

Review Article

Current issues surrounding the definition of *trans*-fatty acids: implications for health, industry and food labels

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Abstract

The definition of *trans*-fatty acids (TFA) was established by the Codex Alimentarius to guide nutritional and legislative regulations to reduce TFA consumption. Currently, conjugated linoleic acid (CLA) is excluded from the TFA definition based on evidence (primarily preclinical studies) implying health benefits on weight management and cancer prevention. While the efficacy of CLA supplements remains inconsistent in randomised clinical trials, evidence has emerged to associate supplemental CLA with negative health outcomes, including increased subclinical inflammation and oxidative stress (particularly at high doses). This has resulted in concerns regarding the correctness of excluding CLA from the TFA definition. Here we review recent clinical and preclinical literature on health implications of CLA and ruminant TFA, and highlight several issues surrounding the current Codex definition of TFA and how it may influence interpretation for public health. We find that CLA derived from ruminant foods differ from commercial CLA supplements in their isomer composition/distribution, consumption level and bioactivity. We conclude that health concerns associated with the use of supplemental CLA do not repudiate the exclusion of all forms of CLA from the Codex TFA definition, particularly when using the definition for food-related purposes. Given the emerging differential bioactivity of TFA from industrial *v.* ruminant sources, we advocate that regional nutrition guidelines/policies should focus on eliminating industrial forms of *trans*-fat from processed foods as opposed to all TFA *per se*.

Key words: *Trans*-fatty acids: Ruminant *trans*-fat: Conjugated linoleic acid: Vaccenic acid

Background and rationale

During the past decade, detrimental health implications of *trans*-fatty acids (TFA) have been extensively studied, particularly in the context of CVD risk. Increased TFA consumption from 'industrial' (iTFA) origin (i.e. partially hydrogenated vegetable oils) has been shown to be positively associated with increased CHD incidence via various mechanisms pertaining to lipid metabolism, insulin sensitivity and inflammation (reviewed in Mozaffarian *et al.*⁽¹⁾ and Brouwer *et al.*⁽²⁾). Emerging epidemiological data have also associated iTFA consumption with an increased risk and/or incidence of breast cancer⁽³⁾, prostate cancer⁽⁴⁾ and colorectal cancer⁽⁵⁾. Consequently, the public has been alerted to restrict the consumption of TFA-containing foods; moreover, TFA content has become a mandatory section on food labels in North America,

some European countries and others. For example, Health Canada currently recommends a TFA limit of 5% of total fat in all products sold to consumers and 2% for commercial margarines and spreads. Denmark has legislated the content of iTFA to be less than 2% of total fat in all oil and fat sold separately or as food ingredients. Notably in 2006, these regulatory bodies agreed to endorse the Codex Alimentarius definition of TFA, with an intent to encourage countries to adopt prudent TFA nutrition labelling and TFA food-related policies.

The Codex definition and differences between ruminant and industrial trans-fatty acids

According to the Codex definition, TFA is defined as 'all the geometrical isomers of monounsaturated and polyunsaturated

Abbreviations: CLA, conjugated linoleic acid; EA, elaidic acid; FSANZ, Food Standards Australia and New Zealand; HDL-C, HDL-cholesterol; iTFA, industrial *trans*-fatty acids; LDL-C, LDL-cholesterol; rTFA, ruminant-derived *trans*-fatty acids; TFA, *trans*-fatty acids; VA, vaccenic acid.

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fatty acids having non-conjugated, interrupted by at least one methylene group, carbon-carbon double bonds in the *trans*-configuration', which excludes all isomers in the family of conjugated linoleic acid (CLA). Such exclusion was established from growing literature suggesting potent body-weight reduction and anti-atherogenic properties of CLA, primarily from cell-culture and animal studies^(6–8). However, a major confusion exists among consumers and industrial bodies to whether or not all *trans*-fats from ruminant sources are equally detrimental to health and should also be eliminated from the diet. Currently, the *trans*-fat content on many food labels (and in legislative documents) does not include ruminant CLA isomers, implying it to have differential properties. We wish to point out that other ruminant fatty acids with one or more *trans* double bonds far more abundant than CLA can remain included on food labels. Indeed, evidence from both epidemiological studies and preclinical experimental models collectively demonstrates the neutral or beneficial health effect of TFA derived from ruminant fat at normal consumption levels⁽⁹⁾. As highlighted in a recent quantitative review of prospective cohort studies by Bendsen *et al.*⁽¹⁰⁾, dietary consumption of ruminant *trans*-fat may be protective against total as well as fatal CHD events. Very recently, Brouwer *et al.*⁽²⁾ updated their previous quantitative review to include new studies and adjustments in data analysis⁽¹¹⁾. Consistent with Bendsen *et al.*⁽¹⁰⁾, ruminant-derived TFA (rTFA) were found to have no adverse effect on biomarkers for CVD at amounts likely to be consumed in the general population (between 2 and 4 g/d)⁽¹¹⁾. Nevertheless, the distinctive health effects of TFA from different food sources (i.e. industrial *v.* ruminant) have not been clarified in the Codex TFA definition.

Issues surrounding supplemental conjugated linoleic acid

The discovery of weight loss as well as other potential health properties of CLA has been the premise for the commercialisation of CLA supplements for weight management. In European countries, CLA has been approved as a novel food ingredient at a dose of 3 g/d up to 6 months⁽¹²⁾; the US Food and Drug Administration has also issued 'Generally Recognized As Safe' notifications on similar CLA products for use in specific foods including meal replacement beverages, milk products and fruit juices at 1.5 g CLA/serving and up to 3 g/d⁽¹³⁾. However, the efficacy associated with its health claims for all populations remains debated⁽¹⁴⁾. Most recently, concerns have surfaced suggesting the potential adverse effect on atherogenic cholesterol profile from supplemental CLA use in select population groups^(2,15–17). As a result, the Food Standards Australia and New Zealand (FSANZ) proposed to re-evaluate their perception regarding the exclusion of CLA from the TFA definition.

Variations in the interpretation of the Codex definition of trans-fatty acids

It is important to appreciate that the current Codex definition for TFA does allow some flexibility within its interpretation.

For example, Canada, the USA, China, South Korea, the Mercosur member countries including Argentina, Brazil, Paraguay, Uruguay and Venezuela, and some European countries such as Denmark, Iceland, Switzerland and Austria have implemented food-labelling regulations based on the current Codex TFA definition; however, variations among these countries exist in the method and type of regulations implemented. The main difference has been whether or not to apply mandates to unprocessed natural foods (e.g. whole-fat dairy products) that do not undergo industrial partial hydrogenation processes. Interestingly, the FSANZ has adopted the definition of TFA as all fatty acids containing *trans* double bond(s) with no exclusion of CLA at all⁽¹⁸⁾.

