yang, 2011) and alleviates the immunosuppression of T lymphocytes in broiler chickens exposed to cyclosporin A by enhancing peripheral blood T lymphocyte proliferation and IL-2 which is mainly attributable to increasing signalling molecules such as phospholipase C and protein kinase C (Long et al., 2012). CLA enhances SIgA expression in the jejunum (middle intestine) and increases the lymphocyte transformation rate and the percentage of CD8 + T lymphocytes in Peyer's node of broiler chickens; this proves that CLA might be used to improve the intestinal mucosal immunity of broiler chickens (Liu, Yang, Tang, & Jiang, 2017). Dietary CLA in piglets increases the number and cytotoxicity of peripheral blood CD8 + T lymphocytes, which may be through changing the fatty acid composition, conformation and signal transduction of peripheral blood lymphocytes (Liu, Liu, Qiu, & Jiang, 2016). Moreover, CLA effectively ameliorates cyclosporin A immunosuppression in piglets via inhibiting the decrease of CD4 + and CD8 + T lymphocytes in the thymus and the decrease of peripheral blood and IL-2 production after cyclosporin A treatment (Liu, Zhu, Liu, & Jiang, 2016). CLA supplementation triggers glycogen storage, providing more glucose, and preferentially uses β-hydroxybutyrate (BHB) as an energy source during the immune response (Gross, Grossen-Rösti, Héritier, Tröscher, & Bruckmaier, 2018). Transcriptome analysis of CLA has shown that 100 μM CLA has an immunoprotective effect on sheep ruminal epithelial cells via inhibiting the pro-inflammatory genes TNF-α, IL-6, CX3CL1, IRF1, ICAM1 and EDN1, and enhancing the cell proliferation-related genes FGF7, FGF21, EREG, AREG and HBEGF, besides regulating the lipid metabolism genes PLIN2, CPT1A, ANGPTL4, ABHD5 and SREBF1 (Yang, Lan, Ye, Zhu, & Fu, 2020). Despite there being no doubt about the impacts of CLA on the innate and adaptive response, the exact mechanism of action on immunity is still unclear.

10.5. Anti-carcinogenic effect

The WHO reported that cancer was the first or second leading cause of death globally and there were 18.1 million new cancer cases and 9.6 million cancer deaths in 2018 (Bray, Ferlay, Soerjomataram, Siegel, Torre, & Jemal, 2018). The persistent generation of ROS/reactive nitrogen species or alterations of the redox state appears to be correlated with tumor promotion (Chaiswing & Oberley, 2010). Thus, the reduction of free radicals and oxidants with antioxidants will antagonize tumor promotion activity (Frei, 1994). The majority of investigations on CLA's anticarcinogenic activity used a mixture of CLA isomers synthesized from vegetable oil, often including two or four main isomers; the 2-isomer mix was virtually always used (Bauman, Lock, Conboy Stephenson, Linehan, Ross, & Stanton, 2020). A higher concentration of

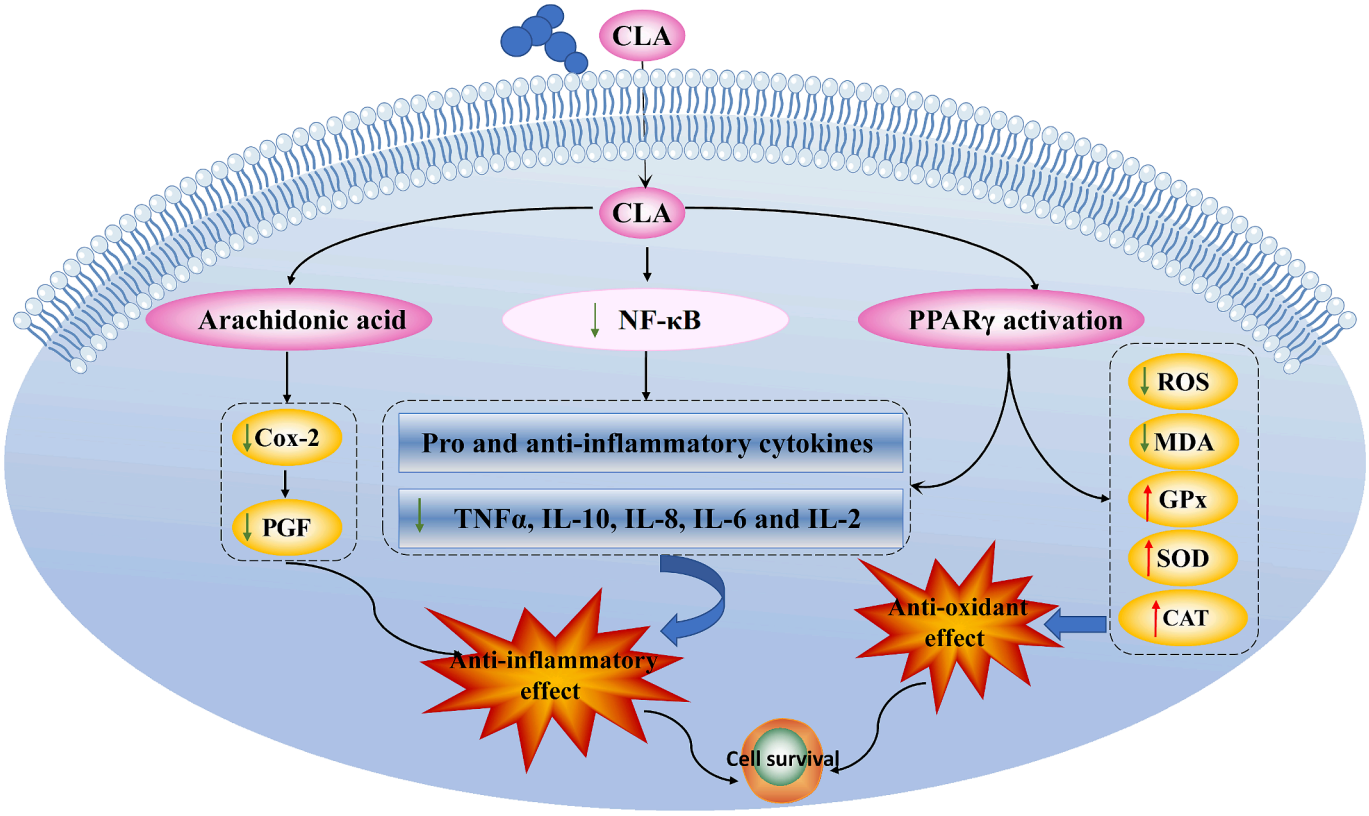

Fig. 4. Possible pathway of CLA antioxidant and anti-inflammatory effect. Dietary CLA reduces prostaglandin E_2 (PGE2) and $F_{2\alpha}$ (PGF2α) derived from arachidonic acid metabolism through inhibition of cyclooxygenase 2 (COX-2). CLA is able to activate PPAR-γ, leading to reduced generation of ROS and lipid peroxidation (malondialdehyde, MDA) and elevated activity of the antioxidant enzymes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx). Inhibition of NF-κB reduces the production of pro-inflammatory cytokines. Altogether, this leads to CLA exerting anti-inflammatory and antioxidant activity and, finally, cell survival.

