British Journal of Nutrition (2013), **110**, 1369–1383
doi:10.1017/S0007114513001086

Review Article

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

Ye Wang and Spencer D. Proctor*

Metabolic and Cardiovascular Diseases Laboratory, Molecular Cell Biology of Lipids Group, Alberta Diabetes and Mazankowski Alberta Heart Institutes, 4-002J Li Ka Shing Centre for Health Research Innovation, University of Alberta, Edmonton, AB, Canada T6G 2E1

(Submitted 15 August 2012 – Final revision received 8 March 2013 – Accepted 8 March 2013 – First published online 18 April 2013)

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.

* **Corresponding author:** Dr S. D. Proctor, fax +1 780 492 9270, email spencer.proctor@ualberta.ca

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

British Journal of Nutrition

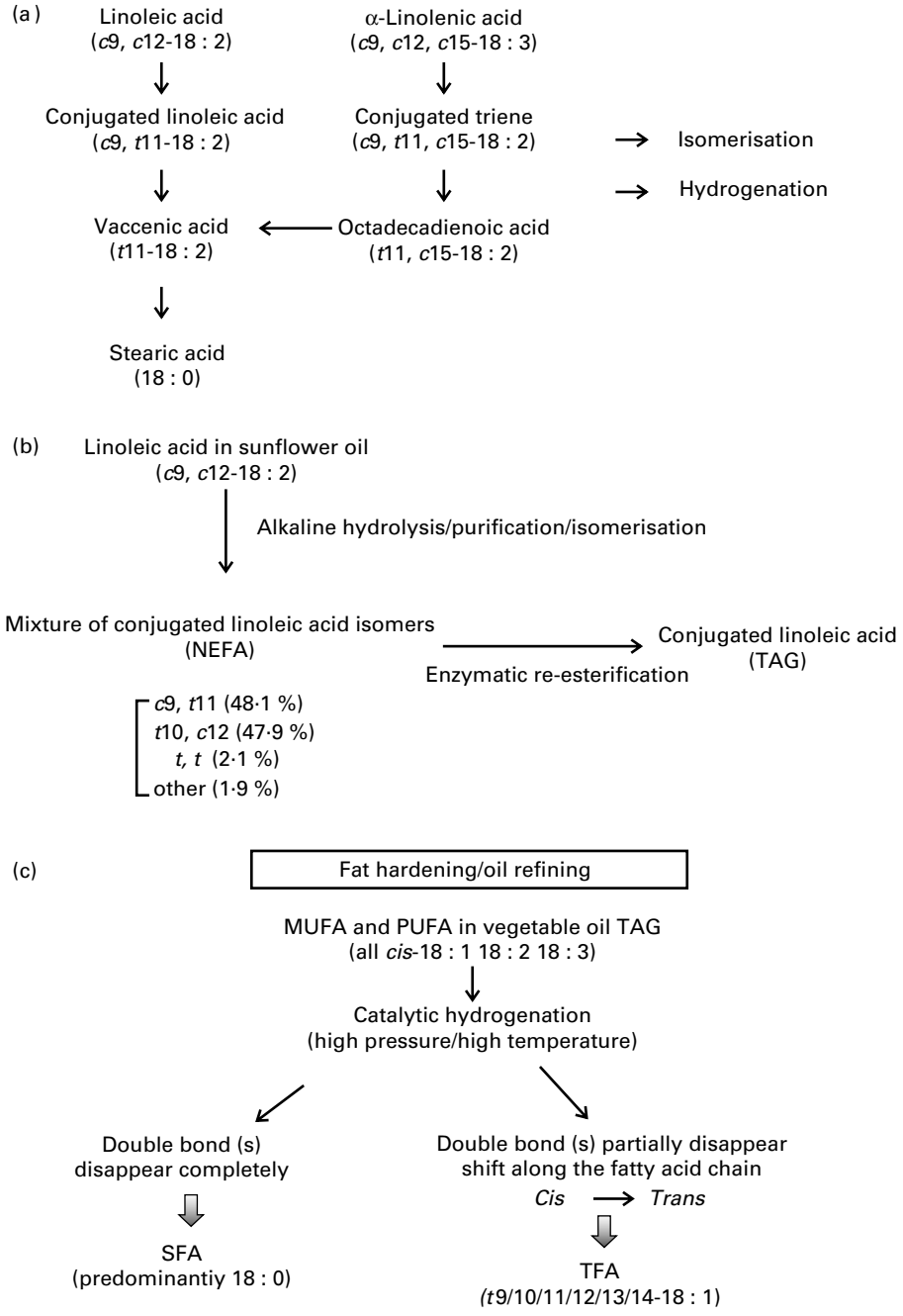

Fig. 1. Schematics of dietary *trans*-fatty acids (TFA) from (a) natural ruminant biohydrogenation, (b) synthetic supplements and (c) industrial partial hydrogenation of vegetable oils.