Section summary. The purpose of the present review is to help clarify some of the major issues surrounding the implications of the Codex TFA definition. In particular, we wish to highlight how supplemental CLA is different from those derived from ruminant fat, and whether or not scientific advances continue to support the exclusion of CLA from the Codex TFA definition. Further, we raise the point that emerging data suggest rTFA differ from iTFA and how this may have an impact on the current interpretations of the Codex TFA definition.

Differences between supplemental conjugated linoleic acid and those derived from ruminant fat

CLA has a similar chemical structure to linoleic acid (*cis*-9, *cis*-12-18:2), except that the conjugated double bonds are predominantly in positions 7 and 9, 8 and 10, 9 and 11, 10 and 12 or 11 and 13 in either the *cis* or *trans* configuration. The family of CLA can include up to twenty-eight possible different isomers, with two of these (i.e. *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA) known to possess bioactivity. *Cis*-9, *trans*-11-CLA is the most predominant isomer, present naturally as esterified fatty acids in the TAG of ruminant fat and dairy products. It is synthesised via biohydrogenation of linoleic/linolenic acid by ruminant bacteria and *in vivo* conversion from *trans*-11-vaccenic acid (VA) in the liver and adipose tissue of ruminant animals⁽¹⁹⁾ (Fig. 1). In addition to its presence in ruminant-derived products, CLA is also available commercially in an enriched supplemental form (usually with a formulation of 80% of the two CLA isomers *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA at a 1:1 ratio) and is typically produced from safflower oil rich in linoleic acid. A common method to produce supplemental CLA is to saponify food-grade safflower oil TAG to NEFA, further isomerised under conditions of high pH and temperature and then inter-esterified with glycerol to re-form TAG⁽²⁰⁾ (Fig. 1). Some manufacturers also provide supplemental CLA in the free acid form. The finished CLA product typically contains a minimum of 78% of total CLA isomers and at least 74% of either a common 50:50 or a less common 80:20 mixture of *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA. In some countries (but not all), supplemental CLA has been accepted as generally safe at 1.5 g/serving up to 3 g/d for 2 years by the US Food and Drug Administration (GRN000232)⁽¹³⁾ and 3–5 g up to 6 months by Health Canada⁽²¹⁾. Although not accepted by the FSANZ, supplemental CLA has also been approved as a novel food ingredient by the



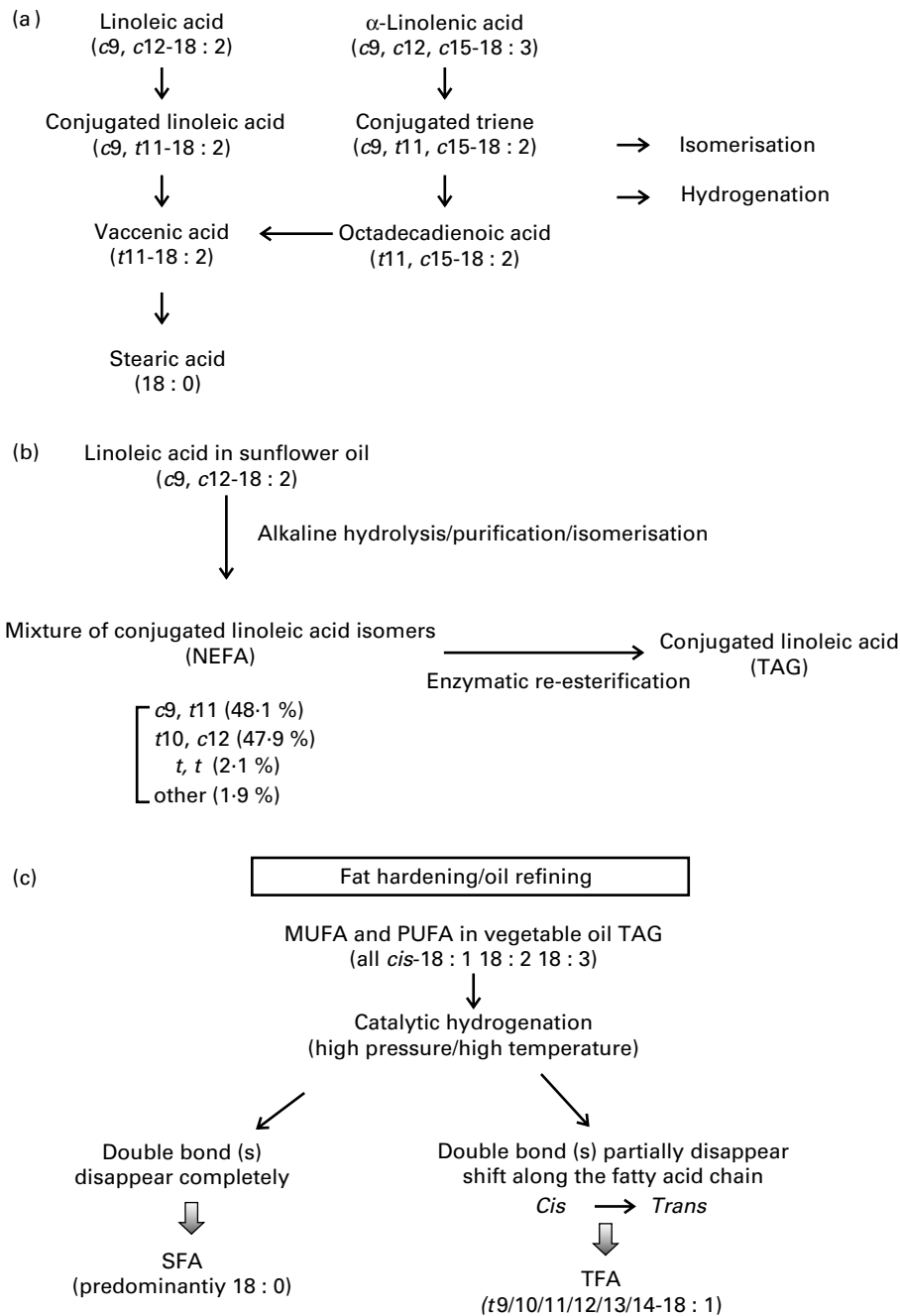


Fig. 1. Schematics of dietary *trans*-fatty acids (TFA) from (a) natural ruminant biohydrogenation, (b) synthetic supplements and (c) industrial partial hydrogenation of vegetable oils.

European Food Safety Authority^(12,14,22) at a dose of 3–3.5 g/d for up to 6 months in the general population, except in subjects diagnosed with type 2 diabetes.

