Effects of Ruminant *trans* Fatty Acids on Cardiovascular Disease and Cancer: A Comprehensive Review of Epidemiological, Clinical, and Mechanistic Studies[1–3]

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ABSTRACT

There are 2 predominant sources of dietary *trans* fatty acids (TFA) in the food supply, those formed during the industrial partial hydrogenation of vegetable oils (iTFA) and those formed by biohydrogenation in ruminants (rTFA), including vaccenic acid (VA) and the naturally occurring isomer of conjugated linoleic acid, *cis*-9, *trans*-11 CLA (c9,t11-CLA). The objective of this review is to evaluate the evidence base from epidemiological and clinical studies to determine whether intake of rTFA isomers, specifically VA and c9,t11-CLA, differentially affects risk of cardiovascular disease (CVD) and cancer compared with iTFA. In addition, animal and cell culture studies are reviewed to explore potential pro- and antiatherogenic mechanisms of VA and c9,t11-CLA. Some epidemiological studies suggest that a positive association with coronary heart disease risk exists between only iTFA isomers and not rTFA isomers. Small clinical studies have been conducted to establish cause-and-effect relationships between these different sources of TFA and biomarkers or risk factors of CVD with inconclusive results. The lack of detection of treatment effects reported in some studies may be due to insufficient statistical power. Many studies have used doses of rTFA that are not realistically attainable via diet; thus, further clinical studies are warranted. Associations between iTFA intake and cancer have been inconsistent, and associations between rTFA intake and cancer have not been well studied. Clinical studies have not been conducted investigating the cause-and-effect relationship between iTFA and rTFA intake and risk for cancers. Further research is needed to determine the health effects of VA and c9,t11-CLA in humans. *Adv. Nutr. 2: 332–354, 2011.*

Introduction

Cardiovascular disease (CVD)[11] and cancer are the leading causes of death in the US (1). Studies have shown that modulation of diet, specifically dietary fat, may be an effective strategy in reducing risk of both CVD and cancer (2–6). In recent years, the implication of *trans* fatty acids (TFA) to public health has received increasing attention. TFA refer to a class of fatty acids that contain one or more double bonds in the *trans* configuration. Most dietary TFA are generated by the partial hydrogenation of vegetable oil and were introduced into commercial solid edible fats as a way to increase shelf life of foods and to replace animal fats (i.e. lard, tallow, and butter). Although observational epidemiological studies strongly support positive associations between coronary heart disease (CHD) and intake of TFA from industrial origin (7–10), associations between intake of industrially produced TFA (iTFA) and cancer have been less consistent (11). The increased risk of CHD has been associated with the

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[11] Abbreviations used: AMI, acute myocardial infarction; CVD, cardiovascular disease; c9, t11-CLA, *cis*-9, *trans*-11 CLA; CHD, coronary heart disease; EA, elaidic acid; ER, estrogen receptor; HDL-C, HDL-cholesterol; iTFA, industrially produced *trans* fatty acid; LDL-C, LDL-cholesterol; LA, linoleic acid; MNU, methylnitrosourea; MI, myocardial infarction; NEFA, nonesterified fatty acid; OA, oleic acid; PHVO, partially hydrogenated vegetable oil; rTFA, ruminant *trans* fatty acid; SMC, smooth muscle cell; TC, total cholesterol; TFA, *trans* fatty acid; t10,c12-CLA, *trans*-10, *cis*-12 CLA; VA, vaccenic acid.

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intake of total TFA, as well as foods known to contain major sources of iTFA, such as margarine, cakes, and cookies, in several studies (7–10). Furthermore, iTFA has been shown to adversely affect multiple CVD risk factors, including increasing plasma concentrations of lipids and lipoproteins and inflammatory markers and impairing endothelial function [reviewed in (12)]. Results of quantitative analyses suggest that if partially hydrogenated vegetable oil (PHVO) were replaced with alternative fats and oils, the risk of CHD may be reduced by as much as 50% (13). As a result of the consistent evidence demonstrating numerous adverse effects of iTFA on various markers of health, efforts have been made in the past decade to remove iTFA from the food supply and restaurants. Despite these advances, a paradox remains in that the scientific literature has begun to differentiate between TFA found in synthetically produced oils and TFA that are produced naturally by ruminant animals, including *trans*-11 18:1 [vaccenic acid (VA)] and the *cis*-9, *trans*-11 isomer of CLA, c9,t11-CLA.