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 rumenic 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 *et al.*[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 *c*9, *t*11 and *t*10, *c*12-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 *et al.*[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 *c*9, *t*11 and *t*10, *c*12-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 *et al.*[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 *c*9, *t*11 and *t*10, *c*12-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 *et al.*[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; *t*10, *c*12-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 *c*9, *t*11/*t*10, *c*12 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; *t*10, *c*12 but not *c*9, *t*11-CLA reduced HDL while raising TAG and the LDL:HDL ratio	Little effect on body composition, insulin sensitivity or immune function; *t*10, *c*12-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 *et al.*[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 *et al.*[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 *et al.*[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 *et al.*[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-$PGF_{2\alpha}$ but not lipid peroxidation	→
Sluijs *et al.*[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 *et al.*[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 *et al.*[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 *et al.*[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-$PGF_{2\alpha}$	→

Table 2. *Continued*

Reference	Study description	Endpoints	Participant characteristics	Intervention*	Results	Summary†
Sato *et al.*[49]	Randomised, double-blind, parallel	CLA absorption and metabolism in human subjects, the effect on blood lipids	Healthy young adults (50 % men), *n* 24, Japanese population	1·8 g/d CLA (50:50, Nisshin OilliO Group), 3 weeks	↑ CLA blood concentration after supplementation; *trans*-10, *cis*-12-CLA was metabolised faster than *cis*-9, *trans*-11-CLA Compared with linoleic acid: → Blood TAG, total, LDL- and HDL-cholesterol → Blood glucose, AST, ALT	→
Asp *et al.*[40]	Randomised, double-blind, cross-over	Glycaemia, blood lipids, inflammation	Obese postmenopausal women, *n* 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 *et al.*[45]	Randomised, single-blind, cross-over	Blood lipids, liver function and body composition	Overweight adults (67 % men) with nearly optimal plasma LDL-cholesterol concentration, *n* 15, Canadian population	1·0 g/d *cis*-9, *trans*-11-CLA from milk or 1·2 g/d *cis*-9, *trans*-11-CLA and 1·1 g/d *trans*-10, *cis*-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 *et al.*[46]	Randomised, single-blind, cross-over	Insulin sensitivity, body composition, blood lipids	Young non-obese women, *n* 18, US population	1·1 g *cis*-9, *trans*-11-CLA from pasture-fed milk, 8 weeks	Compared with 0·35 g/d *cis*-9, *trans*-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 *et al.*[128]	Randomised, double-blind, cross-over	Cholesterol absorption and synthesis	Healthy men, *n* 48, Canadian population	10·2 g/d ruminant TFA (2·0 g *cis*-9, *trans*-11-CLA), 4 weeks	Compared with a diet low in TFA from any source: ↓ Cholesterol absorption → Cholesterol synthesis → Plasma cholesterol concentration	→
Lacroix *et al.*[47]	Randomised, double-blind, cross-over	Plasma lipid profile	Healthy/overweight women, *n* 61, Canadian population	0·7 g/d *cis*-9, *trans*-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 *et al.*[44]	Case–control	Risk of diabetes	Case: diabetic adults (76 % men, *n* 232) Control: healthy volunteers matched for age, sex, area of residence (67 % men, *n* 1512); Costa Rican population		Higher adipose tissue *cis*-9, *trans*-11-CLA was associated with a 43 % lower risk of diabetes (OR 0·48, 95 % CI 0·31, 0·76)	↓

British Journal of Nutrition

Table 2. *Continued*

Reference	Study description	Endpoints	Participant characteristics	Intervention*	Results	Summary†
Smit *et al.*[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 *cis*-9, *trans*-11-CLA	Higher adipose tissue *cis*-9, *trans*-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 *trans*-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 89:11 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 PPARγ 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 antihypertensive 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 ubiquitinylated substrates in one of the most widely used human colorectal adenocarcinoma cell lines ($CaCO_2$ cells)[78]. However, none of these changes was observed when $CaCO_2$ 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

British Journal of Nutrition

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 Δ4 and Δ10 positions, whereas most *trans*-18 : 1 isomers in ruminant fats have their ethylenic bond at position Δ11 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 iTFA and rTFA 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 rTFA consumption and weight change at lower intakes, which plateaued above a daily intake of 1·2 g[94]. When specific iTFA 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 rTFA-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 rTFA 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 rTFA 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 iTFA 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 iTFA-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 iTFA concentrations[102]. Interestingly, non-conjugated 18 : 2 iTFA 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 rTFA and iTFA isomers. Iwata *et al.*[105] assessed two major iTFA (elaidic acid (EA, *trans*-9-18 : 1) and linoelaidic acid (*trans*-9, *trans*-12–18 : 2)) and the most abundant rTFA (i.e. *trans*-11–18 : 1, VA) regarding their individual effect on endothelial function. EA and linoelaidic acid were associated with the increased NF-κB 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 iTFA 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 Δ5 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 PPARα and γ, both of which are common targets for lipid-lowering and anti-diabetic medications such as fenofibrates and thiazolidinedoines, 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 μM) has been shown *in vitro* to effectively attenuate the development cardiomyocyte hypertrophy by activating PPARα/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*.