The differences between supplemental and ruminant sources of CLA can be loosely categorised into the following: (1) isomer distribution; (2) consumption level; (3) regio-specific distribution in TAG molecules; (4) bioavailability. Supplemental CLA contain various isomers, with two being the most abundant (i.e. *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA), and the average recommended daily dose is 1100 mg for each of these two isomers (3 g of total CLA-rich oil). In contrast, CLA

that is present naturally in ruminant-derived foods, such as beef, lamb and dairy products, differs greatly in the proportion of isomers compared with that of supplemental CLA, with the *cis*-9, *trans*-11-CLA isomer (also known as ruminic acid) being predominant (70–90%) and only a trace amount as *trans*-10, *cis*-12-CLA⁽²³⁾. The amount of *cis*-9, *trans*-11-CLA in ruminant sources (e.g. 2% fat milk, butter, beef) can range from 5 mg/g fat with a standard feeding regimen to as high as 47 mg/g fat in enriched products⁽²³⁾. The average dietary intake of ruminant CLA from natural food sources is approximately 100–180 mg/d in the UK and North America⁽²⁰⁾, and may be 2–3-fold

Table 1. Summary of meta-analyses and systematic reviews on the health effect of conjugated linoleic acid (CLA) in human subjects

Reference	Study design	Health effect studied	Dosage and duration	Participant characterisation	Major findings	Conclusions
Meta-analyses						
Onakpoya <i>et al.</i> ⁽¹²¹⁾	Long-term, randomised, double-blind, placebo-controlled clinical trials, published up to October 2010	Body composition	2.4–6 g/d, 50:50 mixture of <i>c9</i> , <i>t11</i> and <i>t10</i> , <i>c12</i> -CLA; longer than 6 months	Healthy overweight/obese adults	Statistically significant reduction in (a) Body weight (kg): OR –0.7 (95% CI –1.09, –0.32); (b) Fat mass (kg): OR –1.33 (95% CI –1.79, –0.86) (c) Waist circumference (cm): OR –0.12 (95% CI –0.82, 0.58) (d) BMI (kg/m ²): OR –0.30 (95% CI –0.44, –0.16) No dose response observed	No clinically relevant effect on body composition on the long term
Schoeller <i>et al.</i> ⁽¹²²⁾	Randomised, double-blind, placebo-controlled clinical trials, published between 1999 and 2007	Body fat reduction	1–6.8 g/d, 50:50 mixture of <i>c9</i> , <i>t11</i> and <i>t10</i> , <i>c12</i> -CLA; 12 weeks or less	Normal-weight, overweight and obese subjects of any age	Fat mass loss for the first 6 months of treatment: (a) CLA –0.05 (SD 0.05) kg/week CLA relative to placebo: –0.09 (SD 0.07) kg/week Weak dose effect: –0.024 kg fat/g CLA per week	Weak effect on fat mass
Whigham <i>et al.</i> ⁽¹²³⁾	Randomised, double-blind, placebo-controlled clinical trials, published between 1999 and 2007	Change in fat-free mass	1–6.8 g/d, 50:50 mixture of <i>c9</i> , <i>t11</i> and <i>t10</i> , <i>c12</i> -CLA; 12 weeks or less	Normal-weight, overweight and obese subjects of any age	Fat-free mass increased with CLA treatment (0.3 (SD 0.7) kg) but change is small (<1%), no dose or time effect	Weak effect on fat mass or fat-free mass
Systematic reviews						
Lenz & Hamilton ⁽¹²⁴⁾	Blinded, placebo-controlled, randomised clinical trials, up until May 2003	Weight loss	1.7–6.8 g/d; 12 weeks or less	Overweight and obese subjects	Reduced body fat, no change in body weight or BMI	CLA appears to be safe for short-term use
Salas-Salvadó <i>et al.</i> ⁽¹²⁵⁾	Double-blind, placebo-controlled, randomised clinical trials, between 2000 and 2005	Body composition, glucose, lipid, insulin sensitivity	1.4–6.8 g/d, 50:50 or other mixtures of CLA isomers; 4–52 weeks	Normal-weight, overweight and obese subjects; either healthy or with the metabolic syndrome/type 2 diabetes	No significant change in body weight; body fat loss with exercise; inconsistent changes in TAG cholesterol, glucose or insulin; induced lipid peroxidation; <i>t10</i> , <i>c12</i> -CLA tends to be more harmful	Insufficient evidence to support the effect on weight/body composition in human subjects; larger randomised controlled trials needed to clarify safety or efficacy
Tricon & Yaqoob ⁽¹²⁶⁾	Randomised clinical trials	Body composition, blood lipids, liver metabolism, insulin sensitivity, immune function	0.7–6.8 g/d, CLA isomer mixtures or single <i>c9</i> , <i>t11</i> / <i>t10</i> , <i>c12</i> isomers	Normal-weight, overweight and obese subjects; either healthy or with the metabolic syndrome/type 2 diabetes	No effect on body composition, glucose, insulin or immune function with either CLA mixture or single isomers; <i>t10</i> , <i>c12</i> but not <i>c9</i> , <i>t11</i> -CLA reduced HDL while raising TAG and the LDL:HDL ratio	Little effect on body composition, insulin sensitivity or immune function; <i>t10</i> , <i>c12</i> -CLA had a relative detrimental effect on blood lipids

higher in certain European countries such as Germany, Denmark and The Netherlands (depending on population dietary patterns, geographical locations, forage conditions and other factors)^(24,25). The highest level reported (1000 mg/d) was observed in a Hare Krishna community in Australia due to a high consumption of ghee and butter⁽²⁴⁾. It is also important to note that isomers from supplemental CLA can be present as either free acids or inter-esterified TAG at various *sn* positions based on synthetic conditions and substrate ratios⁽²⁶⁾. In contrast, ruminant-derived CLA is incorporated considerably into the *sn*-1 position in phospholipids and over 50% in the *sn*-3 position in milk TAG^(27,28). The positional distribution of CLA in ruminant muscle or adipose tissue can differ, with more incorporation into the *sn*-2 position of the TAG⁽²⁹⁾.

Variations in the bioavailability of CLA from supplemental and ruminant sources have been attributed to their presence as a free or esterified acid, the *sn* position of TAG as well as the characteristics of the food matrix they are consumed with. A number of studies have compared intestinal absorption of supplemental CLA isomers in different forms (i.e. NEFA, TAG or fatty acid ethyl esters) in rodents and human subjects. It has been reported that CLA is better absorbed as a TAG than a NEFA (which also tends to be more susceptible to oxidation)^(30–32). Moreover, fatty acids incorporated into the *sn*-2 position of TAG tend to be more absorbed than either the *sn*-1 or -3 position⁽³³⁾; but opposing results have also been reported for ruminant CLA, which has been found to be more bioavailable when in the external position (*sn*-1/3) than in the internal *sn*-2 position⁽²⁷⁾. Gervais *et al.*⁽³⁴⁾ further reported that *cis*-9, *trans*-11-CLA was highly bioavailable from milk and the specific regiodistribution did not affect its intestinal digestibility.