Ruminant TFA (rTFA) are naturally occurring fatty acids that are synthesized via bacterial metabolism of unsaturated fatty acids in ruminant animals and found in ruminant-derived foods (i.e. beef, lamb, and dairy). Both c9,t11-CLA and VA are produced during the biohydrogenation of C18 unsaturated fatty acids, primarily linoleic acid (LA), and α-linolenic acid (14). The c9,t11-CLA isomer comprises ~90% of total CLA isomers (15). VA is the predominant *trans* monoene isomer in ruminant fats (50–80% of total *trans* fat) (14). It is estimated that over 80% of c9,t11-CLA in ruminant fat is endogenously synthesized by Δ9-desaturase using VA as the substrate (16). Humans and rodents also possess the ability to desaturate VA to c9,t11-CLA. In the largest study in healthy adults, the average estimate for conversion in humans was 19% (with inter-individual differences depending on intake of VA and other fatty acids) (17) and has been reported to range from 0 to >30% (17–19). Despite the potential beneficial effects of c9,t11-CLA that have been demonstrated in some studies, very few studies have investigated the effects of VA, specifically, on health indices. Recently, agricultural scientists have made efforts to increase the c9,t11-CLA content of ruminant fats, which has resulted in simultaneous elevated VA production and decreased SFA (20–22). VA also is present in hydrogenated plant oils, contributing to ~13–17% of total VA intake (23). The bioactivity of VA per se and how it could affect chronic disease remains unclear. The increase in the proportion of rTFA in dairy-derived products has complicated the recommendations to minimize total dietary TFA; thus, it is essential to better understand the bioactivity, as well as the health effects, of specific rTFA isomers.

Although there is considerable evidence supporting a positive association between TFA intake and CHD risk, there are only a limited number of studies that have attempted to distinguish the association of CHD risk between iTFA and rTFA. Some epidemiological studies suggest that a positive association with CHD risk exists between TFA isomers generated by industrial means and not isomers formed through biohydrogenation reactions. There has been some acknowledgment that rTFA isomers, including c9,t11-CLA, exhibit differential health effects than PHVO-derived iTFA; the definition of TFA in the Codex Alimentarius standard, as well as official dietary recommendations of countries such as the US, Canada, and Denmark, have been amended to exclude TFA isomers with conjugated double bonds for labeling purposes. Despite the recognition that some rTFA may elicit differential biological effects, the data to date have not been sufficiently comprehensive. In particular, few studies have investigated the effects of VA.

In this paper, we review the evidence base from epidemiological and clinical studies to determine whether intake of rTFA isomers, specifically VA and c9,t11-CLA (when data are available), differentially affects risk of CVD and cancer compared with iTFA. In addition, animal and cell culture studies are reviewed to explore potential pro- and antiatherogenic mechanisms of VA and c9,t11-CLA.

Current status of knowledge

Food composition and dietary intake of ruminant TFA

Ruminant TFA constitute a relatively small portion of the fat in dairy products (typically 2–5% of total fatty acids) and beef and lamb (3–9% of total fatty acids) (24,25), with variations in fatty acid compositions due to feeding practices as well as geographical and seasonal change (25,26). In contrast, PHVO can consist of up to 60% of total fatty acids as TFA (27). The amounts of TFA in commonly consumed

Table 1. Amounts of TFA in commonly consumed ruminant products[1]

Food	Total fat (g/100 g)	TFA (g/100 g)	TFA (% total fat)	TFA (g/serving)	Total CLA (mg/g fat)[2]
Dairy products					
Cheese, cheddar (28 g, 1 oz)	36.4	0.87	2.39	0.24	3.6 (93)
Milk, whole (244 g, 1 cup)[3]	3.10	0.09	2.90	0.21	5.5 (92)
Yogurt, plain, low-fat (255 g, 1 cup)	1.16	0.03	2.59	0.06	4.4 (86)
Meat					
Meat, beef, ground, 20.8% fat, raw (115 g, 4 oz)	21	0.79	3.76	0.91	4.3 (85)
Meat, beef, ground, 22.1% fat, raw (115 g, 4 oz)	22.1	0.93	4.21	1.07	4.3 (85)

[1] Source of data, except for CLA: (173).
[2] Source of CLA data: (174). Values in parentheses represent percentage of CLA as c9,t11 isomer. Percentage fat of ground beef was not specified in this study.
[3] Values were averaged from April, January, July, and November.

ruminant products are presented in **Table 1**. As is evident from the food composition data and dietary intake data (below), it is difficult to consume very high amounts of rTFA in a typical diet.

In 2002, the panel on Macronutrients of the U.S. National Academies, Institute of Medicine recommended that TFA consumption be as low as possible in a nutritionally adequate diet (28). Subsequently, in 2003, the WHO recommended that TFA intake be limited to <1% of overall energy consumption (29). As a result, recent consensus indicates a decline of total TFA consumption from an average of 10 g/d worldwide (equivalent to 4.5% of energy within a 2000-kcal diet) to 3–4 g/d in North America, 2–4 g/d in northern European countries such as Denmark and The Netherlands, 1–3 g/d in Mediterranean countries, and <1 g/d in eastern Asian countries (equivalent to <0.45–1.8% of energy within a 2000-kcal diet) (30,31). It is important to note that estimates of dietary intake vary depending on the method used to assess consumption (i.e. food disappearance data, dietary consumption, analysis of composite diets, or TFA content of biological tissues/biomarker data) (30). Intake estimates are further complicated by inaccurate food databases due to quickly changing products on the market.