Acknowledgements

S. D. P. received research grants from the Alberta Meat and Livestock Agency and the Dairy Farmers of Canada. He also received recognition for his research from the International Dairy Federation. S. D. P. holds a New Investigator Award from the Heart and Stroke Foundation of Canada. Both authors contributed equally to the present review. Neither author has a conflict of interest with the work described herein.

References

1. Mozaffarian D, Aro A & Willett WC (2009) Health effects of *trans*-fatty acids: experimental and observational evidence. *Eur J Clin Nutr* **63**, Suppl. 2, S5–S21.
2. Brouwer IA, Wanders AJ & Katan MB (2010) Effect of animal and industrial *trans* fatty acids on HDL and LDL cholesterol levels in humans – a quantitative review. *PloS one* **5**, e9434.
3. Voorrips LE, Brants HA, Kardinaal AF, *et al.* (2002) Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer. *Am J Clin Nutr* **76**, 873–882.
4. Chavarro JE, Stampfer MJ, Campos H, *et al.* (2008) A prospective study of *trans*-fatty acid levels in blood and risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* **17**, 95–101.
5. Vinikoor LC, Millikan RC, Satia JA, *et al.* (2010) *Trans*-fatty acid consumption and its association with distal colorectal cancer in the North Carolina Colon Cancer Study II. *Cancer Causes Control* **21**, 171–180.
6. Stachowska E (2008) Conjugated dienes of linoleic acid and tumorigenesis. *Ann Acad Med Stetin* **54**, 122–125.
7. Kennedy A, Martinez K, Schmidt S, *et al.* (2010) Antiobesity mechanisms of action of conjugated linoleic acid. *J Nutr Biochem* **21**, 171–179.
8. Reynolds CM & Roche HM (2010) Conjugated linoleic acid and inflammatory cell signalling. *Prostaglandins Leukot Essent Fatty Acids* **82**, 199–204.
9. Gebauer SK, Chardigny JM, Jakobsen MU, *et al.* (2011) Effects of ruminant *trans* fatty acids on cardiovascular disease and cancer: a comprehensive review of epidemiological, clinical, and mechanistic studies. *Adv Nutr* **2**, 332–354.
10. Bendsen NT, Christensen R, Bartels EM, *et al.* (2011) Consumption of industrial and ruminant *trans* fatty acids and risk of coronary heart disease: a systematic review and meta-analysis of cohort studies. *Eur J Clin Nutr* **65**, 773–783.
11. Brouwer IA, Wanders AJ & Katan MB (2013) *Trans* fatty acids and cardiovascular health-research completed? *Eur J Clin Nutr* (epublication ahead of print version 27 March 2013).
12. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2012) Statement on the safety of the "conjugated linoleic acid (CLA)-rich oils" Clarinol and Tonalin TG80 as Novel Food ingredients. *EFSA J* **10**, 2700.
13. United States Food and Drug Administration (2007) GRAS notice inventory-GRN No.232 – conjugated linoleic acid isomers. http://www.accessdata.fda.gov/scripts/fcn/fcnDetailNavigation.cfm?rpt=grasListing&id=232
14. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) (2010) Scientific opinion on the substantiation of health claims related to conjugated linoleic acid (CLA) isomers. *EFSA J* **8**, 1794.
15. Riserus U, Arner P, Brismar K, *et al.* (2002) Treatment with dietary *trans*10*cis*12 conjugated linoleic acid causes isomer-specific insulin resistance in obese men with the metabolic syndrome. *Diabetes Care* **25**, 1516–1521.