Section summary. Differences exist between supplemental and ruminant sources of CLA including: isomer distribution, consumption level, regio-specific distribution in TAG and possibly bioavailability. Supplemental CLA contains two abundant isomers (i.e. *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA), whereas in ruminant-derived foods (such as beef, lamb and dairy products), the predominant isomer is *cis*-9, *trans*-11-CLA and only a trace amount as *trans*-10, *cis*-12-CLA⁽²³⁾. The differences between these two forms/sources of CLA suggest that they should not be considered equal with respect to health regulations and/or nutritional guidelines.

Does the current literature (clinical and preclinical studies) suggest whether conjugated linoleic acid should be excluded from the Codex *trans*-fatty acid definition?

Independent reviews published before April 2010 to elucidate the effect of CLA in human subjects, with a primary focus on body-weight/fat reduction, are summarised in Table 1. A number of government regulatory bodies such as the FSANZ and the European Food Safety Authority have also generated reports on the safety of supplemental CLA as a potential ingredient for novel foods^(14,22). Despite different recommendations provided by these government reports, they are consistent in that CLA supplementation at a daily

dose of less than 7 g showed little effect on clinically meaningful reduction in body weight or fat mass. In order to gather the most recent literature on CLA (with a specific focus for the definition of TFA), we have reviewed research published from April 2010 to November 2012 that have advanced this field (by searching the PubMed database using 'conjugated linoleic acid' and 'CLA'). Only human studies using CLA as the primary investigating agent and with a focus of obesity and CVD-related endpoints were included in the following discussion (fourteen randomised clinical trials and two retrospective case–control studies; Table 2).

Supplemental conjugated linoleic acid and human health

The majority of recent clinical intervention studies have focused on the effect of supplemental CLA on cardiovascular risk parameters. Of these, four publications were generated from an intervention trial conducted in a group of healthy Dutch adults, each focusing on a different risk factor for CVD. Collectively, the results demonstrated that supplemental CLA (*cis*-9, *trans*-11:*trans*-10, *cis*-12-CLA, 80:20), relative to sunflower oil high in oleic acid, had no effect on blood pressure⁽³⁵⁾, insulin sensitivity⁽³⁶⁾, plasma proteome⁽³⁶⁾, inflammatory markers or oxidative stress⁽³⁷⁾ at a dose of 20.2 g/d for 3 weeks. Negative effects on lipoprotein profiles were observed in the same study, which include increased total cholesterol, LDL-cholesterol (LDL-C) and total:HDL-cholesterol (HDL-C) ratio compared with sunflower oil^(36,38). These adverse effects may probably be attributed to the high dosage (20.2 g/d, equivalent to about 9% daily energy), since another Dutch study showed no such adverse outcomes using the same CLA preparation for a 7-fold longer duration (6 months) but at a lower dose (3.1 g/d, 1.1% daily energy)⁽³⁹⁾. Interestingly, a neutral effect was reported for supplemental CLA with a different isomer profile (*cis*-9, *trans*-11:*trans*-10, *cis*-12-CLA, 50:50) on body composition, blood lipid profile, endothelial function and inflammatory markers with effective doses varying from 1.8 to 6.4 g/d when compared with safflower oil^(40–42).

Ruminant conjugated linoleic acid and human health

In two retrospective case–control studies, it was suggested that the adipose enrichment of *cis*-9, *trans*-11-CLA appeared to be protective against the future risk of non-fatal acute myocardial infarction and diabetes^(43,44). In a number of clinical intervention studies, *cis*-9, *trans*-11-CLA-enriched dairy fat at doses between 0.7 and 1.0 g/d did not appear to affect serum lipid or lipoprotein profile in normolipidaemic, yet overweight human subjects when consumed in moderation^(45–47). Venkatramanan *et al.*⁽⁴⁵⁾ compared the effect of milk naturally or synthetically enriched with *cis*-9, *trans*-11-CLA (1.1 g/d) on blood lipid indices, liver function and body composition in overweight human subjects. In this 8-week intervention study, conventional milk (0.2 g/d *cis*-9, *trans*-11-CLA) was used as the control and no significant changes were observed in both CLA-supplemented groups on all parameters measured. Similarly, neutral effects of ruminant CLA (from pasture-fed beef) on blood lipids and body composition were observed

Table 2. Summary of observational and intervention studies on the health effect of conjugated linoleic acid (CLA) in human subjects

Reference	Study description	Endpoints	Participant characteristics	Intervention*	Results	Summary†
Clinical trials using synthetic CLA supplements						
Wanders <i>et al.</i> ⁽³⁸⁾	Randomised, single-blind, multiple cross-over	Atherogenic lipoprotein profile Commercial CLA supplements	Healthy adults (41 % men), BMI < 30 kg/m ² , n 61, Dutch population	20.2 g/d CLA (80:20, Lipid Nutrition), 9 %en, 3 weeks	Compared with sunflower oil: ↑ LDL, total:HDL-cholesterol ratio ↓ HDL → TAG, body weight, total cholesterol	↑
De Roos <i>et al.</i> ⁽³⁶⁾	Randomised, single-blind, multiple cross-over	Insulin resistance, plasma proteome	Healthy adults (41 % men), BMI < 30 kg/m ² , n 61, Dutch population	20.2 g/d CLA (80:20, Lipid Nutrition), 9 %en, 3 weeks	Compared with sunflower oil: → Glucose, insulin, HOMA → Plasma protein profiles by 2-DE	→
Engberink <i>et al.</i> ⁽³⁵⁾	Randomised, single-blind, multiple cross-over	Blood pressure	Healthy adults (41 % men), BMI < 30 kg/m ² , n 61, Dutch population	20.2 g/d CLA (80:20, Lipid Nutrition), 9 %en, 3 weeks	Compared with sunflower oil: → Systolic and diastolic blood pressure	→
Smit <i>et al.</i> ⁽³⁷⁾	Randomised, single-blind, multiple cross-over	Inflammation, oxidative stress	Healthy adults (41 % men), BMI < 30 kg/m ² , n 61, Dutch population	20.2 g/d CLA (80:20, Lipid Nutrition), 9 %en, 3 weeks	Compared with sunflower oil: → Plasma inflammatory markers (CRP, IL-6, E-selectin, MCP-1, TNF-RI/II) ↑ Urinary 8-iso-PGF _{2α} but not lipid peroxidation	→
Sluijs <i>et al.</i> ⁽³⁹⁾	Randomised, double-blind, parallel	Aortic stiffness, insulin resistance, blood lipids, CRP, body composition	Healthy overweight/obese adults (48 % men), n 346, 40–70 years, Dutch population	3.1 g/d CLA (80:20, Lipid Nutrition), 1.1 %en, 6 months	Compared with a mixture of palm oil (80 %) and soyabean oil (20 %): → Aortic pulse wave velocity → Body composition, blood pressure, blood lipids, insulin resistance or CRP	→
Bachmair <i>et al.</i> ⁽¹²⁷⁾	Randomised, double-blind, parallel	Platelet proteome	Healthy overweight/obese adults (48 % men), n 40, 40–70 years, Dutch population	3.1 g/d CLA (80:20, Lipid Nutrition), 1.1 %en, 3 months	Compared with a mixture of palm oil (80 %) and soyabean oil (20 %): Changed abundance of seventy-four proteins, forty of which identified as being associated with platelet structure, receptor action and cell signalling	NA
Joseph <i>et al.</i> ⁽⁴¹⁾	Randomised, double-blind, cross-over	Body composition, blood lipids, safety biomarkers	Overweight, borderline hypercholesterolaemic men (n 27), Canadian population	2.8 g/d CLA (50:50, Clarinol G-80) or 2.7 g/d CLA (89:11), 8 weeks	Compared with safflower oil: → Body composition → Blood lipids, ox-LDL, → HOMA-IR → Inflammation (hs-CRP, TNF-α, IL-6) → Lipid oxidation rate	→
Pfeuffer <i>et al.</i> ⁽⁴²⁾	Randomised, double-blind, parallel	Endothelial function, metabolic syndrome	Overweight/obese men (45–68 years), 75 % diagnosed with the metabolic syndrome, n 85, German population	3.4 g/d CLA (50:50, Tonalin G-80), 4 weeks	Compared with safflower oil: → Endothelial function, total, LDL- or HDL-cholesterol, TAG, insulin sensitivity, CRP, soluble adhesion molecules, ox-LDL, Lp(a); ↓ Body weight (– 1.13 (sd 1.65) kg), arylesterase activity; ↑ 8-Iso-PGF _{2α}	→