Traditionally, intake of total TFA was considered independent of the origin (ruminant vs. industrially produced) (32); however, more recent studies have separately estimated intake of rTFA. In the TRANSFAIR study, TFA derived from milk and ruminant fat ranged from 28 to 79% in the 14 western European countries studied (31). In the Mediterranean countries, >50% of total TFA intake came from ruminant sources. More recent studies have published national data of individual countries (10,33–37). The dietary intake of rTFA from various countries is presented in **Table 2** (intake data for VA and c9,t11-CLA are presented when available). Intake of VA and c9,t11-CLA ranges from 0.4 to 0.8 g/d and 0.14 to 0.33 g/d, respectively. The average intake of CLA varies considerably due to distinctive dietary patterns and variations in fatty acid composition of dairy products. In European and Australian populations, rTFA intake represents ~63–75% of total TFA intake. In the US, intake of rTFA represents ~20% of total TFA intake, with >85% of rTFA coming from milk fat (38). More recently, it has been recognized that the endogenous in vivo synthesis of c9,t11-CLA also may contribute to whole body concentrations of this isomer. Data suggest that the total contribution of ruminant fat to body CLA status may be on average 1.5 times the CLA content because of endogenous synthesis from VA (17). Thus, the estimation of dietary intake of c9,t11-CLA alone may not adequately represent the endogenous synthesis from VA. It is important to note that a lack of standardization may affect previous estimations of TFA content in foods. Many of the earlier studies may have underestimated the intake of c9,t11-CLA and VA due to a lack of separation and resolution of the *trans* isomers by GC. Conversely, it also is possible that intake may have been overestimated if other CLA isomers coeluted with c9,t11-CLA.

Table 2. Dietary intake of ruminant TFA (individual isomer data reported when available)[1]

	Ruminant TFA	VA	c9,t11-CLA
		g/d	
Denmark	1.7 (33)	0.6 (175)	0.25 (175)
France	1.7 (31), 1.3 (34)	0.7 (175)	0.30 (31)
Germany	1.7 (31)	0.7 (175)	0.28 (175), 0.39 (176)
Netherlands	1.2 (31), 1.7 (10)	0.6 (175)	0.23 (175)
New Zealand	1.1 (35)	—	—
Australia	1.0 (35)	—	—
Spain	1.3 (31)	0.4 (175)	0.14 (175)
Sweden	1.3 (31)	0.8 (30)	0.33 (175)
Italy	1.2 (31)	0.5 (175)	0.22 (175)
United States	1.2 (177)	–	0.18 (37)
United Kingdom	0.98 (31)	0.5 (175)	0.21 (175)
Greece	0.8 (31)	0.4 (175)	0.15 (175)

[1] Intake of ruminant TFA from the TRANSFAIR study (31) was calculated by summation of the percentage of energy of TFA from each category of ruminant food sources (milk and milk products, cheese, meat and meat products, and butter) multiplied by total TFA intake (g/d).

Cardiovascular disease

Review of epidemiological studies. Numerous epidemiological studies have investigated the associations of intake of rTFA and CHD (**Table 3**). In the Scottish Heart Health Study (39), a cross-sectional study, energy-adjusted intake of rTFA and iTFA was not statistically associated with CHD after controlling for CHD risk factors among women. The OR for intake of rTFA and iTFA, when comparing the highest and lowest quintiles of intake, were 0.96 (95% CI = 0.58–1.59; *P*-trend = 0.78) and 1.26 (95% CI = 0.92–1.72; *P*-trend = 0.11), respectively. Interestingly, in men, the intake of rTFA was inversely associated with CHD risk (highest vs. lowest quintile; OR = 0.65; 95% CI = 0.41–1.04; *P*-trend = 0.02) after controlling for CHD risk factors.

There have been multiple case-control studies that have investigated the associations of rTFA and CHD. Ascherio et al. (40) found no association between the intake of rTFA and risk of first acute myocardial infarction (AMI) but did find a direct relationship between iTFA and AMI risk after controlling for other risk factors. In this study, there was a trend for a positive association between absolute intake of rTFA and risk of AMI but not for energy-adjusted intake. In a nested case-control study from the Nurses' Health Study population, concentrations of VA in erythrocytes were higher in cases than in controls after controlling for CHD risk factors [0.40 ± 0.10 vs. 0.38 ± 0.11% of total fatty acids, respectively) ($P = 0.05$) (8). In a more recent case-control study in Costa Rica, the concentration of c9,t11-CLA in adipose tissue was associated with a lower risk of myocardial infarction (MI; highest vs. lowest quintile; OR = 0.51; 95% CI = 0.36–0.71; *P*-trend < 0.0001) (41). There was a strong association of SFA intake and risk of MI (highest vs. lowest quintile; OR = 1.68; 95% CI = 1.29–2.19; *P*-trend = 0.0001); however, interestingly, there was an inverse association of dairy intake and risk of MI after multivariate adjustment (highest vs. lowest quintile; OR = 0.75; 95% CI = 0.58–0.98; *P*-trend = 0.03). After further adjustment for c9,t11-CLA in adipose tissue, the association

Table 3. Associations of intake of rTFA with CHD and cancer: summary of epidemiological studies[1]