16. Riserus U, Basu S, Jovinge S, *et al.* (2002) Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin resistance. *Circulation* **106**, 1925–1929.
17. Riserus U, Vessby B, Arner P, *et al.* (2004) Supplementation with *trans*10*cis*12-conjugated linoleic acid induces hyperproinsulinaemia in obese men: close association with impaired insulin sensitivity. *Diabetologia* **47**, 1016–1019.
18. Food Standards Australia New Zealand (FSANZ) (2009) Intake of *trans* fatty acids in New Zealand and Australia review report-2009 assessment. http://www.foodstandards.gov.au/_srcfiles/TFAs_Aus_NZ_Food%20_Supply.pdf
19. Palmquist DL, Lock AL, Shingfield KJ, *et al.* (2005) Biosynthesis of conjugated linoleic acid in ruminants and humans. *Adv Food Nutr Res* **50**, 179–217.
20. McCrorie TA, Keaveney EM, Wallace JM, *et al.* (2011) Human health effects of conjugated linoleic acid from milk and supplements. *Nutr Res Rev* **24**, 206–227.
21. Health Canada (2010) Conjugated Linoleic Acid. http://www.hc-sc.gc.ca/dhp-mps/prodnatur/applications/licen-prod/monograph/mono_cla-alc-eng.php
22. Tetens I (2010) Scientific Opinion on the safety of "conjugated linoleic acid (CLA)-rich oil" (Tonalin® TG 80) as a Novel Food ingredient. *EFSA J* **8**, 1600.
23. Lock AL & Bauman DE (2004) Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. *Lipids* **39**, 1197–1206.
24. Parodi PW (1999) Conjugated linoleic acid: an anticarcinogenic fatty acid present in milk fat. *J Dairy Sci* **82**, 1339–1349.
25. Fritsche J & Steinhart H (1998) Amounts of conjugated linoleic acid (CLA) in German foods and evaluation of daily intake. *Z Lebensm Unters Forsch* **2065**, 77–82.
26. Maurelli S, Blasi F, Cossignani L, *et al.* (2009) Enzymatic synthesis of structured triacylglycerols containing CLA isomers starting from *sn*-1,3-diacylglycerols. *J Am Oil Chem Soc* **86**, 127–133.
27. Chardigny JM, Masson E, Sergiel JP, *et al.* (2003) The position of rumenic acid on triacylglycerols alters its bioavailability in rats. *J Nutr* **133**, 4212–4214.
28. Valeille K & Martin JC (2004) Complete stereospecific determination of conjugated linoleic acids in triacylglycerol of milk-fat. *Reprod Nutr Dev* **44**, 459–464.
29. Paterson LJ, Weselake RJ, Mir PS, *et al.* (2002) Positional distribution of CLA in TAG of lamb tissues. *Lipids* **37**, 605–611.
30. Yurawecz MP, Hood JK, Mossoba MM, *et al.* (1995) Furan fatty acids determined as oxidation products of conjugated octadecadienoic acid. *Lipids* **30**, 595–598.
31. Sugano M, Tsujita A, Yamasaki M, *et al.* (1997) Lymphatic recovery, tissue distribution, and metabolic effects of conjugated linoleic acid in rats. *J Nutr Biochem* **8**, 38–43.
32. Martin JC, Sebedio JL, Caselli C, *et al.* (2000) Lymphatic delivery and *in vitro* pancreatic lipase hydrolysis of glycerol esters of conjugated linoleic acids in rats. *J Nutr* **130**, 1108–1114.
33. Bracco U (1994) Effect of triglyceride structure on fat absorption. *Am J Clin Nutr* **60**, 1002S–1009S.
34. Gervais R, Gagnon F, Kheadr EE, *et al.* (2009) Bioaccessibility of fatty acids from conjugated linoleic acid-enriched milk and milk emulsions studied in a dynamic *in vitro* gastrointestinal model. *Int Dairy J* **19**, 574–581.
35. Engberink MF, Geleijnse JM, Wanders AJ, *et al.* (2012) The effect of conjugated linoleic acid, a natural *trans* fat from milk and meat, on human blood pressure: results from a randomized crossover feeding study. *J Hum Hypertens* **26**, 127–132.
36. de Roos B, Wanders AJ, Wood S, *et al.* (2011) A high intake of industrial or ruminant *trans* fatty acids does not affect the plasma proteome in healthy men. *Proteomics* **11**, 3928–3934.
37. Smit LA, Katan MB, Wanders AJ, *et al.* (2011) A high intake of *trans* fatty acids has little effect on markers of inflammation and oxidative stress in humans. *J Nutr* **141**, 1673–1678.
38. Wanders AJ, Brouwer IA, Siebelink E, *et al.* (2010) Effect of a high intake of conjugated linoleic acid on lipoprotein levels in healthy human subjects. *PloS one* **5**, e9000.
39. Sluijs I, Plantinga Y, de Roos B, *et al.* (2010) Dietary supplementation with *cis*-9, *trans*-11 conjugated linoleic acid and aortic stiffness in overweight and obese adults. *Am J Clin Nutr* **91**, 175–183.
40. Asp ML, Collene AL, Norris LE, *et al.* (2011) Time-dependent effects of safflower oil to improve glycemia, inflammation and blood lipids in obese, post-menopausal women with type 2 diabetes: a randomized, double-masked, crossover study. *Clin Nutr* **30**, 443–449.
41. Joseph SV, Jacques H, Plourde M, *et al.* (2011) Conjugated linoleic acid supplementation for 8 weeks does not affect body composition, lipid profile, or safety biomarkers in overweight, hyperlipidemic men. *J Nutr* **141**, 1286–1291.
42. Pfeuffer M, Fielitz K, Laue C, *et al.* (2011) CLA does not impair endothelial function and decreases body weight as compared with safflower oil in overweight and obese male subjects. *J Am Coll Nutr* **30**, 19–28.
43. Smit LA, Baylin A & Campos H (2010) Conjugated linoleic acid in adipose tissue and risk of myocardial infarction. *Am J Clin Nutr* **92**, 34–40.
44. Castro-Webb N, Ruiz-Narvaez EA & Campos H (2012) Cross-sectional study of conjugated linoleic acid in adipose tissue and risk of diabetes. *Am J Clin Nutr* **96**, 175–181.
45. Venkatramanan S, Joseph SV, Chouinard PY, *et al.* (2010) Milk enriched with conjugated linoleic acid fails to alter blood lipids or body composition in moderately overweight, borderline hyperlipidemic individuals. *J Am Coll Nutr* **29**, 152–159.
46. Brown AW, Trenkle AH & Beitz DC (2011) Diets high in conjugated linoleic acid from pasture-fed cattle did not alter markers of health in young women. *Nutr Res* **31**, 33–41.
47. Lacroix E, Charest A, Cyr A, *et al.* (2012) Randomized controlled study of the effect of a butter naturally enriched in *trans* fatty acids on blood lipids in healthy women. *Am J Clin Nutr* **95**, 318–325.
48. Zlatanos SN, Laskaridis K & Sagredos A (2008) Conjugated linoleic acid content of human plasma. *Lipids Health Dis* **7**, 34.
49. Sato K, Shinohara N, Honma T, *et al.* (2011) The change in conjugated linoleic acid concentration in blood of Japanese fed a conjugated linoleic acid diet. *J Nutr Sci Vitaminol (Tokyo)* **57**, 364–371.
50. Declercq V, Taylor CG & Zahradka P (2011) Isomer-specific effects of conjugated linoleic acid on blood pressure, adipocyte size and function. *Br J Nutr* **107**, 1413–1421.
51. Park Y, Terk M & Park Y (2011) Interaction between dietary conjugated linoleic acid and calcium supplementation affecting bone and fat mass. *J Bone Miner Metab* **29**, 268–278.
52. Lasa A, Simon E, Churruca I, *et al.* (2011) Effects of *trans*-10, *cis*-12 CLA on liver size and fatty acid oxidation under