Table 2. Continued

Reference	Study description	Endpoints	Participant characteristics	Intervention*	Results	Summary†
Sato <i>et al.</i> ⁽⁴⁹⁾	Randomised, double-blind, parallel	CLA absorption and metabolism in human subjects, the effect on blood lipids	Healthy young adults (50% men), <i>n</i> 24, Japanese population	1.8 g/d CLA (50:50, Nisshin OilliO Group), 3 weeks	↑ CLA blood concentration after supplementation; <i>trans</i> -10, <i>cis</i> -12-CLA was metabolised faster than <i>cis</i> -9, <i>trans</i> -11-CLA Compared with linoleic acid: → Blood TAG, total, LDL- and HDL-cholesterol → Blood glucose, AST, ALT	→
Asp <i>et al.</i> ⁽⁴⁰⁾	Randomised, double-blind, cross-over	Glycaemia, blood lipids, inflammation	Obese postmenopausal women, <i>n</i> 35, US population	6.4 g/d CLA (50:50, Cognis Corporation), 16 weeks	Compared with safflower oil: → Glycaemia (glucose AUC, 2 h post-OGTT glucose, insulin AUC, HOMA1 β-cell) → Total, LDL- or HDL-cholesterol, TAG, NEFA or CRP	→
Clinical trials using CLA-enriched dairy fat Venkatramanan <i>et al.</i> ⁽⁴⁵⁾	Randomised, single-blind, cross-over	Blood lipids, liver function and body composition	Overweight adults (67% men) with nearly optimal plasma LDL-cholesterol concentration, <i>n</i> 15, Canadian population	1.0 g/d <i>cis</i> -9, <i>trans</i> -11-CLA from milk or 1.2 g/d <i>cis</i> -9, <i>trans</i> -11-CLA and 1.1 g/d <i>trans</i> -10, <i>cis</i> -12-CLA from supplements, 4 weeks	Compared with control untreated milk: → Plasma TAG, total, HDL- or LDL-cholesterol → Body weight or fat composition → ALT, total bilirubin, CRP, TNF-α	→
Brown <i>et al.</i> ⁽⁴⁶⁾	Randomised, single-blind, cross-over	Insulin sensitivity, body composition, blood lipids	Young non-obese women, <i>n</i> 18, US population	1.1 g <i>cis</i> -9, <i>trans</i> -11-CLA from pasture-fed milk, 8 weeks	Compared with 0.35 g/d <i>cis</i> -9, <i>trans</i> -11-CLA from grain-fed cattle → Blood VLDL, LDL, HDL, TAG or total cholesterol → Glucose, insulin or glucagon response after the OGTT → AST, ALT → Body composition	→
Labonte <i>et al.</i> ⁽¹²⁸⁾	Randomised, double-blind, cross-over	Cholesterol absorption and synthesis	Healthy men, <i>n</i> 48, Canadian population	10.2 g/d ruminant TFA (2.0 g <i>cis</i> -9, <i>trans</i> -11-CLA), 4 weeks	Compared with a diet low in TFA from any source: ↓ Cholesterol absorption → Cholesterol synthesis → Plasma cholesterol concentration	→
Lacroix <i>et al.</i> ⁽⁴⁷⁾	Randomised, double-blind, cross-over	Plasma lipid profile	Healthy/overweight women, <i>n</i> 61, Canadian population	0.7 g/d <i>cis</i> -9, <i>trans</i> -11-CLA from enriched butter	Compared with the control diet low in rTFA: → Blood total, LDL-cholesterol, TAG, ApoB, ApoA-1 ↓ HDL-cholesterol in overweight but not healthy women	→
Observational studies Castro-Webb <i>et al.</i> ⁽⁴⁴⁾	Case-control	Risk of diabetes	Case: diabetic adults (76% men, <i>n</i> 232) Control: healthy volunteers matched for age, sex, area of residence (67% men, <i>n</i> 1512); Costa Rican population		Higher adipose tissue <i>cis</i> -9, <i>trans</i> -11-CLA was associated with a 43% lower risk of diabetes (OR 0.48, 95% CI 0.31, 0.76)	↓

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Table 2. Continued

Reference	Study description	Endpoints	Participant characteristics	Intervention*	Results	Summary†
Smit <i>et al.</i> ⁽⁴³⁾	Case-control	Risk of MI	Cases: first non-fatal acute MI (n 1813); Control: healthy volunteers matched for age, sex, area of residence (n 1813); Costa Rican population	Subcutaneous adipose tissue <i>cis</i> -9, <i>trans</i> -11-CLA	Higher adipose tissue <i>cis</i> -9, <i>trans</i> -11-CLA was associated with a 43% lower risk of MI (OR 0.57, 95% CI 0.45, 0.71) Inverse association between dairy intake and the risk of MI after being adjusted for adipose tissue saturated fat and <i>trans</i> -fat	↓

%en, Percentage of energy; HOMA, homeostatic model assessment; 2-DE, two-dimensional electrophoresis; CRP, C-reactive protein; MCP-1, monocyte chemoattractant protein-1; TNF-R, TNF receptor; NA, not available; ox-LDL, oxidised LDL; HOMA-IR, HOMA of insulin resistance; hs-CRP, high-sensitivity CRP; Lp (a), lipoprotein (a); AST, aspartate aminotransferase; ALT, alanine aminotransferase; OGTT, oral glucose tolerance test; TFA, *trans*-fatty acids; rTFA, ruminant-derived TFA; MI, myocardial infarction.