CLA in the serum is associated with a lower risk of mammary carcinogenesis in rats (Białek, Tokarz, & Zagrodzki, 2014, 2015; Polidori, Vincenzetti, Pucciarelli, & Polzonetti, 2018). However, the exact mechanism of the anti-carcinogenic action of CLA has not been fully explained. It could be related to antioxidant and anti-inflammatory properties, and a reduction of cell proliferation (Fuke & Nornberg, 2017; Oraldi, Maggiora, Paiuzzi, Canuto, & Muzio, 2013). It may also be related to the induction of the mitochondrial apoptotic pathway (Koronowicz, Drozdowska, Banks, Piasna-Słupecka, Domagała, & Leszczyńska, 2018). CLA promotes mammary tumorigenesis by affecting the mammary stromal environment, leading to tumor progression and cellular expansion in a mouse model of invasive breast cancer (Flowers et al., 2010) and the interactions with PUFAs in the lipoxygenase (LOX) and cyclooxygenase (COX) pathways could also explain the anti-carcinogenic activity of CLA (Białek, Jelińska, & Tokarz, 2015). Moreover, dietary c9,t11-CLA reduces the incidence of breast cancer by up to 50%, affecting cell proliferation (decreasing the weight and volume of the tumor) and the level of hormonal receptors (significantly lowering the expression of progesterone receptor and Ki-67 (a cell proliferation marker) in female Sprague Dawley rats (Zeng et al., 2020). CLA isomers can affect the viability, growth and fatty acid metabolism of the human colon cell line HT-29 by converting t11,t13-CLA into its isomer c9,t11-CLA which can contribute to the potent antineoplastic properties of CLA (Degen, Ecker, Piegholdt, Liebisch, Schmitz, & Jahreis, 2011). Furthermore, it decreases the levels of angiogenesis and tumor invasion biomarkers such as TNF-α, IL-1β, hs-CRP, MMP-2 and MMP-9. Therefore, it is effective in reducing tumor invasion and resistance to cancer treatment in rectal cancer patients (Mohammadzadeh, Faramarzi, Mahdavi, Nasirimotlagh, & Asghari Jafarabadi, 2013; Oleszczuk, Oleszczuk, Siwicki, & Skopińska-Skopińska, 2012). An *in vitro* study on the ovarian cancer cell line TOV-21G and *in vivo* data (in mice) demonstrate that t10,c12-CLA diminishment of tumor growth may be via cell cycle arrest in the S phase and this effect is dose and time-dependent (Thuillier et al., 2013). Thus, the supplementation time of CLA is crucial and CLA is most effective as an anti-carcinogen when administered during early tumorigenesis and less effective in models of established tumors (den Hartigh, 2019) (Fig. 5).

The activation of the mitochondrial apoptotic pathway, supported by the JNK signaling pathway could be a mechanism of CLA-enriched egg activity in MCF-7 breast cancer cells. Thus, CLA-enriched eggs could be a readily available functional food product with a potential chemopreventative activity (Koronowicz, Drozdowska, Banks, Piasna-Słupecka, Domagała, & Leszczyńska, 2018). CLA-enriched food products including dairy products, meat and eggs limit cancer cell survival and proliferation more efficiently than synthesized CLA isomers. However, it should be noted that CLA-enriched dietary products also contain other fatty acids and nutrients, which should be considered while researching the overall health impacts (Koronowicz & Banks, 2018). Finally, the anti-proliferative and anti-cancer effects of CLA recommend its use in the future as a treatment in cancer therapy either alone or in combination with other chemotherapeutic agents. There is an urgent demand for more clinical studies to confirm its anti-carcinogenic efficacy.

10.6. Anti-atherosclerotic effect

The WHO has estimated that cardiovascular disease (CVD) caused 17.9 million deaths around the world in 2019 (WHO, 2021). Atherosclerosis is a common cause of CVD and is characterized by slow-progressing inflammation in conductance and resistance arteries, in which particles containing LDL cholesterol accumulate below the

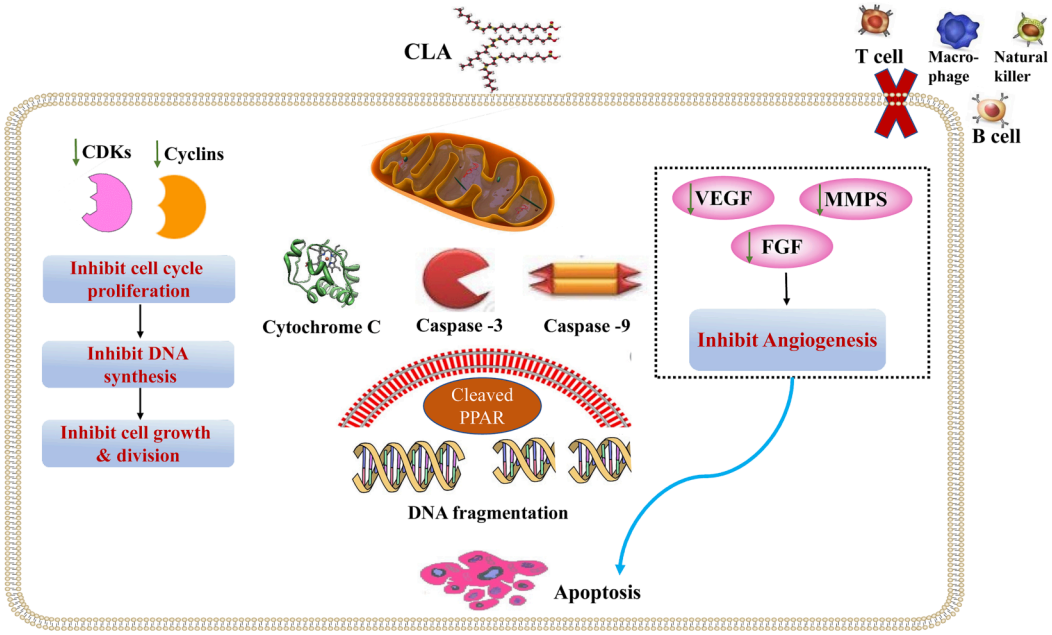

Fig. 5. CLA promising anticarcinogenic properties. Dietary CLA prevents the development and progression of cancer by inhibiting cell cycle growth, division and proliferation and inducing apoptosis by activating the caspase cascade pathway. CLA suppress angiogenesis by inhibiting the expression of pro-angiogenic factors such as Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factor (FGF), and matrix metalloproteinases (MMPs) which are crucial for the proliferation and migration of endothelial cells that form blood vessels and can prevent the formation of new blood vessels in tumors. CLA can also modulate the immune system by enhancing the production of cytokines and chemokines involved in the immune response against cancer cells.

endothelial layer. Currently, despite increasing incidence and significant expenses borne by the healthcare systems in both western and developing countries, there is no curative therapy for atherosclerosis (Albany, Trevelin, Giganti, Lombardi, & Scottà, 2019). CLA ameliorated acrolein-inducing cardiotoxicity in rats by reducing oxidative stress and mitochondrial dysfunction (Aydın, Şekeroğlu, & Şekeroğlu, 2018a, 2018b). CLA improves organ damage associated with metabolic syndrome in spontaneously hypertensive rats (Soto-Rodríguez, Pulido-Camarillo, Hernández-Diaz, Alexander-Aguilera, & Garcia, 2011). CLA feeding was associated with significant reductions in total cholesterol, LDL-cholesterol, and plasma triacylglycerol concentrations (Polidori, Vincenzetti, Pucciarelli, & Polzonetti, 2018). The significant reduction in inflammation and improvement in plasma lipid profiles in people with risk for CVD consuming naturally enriched goat cheese with CLA could play a function as a high-nutritional food to enhance the condition of health and could replace other foods with high nutritional value in a diet (Santurino et al., 2020). CLA-enriched ghee (clarified butter) is reported to have antioxidant and anti-atherogenic activity in female Wistar rats by increasing the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione S-transferase (GST) besides decreasing cholesterol, TG and HDL, leading to a reduction in the atherogenic index, suggesting that high-CLA ghee can be used as potential food for decreasing the risk of CVD (Chinnadurai, Kanwal, Tyagi, Stanton, & Ross, 2013). CLA-rich eggs may potentially be used in a diet to prevent or slow the progression of CVD by combating obesity. Consumption of CLA in diets can be enhanced by providing access to eggs supplemented with one or more beneficial fatty acids, or by incorporating these eggs into other foods such as mayonnaise, pasta, salad dressings, or baked goods. However, the success of enriched eggs and egg products will be determined by economic feasibility, acceptable sensory features, and stability during cooking, storage, and processing, all of which must be thoroughly researched (Shinn, 2016).

The beneficial effects of the t10,c12-CLA isomer on blood pressure and adipocyte size in *fa/fa* Zucker rats may be due to its ability to reduce the number of large adipocytes, which alters the levels of vasoactive molecules secreted from adipose tissue (DeClercq, Taylor, & Zahradka, 2012). The t10,c12-CLA isomer attenuates the development of obesity-related hypertension in *fa/fa* Zucker rats, by stimulating adiponectin production, which subsequently activates vascular endothelial nitric oxide synthase (eNOS) (DeClercq, Taylor, Wigle, Wright, Tworek, & Zahradka, 2012).