energy restriction conditions in hamsters. *Nutrition* **27**, 116–121.
53. Obsen T, Faergeman NJ, Chung S, *et al.* (2012) *Trans*-10, *cis*-12 conjugated linoleic acid decreases *de novo* lipid synthesis in human adipocytes. *J Nutr Biochem* **23**, 580–590.
54. Lasa A, Miranda J, Churruca I, *et al.* (2011) The combination of resveratrol and CLA does not increase the delipidating effect of each molecule in 3T3-L1 adipocytes. *Nutr Hosp* **26**, 997–1003.
55. Ashwell MS, Ceddia RP, House RL, *et al.* (2010) *Trans*-10, *cis*-12-conjugated linoleic acid alters hepatic gene expression in a polygenic obese line of mice displaying hepatic lipidosis. *J Nutr Biochem* **21**, 848–855.
56. Yu J, Yu B, Jiang H, *et al.* (2012) Conjugated linoleic acid induces hepatic expression of fibroblast growth factor 21 through PPAR-alpha. *Br J Nutr* **107**, 461–465.
57. Hommelberg PP, Plat J, Remels AH, *et al.* (2010) *Trans*-10, *cis*-12 conjugated linoleic acid inhibits skeletal muscle differentiation and GLUT4 expression independently from NF-kappaB activation. *Mol Nutr Food Res* **54**, 1763–1772.
58. Jiang S, Chen H, Wang Z, *et al.* (2011) Activated AMPK and prostaglandins are involved in the response to conjugated linoleic acid and are sufficient to cause lipid reductions in adipocytes. *J Nutr Biochem* **22**, 656–664.
59. Martinez K, Kennedy A & McIntosh MK (2011) JNK inhibition by SP600125 attenuates *trans*-10, *cis*-12 conjugated linoleic acid-mediated regulation of inflammatory and lipogenic gene expression. *Lipids* **46**, 885–892.
60. Zhai JJ, Liu ZL, Li JM, *et al.* (2010) Different mechanisms of *cis*-9, *trans*-11- and *trans*-10, *cis*-12- conjugated linoleic acid affecting lipid metabolism in 3T3-L1 cells. *J Nutr Biochem* **21**, 1099–1105.
61. Stringer DM, Zahradka P, Declercq VC, *et al.* (2010) Modulation of lipid droplet size and lipid droplet proteins by *trans*-10, *cis*-12 conjugated linoleic acid parallels improvements in hepatic steatosis in obese, insulin-resistant rats. *Biochim Biophys Acta* **1801**, 1375–1385.
62. DeClercq V, Zahradka P & Taylor CG (2010) Dietary *t*10, *c*12-CLA but not *c*9, *t*11 CLA reduces adipocyte size in the absence of changes in the adipose renin–angiotensin system in fa/fa Zucker rats. *Lipids* **45**, 1025–1033.
63. Stachowska E, Siennicka A, Baskiewcz-Halasa M, *et al.* (2012) Conjugated linoleic acid isomers may diminish human macrophages adhesion to endothelial surface. *Int J Food Sci Nutr* **63**, 30–35.
64. Rungapamestry V, McMonagle J, Reynolds C, *et al.* (2012) Inter-organ proteomic analysis reveals insights into the molecular mechanisms underlying the anti-diabetic effects of *cis*-9, *trans*-11-conjugated linoleic acid in ob/ob mice. *Proteomics* **12**, 461–476.
65. Hsu YC, Meng X, Ou L, *et al.* (2010) Activation of the AMP-activated protein kinase-p38 MAP kinase pathway mediates apoptosis induced by conjugated linoleic acid in p53-mutant mouse mammary tumor cells. *Cell Signal* **22**, 590–599.
66. Stachowska E, Kijowski J, Dziedziejko V, *et al.* (2011) Conjugated linoleic acid regulates phosphorylation of PPAR-gamma by modulation of ERK 1/2 and p38 signaling in human macrophages/fatty acid-laden macrophages. *J Agric Food Chem* **59**, 11846–11852.
67. Declercq V, Taylor CG, Wigle J, *et al.* (2012) Conjugated linoleic acid improves blood pressure by increasing adiponectin and endothelial nitric oxide synthase activity. *J Nutr Biochem* **23**, 487–493.