Reference	Study description	Participant characteristics	Assessment of intake	Endpoints	Results[2]	Conclusions/comments	Summary[3]
CHD							
Bolton-Smith et al. (39)	Scottish Heart Study; cross-sectional	*n* = 10,359; Scottish women and men	Energy-adjusted intake of rTFA and iTFA (FFQ)	Risk of CHD	rTFA, men: OR = 0.65 (0.41, 1.04); *P* = 0.02. rTFA, women: OR = 0.96, 95% CI = 0.58–1.59; *P*-trend = 0.78.	Inverse association between rTFA and CHD in men. No association between rTFA and CHD among women.	↓ in men → in women
Ascherio et al. (40)	Case-control	*n* = 239 Men and women with first AMI (cases), *n* = 282 controls; U.S. population	Absolute and energy-adjusted intake of rTFA (FFQ)	Risk of first AMI	Absolute rTFA: OR = 1.23 (0.60, 2.50); *P* = 0.09. Energy-adjusted rTFA: OR = 1.02, 95% CI = 0.43–2.41; *P*-trend = 0.57.	Trend for a positive association between absolute intake of rTFA and risk of AMI. No association between energy-adjusted intake of rTFA and risk of AMI.	→
Sun et al. (8)	Nurses' Health Study; nested case-control	*n* = 166 Incident cases with CHD, *n* = 327 controls; U.S. women	Concentration of VA in erythrocytes	Risk of CHD	% of total fatty acids: 0.40 ± 0.10 (cases) vs. 0.38 ± 0.11 (controls); *P* = 0.05. Total 18:1t associated with increased risk of CHD; *P*-trend < 0.01. Associations for individual 18:1t isomers were similar to total 18:1t.	Concentration of VA in erythrocytes was higher in cases than controls. Direct association between VA and CHD.	→/↑
Smit et al. (41)	Case-control	*n* = 1813 Incident cases of a first AMI, *n* = 1813 controls; Costa Rican population	Concentration of c9,t11-CLA in adipose tissue	Risk of MI	OR = 0.51, 95% CI = 0.36–0.71; *P*-trend < 0.0001.	Adipose c9,t11-CLA associated with lower risk of MI.	↓
Willett et al. (7)	Nurses' Health Study; prospective	*n* = 69,181 Women; U.S. population	Energy-adjusted dietary intake of rTFA (FFQ)	Risk of CHD	RR = 0.59, 95% CI = 0.30–1.17; *P*-trend = 0.230.	No significant association between rTFA and CHD. Trend for inverse association.	→
Oomen et al. (10)	Zutphen Elderly Study; prospective	*n* = 667 Men (64–84 y); Dutch population	Energy-adjusted dietary intake of rTFA (dietary surveys)	Risk of CHD	RR (0.5 E%) = 1.17, 95% CI = 0.69–1.98.	No significant association between rTFA and CHD. Trend for a direct association.	→
Jakobsen et al. (43)	Prospective	*n* = 3686 Men and women; Danish population	Absolute and energy-adjusted dietary intake of rTFA (7-d weighed food records, dietary interviews)	Risk of CHD	Absolute rTFA: RR (0.5 E%) = 0.97 (0.91, 1.04). Energy-adjusted rTFA: RR (0.5 E%) = 1.05, 95% CI = 0.92–1.19.	No significant association between rTFA and CHD. Trend for inverse association among women.	→
Pietinen et al. (42)	The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study; prospective	*n* = 21,930 Male smokers; Finnish population	Energy-adjusted dietary intake of rTFA (FFQ)	Risk of coronary death	RR = 0.83 (0.62–1.11); *P*-trend = 0.035.	Inverse association between rTFA intake and CHD.	↓

(Continued)

Table 3. (*Continued*)

Reference	Study description	Participant characteristics	Assessment of intake	Endpoints	Results[2]	Conclusions/comments	Summary[3]
Cancer							
Aro et al. (104)	Case-control	n = 195 Consecutive women with breast cancer, n = 208 (controls); Finnish population	Dietary intake (FFQ) and serum concentrations of VA and CLA	Risk of breast cancer	Serum VA: OR = 0.3, 95% CI = 0.1–0.7. Serum CLA: OR = 0.4, 95% CI = 0.2–0.9. Similar OR after adjustment for known risk factors of breast cancer.	Inverse association of serum VA and breast cancer among postmenopausal women. Significantly lower dietary CLA and serum VA and CLA in cases among postmenopausal women.	↓ postmenopausal women
Voorrips et al. (105)	Netherlands Cohort Study on Diet and Cancer; prospective	n = 941 Incident cases of breast cancer; Dutch population	Energy-adjusted dietary intake of VA and CLA (FFQ)	Risk of breast cancer	VA: RR = 1.34, 95% CI = 0.98–1.82; P-trend = 0.006. CLA: RR = 1.24, 95% CI = 0.91, 1.69; P-trend = 0.02.	VA intake associated with an increased risk of breast cancer. CLA intake weakly associated with breast cancer incidence. Note: no relationship between breast cancer incidence and intake of animal fat intake or milk/milk products and meat from ruminants.	↑
Rissanen et al. (106)	Nested case-control	n = 127 Incident breast cancer cases, n = 242 controls; Finnish population	Serum VA	Risk of breast cancer	OR = 3.69, 95% CI = 1.35–10.06.	Direct association of serum VA and breast cancer risk. Note: association stronger in postmenopausal women.	↑
King et al. (107)	β-Carotene and Retinol Efficacy Trial (CARET); nested case-control	n = 272 Men (cases), n = 426 men (controls); U.S. population	Concentration of serum phospholipid VA	Risk of prostate cancer	OR = 1.69, 95% CI = 1.03–2.77; P-trend = 0.04.	Direct association of serum VA and prostate cancer risk.	↑
Shannon et al. (108)	Case-control	n = 322 Women with breast cancer, n = 1030 (controls); Chinese population	Concentration of VA in erythrocytes	Risk of breast cancer	OR 2.21, 95% CI = 1.25, 3.88; P-trend = 0.002.	Direct association of VA in erythrocytes and breast cancer risk.	↑
Chajes et al. (2002) (109)	Case-control	n = 241 with invasive breast cancer (cases), n = 88 with benign breast pathologies; French population	Concentration of CLA in adipose tissue	Risk of breast cancer	OR = 1.83, 95% CI = 0.77–2.65; P-trend = 0.27.	No significant association of CLA and breast cancer risk.	→
Larsson et al. (110)	Swedish Mammography Cohort; case-control	n = 798 Incident cases of colorectal cancer; Swedish population	Dietary intake of CLA (FFQ)	Risk of colorectal cancer	RR = 0.71, 95% CI = 0.55–0.91; P-trend = 0.004.	Inverse association of dietary intake of CLA and colorectal cancer risk.	↓