Dietary supplementation with t10,c12-CLA inhibits atherosclerosis in ApoE−/− and LDLr−/− mice, with changes in plasma and liver markers (Mitchell, Karakach, Currie, & McLeod, 2012). The potential anti-atherosclerotic effect of c9,t11-CLA is through reduced adhesion of macrophages to human vein endothelial cells in cultured peripheral blood mononuclear cells that occur via the reduction of vascular cell adhesion molecule 1 (VCAM-1) and intracellular cell adhesion molecule 1 (ICAM-1) expressed on the endothelium surface (Stachowska, Siennicka, Baśkiewcz-Hałasa, Bober, Machalinski, & Chlubek, 2012); and its effect on the platelet proteome in humans has revealed that c9,t11-CLA regulates the proteins CDC42Hs, α-actinin-1 and integrin α-IIb precursor which assess it is effects on the platelet and the mechanisms involved in atherogenesis (Bachmair et al., 2012). CLA isomers do not unequivocally present as protective against atherosclerosis, but they have potentially beneficial effects through inhibition of monocyte migration, inflammatory mediator expression and foam cell formation which are critical cellular targets of CLA (Mooney, McCarthy, & Belton, 2012). The t10, c12-CLA supplementation in male LDL receptor-deficient (Ldlr−/−) mice increases macrophage content in the surrounding perivascular adipose tissue, suggesting that alterations to the aortic microenvironment may contribute to the anti-atherosclerotic effect of CLA (Kanter et al., 2018). Co-administration of CLA and rosiglitazone (an anti-hyperglycaemic drug used to treat type 2 diabetes mellitus) increases the atherogenic co-efficient, coupled with vascular resistance in the context of endothelial loss, and alters isoprenaline-induced vasodilatation in rats (Chai et al., 2018). CLA supplementation modifies the FAs accumulated in cardiac tissue through the significant suppression of PUFA oxidation, as evidenced by the lower content of MDA, besides significantly inhibiting the oxidation of cholesterol which provides possible functional and/or structural modifications in cardiac tissue (Białek, Białek, & Czauderna, 2019) (Fig. 6). Overall, these mechanisms

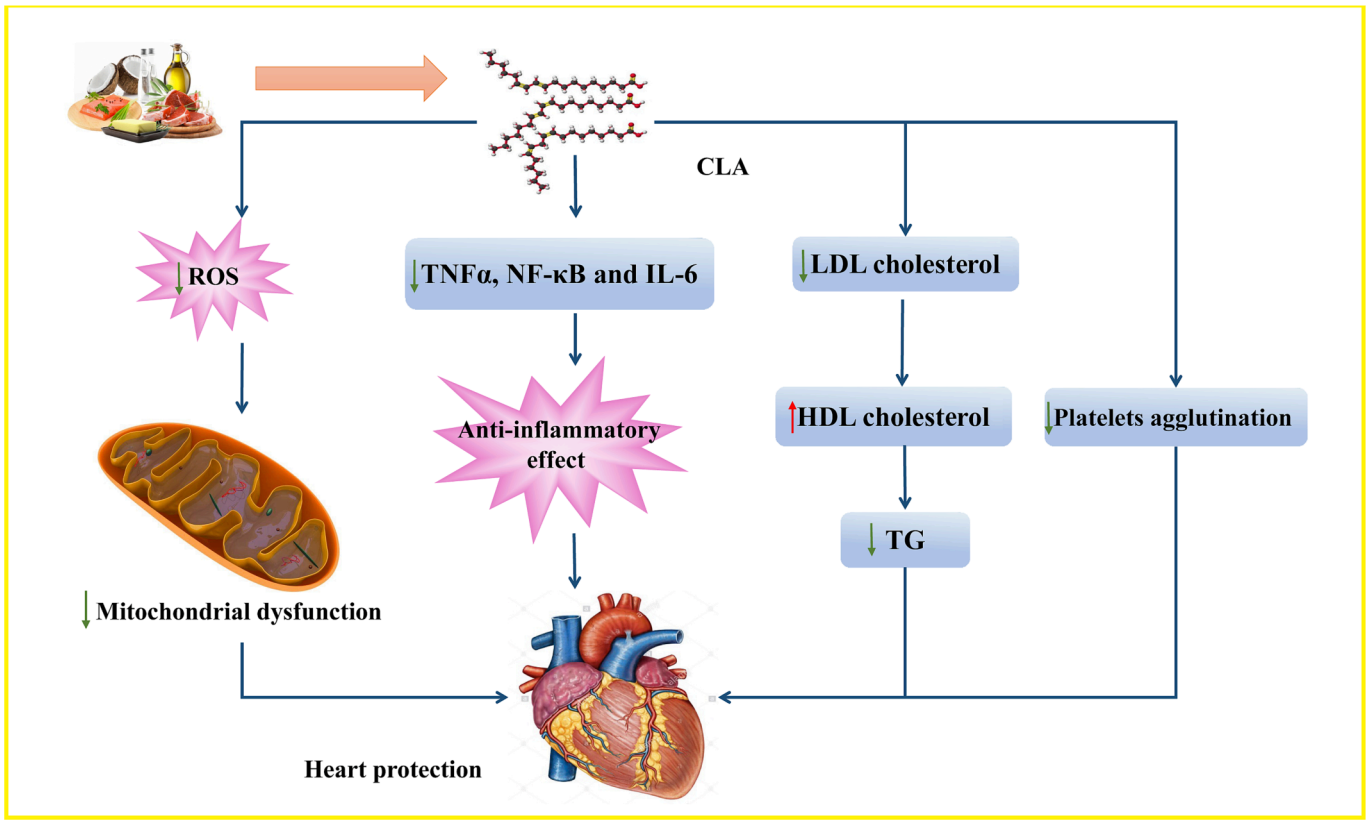

Fig. 6. CLA has a cardioprotective activity that can help prevent cardiovascular diseases such as atherosclerosis and coronary heart disease. CLA can scavenge free radicals and prevent lipid peroxidation, leading to a decrease in oxidative stress and inflammation that contribute to atherosclerosis. CLA can improve lipid metabolism by decreasing the levels of triglycerides and LDL cholesterol and increasing the levels of HDL cholesterol, which is considered "good" cholesterol. By improving lipid metabolism, CLA can decrease the risk of developing cardiovascular diseases. Moreover, CLA may help reduce platelet aggregation and lower the risk of heart attacks and strokes.

could contribute to the potent anti-atherosclerotic effects of CLA.

10.7. Beneficial effect on bone, joint and skeletal muscle health

CLA is well known to enhance bone mineralization. The t10,c12-CLA, but not c9,t11-CLA, has an anti-osteoporotic effect by modulating osteoclastogenesis, significantly inhibiting adipogenesis and promoting osteoblastogenesis from mesenchymal stem cells via PPAR-γ-mediated mechanisms and mothers against decapentaplegic (MAD)-related family transcription factors of molecules 8 (SMAD8)-mediated mechanism (Kim, Park, Lee, & Park, 2013; Rahman, Halade, Williams, & Fernandes, 2011; Reynolds, Segovia, Zhang, Gray, & Vickers, 2015). Prolonged dietary intake of c9,t11-CLA for around 4 months in healthy men positively correlates to BMD but does not affect parathyroid hormone (PTH) (DeGuire, Makarem, Vanstone, Morin, Duque, & Weiler, 2012). CLA administration significantly prevents alveolar bone loss via increasing osteoblastic activity and counts besides decreasing osteoclastic activity along with inflammatory cell infiltration in diabetic Wistar rats (Balci Yuce, Akbulut, Ocakli, Kayir, & Elmastas, 2017). CLA also prevents postmenopausal bone loss in C57BL/6 female mice not only by inhibiting excessive bone resorption due to oestrogen deficiency but also by stimulating new bone formation (Rahman, Fernandes, & Williams, 2014). It also minimizes some of the androgen deficiency that has adverse effects on BMD and preserves bone strength in male retired guinea pigs (DeGuire, Mak, Lavery, Agellon, Wykes, & Weiler, 2015). Moreover, co-administration of calcium with CLA maximizes its beneficial effects on bone health via maintaining bone weight and promoting the expression of bone formation genes such as bone gamma-carboxyglutamate protein 2 (Bglap2) and collagen Iα1 (Col1α1) with a significant effect on key players in energy metabolism, leptin and adiponectin tibia receptors which support their beneficial effects on bone metabolism (Chaplin, Palou, & Serra, 2015; Park, Kim, Scrimgeour, Condlin, Kim, & Park, 2013; Park, Pariza, & Park, 2008; Park, Terk, & Park, 2011). Further, dietary CLA has a potentially beneficial effect on bone markers in patients with rheumatoid arthritis. Herein, it may be useful in the prevention and reduction of osteoporosis in rheumatoid arthritis patients (Aryaeian, Shahram, & Djalali, 2016). Additionally, vitamin E co-supplementation for 3 months increases the anti-inflammatory effects of CLA on active rheumatoid arthritis (Aryaeian, Djalali, Shahram, Djazayery, & Eshragian, 2014). CLA may protect against bone loss in postmenopause and during aging so it might be a potential alternative therapy against osteoporotic bone loss.