68. Kim DI, Kim KH, Kang JH, *et al.* (2011) *Trans*-10, *cis*-12-conjugated linoleic acid modulates NF-kappaB activation and TNF-alpha production in porcine peripheral blood mononuclear cells via a PPARgamma-dependent pathway. *Br J Nutr* **105**, 1329–1336.
69. Kim KH, Kim DI, Kim SH, *et al.* (2011) *Trans*-10, *cis*-12-conjugated linoleic acid attenuates tumor necrosis factor-alpha production by lipopolysaccharide-stimulated porcine peripheral blood mononuclear cells through induction of interleukin-10. *Cytokine* **56**, 224–230.
70. Perdomo MC, Santos JE & Badinga L (2011) *Trans*-10, *cis*-12 conjugated linoleic acid and the PPAR-gamma agonist rosiglitazone attenuate lipopolysaccharide-induced TNF-alpha production by bovine immune cells. *Domest Anim Endocrinol* **41**, 118–125.
71. Paek J, Kang JH, Kim SS, *et al.* (2010) *Trans*-10, *cis*-12 conjugated linoleic acid directly enhances the chemotactic activity of porcine peripheral blood polymorphonuclear neutrophilic leukocytes by activating F-actin polymerization *in vitro*. *Res Vet Sci* **89**, 191–195.
72. Kelley NS, Hubbard NE & Erickson KL (2007) Conjugated linoleic acid isomers and cancer. *J Nutr* **137**, 2599–2607.
73. Bialek A, Tokarz A, Dudek A, *et al.* (2010) Influence of diet enriched with conjugated linoleic acids on their distribution in tissues of rats with DMBA induced tumors. *Lipids Health Dis* **9**, 126.
74. Bougnoux P, Hajjaji N, Maheo K, *et al.* (2010) Fatty acids and breast cancer: sensitization to treatments and prevention of metastatic re-growth. *Prog Lipid Res* **49**, 76–86.
75. Heinze VM & Actis AB (2012) Dietary conjugated linoleic acid and long-chain *n*-3 fatty acids in mammary and prostate cancer protection: a review. *Int J Food Sci Nutr* **63**, 66–78.
76. Hsu YC & Ip MM (2011) Conjugated linoleic acid-induced apoptosis in mouse mammary tumor cells is mediated by both G protein coupled receptor-dependent activation of the AMP-activated protein kinase pathway and by oxidative stress. *Cell Signal* **23**, 2013–2020.
77. Rakib MA, Kim YS, Jang WJ, *et al.* (2010) Attenuation of 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced gap junctional intercellular communication (GJIC) inhibition in MCF-10A cells by *c*9, *t*11-conjugated linoleic acid. *J Agric Food Chem* **58**, 12022–12030.
78. Palmieri G, Bergamo P, Luini A, *et al.* (2011) Acylpeptide hydrolase inhibition as targeted strategy to induce proteasomal down-regulation. *PloS one* **6**, e25888.
79. Degen C, Lochner A, Keller S, *et al.* (2011) Influence of *in vitro* supplementation with lipids from conventional and Alpine milk on fatty acid distribution and cell growth of HT-29 cells. *Lipids Health Dis* **10**, 131.
80. Bassaganya-Riera J & Hontecillas R (2010) Dietary conjugated linoleic acid and *n*-3 polyunsaturated fatty acids in inflammatory bowel disease. *Curr Opin Clin Nutr Metab Care* **13**, 569–573.
81. Gorissen L, Raes K, Weckx S, *et al.* (2010) Production of conjugated linoleic acid and conjugated linolenic acid isomers by *Bifidobacterium* species. *Appl Microbiol Biotechnol* **87**, 2257–2266.
82. Martin CA, Milinsk MC, Visentainer JV, *et al.* (2007) *Trans* fatty acid-forming processes in foods: a review. *An Acad Bras Cienc* **79**, 343–350.
83. Kemeny Z, Recseg K, Henon G, *et al.* (2001) Deodorization of vegetable oils: prediction of *trans* polyunsaturated fatty acid content. *J Am Oil Chem Soc* **78**, 973–979.
84. Mozaffarian D (2008) Commentary: ruminant *trans* fatty acids and coronary heart disease – cause for concern? *Int J Epidemiol* **37**, 182–184.