(Continued)

Table 3. (*Continued*)

Reference	Study description	Participant characteristics	Assessment of intake	Endpoints	Results[2]	Conclusions/comments	Summary[3]
McCann et al. (111)	Western New York Exposures and Breast Cancer Study (WEB Study); case-control	*n* = 1122 Women with primary, incident, breast cancer (cases), *n* = 2036 (controls)	Dietary intake of CLA (FFQ, in-person interviews)	Risk of breast cancer	In postmenopausal women: OR = 1.17 (0.85–1.62). In premenopausal women: OR = 0.84 (0.50–1.43). In premenopausal women, ER-negative tumors: OR = 0.40, 95% CI = 0.16–1.01.	No significant association of c9,t11-CLA and overall risk of premenopausal and postmenopausal breast cancer. Reduced risk of having ER-negative tumors in premenopausal women.	→ overall ↓ ER-negative, premenopausal
Chajes et al. (2003) (112)	Prospective	*n* = 209 Patients with initially localized breast cancer; French population	Concentration of CLA in breast adipose tissue	Risk of metastasis or death	Breast adipose tissue CLA was low with narrow range: 0.44% of total fatty acids (0.19–0.85). No significant association with CLA and prognostic factors, metastasis, death	No significant association of CLA and risk of metastasis or death.	→

[1] Values are reported from multivariate analyses unless otherwise indicated. IHD, ischemic heart disease.
[2] OR and RR are reported for highest vs. lowest quintiles/quartiles of intake.
[3] →, no association; ↓, inverse association; ↑, direct association.

between dairy intake and MI was no longer significant (*P*-trend = 0.28).

There are 4 prospective studies that have investigated the association between intake of TFA and CHD (7,10,42,43). Of these studies, one demonstrated a significant inverse association between energy-adjusted intake of rTFA and risk of CHD after controlling for CHD risk factors (42). The other 3 studies found no significant associations (7,10,43); however, one study indicated a trend for an inverse association (7) and one study indicated a trend for a direct association (10). Three of the 4 studies also investigated associations between intake of iTFA and risk of CHD. Two studies found significant direct associations with energy-adjusted intake of iTFA and risk of CHD (7,42), whereas one study showed no significant association (10).

Results from a recent meta-analysis of prospective cohort studies suggest that iTFA may increase the risk of CHD, whereas rTFA does not (44). The pooled RR estimates for total TFA intake (ranging from 2.8 to 10 g/d) were 1.22 for CHD events (95% CI = 1.08–1.38; $P = 0.002$) and 1.24 for fatal CHD (95% CI = 1.07–1.43; $P = 0.003$). Intake of rTFA (0.5–1.9 g/d) was not significantly associated with risk of CHD (RR = 0.92; 95% CI = 0.76–1.11; $P = 0.36$), whereas there was a trend for a positive association between iTFA intake and CHD risk (RR = 1.21; 95% CI = 0.97–1.50; $P = 0.09$). The authors concluded that the limited number of available studies prohibits any firm conclusions as to whether source of TFA is important (44).

Review of clinical studies. There have been very few highly controlled clinical studies that have compared the effects of rTFA and iTFA on risk factors of CVD (**Table 4**). The development of special feeding practices of dairy cows in recent years has resulted in the use of dairy products in clinical trials that are rich in rTFA (45) and has made it possible to achieve diets containing comparable amounts of rTFA and iTFA. In a double-blind, randomized, 5-wk parallel intervention study, healthy young men ($n = 42$) were given 115 g fat/d from test butter that was enriched in VA (3.6 g/100 g) or a control butter low in VA (0.4 g/100 g) (46). Following consumption of the VA-rich butter, plasma total cholesterol (TC) was reduced by 6% ($P = 0.05$) and HDL-cholesterol (HDL-C) was reduced by 9% ($P = 0.002$) compared with the low-VA control butter. The ratio of TC:HDL-C did not differ when comparing treatment and control. Compared to the control butter, the VA-enriched butter contained higher amounts of MUFA and lower amounts of SFA; thus, the changes in lipids and lipoproteins may be due to differences in fatty acid composition between treatments rather than differences in VA intake alone.