The transcriptomic analysis of skeletal muscle genes revealed a nine-fold increase in stearoyl-coenzyme A desaturase 1 (SCD1) by CLA. This high induction is associated with a protective role in the reduced expression of endoplasmic reticulum stress markers in CLA-treated male C57BL/6J mice (Parra, Serra, & Palou, 2012). CLA increases LBM and enhances exercise performance by increasing fat utilization and reducing the consumption of stored liver glycogen (Kim et al., 2010). CLA improves voluntary activity and endurance capacity, accompanied with modulation of CPT1, UCP2 and PPAR-δ in skeletal muscle (Kim, Kim, & Park, 2012; Park & Park, 2012). CLA supplementation significantly reduces BW and FM independent of exercise through activation of AMPK, mitochondrial biogenic markers, PPAR-γ coactivator 1α (PGC-1α), nuclear respiratory factor 1 (NRF-1) and mitochondrial transcription factor A (Tfam) signalling molecules to stimulate mitochondrial biogenesis, resulting in increased voluntary activity and muscle mass, potentially contributing to the regulation of weight gain. Moreover, it significantly induces overall genes associated with muscle fibers, such as type I (slow twitch) and type II (fast twitch). That is why it could be

useful to improve muscle metabolism, which would have a significant health impact (Kim, Kim, & Park, 2016; Kim, Kim, Whang, & Park, 2016; Kim & Park, 2015). Collectively, these findings indicate that CLA improves endurance capacity independent of mild-intensity exercise via a PPAR-δ-mediated mechanism.

10.8. Beneficial effect on fertility

The t10,c12-CLA supplementation during maturation *in vitro* improves the competence of bovine oocytes to develop into higher quality embryos (Dalbies-Tran et al., 2020; González-Serrano et al., 2016; Lapa et al., 2011) and is also capable of modifying the distribution and morphology of cytoplasmic lipid droplets during maturation of porcine oocytes, thus reducing their lipid content in a time-dependent manner (Prates et al., 2013). Besides that, it enhances nuclear and cytoplasmic maturation, which subsequently enhances *in vitro* embryo development in porcine oocytes during maturation (Jia et al., 2014). CLA ameliorates the undesirable effect of heat stress on maturation and embryo development due to it improving the antioxidative potential of the oocytes which is associated with lower levels of ROS and a higher content of intracellular glutathione. However, CLA cannot change the ratio of BAX to BCL_2 expression levels and thus fails to prevent blastocyst apoptosis (Abazarikia, Zhandi, Shakeri, Towhidi, & Yousefi, 2020). Supplementation of lactating sows with 2% CLA can decrease losses of piglets from birth to weaning and shorten the weaning-to-estrous interval due to better estrous onset but does not affect the growth performance of piglets from birth to weaning. Therefore, CLA can be used as an effective nutritional supplement for improving pig reproduction (Hadaš, Čechová, & Nevrkla, 2015).

CLA supplementation induces an increase in testosterone levels in Leydig cells suggesting that CLA supplementation may promote testosterone synthesis through a molecular pathway; in young healthy physically active men (Macaluso et al., 2012). In an *in vitro* study, Leydig tumor rat cells (R2C) supplemented with different concentrations of CLA (0 to 7.5 μM) exhibited increasing testosterone biosynthesis due to rising levels of cytochrome P450 family 17 subfamily A member 1 (CYP17A1) mRNA and protein. *In vivo*, 32 male mice (BALB/cAnNHsd) showed an increase in free plasma testosterone and upregulation of CYP17A1 mRNA and protein, demonstrating that CLA stimulates testosterone biosynthesis via CYP17A1 in the Leydig cells of the testis (Barone et al., 2013). Moreover, CLA has a protective effect on the lipid parameters induced by orchidectomy. In Rojas, Villalpando, Ferrer, Alexander-Aguilera, and García (2020) study using rats, similar protective effects were observed for biomarkers of cardiac and renal damage also prevented the reduction of total protein and total bilirubin concentrations that were modified by the loss of gonadal function in the orchidectomized group. When CLA was fed with dietary oils it can mitigate some of the negative reproductive and metabolic characteristics (Safari Hasanabad et al., 2022).

11. Adverse effects of CLA

Despite the numerous beneficial effects of CLA such as anti-obesity, anti-inflammatory, antioxidant, immunomodulatory, anti-carcinogenic and anti-atherosclerotic effects, it has raised some health concerns. Most of these risks are attributable to the t10,c12-CLA isomer, while the c9,t11-CLA may be without hazard to human consumption, which must be confirmed as well (Yang et al., 2015). Furthermore, consuming too much CLA through supplements can have negative health consequences, so it's best to get it naturally. Some adverse effects have been reported, as follows (Fig. 7).