* Doses refer to total CLA isomers as unesterified fatty acids: 50:50, 80:20 or 88:11 indicates the ratio of *cis*-9, *trans*-11-CLA:*trans*-10, *cis*-12-CLA in CLA supplements.

† Effect on the endpoints: ↑ increased; ↓ decreased; → neutral effect.

in healthy women in a US intervention study at the same dose and duration (*cis*-9, *trans*-11-CLA: 1.17 g/d for 8 weeks) relative to grain-fed ground beef⁽⁴⁶⁾. In a group of healthy Canadian women, a *cis*-9, *trans*-11-CLA of 0.7 g/d for 4 weeks from rTFA-enriched butter showed the neutral effect on LDL relative to regular butter containing one-third of the rTFA content in enriched butter. However, we note that only one-quarter of the dose and half the duration were used in this Canadian study compared with the two clinical trials discussed earlier⁽⁴⁷⁾. Further, the baseline characteristics of participants involved in the clinical trials indicate that fasting blood TAG, total cholesterol and LDL-C were well within the desirable or near optimal range according to the International Diabetes Federation and National Cholesterol Education Panel – Adult Treatment Panel (NCEP-ATP) III guidelines. The observed lack of the efficacy of *cis*-9, *trans*-11-CLA may possibly be due to: the relatively low consumption level of this isomer from food; the putative beneficial effects from control fats (e.g. sunflower oil high in oleic acid) on the same parameters measured; the lack of predisposed metabolic disorders in the studied population. We also acknowledge that the enrichment of *cis*-9, *trans*-11-CLA in dairy fat is accompanied by changes in other potentially bioactive fatty acids (e.g. *trans*-11-VA); potential healthy implications associated with such products could not be ascribed solely to *cis*-9, *trans*-11-CLA (discussed below).

Isomer-specific effect of conjugated linoleic acid from preclinical studies

One of the major differences between supplemental and ruminant CLA is the isomer composition. In order to delineate differential health effects associated with specific CLA isomers, publications included in the following discussion focused exclusively on individual isomers (i.e. *cis*-9, *trans*-11-CLA and *trans*-10, *cis*-12-CLA) rather than mixtures of CLA isomers. The studies currently available are predominantly from preclinical models rather than from human subjects. Notably, the general dose used in cited animal studies was 0.5% (w/w) for each isomer (equivalent to approximately 1% of daily energy), which appears to be much higher than the common doses used in human clinical trials (e.g. 3.1 g/d of 50:50 isomer mixture, 0.5% daily energy for each isomer based on a 10 460 kJ (2500 kcal) diet). Similarly, the *in vitro* studies cited below generally used supraphysiological doses between 50 and 200 μmol/l, which are difficult to achieve even with supplementation^(48,49). Therefore, caution should be applied when examining these preclinical data so as to avoid over-interpretation.

Anti-obesity effects. The potent effect of *trans*-10, *cis*-12-CLA present in supplemental CLA has been associated with reduced lipid content, the size and number of adipocytes in rats, mice and human subjects, as discussed in Declercq *et al.*⁽⁵⁰⁾ and Park *et al.*⁽⁵¹⁾, but not in hamsters⁽⁵²⁾. The increased mobilisation of fatty acids from adipose tissue was found to be commonly associated with hepatic hypertrophy and steatosis, insulin resistance as well as increased inflammation and decreased *de novo* adipocyte lipogenesis⁽⁵³⁾,

without affecting adipose TAG lipase activity or fatty acid synthesis in mature adipocytes⁽⁵⁴⁾. These changes appear to be mediated by a select expression pattern of key metabolic regulators including: increased proliferative signals in the liver^(55,56), suppressed myogenic differentiation and GLUT4 expression in the muscle⁽⁵⁷⁾ as well as activated AMP-activated protein kinase and c-Jun N-terminal kinase signalling pathways in adipocytes^(58,59) upon supplementation of *trans*-10, *cis*-12-CLA. However, no such effects were reported for the ruminant isomer *cis*-9, *trans*-11-CLA⁽⁶⁰⁾. Interestingly, *trans*-10, *cis*-12-CLA appeared to have an inconsistent effect on the content of lipid in the liver and systemic inflammation in *fa/fa* Zucker rats. Although one study suggested that *trans*-10, *cis*-12-CLA appeared to be beneficial⁽⁶¹⁾, another two studies suggested adverse implications on liver morphology and function^(50,62).

Anti-cardiovascular effects. The *cis*-9, *trans*-11-CLA isomer, which is typically found in dairy products and beef, has recently been shown to reduce the expression of intercellular adhesion molecule 1 and vascular cell adhesion molecule 1 on the surface of endothelial cells as well as to reduce macrophage adhesion to human umbilical vein endothelial cells in cell culture⁽⁶³⁾. *Cis*-9, *trans*-11-CLA has also been found to reduce insulin resistance and associated inflammation in *ob/ob* mice, possibly by improving cellular endoplasmic reticulum stress and redox status⁽⁶⁴⁾. In addition, this 'naturally occurring' isomer has been shown to increase PPAR γ activation and adipocyte differentiation by inhibiting extracellular signal-regulated protein kinases 1 and 2 phosphorylation, whereas *trans*-10, *cis*-12-CLA regulates macrophage metabolism via a different pathway (i.e. p38 phosphorylation) and mediates its apoptotic effect on mammary epithelial cells^(65,66). On the other hand, certain bioactivities in attenuating CVD risk have been associated with the *trans*-10, *cis*-12-CLA isomer but not the *cis*-9, *trans*-11-CLA isomer. Declercq *et al.*^(50,62,67) published a series of studies using the *fa/fa* Zucker rat model (that have established obesity and hypertension). The authors have reported that purified *trans*-10, *cis*-12-CLA (but not *cis*-9, *trans*-11-CLA) effectively reduced systolic blood pressure by 17 mmHg at a dose of 0.4% (w/w) for 8 weeks⁽⁶⁷⁾. Changes in adiponectin levels further induced increased phosphorylated endothelial NO synthase in adipose tissue and the aorta⁽⁶⁷⁾. Similar anti-hypertensive effects have also been observed in young Zucker rats in which *trans*-10, *cis*-12-CLA prevented the increase in systolic blood pressure⁽⁵⁰⁾. *Trans*-10, *cis*-12-CLA has also been associated with the potent immunoregulatory effect on inflammatory cells such as monocytes^(68–70) and polymorphonuclear neutrophilic leucocytes⁽⁷¹⁾ in pigs and bovine animals.