In a crossover study by Motard-Belanger et al. (47), healthy men ($n = 38$) consuming a high-iTFA diet (10.2 g/2500 kcal, 3.7% of energy) led to significantly higher LDL-cholesterol (LDL-C) compared with moderate intake of rTFA (4.2 g/2500 kcal, 1.5% of energy). High intake of rTFA (10.2 g/2500 kcal) also led to significantly higher LDL-C compared to a diet low in TFA from any source (control; 2.2 g/2500

Table 4. Effects of rTFA on lipids and lipoproteins: summary of clinical studies

Reference	Study description	Participant characteristics	Intervention	Results[1]	Summary[2]
Tholstrup et al. (46)	Randomized, double-blind, parallel design	Healthy young men (n = 42); Danish population	Test butter high in VA (3.6 g/d) or control butter low in VA. 5 wk	VA butter decreased plasma TC and HDL-C vs. control. No differences in TC/HDL-C. Note: there were differences in MUFA and SFA between treatments, which may have confounded results; basal diets were not controlled.	→
Kuhnt et al. (50)	Randomized, parallel design	Healthy men and women (n = 24); German population	Test group (n = 12): VA (t11–18:1), t12–18:1 in equal amounts (6.0 g/d). Control group (n = 12): oil without TFA or CLA. 6 wk	No effect of 18:1 supplementation on inflammatory markers (IL-6, IL-8, TNFα) or immune function. Note: basal diets were not controlled.	→
Naumann et al. (54)	Parallel design	Men and women with LDL phenotype B; Dutch population	Dairy product enriched with either 3 g c9,t11-CLA (n = 34), 3 g t10,c12-CLA (n = 19), or not enriched with CLA (placebo, n = 34). 13 wk	No effects of c9,t11-CLA or t10,c12-CLA on LDL-C, HDL-C, and TG.	→
Riserus et al. (55)	Randomized, double-blind, placebo-controlled parallel design	Obese men (n = 25); Swedish population	3 g/d c9,t11-CLA or placebo (olive oil). 3 mo	No effects of c9,t11-CLA on lipids and lipoproteins. Note: c9,t11-CLA increased urinary 8-*iso*-PG $F_{2\alpha}$.	→
Sluijs et al. (56)	Randomized, double-blind, placebo-controlled parallel design	Overweight and obese men; Dutch population	4 g/d CLA (2.5 g/d c9,t11-CLA, 0.6 g/d t10,c12-CLA) or placebo 6 mo	No effects of c9,t11-CLA on lipids and lipoproteins.	→
Tardy et al. (49)	Randomized, double-blind, parallel design	Overweight women (n = 63); French population	60 g of low-TFA lipids (0.54 g/d; n = 21), rTFA lipids (4.86 g/d; n = 21), or iTFA lipids (5.58 g/d; n = 21). 4 wk	No effects of rTFA blend (enriched in VA) on lipids and lipoproteins. Note: fatty acid composition of diets was not matched, which may have confounded results.	→
Chardigny et al. (48)	Randomized, double-blind, crossover design	Healthy men and women (n = 40); French population	Food items containing rTFA or iTFA (11–12 g/d, ~5% of daily energy). 3 wk, 1-wk washout	rTFA diet significantly increased LDL-C and HDL-C vs. iTFA diet, in women only. Note: differences in MUFA and SFA between treatments, which may have confounded results; basal diets not controlled; high amount of rTFA fed.	↑ among women
Tricon et al. (53)	Randomized double-blind, crossover design	Healthy men (n = 49); British population	3 doses of highly enriched c9,t11-CLA (0.59, 1.19, and 2.38 g/d) or t10, c12-CLA (0.63, 1.26, and 2.52 g/d). 8 wk each, 6-wk washout	c9,t11-CLA significantly decreased TC:HDL-C ratio relative to baseline. t10,c12 significantly increased TC:HDL-C ratio relative to baseline. TG, LDL-C:HDL-C ratio, and TC:HDL-C ratio were significantly higher after t10,c12 vs. c9,t11-CLA.	↓

(Continued)

Table 4. (*Continued*)