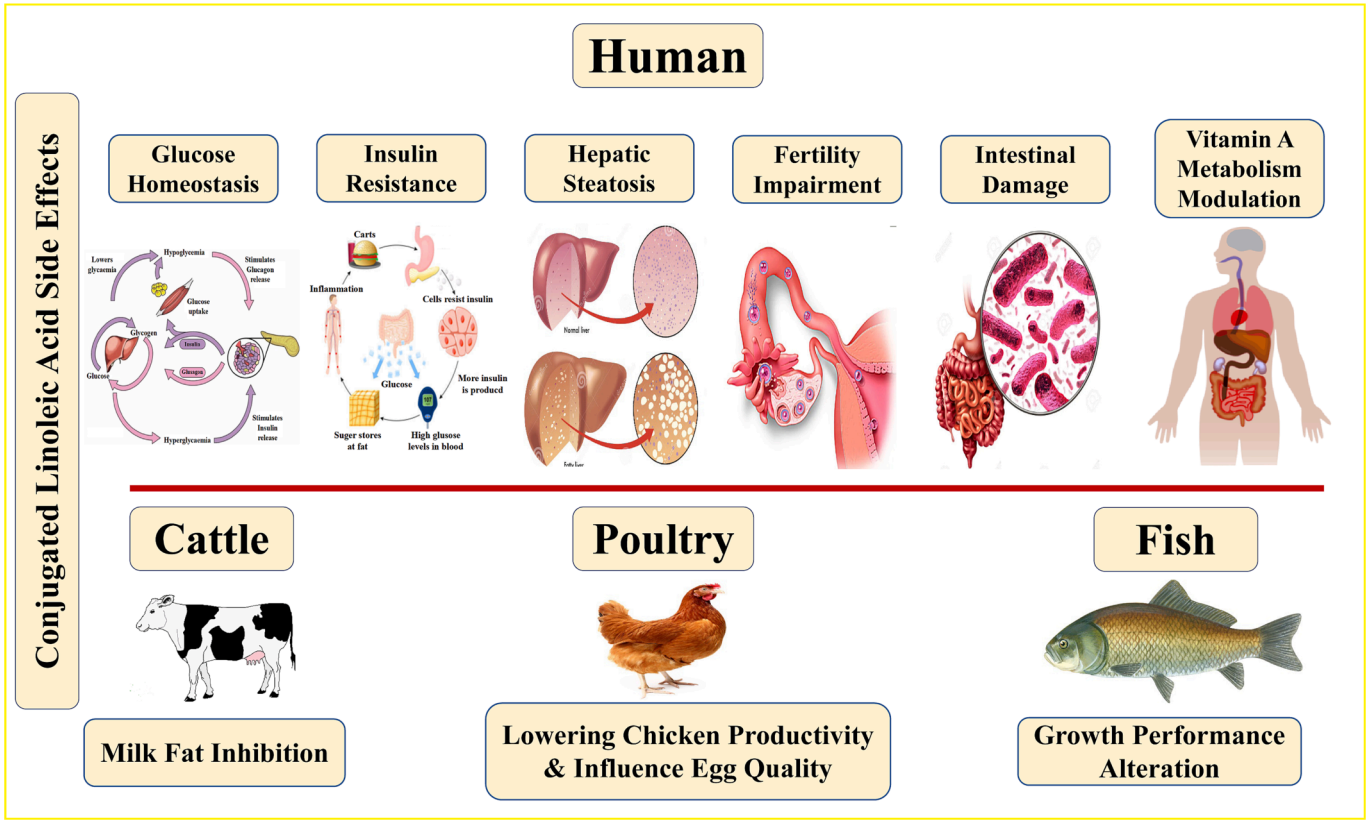

Fig. 7. CLA supplements in humans may worsen insulin resistance or affect how the body absorbs sugar, mainly in people with diabetes or metabolic syndrome. It may cause dangerous effects on the liver and leads to hepatic steatosis. Besides that, it may impact the intestines, fertility and vitamin A metabolism. CLA's adverse effects in animals mainly involve the lowering of milk fat production and chicken productivity, influencing egg quality and altering fish performance.

11.1. Glucose homeostasis

The use of CLA as a weight loss supplement in animals and humans poses a particularly complex safety concern. Studies show that CLA does not seem to lower a person's weight or BMI. There is not enough evidence that CLA intake will help in weight loss. As well as that, it has less effect on obesity-associated syndromes, such as atherosclerosis and type 2 diabetes, especially in humans. According to EFSA (2010b), there is no compelling evidence that any of the CLA isomers contribute to the prevention or promotion of diet-related diseases. Thus, special attention should be paid to its negative adverse health effects such as insulin resistance and increased LDL: HDL cholesterol ratios(den Hartigh, 2019; Moon, 2014). CLA supplementation does not affect fasting blood glucose or waist circumference (WC) in healthy humans (Rahbar, Ostovar, Derakhshandeh-Rishehri, Janani, & Rahbar, 2017) or healthy obese men and women (Ormsbee et al., 2014). CLA does not affect blood lipids nor any biomarkers of metabolic control in overweight type 2 diabetic patients (Shadman, Taleban, Saadat, & Hedayati, 2013) or obese, postmenopausal women with type 2 diabetes (Asp et al., 2011). The same effects have been observed in obese children (6–10 years old) who exhibit decreased body fat without improving plasma lipids or glucose (Racine et al., 2010). Furthermore, high amounts of the c9,t11-CLA isomer in adipose tissue may play a role in the development of diabetes as it has the opposite relation with fasting plasma TG and blood glucose concentrations (Castro-Webb, Ruiz-Narváez, & Campos, 2012). Studies on the effect of naturally enriched or synthetically CLA in overweight, hyperlipidaemic humans, revealed that CLA does not change the BW, fat composition, or blood lipids and does not affect the β-oxidation rate of fatty acids or induce significant alterations in the safety markers (high sensitive-CRP, TNF-α and IL-6) (Joseph, Jacques, Plourde, Mitchell, McLeod, & Jones, 2011; Venkatramanan, Joseph, Chouinard, Jacques, Farnworth, & Jones, 2010). Also, CLA supplementation (2.4 g/day) for 8 weeks does not have a significant effect on lipid peroxidation and antioxidant metabolism in healthy overweight/obese Korean individuals (Kim, Paik, Shin, & Park, 2012). In addition, a diet naturally enriched with over a three-fold increase in CLA for 8 weeks does not cause differences in insulin sensitivity, body composition, circulating blood lipids or other measured disease risk factors in young women (20 to 39 years old) (Brown, Trenkle, & Beitz, 2011). On the other hand, Pfeuffer et al. (2011) found that CLA mixture at 4.5 g/day for 28 days in overweight men decreases BW without changing LDL or HDL cholesterol, TG, or insulin sensitivity indices. The same findings were reported by Bulut, Bodur, Colak, and Turnagol (2013) who found that CLA supplementation and exercise can change body composition and insulin sensitivity positively but not all blood components of the lipid profile. The naturally enriched cheese with CLA possesses beneficial properties because it improves the plasma lipid profile, and more noticeably reduces endocannabinoid biosynthesis. Synthetic CLA consumption led to plasma levels of CLA and its metabolites similar to those found in enriched cheese, but neither the endocannabinoid levels nor the plasma lipid profile significantly changed (Pintus et al., 2013). Based on a systematic review and *meta*-analysis, CLA supplementation can slightly significantly reduce BW, BMI and FM with increasing LBM in overweight and obese subjects (Namazi, Irandoost, Larijani, & Azadbakht, 2019). Nevertheless, its effects on WC are not statistically significant.

Furthermore, the output data for CLA supplementation in humans and research on an experimental animal model elucidate that a CLA-enriched diet enhances lipotoxicity in peripheral organs by increasing the expression of lipogenic genes, resulting in inefficient fatty acid storage which leads to profound metabolic dysfunction in ApoE−/− mice (Reynolds et al., 2013). The t10,c12-CLA isomer seems not to be useful for increasing body fat reduction associated with restricted feeding in hamsters as it elevates liver CPT1 and acetyl-CoA oxidase (ACO) activity and does not reduce hepatic TG content or decrease adipose tissue size (Lasa et al., 2011). Another research article reported that the weight loss due to t10,c12-CLA in mice exerts profound effects on adiposity by redistributing fat stores away from healthy subcutaneous regions, suggesting that weight loss achieved by t10,c12-CLA is not metabolically 'healthy' (den Hartigh et al., 2017). CLA mixture supplementation in rats shows an elevation in plasma 8-isoprostane levels and CAT activity, these results suggesting that CLA could act as a pro-oxidant agent and increase lipid oxidation (da Silva Marineli, Marques, Furlan, & Maróstica Jr, 2012). The t10,c12-CLA promotes dysregulation of lipid and glucose metabolism, at least in part, by an isomer-specific modulation of hepatic expression of NR4A receptors in ApoE-deficient mice (Navarro et al., 2010). In both obese and lean mice, t10,c12-CLA reduces the whole-body FM by decreasing all fat depots, with blood glucose elevation rise (Yeganeh, Zahradka, & Taylor, 2017). A recent systematic review and *meta*-analysis study indicate that CLA supplementation has no significant effect on blood levels of triglycerides, total cholesterols, LDL, apolipoprotein A, and apolipoprotein B. On the other hand, CLA supplementation decreases serum concentrations of HDL (Asbaghi et al., 2022). Together, these research studies indicate that the effects of specific CLA isomers on blood glucose, BW and lipid profile levels in human and animal studies are controversial and still need further investigation.