85. Santora JE, Palmquist DL & Roehrig KL (2000) *Trans*-vaccenic acid is desaturated to conjugated linoleic acid in mice. *J Nutr* **130**, 208–215.
86. Turpeinen AM, Mutanen M, Aro A, *et al.* (2002) Bioconversion of vaccenic acid to conjugated linoleic acid in humans. *Am J Clin Nutr* **76**, 504–510.
87. Bhattacharya A, Banu J, Rahman M, *et al.* (2006) Biological effects of conjugated linoleic acids in health and disease. *J Nutr Biochem* **17**, 789–810.
88. Wolff RL, Combe NA, Destaillats F, *et al.* (2000) Follow-up of the delta4 to delta16 *trans*-18 : 1 isomer profile and content in French processed foods containing partially hydrogenated vegetable oils during the period 1995–1999. Analytical and nutritional implications. *Lipids* **35**, 815–825.
89. Micha R, King IB, Lemaitre RN, *et al.* (2010) Food sources of individual plasma phospholipid *trans* fatty acid isomers: the Cardiovascular Health Study. *Am J Clin Nutr* **91**, 883–893.
90. Sartika RA (2011) Effect of *trans* fatty acids intake on blood lipid profile of workers in East Kalimantan, Indonesia. *Malays J Nutr* **17**, 119–127.
91. Varraso R, Kabrhel C, Goldhaber SZ, *et al.* (2012) Prospective study of diet and venous thromboembolism in US women and men. *Am J Epidemiol* **175**, 114–126.
92. Vesper HW, Kuiper HC, Mirel LB, *et al.* (2012) Levels of plasma *trans*-fatty acids in non-Hispanic white adults in the United States in 2000 and 2009. *JAMA* **307**, 562–563.
93. Laake I, Pedersen JI, Selmer R, *et al.* (2011) A prospective study of intake of *trans*-fatty acids from ruminant fat, partially hydrogenated vegetable oils, and marine oils and mortality from CVD. *Br J Nutr* **108**, 743–754.
94. Hansen CP, Berentzen TL, Halkjaer J, *et al.* (2012) Intake of ruminant *trans* fatty acids and changes in body weight and waist circumference. *Eur J Clin Nutr* **66**, 1104–1109.
95. Baylin A, Kabagambe EK, Ascherio A, *et al.* (2003) High 18 : 2 *trans*-fatty acids in adipose tissue are associated with increased risk of nonfatal acute myocardial infarction in Costa Rican adults. *J Nutr* **133**, 1186–1191.
96. Lemaitre RN, King IB, Mozaffarian D, *et al.* (2006) Plasma phospholipid *trans* fatty acids, fatal ischemic heart disease, and sudden cardiac death in older adults: The Cardiovascular Health Study. *Circulation* **114**, 209–215.
97. Kraft J, Spiltoir JI, Salter AM, *et al.* (2011) Differential effects of the *trans*-18 : 1 isomer profile of partially hydrogenated vegetable oils on cholesterol and lipoprotein metabolism in male F1B hamsters. *J Nutr* **141**, 1819–1826.
98. Teixeira AM, Dias VT, Pase CS, *et al.* (2012) Could dietary *trans* fatty acids induce movement disorders? Effects of exercise and its influence on NaK-ATPase and catalase activity in rat striatum. *Behav Brain Res* **226**, 504–510.
99. Collison KS, Zaidi MZ, Saleh SM, *et al.* (2012) Nutrigenomics of hepatic steatosis in a feline model: effect of monosodium glutamate, fructose, and *trans*-fat feeding. *Genes Nutr* **7**, 265–280.
100. Dhibi M, Brahmi F, Mnari A, *et al.* (2011) The intake of high fat diet with different *trans* fatty acid levels differentially induces oxidative stress and non alcoholic fatty liver disease (NAFLD) in rats. *Nutr Metab* **8**, 65.
101. Pimentel GD, Lira FS, Rosa JC, *et al.* (2012) Intake of *trans* fatty acids during gestation and lactation leads to hypothalamic inflammation via TLR4/NFkappaBp65 signaling in adult offspring. *J Nutr Biochem* **23**, 265–271.
102. Bowman GL, Silbert LC, Howieson D, *et al.* (2012) Nutrient biomarker patterns, cognitive function, and MRI measures of brain aging. *Neurology* **78**, 241–249.
103. Harvey KA, Arnold T, Rasool T, *et al.* (2008) *Trans*-fatty acids induce pro-inflammatory responses and endothelial cell dysfunction. *Br J Nutr* **99**, 723–731.
104. Kummerow FA, Zhou Q & Mahfouz MM (1999) Effect of *trans* fatty acids on calcium influx into human arterial endothelial cells. *Am J Clin Nutr* **70**, 832–838.
105. Iwata NG, Pham M, Rizzo NO, *et al.* (2011) *Trans* fatty acids induce vascular inflammation and reduce vascular nitric oxide production in endothelial cells. *PloS one* **6**, e29600.
106. Mozaffarian D (2006) *Trans* fatty acids – effects on systemic inflammation and endothelial function. *Atheroscler Suppl* **7**, 29–32.
107. Fournier N, Attia N, Rousseau-Ralliard D, *et al.* (2012) Deleterious impact of elaidic fatty acid on ABCA1-mediated cholesterol efflux from mouse and human macrophages. *Biochim Biophys Acta* **1821**, 303–312.
108. Enke U, Jaudszus A, Schleussner E, *et al.* (2011) Fatty acid distribution of cord and maternal blood in human pregnancy: special focus on individual *trans* fatty acids and conjugated linoleic acids. *Lipids Health Dis* **10**, 247.
109. Rosenthal MD & Doloresco MA (1984) The effects of *trans* fatty acids on fatty acyl delta 5 desaturation by human skin fibroblasts. *Lipids* **19**, 869–874.
110. Tyburczy C, Major C, Lock AL, *et al.* (2009) Individual *trans* octadecenoic acids and partially hydrogenated vegetable oil differentially affect hepatic lipid and lipoprotein metabolism in golden Syrian hamsters. *J Nutr* **139**, 257–263.
111. Wang Y, Jacome-Sosa MM, Ruth MR, *et al.* (2009) *Trans*-11 vaccenic acid reduces hepatic lipogenesis and chylomicron secretion in JCR:LA-cp rats. *J Nutr* **139**, 2049–2054.
112. Bassett CM, Edel AL, Patenaude AF, *et al.* (2010) Dietary vaccenic acid has antiatherogenic effects in LDLr − /− mice. *J Nutr* **140**, 18–24.
113. Jacome-Sosa MM, Lu J, Wang Y, *et al.* (2010) Increased hypolipidemic benefits of *cis*-9, *trans*-11 conjugated linoleic acid in combination with *trans*-11 vaccenic acid in a rodent model of the metabolic syndrome, the JCR:LA-cp rat. *Nutr Metab* **7**, 60.
114. Anadon A, Martinez-Larranaga MR, Martinez MA, *et al.* (2011) A 4-week repeated oral dose toxicity study of dairy fat naturally enriched in vaccenic, rumenic and alpha-linolenic acids in rats. *J Agric Food Chem* **59**, 8036–8046.
115. Sun X, Zhang J, Macgibbon AK, *et al.* (2011) Bovine milk fat enriched in conjugated linoleic and vaccenic acids attenuates allergic dermatitis in mice. *Clin Exp Allergy* **41**, 729–738.
116. Van Nieuwenhove CP, Cano PG, Perez-Chaia AB, *et al.* (2011) Effect of functional buffalo cheese on fatty acid profile and oxidative status of liver and intestine of mice. *J Med Food* **14**, 420–427.
117. Wang Y, Jacome-Sosa MM, Ruth MR, *et al.* (2012) The intestinal bioavailability of vaccenic acid and activation of peroxisome proliferator-activated receptor-α and -γ in a rodent model of dyslipidemia and the metabolic syndrome. *Mol Nutr Food Res* **56**, 1234–1246.
118. Chardigny JM, Destaillats F, Malpuech-Brugere C, *et al.* (2008) Do *trans* fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the *trans* Fatty Acids Collaboration (TRANSFACT) study. *Am J Clin Nutr* **87**, 558–566.