Reference	Study description	Participant characteristics	Intervention	Results[1]	Summary[2]
Motard-Belanger et al. (47)	Randomized, double-blind, crossover design; controlled-feeding	Healthy men (n = 48); Canadian population	4 isocaloric diets: high in rTFA (10.2 g/2500 kcal), moderate in rTFA (4.2 g/2500 kcal), high in iTFA (10.2 g/2500 kcal), low in TFA from any source (2.2 g/2500 kcal) (control diet). 4 wk each	LDL-C significantly higher after high-rTFA diet vs. control or moderate-rTFA diet. HDL-C significantly lower after high-rTFA vs. moderate-rTFA diet. No significant differences between moderate rTFA diet and control.	→moderate rTFA ↑ high rTFA
Wanders et al. (52)	Randomized, crossover design; controlled-feeding	Healthy men and women (n = 61); Dutch population	Isocaloric diets with 7% of energy (~20 g/d) from OA, iTFA, or mixture of 80% c9,t11-CLA and 20% t10,c12-CLA. 3 wk each	Compared to OA diet, TC:HDL-C ratio was significantly higher after iTFA and CLA diets. Note: high amount of rTFA was fed.	↑

[1] Effects are considered significant at $P < 0.05$.
[2] Effect on lipoprotein risk factors: →, no effect; ↓, decrease; ↑, increase.

kcal, 0.8% of energy) or moderate rTFA. Moderate intake of rTFA did not significantly differ from the low-TFA control diet. Following consumption of the high-rTFA diet, HDL-C was significantly lower compared with the moderate rTFA diet. These results suggest that at high intakes, the effects of rTFA on CVD risk factors are comparable to iTFA; however, at moderate intakes, which would be potentially attainable with consumption of a very high-dairy diet, rTFA may not significantly affect CVD risk factors in healthy individuals. A strength of this study was that specific vegetable and animal oils and fat were incorporated into each diet to minimize differences in the amounts of SFA and unsaturated fatty acids between treatments. As a result, the treatment diets contained comparable amounts of SFA, MUFA, and PUFA. In addition, all foods were provided to the study participants throughout the duration of the study. A limitation of this study was that it was not statistically powered to detect changes between all of the diets.

In another crossover study directly comparing the 2 sources of TFA (n = 40; 11–12 g/d, ~5% of energy), the rTFA diet significantly increased LDL-C and HDL-C compared with the iTFA diet, but this effect was observed in women only (48). Unexpectedly, consumption of the iTFA diet did not affect LDL-C, which may suggest that the isomer profile of iTFA is of importance. A limitation of this study is that there was no control diet to which to compare the iTFA and rTFA diets. Further, the study was not a controlled-feeding intervention, but rather dietary instruction was provided to the participants by a dietitian and dietary records were collected and analyzed. In addition, there were differences in the SFA and MUFA content of the treatment diets, which may have influenced the results, and the amounts of rTFA fed were well beyond the current consumption of rTFA in any population.

Tardy et al. (49) measured the effects of rTFA (2% energy intake) and iTFA (2.6% energy intake) on insulin sensitivity, measured by hyperinsulinemic-euglycemic clamp, in overweight women (n = 63). The rTFA and iTFA diets did not alter lipid profile compared to the low-TFA control. However, there was a significant decrease in HDL-C, regardless of treatment group, following the 4-wk intervention. In addition, there was a significant decrease in LDL-C following consumption of the low-TFA control group (no changes in LDL-C were observed in the rTFA or iTFA groups). A limitation of the study was that the fatty acid composition of the diets was not matched, except for *trans*-MUFA and *cis*-MUFA, which may have influenced the results.

A study by Kuhnt et al. (50) investigated the effects of 6 wk of supplementation with equal amounts of the isomers t11–18:1 and t12–18:1 (6 g/d) in men and women (n = 24) on several markers of inflammation. Supplementation with t11–18:1 and t12–18:1 was associated with a significant increase in the incorporation of c9,t11-CLA in peripheral blood mononuclear cells. There were no significant differences in biomarkers of inflammation and immune function when comparing the treatment and placebo groups. However, t11–18:1 and t12–18:1 supplementation did lead to a significantly

Table 5. Potential mechanisms of rTFA isomers: summary of experimental evidence from animal studies[1]