Anti-carcinogenic effects. Preclinical studies that have used a synthetic mixture of CLA isomers during or after chemical carcinogen-induced tumorigenesis have implied anti-cancer efficacy in the mammary gland, colon and skin^(72–75). These differential effects of CLA on tumorigenesis have been primarily demonstrated in *in vitro* models including: colorectal cancer cells; MG63 osteosarcoma cells; MCF-7 breast cancer cells. In terms of isomer-specific effects, *trans*-10, *cis*-12-CLA (but not *cis*-9, *trans*-11-CLA) induced apoptosis

via enhanced AMPK pathways independent of nutrient/energy depletion^(65,76) in a *p53*-mutant rat mammary tumour cell model; however, in a different mammary cell line (MCF-10A), *cis*-9, *trans*-11-CLA has been shown to be a more effective anti-carcinogenic than *trans*-10, *cis*-12-CLA⁽⁷⁷⁾. In the case of colorectal cancers, treatment of *trans*-10, *cis*-12-CLA was associated with suppressed proteasome activity and the accumulation of ubiquitinated substrates in one of the most widely used human colorectal adenocarcinoma cell lines (CaCO₂ cells)⁽⁷⁸⁾. However, none of these changes was observed when CaCO₂ cells were treated with the *cis*-9, *trans*-11-CLA isomer at the same dose and duration. In a different colon cancer cell model, the enrichment of *cis*-9, *trans*-11-CLA in alpine milk lipids (2.7% of fat as *cis*-9, *trans*-11-CLA) showed no additional growth-inhibitory effect in highly transformed HT-29 adenocarcinoma cells relative to conventional milk (0.3% of total fat as *cis*-9, *trans*-11-CLA)⁽⁷⁹⁾. An interesting study was conducted by Bassaganya-Riera & Hontecillas⁽⁸⁰⁾ that assessed the immunoregulatory mechanism of CLA in colorectal cancer, using either a commercial 50:50 CLA mixture or a probiotic mixture that synthesises predominantly *cis*-9, *trans*-11-CLA in the gut lumen of C57BL/6 wild-type mice⁽⁸¹⁾. This study showed that the probiotic mixture (with undetectable amounts of *trans*-10, *cis*-12-CLA) was more effective in decreasing inflammation and reducing disease activity in two colon carcinoma mouse models compared with the commercial CLA product⁽⁸⁰⁾. Nevertheless, data from human subjects on isomer-specific bioactivities remain to be limited and require further investigation.

Section summary. Literature published over the last 2–3 years remains consistent with earlier findings (i.e. before April 2010) that supplemental CLA regimens have shown little effectiveness to the reduction of body fat or CVD risk markers. This may be particularly relevant at higher doses or select population groups. In contrast, the *cis*-9, *trans*-11-CLA isomer from ruminants (in the form of conventional or moderately enriched dairy fat preparations) appears to be associated with neutral to beneficial health outcomes in humans. The consequence of these diverging observations underpins the increasing confusion for public health messaging and food labelling. Since supplemental CLA preparations are fundamentally different from CLA associated with food (and are usually consumed at substantially higher doses), we propose that concerns pertaining to CLA supplementation should be addressed separately from food-related issues and its usage be regulated independently as a nutraceutical or natural health product.

Do trans-fatty acids from ruminant and industrial sources have differential bioactivity?

iTFA isomers, often in the form of *trans*-18:1, originate from the refining process of vegetable oils or fat hardening, aiming at producing edible fat with a more pleasant colour, neutral flavour and odour⁽⁸²⁾ (Fig. 1). However, there are also various *trans*-18:2 fatty acids formed during the heating of vegetable oils in the refinery (e.g. during deodorisation)⁽⁸³⁾. Industrial fats/oils contain appreciable amounts of non-conjugated *trans*-18:2 fats, whereas on the

contrary, ruminant-derived fats contain only traces^(1,84). *Trans*-11–18:1 (VA) is the most predominant TFA isomer in ruminant fat when feeding a high proportion of forage, generally accounting for approximately 70% of the total ruminant *trans*-fat⁽²³⁾. Interestingly, in ruminants, rodents and humans, VA is also the major precursor for the endogenous synthesis of *cis*-9, *trans*-11-CLA^(85,86). In humans, approximately 19–30% of dietary VA is converted to this natural CLA isomer^(86,87). Although VA is also present in industrial fats, the contribution from these commercial sources to the total intake of VA is far below that attributable to seasonal variations of VA in ruminant fat⁽⁸⁸⁾. While it is true that select TFA isomers are found in industrial partially hydrogenated vegetable oils as well as natural ruminant fat, the relative abundance of these individual fatty acid isomers differs significantly. In addition, we note that the majority of *trans*-18:1 isomers in industrial fats have their ethylenic bond between the $\Delta 4$ and $\Delta 10$ positions, whereas most *trans*-18:1 isomers in ruminant fats have their ethylenic bond at position $\Delta 11$ and beyond⁽⁸⁸⁾. It is generally accepted that the TFA profiles of industrial and ruminant *trans*-fat are fundamentally different in their isomer distribution, stereochemistry, physical property as well as their abundance in food sources⁽⁹⁾.