119. Motard-Belanger A, Charest A, Grenier G, *et al.* (2008) Study of the effect of *trans* fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. *Am J Clin Nutr* **87**, 593–599.
120. Tardy AL, Lambert-Porcheron S, Malpuech-Brugere C, *et al.* (2009) Dairy and industrial sources of *trans* fat do not impair peripheral insulin sensitivity in overweight women. *Am J Clin Nutr* **90**, 88–94.
121. Onakpoya IJ, Posadzki PP, Watson LK, *et al.* (2012) The efficacy of long-term conjugated linoleic acid (CLA) supplementation on body composition in overweight and obese individuals: a systematic review and meta-analysis of randomized clinical trials. *Eur J Nutr* **51**, 127–134.
122. Schoeller DA, Watras AC & Whigham LD (2009) A meta-analysis of the effects of conjugated linoleic acid on fat-free mass in humans. *Appl Physiol Nutr Metab* **34**, 975–978.
123. Whigham LD, Watras AC & Schoeller DA (2007) Efficacy of conjugated linoleic acid for reducing fat mass: a meta-analysis in humans. *Am J Clin Nutr* **85**, 1203–1211.
124. Lenz TL & Hamilton WR (2004) Supplemental products used for weight loss. *J Am Pharm Assoc* **44**, 59–67, quiz 67–58.
125. Salas-Salvadó J, Marquez-Sandoval F & Bullo M (2006) Conjugated linoleic acid intake in humans: a systematic review focusing on its effect on body composition, glucose, and lipid metabolism. *Crit Rev Food Sci Nutr* **46**, 479–488.
126. Tricon S & Yaqoob P (2006) Conjugated linoleic acid and human health: a critical evaluation of the evidence. *Curr Opin Clin Nutr Metab Care* **9**, 105–110.
127. Bachmair EM, Bots ML, Mennen LI, *et al.* (2012) Effect of supplementation with an 80:20 *cis*9, *trans*11 conjugated linoleic acid blend on the human platelet proteome. *Mol Nutr Food Res* **56**, 1148–1159.
128. Labonte ME, Couture P, Paquin P, *et al.* (2011) Comparison of the impact of *trans* fatty acids from ruminant and industrial sources on surrogate markers of cholesterol homeostasis in healthy men. *Mol Nutr Food Res* **55**, Suppl. 2, S241–S247.

British Journal of Nutrition