11.2. Insulin resistance

Insulin resistance is a primary defect leading to the development of type 2 diabetes (Henriksen, 2010). Dietary t10,c12-CLA mediates insulin resistance and increases prostaglandin $F_{2\alpha}$ (PGF2α) *in vitro* in primary human adipocytes via activation of NF-κB, an extracellular signal-related kinase (ERK), and elevation of Ca^{2+} levels (Kennedy et al., 2009, 2010). It has also been found to reduce the FM and increase the lean mass, but significantly contributes to increased insulin resistance and liver steatosis in 12-month-old C57Bl/6J mice, whereas the c9,t11 isomer prevents insulin resistance (Halade, Rahman, & Fernandes, 2010). Both CLA isomers could have a significant role in the development of insulin resistance in hepatic C9 cells through IRS-1 serine phosphorylation, protein kinase C epsilon (PKCε) activation and hepatic lipid accumulation (Roura-Guiberna et al., 2019). In addition, studies have shown that dietary t10,c12 CLA may alter hepatic glucose and lipid metabolism indirectly, in response to the loss of adipose tissue (Cordoba-Chacon et al., 2019). Feeding rats, a high-fat diet with equal parts of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA has no effect on body composition with potential negative effects on insulin sensitivity and induce insulin resistance caution should be taken before synthetic CLA supplements (de Almeida et al., 2015). Moreover, rosiglitazone improves insulin resistance that is adversely affected by t10,c12-CLA supplementation alone in a male mouse model of metabolic syndrome which maintains significant weight and fat loss (Wang, Goodspeed, Turk, Houston, & den-Hartigh, 2017).

11.3. Hepatic toxicity

Nowadays, there is such an increase in the use of herbal and dietary supplements worldwide that it is unfortunately considered the second most common cause of drug-induced liver injury (DILI) in the USA and about 20% of patients have a DILI with serious outcomes, either liver transplantation or death, as reported by the Drug-Induced Liver Injury Network (DILIN) (Navarro et al., 2014; Navarro, Khan, Björnsson, Seeff, Serrano, & Hoofnagle, 2017). The first case report of CLA-induced hepatotoxicity (asthenia, jaundice and pruritus) was recorded in a 46-year-old female patient who self-medicated with CLA for 2 weeks for weight loss (Ramos, Mascarenhas, Duarte, Vicente, & Casteleiro, 2009). Meanwhile, the first case in the USA and the third case in the world was reported in an obese 26-year-old woman who suffered from acute hepatitis and elevation of serum bilirubin as observed in the other two cases. The conclusion is that the severity of hepatitis is correlated with serum bilirubin rather than aminotransferases (ATs) but further investigations

are needed to prove this (Bilal, Patel, Burkitt, & Babich, 2015). Go et al. (2013) investigated the mechanism that underlies the t10,c12-CLA hepatoxicity in human hepatoma HepG2 cells, that is through stimulating the hepatic *de novo* lipogenesis and TG synthesis with consequent lipid accumulation via activation of the mTOR/SREBP1 pathway. The c9,t11-CLA can exert an insulin-like effect directly on the liver to bring about a reduction in hepatic gluconeogenesis, which is mediated by a reduction in PCK1 and G6PC expression. In contrast, t10,c12-CLA increases hepatic gluconeogenesis by increasing G6PC expression at the transcriptional level (Chai et al., 2019).

In preclinical studies, dietary t10,c12-CLA induces severe hepatic steatosis in mice more than in other animal species. Regardless of species, the potential role of liver fatty acid (FA) composition, insulin secretion and sensitivity, and adipokine and inflammatory responses are the potential mechanisms behind CLA-induced hepatic steatosis (Vyas, Kadegowda, & Erdman, 2012). CLA induces liver enlargement, hepatic steatosis and insulin resistance in mice by elevating hepatic TG and diacylglycerol concentrations and membrane associated PKCε which is correlated with increased hepatic gluconeogenic gene expression (Fedor, Adkins, Mackey, & Kelley, 2012; Kostogrys, Franczyk-Żarów, Maslak, Gajda, Mateuszuk, & Chłopicki, 2012; Stout, Liu, & Belury, 2011; Wendel, Purushotham, Liu, & Belury, 2008). The addition of CLA mixture to a high-fructose diet results in the development of liver steatosis without altering the plasma lipid profile or level of atherosclerosis in apolipoprotein E and LDL receptor double-knockout mice (Kostogrys, Franczyk-Żarów, Maślak, Gajda, Mateuszuk, & Chłopicki, 2010). Moreover, t10,c12-CLA and t10,c12-CLA alter bile acid homeostasis and increases the risk of cholelithiasis in female C57BL/6J mice (Letona et al., 2011) and a high dose of CLA causes lipoatrophy, leading to steatosis and marked inflammation in the WAT of mice (Shen et al., 2013). Chronic administration of a diet supplemented with 0.3% t10, c12-CLA for 6 weeks in male CD-1 mice increases the liver mass and lipid accumulation due to activation of lipogenesis, insufficient induction of FA oxidation and LDL secretion, enhancing TG accumulation and producing greater liver mass (Li, Viswanadha, & Loor, 2012). CLA isomers have different steatogenic effects, the most evident effect being due to the t10,c12-CLA isomer. Therefore, caution should be exercised in using it as a supplement in the human diet considering the presence of a species-specific effect, as adverse effects might occur in long-term supplementation (Della Casa, Rossi, Romanelli, Gibellini, & Iannone, 2016). We can assume that t10,c12-CLA exerts contrary effects on hepatic lipid metabolism, increases risk factors for steatosis and enhances FM in the liver. However, the mechanisms that underlie CLA-induced hepatic de novo lipogenesis and lipid synthesis are largely unknown (Fig. 8).

11.4. Failure of anti-atherosclerotic effects

CLA mixture (5.5 g/day for 16 weeks) elicits higher levels of CRP in serum and 8-*iso*-PGF2α in the urine of healthy postmenopausal women and has several adverse effects on classical and novel biomarkers of coronary vascular disease (Tholstrup, Raff, Straarup, Lund, Basu, & Bruun, 2008). The c9,t11-CLA supplementation does not affect blood pressure, body composition, insulin resistance, or concentrations of lipid, glucose and CRP, which does not support an anti-atherosclerotic effect or an effect on cardiovascular risk factors in humans (Sluijs, Plantinga, de Roos, Mennen, & Bots, 2010; Wanders, Brouwer, Siebelink, & Katan, 2010). CLA supplementation does not affect selected biomarkers of atherosclerosis in obese and overweight women (Dus-Zuchowska, Madry, Krzyzanowska, Bogdanski, & Walkowiak, 2016) and high short-term (3 weeks) intake of c9,t11-CLA from dairy products does not affect blood pressure or heart rate in healthy humans (Engberink, Geleijnse, Wanders, & Brouwer, 2012). A *meta*-analysis study on the effect of CLA on blood pressure did not support the overall favorable effect of CLA supplementation on blood pressure regulation (Yang,