Ruminant-derived trans-fatty acids v. industrial trans-fatty acids: epidemiological and clinical studies

Several epidemiological studies in Europe and the USA have released their latest findings on TFA intake and cardiovascular health outcomes. A few cross-sectional studies have reported a positive association between CVD incidence/major risk factors and *trans*-fat consumption primarily from processed vegetable oils^(89–91). In the National Health and Nutrition Examination Survey (NHANES) cohort, plasma concentrations of all major TFA (both industrial and ruminant) and corresponding LDL-C have declined significantly following the successful implementation of *trans*-fat regulations⁽⁹²⁾. Unfortunately, a more detailed assessment of the association between LDL-C and individual TFA isomers using the NHANES cohort was not feasible due to limited information. However, a large-scale prospective cohort study in Norwegian counties conducted by Laake *et al.*⁽⁹³⁾ has followed 70 000 people over 20 years, and the association of TFA from *i*TFA and *r*TFA with cardiovascular mortality assessed. The authors have reported that dietary TFA intake increased CVD risk irrespective of source, but that the association was not significant for ruminant *trans*-fat in either men or women after several major confounders were accounted for (e.g. dietary saturated fat and cholesterol)⁽⁹³⁾. A recently published prospective cohort study in Denmark has further revealed a weak but significantly inverse association between *r*TFA consumption and weight change at lower intakes, which plateaued above a daily intake of 1.2 g⁽⁹⁴⁾. When specific *i*TFA isomers were studied, non-conjugated *trans*-18:2 have been shown to have a stronger positive relationship with CHD than for other *trans*-fats^(89,95,96). On the contrary, *cis*-9, *trans*-11-CLA in adipose tissue that is linearly correlated with dairy intake⁽⁴³⁾ was significantly lower in patients with diabetes (n 1512) relative

to controls (n 232)⁽⁴⁴⁾. Only a few randomised controlled trials have ever been published using *r*TFA-enriched dairy fat, which collectively appear to have neutral health effects in normolipidaemic subjects (as discussed in the section 'Ruminant conjugated linoleic acid and human health'). Unfortunately, no data have been published thus far using purified preparations of individual *r*TFA isomers in people with increased CVD risk.

Section summary. The findings from recent prospective cohort studies and randomised clinical trials are consistent with earlier systematic reviews^(2,10), showing that moderate consumption of *r*TFA at doses achievable by the diet alone has no adverse effect on CVD risk.

Ruminant-derived trans-fatty acids v. industrial trans-fatty acids: preclinical studies

The consumption of partially hydrogenated vegetable oil as the major source of *i*TFA in animal models has been shown to increase the atherogenic lipoprotein profile⁽⁹⁷⁾, blunt brain neurochemical synthesis⁽⁹⁸⁾ and induce hepatic steatosis, lipid peroxidation and hypertrophy^(99,100). A high consumption of hydrogenated vegetable fat during pregnancy and lactation has also been shown to lead to hypothalamic inflammation and impaired satiety sensing, which promotes deleterious metabolic consequences such as obesity⁽¹⁰¹⁾. Impairment in brain function in *i*TFA-fed rats appears to be consistent with a cross-sectional clinical study that reported a decreased cerebral brain volume and worse cognitive function among those with higher plasma *i*TFA concentrations⁽¹⁰²⁾. Interestingly, non-conjugated 18:2 *i*TFA have been associated with the induction of pro-inflammatory response, endothelial dysfunction⁽¹⁰³⁾ and endothelial cell calcification⁽¹⁰⁴⁾, which in turn could accelerate the development of CVD.

A number of recent *in vitro* cell-culture studies have provided an updated perspective in support of the discretionary bioactivity on cellular metabolic pathways between major *r*TFA and *i*TFA isomers. Iwata *et al.*⁽¹⁰⁵⁾ assessed two major *i*TFA (elaidic acid (EA, *trans*-9-18:1) and linoelaidic acid (*trans*-9, *trans*-12–18:2)) and the most abundant *r*TFA (i.e. *trans*-11–18:1, VA) regarding their individual effect on endothelial function. EA and linoelaidic acid were associated with the increased NF- κ B activation and impairment of endothelial insulin signalling and NO production, consistent with previously reported endothelial dysfunction for industrial *trans*-fat in human subjects^(103,106). On the contrary, such adverse effects were not observed in cells treated with VA. In another *in vitro* study, treatment of EA (but not VA) was associated with impaired cholesterol efflux from mouse and human macrophages⁽¹⁰⁷⁾. The authors have accredited the changes to reduced long-chain PUFA incorporation into membrane phospholipids, thus altered membrane fluidity in EA-treated macrophages⁽¹⁰⁷⁾. The negative effect of EA on *n*-3 long-chain PUFA incorporation is consistent with a recent cross-sectional study assessing maternal *trans*-fat intake and corresponding fetal blood fatty acid composition⁽¹⁰⁸⁾. The distinctive bioactivity on membrane PUFA incorporation between VA and major *i*TFA isomers and subsequent changes in cell signalling pathways may be explained by earlier studies that have

demonstrated that EA (and to a lesser extent linoelaidic acid) are potent inhibitors of $\Delta 5$ desaturation (critical for the biosynthesis of *n*-3 and *n*-6 PUFA). No such effect was shown for VA⁽¹⁰⁹⁾.

Bioactivity of ruminant trans-fatty acids—trans-11-vaccenic acid

There is consistent evidence that purified VA supplementation (6.7% of total fat) substantially improves atherogenic lipid profiles (e.g. TAG, LDL-C, total cholesterol) and improves hepatic steatosis in animal models of dyslipidaemia and the metabolic syndrome^(110–116). It has been further proposed that VA binds to and functionally activates PPAR α and γ , both of which are common targets for lipid-lowering and anti-diabetic medications such as fenofibrates and thiazolidinediones, respectively⁽¹¹⁷⁾. *In vitro* cell-culture studies have also confirmed that VA does not have the same bioactivity as those from partially hydrogenated vegetable oils, such as EA^(105,107). Furthermore, treatment of purified VA at physiological doses (40 μ M) has been shown *in vitro* to effectively attenuate the development of cardiomyocyte hypertrophy by activating PPAR α / γ -dependent pathways⁽¹¹⁷⁾. As discussed earlier in the present review, evidence from randomised clinical trials so far has indicated that CLA/VA-enriched dairy fat can elicit neutral effects on blood lipid variables (LDL-C, HDL-C, total:HDL-C ratio) relative to iTFA in healthy individuals^(47,118,119). Most recently, VA/CLA-enriched dairy fat has been shown to exert a neutral impact on peripheral insulin sensitivity in overweight women, but not significantly different from industrial sources of trans-fat⁽¹²⁰⁾.

Section summary. Recent clinical and preclinical data continue to demonstrate a positive correlation between the consumption of industrial trans-fats and CVD risk measures, whereas this is not the case with a moderate intake of TFA from ruminant sources.

Concluding remarks

As the intake of dietary iTFA gradually declines, the proportion of rTFA to total TFA consumption will subsequently increase, suggesting that a clear understanding of both these forms of TFA will be critical for accurate public health policy. The current Codex definition of TFA encompasses the mandate to reduce the dietary intake of deleterious iTFA, but does not necessarily reflect emerging evidence suggesting differential health implications between iTFA and rTFA. We conclude that health concerns associated with the use of supplemental CLA do not repudiate the exclusion of all forms of CLA from the Codex TFA definition, particularly when using the definition for food-related purposes. Given the emerging differential bioactivity of TFA from industrial *v.* ruminant sources, we advocate that regional nutrition guidelines/policies should focus on eliminating industrial forms of trans-fat from processed foods as opposed to all TFA *per se*.

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