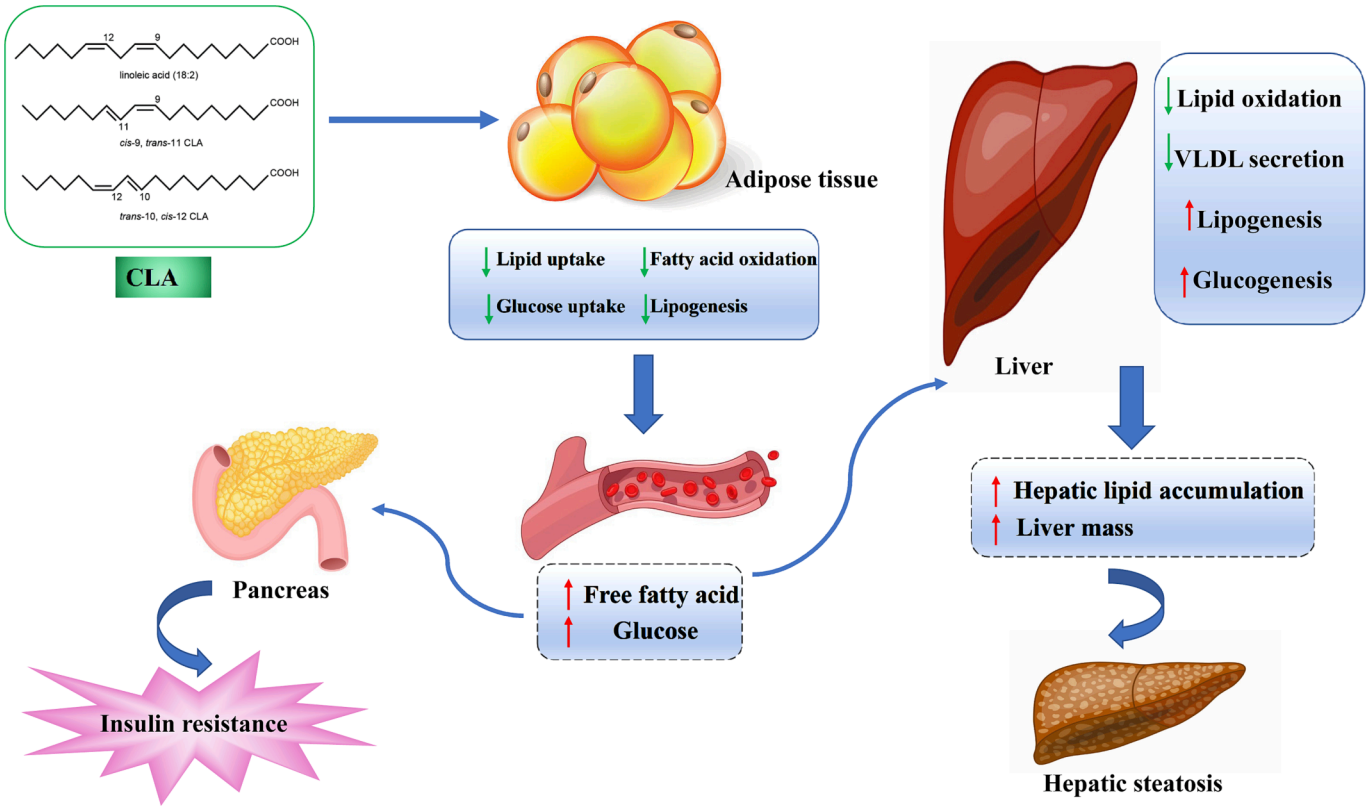

Fig. 8. Possible pathways by which CLA induces hepatic steatosis. (1) CLA in adipose tissue causes a reduction in fatty acid oxidation, lipogenesis and uptake of lipid and glucose, leading to higher circulatory levels of free fatty acids and glucose. (2) Pancreas-induced systemic insulin resistance. (3) Alterations in hepatic lipid metabolism and increased hepatic lipid accumulation and liver mass, leading to hepatic steatosis.

Wang, Zhou, Zhou, Chen, & Qin, 2015). Dietary supplementation with 4 g/day of c9, t11-CLA has a minor clinical effect on platelet function in healthy humans. However, in women, it causes a small inhibition of collagen-induced platelet aggregation which may provide evidence of the sex-dependent effects of CLA on platelet function (Bachmair, Wood, Keizer, Horgan, Ford, & de Roos, 2015). Although the t10,c12-CLA isomer has beneficial effects on reducing adipocyte size in obese rats, this does not translate into changes in the local renin–angiotensin system (RAS) or associated adipokines (DeClercq, Zahradka, & Taylor, 2010) and in mice, CLA supplementation has no anti-atherosclerotic effect (Kostogrys, Franczyk-Żarów, Maślak, Gajda, Mateuszuk, & Chłopicki, 2010, 2012). However, CLA has beneficial effects on acrolein-induced oxidative damage in rat heart mitochondria by increasing GSH and TAC levels. Meanwhile, CLA may have a dyslipidaemic effect because of the highest TG and LDL levels. Whereas CLA can reduce acrolein toxicity, using these food supplements together with acrolein may cause adverse effects on some biochemical parameters related to the cardiovascular system (Aydın, Şekeroğlu, & Şekeroğlu, 2018a, 2018b). Accordingly, CLA supplementation for CVD patients' needs special considerations to ensure its effect and safety.

11.5. Intestinal damage

To date, the effects of CLA on the intestinal epithelial barrier function remain largely unknown. However, CLA-enriched butter has been found to exacerbate 5-fluorouracil (5-FU)-induced intestinal damage (Barros et al., 2017). The c9,t11-CLA isomer, but not the t10,c12-CLA isomer, impair the intestinal epithelial barrier function in IPEC-J2 cells and mice via the activation of G protein-coupled receptor 120 (GPR120), a receptor for long-chain MUFAs that play important roles in appetite control, insulin sensitivity and inflammation regulation through the MLCK signalling pathway, which provides new insight into the regulation of the intestinal epithelial barrier function by different CLA isomers and about the potential application of CLA in human health and animal production (Su et al., 2020). CLA supplementation in food significantly worsens colorectal tumor formation induced by azoxymethane and DSS by inducing macrophage and T-cell-producing TGF-β via PPAR-γ activation. Thus, the anti-inflammatory properties of CLA are associated with the prevention of colitis but also with the development of colorectal cancer (Moreira et al., 2019). Therefore, further investigations are needed to demonstrate both the short- and long-term effects of CLA supplementation in clinical trials, determine the effects of each isomer of CLA and investigate its safety for applications to humans.

11.6. Lack of ergogenic effects

CLA has no ergogenic benefits in a model of aerobic training-induced improvements in neuromuscular fatigue or on field tests of muscle endurance (Jenkins et al., 2014a). CLA and aerobic exercise may have synergistic effects on lowering blood TG, but CLA is not efficacious for enhancing aerobic exercise performance (does not affect peak oxygen uptake or cardiorespiratory fatigue thresholds) during the 6 weeks of an aerobic exercise training program in college-age men (Jenkins et al., 2014b). CLA supplementation in young healthy men for 8 weeks does not alter oxygen consumption (VO_2 max), time to exhaustion, weight, BMI, or WC. The evidence shows that CLA supplementation might not be effective for the improvement of exercise performance or weight loss in healthy men with normal weight (Tajmanesh, Aryaeian, Hosseini, Mazaheri, & Kordi, 2015). Short-term CLA supplementation for 6 weeks associated with endurance exercise or not in mice does not stimulate mitochondrial biogenesis but does not change the expression of the PGC-1α protein or its isoforms (α1, α2 and α3) (Barone et al., 2017). CLA supplementation with and without physical activity reduces body fat accumulation in both sexes of Wistar rats without any evidence of LBM enhancement in exercised rats (Salgado et al., 2012). CLA treatment does not affect the activity of PGC-1α, a primary regulator in mitochondrial biogenesis, even when the mitochondrial content is significantly increased in human skeletal muscle cells by CLA (Vaughan, Garcia-Smith, Bisoffi, Conn, & Trujillo, 2012).

11.7. Impairment of fertility

CLA adversely affects female reproduction and has negative effects on systemic and local hormones involved in ovulation. It significantly decreases the serum levels of FSH, LH, oestradiol, NO, leptin and TNF-α. Besides that, it significantly reduces ovarian production of PGE2 and PGF2α. Moreover, it has no stimulatory effect on the ovulation rate in mice (Khodaei, Chamani, Sadeghi, & Hejazi, 2009). CLA in dairy cows affects the morphology and function of follicular granulosa cells, resulting in compromised ovarian cyclicity and impairment of fertility (Bionaz, Vargas-Bello-Pérez, & Busato, 2020; Sharma et al., 2020). In addition to its harmful impacts on female fertility, CLA does not improve boar fertility or semen quality, or the FA profiles of spermatozoa (Karimi et al., 2017; Zamora-Zamora et al., 2017). Long-term (26 weeks) supplementation with CLA mixture in male rabbits modestly enhances growth but negatively affects male reproduction. It reduces sperm concentration and, contrarily, affects sperm motility and sperm nutrition which might be due to changes in epididymal histological structure (disappearance of fat globules in the epididymis), FA profile and gene expression. Leydig cell hyperplasia could be due to hormonal imbalance and testicular tissue apoptosis which consequently cause a reduction of spermatogenesis (Abdelatty et al., 2020). A recent review article concluded that CLA has minimal or even negative effects on sperm cryopreservation, especially when supplemented in the diet (Freitas, Lopes, Nascimento, Pereira, Batista, & Campos-Junior, 2020).

11.8. Modulation of vitamin A metabolism

Dietary CLA affects retinoid metabolism. However, the exact mechanism has not yet been elucidated. Chronic intake of c9,t11 and t10,c12-CLA alters hepatic retinol secretion, storage and delivery in mice and also redistributes retinoids from hepatic stores toward adipose tissue via retinol-binding protein (RBP) (Ortiz, Wassef, Shabrova, Cordeddu, Banni, & Quadro, 2009). Moreover, acute administration of both CLA isomers influences vitamin A metabolism by rapidly enhancing hepatic uptake of dietary vitamin A and its secretion from the liver in the form of retinol bound to RBP and might rapidly enhance intestinal absorption of dietary vitamin A (Giordano, Banni, & Quadro, 2011). One of the possible mechanisms is competition between retinol and CLA by modulating the activity of PPAR-α and retinoid X receptor (RXR) heterodimer in different tissues (Carta et al., 2014). CLA supplementation in pregnant and lactating rats is non-critical in terms of the tocopherol status of newborns; it does not affect feed intake or BW development of nursing rats and their pups (Zeitz, Most, & Eder, 2016). Dietary CLA has little effect on vitamin A concentrations and metabolism in lactating rats and does not influence tissue vitamin A concentrations in their offspring. Thus, it seems unnecessary to use dietary CLA to increase vitamin A concentrations in milk (Zeitz, Most, & Eder, 2018). There are no significant effects of CLA supplementation during the first weeks after calving, either on milk yield and composition or on metabolic key parameters in the blood, indicating that CLA supplementation during the first 4 weeks of lactation does not affect massive peripheral lipomobilization (Sigl, Schlamberger, Kienberger, Wiedemann, Meyer, & Kaske, 2010).

12. Toxicity of CLA

Toxicity studies have been conducted using novel foods containing different percentages of CLA, for example with Tonalin^R TG 80, CLA-rich oil, as a food ingredient in the context of Regulation (EC) No. 258/97. Tonalin^R TG 80 consists of approximately 80% of the two CLA isomers c9,t11 and t10,c12 (1:1) (EFSA, 2010a).

In recent years, studies have been carried out to assess the safety of diacylglycerol (DAG) oil containing oleic and LAs, focusing primarily on acute and repeated toxicity, developmental and reproductive toxicity, genotoxicity, carcinogenicity and high-dose dietary DAG oil clinical studies, all of which have demonstrated no adverse effect (Honda, Fujita, Hayashi, Ikeda, Ito, & Morita, 2016).

12.1. Acute toxicity studies

An acute oral toxicity study in rats (strain unspecified) was performed using commercial beadlets of CLA methyl esters of unknown purity (Berven, Gaullier, & Gudmundsen, 2002). The authors concluded that oral administration of CLA methyl esters is 'non-toxic' based on an oral lethal dose (LD50) greater than 2 g/kg BW. The acute oral toxicity of a t11 C18:1 + c9,t11 C18:2-rich milk fat (t11-CLA; 14% and 4.8% of total FAs, respectively) was studied in rats receiving a single oral dose of 2000 mg/kg BW. Two weeks following the administration of t11-CLA milk fat, there were no changes in hematological and serum chemistry parameters (excepting plasma lipids), organ weights, gross pathology, and histopathology. t11-CLA has a low order of acute toxicity; the LD50 for male and female rats is more than 2000 mg/kg BW (Anadón et al., 2010).

12.2. Subchronic and chronic toxicity studies

A 90-day chronic toxicity study was conducted in 4-week-old male and female Sprague Dawley rats where three diets supplemented with linolenic acid and enriched with DAG containing, among other FAs, 19.7%, 15.1% and 16.5% CLA, respectively, were evaluated for possible adverse effects; no toxicologically significant treatment-related changes were found, assessed by clinical signs, functional observation battery, BW, food consumption, ophthalmology, urinalysis, hematology, clinical chemistry, organ weights, necropsy and histopathology (Bushita et al., 2018).

12.3. Genotoxicity

For characterization of two FA hydroperoxides from oxidation of LA and α-linolenic acid by electrospray ionization mass spectrometry (ESI-MS), the genotoxic effects of these hydroperoxides were studied by generating nucleotide adducts, which are the basic building blocks of DNA and RNA. Previous research studies have indicated that a range of carcinogenic compounds, such as polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers, acrylamides and aristolochic acids, can covalently bind to nucleosides to form relevant adducts which need to be further studied in cell and animal models. In addition, we investigated the reactive activity of two FA hydroperoxides with nucleosides, which are the basic building blocks of DNA and RNA (Cao et al., 2021).

Alpha-linolenic acid (ALA)-DAG oil is an edible oil enriched with DAG (>80%) and ALA (>50%). DAG mainly consists of oleic and LAs. In one study, ALA-DAG oil showed negative results in the bacterial reverse mutation test (Ames test) and in vitro micronucleus test in cultured Chinese hamster lung cells with and without metabolic activation, and in the in vivo bone marrow micronucleus test in mice. These results did not show any genotoxicity, suggesting that the FA composition has no deleterious effects (Honda, Fujita, Hayashi, Ikeda, Ito, & Morita, 2016).

12.4. Reproductive and developmental toxicity studies

In a study investigating the effects of vancomycin–LA nanoparticles on testicular tissue in an experimental animal model in 25 adult male adult Sprague Dawley rats, one group was administered with LA nanoparticles. Alterations in seminal fluid parameters showed a statistically significant reduction in sperm count. In the treated groups there was a thickening of the basement membrane. In the vancomycin–LA-treated group, testicular morphometry and hormonal milieu were altered sufficiently to induce an alteration of reproductive function (Naidu, Olojede, Lawal, Peter, Akang, & Azu, 2021).

12.5. Allergenicity

Patients with atopic dermatitis (AD) have been reported to show imbalances in the levels of omega-6 PUFAs and nutritional supplementation with omega-6 PUFAs is of potential interest in the treatment of AD. Although there is no direct evidence, it has been reported that LA metabolites decrease in patients with AD due to impairment of the activity of delta-6-desaturase involved in the conversion of LA to gamma linolenic acid. A research study with 71 six-month-old infants (39 healthy controls and 32 infants with AD) from the Cohort for Childhood Origin of Asthma and Allergic Diseases (COCOA) birth cohort study showed that the reduction in gut LA may increase the severity of AD during infancy, possibly by inhibiting the anti-inflammatory effects of gut LA. To confirm the role of gut LA in AD, it is necessary to further investigate the association with changes in blood metabolites (Lee et al., 2021).

13. Conclusion

The present review highlights the CLA's therapeutic effects and safety. CLA has gained a lot of global interest over the past few decades due to it providing numerous health benefits including anti-carcinogenic, anti-diabetic, anti-inflammatory, anti-obesity and anti-atherogenic effects, which have been widely confirmed *in vivo* as well as *in vitro*. Given the biological properties of CLA, there has been a great focus on enriching egg, meat and dairy products for human consumption. It is possible to change the lipid composition of food products, such as eggs, milk, or meat, easily by modifying the diet of the animals. Meanwhile, the influence of CLA on humans has been much less than that observed in animals; the effective dose for CLA in animals and humans is still unclear and the mechanism of action is not fully explained. Moreover, several adverse effects have now arisen which are not yet completely understood because of the lack of human studies and the absence of enough scientific information to determine if these adverse effects are related to the CLA dosage or duration of administration. Interestingly, the presence of drug interactions, if CLA is co-administered with various kinds of medicinal drugs generally used in clinical practice or with other natural food supplements or functional foods, is worthy of further investigation. Besides that, the different beneficial health consequences and potential health risks of CLA isomers are not the same due to their physiological properties meaning individual responses to CLA consumption may vary, therefore it's best to seek personalized guidance from a healthcare expert or qualified dietitian based on your specific needs and health goals. In a word, CLA has been linked to several health benefits, more research is needed to fully understand its effects on human health and establish the proper, effective and safe doses of both isomers and CLA mixture with minimum adverse effects before CLA is recommended for human use.

Author contribution

All authors contributed in performing data curation, investigation, validation, and writing original draft. Xu Wang, Arturo Anadón and María-Aránzazu Martínez were responsible for critically reading the manuscript, revision the original draft, review and editing. All authors read and approved the final version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgement

This work was supported by National Natural Science Foundation of China (NSFC) (32072925), the Fundamental Research Funds for the Central Universities of China (2662020DKPY020), and Project Ref. PID2020-115979RR-C33 from Ministerio de Ciencia e Innovación, Spain

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