Reference	Animal model	Intervention	Results[2]	Summary
CVD				
Kritchevsky et al. 2004 (68)	Rabbits bearing atheromatous lesions	0.5% c9,t11-CLA, t10,c12-CLA, or mixture for 90 d	Both c9,t11-CLA and mixture decreased atherosclerotic lesions in arch and thoracic area of aorta. t10,c12-CLA only reduced lesions in aortic arch.	CLA improved atherosclerotic lesions; efficacy was higher in high-cholesterol diet.
Bauchart et al. (85)	Male New Zealand white rabbits	One of 3 butters: t10–18:1 (11.8% FA as t10–18:1), VA+CLA (7% FA as t11–18:1, 2.6% FA as c9,t11-CLA), or control diet low in all 3 fatty acids; 12 wk	Compared to t10-18:1 diet and control diet, VA+CLA butter resulted in lower VLDL-C, LDL-C, TG, plasma TC, phospholipids, and apoB.	VA/CLA butter improved plasma risk factors for CHD in rabbits. Results suggest a neutral effect of c9,t11-CLA, and possible adverse effect of t10,c12 isomers.
Roy et al. (86)	Male New Zealand white rabbits	One of 3 butters: t10–18:1 (11.8% t10–18:1, 1.8% t11–18:1 of total FA), VA+CLA (7% t11–18:1, 2.2% t10–18:1, 2.6% c9,t11-CLA), or control low in all 3 fatty acids; high-fat cholesterol diets; 12 wk	t10–18:1 raised VLDL-C more than the other butters. t10–18:1 raised TC, LDL-C, non-HDL-C/HDL-C, and aortic lipid deposition vs. t11–18:1 butter. t11–18:1 decreased HDL and increased liver TG vs. other butters.	VA had a neutral effect on CVD risk factors and tended to decrease atherosclerotic lesion development in rabbits. Effects of VA and c9,t11-CLA were not significantly different.
LeDoux et al. (73)	Cholesterol-fed hamsters	1% wt:wt of c9,t11-CLA, t10,c12-CLA, or CLA mixture; 12 wk	c9,t11-CLA reduced TC, HDL-C, LDL-C, and small dense LDL. No significant changes in the t10,c12-CLA or the mixture group.	c9,t11-CLA had a beneficial effect on lipoprotein profile in this model. t10,c12-CLA and CLA mixture were less active.
Wilson et al. (70)	Cholesterol-fed hamsters	0.5% wt:wt c9,t11-CLA, t10,c12-CLA, or LA; 12 wk	c9,t11-CLA decreased plasma TC, HDL-C, non-HDL-C, TG, and cholesterol accumulation in aortic arch	c9,t11-CLA improved CVD risk factors.
Mitchell et al. (69)	High-fat, high-cholesterol–fed hamsters	1% wt:wt c9,t11-CLA, t10,c12-CLA, or LA; 12 wk	c9,t11-CLA decreased non-HDL-C/HDL-C and aortic fatty streak lesion. No effect of c9,t11-CLA on plasma TG and LDL-C.	Individual CLA isomers beneficially affect lipoprotein profile and reduce atherosclerotic lesion development, but not different than LA.
Tyburczy et al. (61)	Cholesterol-fed hamsters	Control Western diet or 2% supplementation of PHVO, VA, or EA; 4 wk	VA and EA decreased plasma TC:HDL-C and non-HDL-C:HDL-C ratios, while PHVO increased these parameters	Hypercholesterolemic effect of PHVO is not dependent on EA or VA.
Valeille et al. 2004 (81)	Cholesterol-fed hamsters	Control diet, 0.6% c9,t11-CLA, 1.2% CLA mixture, 1.2% CLA mixture +1.2% fish oil, or control diet + 1.2% fish oil; 8 wk	c9,t11-CLA increased LDL-receptor and scavenger receptor type B-1	Part of the beneficial effects of CLA can be ascribed to c9,t11-CLA and are boosted by fish oil.
Meijer et al. (60)	Cholesterol-fed hamsters	MCFA (C8:0+C10:0), SFA (C16:0), MUFA (C18:1 9c), EA, VA; 5 wk	VA decreased LDL-C and TG compared to SFA. No difference in LDL-C, VLDL-C, TG, or TC compared to MUFA, EA, or MCFA. VA increased LDL-C:HDL-C ratio compared to EA.	Both EA and VA lowered CVD risk factors, but this study does not support that EA is more detrimental than VA.
Valeille et al. 2005 (71)	Cholesterol-fed hamsters	20% butter fat (1% VA + 0.4% c9,t11-CLA; B diet); 20% butter fat + 1% c9,t11-CLA (BR diet); 20% butter fat+1% fish oil (BF diet); 12 wk	• BR diet had lowest aortic lipid deposition; reduced plasma non-HDL/HDL; improved antioxidized LDL paraoxonase activity; and down-regulated expression of inflammatory-related genes (TNF-α, IL-1β, COX-2)	Milk fat rich in c9,t11-CLA reduced atherogenic process in hyperlipidemic hamsters.

(Continued)

Table 5. (*Continued*)

Reference	Animal model	Intervention	Results[2]	Summary
Lock et al. 2005 (84)	Cholesterol-fed hamsters	20% standard butter, 5% standard butter+15% VA/CLA butter, 15% standard butter+5% PHVO; 4 wk	VA/CLA butter decreased plasma cholesterol, VLDL-C, and ratio of (VLDL-C+LDL-C):HDL-C	VA/CLA butter improved plasma lipoprotein profile and reduced risk of atherosclerosis.
Gavino et al. (80)	Male golden Syrian hamsters	1% CLA mixture, 0.2% c9,t11-CLA, or 0.2% LA	CLA mixture led to lower plasma TC and TG vs. c9,t11-CLA and LA. No differences between c9,t11-CLA and LA.	Effects of CLA mixture on CVD risk factors were greater than c9,t11-CLA alone.
Rice et al. (63)	Male Hartley guinea pigs	Hypercholesterolemic diet containing PHVO (iTFA), butter oil (rTFA), coconut oil, or soybean oil (9% by weight for each) for 8 or 12 wk	Compared with soybean oil group, rTFA and iTFA groups had higher TC, LDL-C, and HDL-C. No differences between rTFA and iTFA for plasma TC, LDL-C, HDL-C, or TG. Total and small HDL particles were significantly higher in rTFA group vs. iTFA group.	At higher doses, iTFA and rTFA may have similar effects on traditional risk factors of CHD.
Cooper et al. (82)	ApoE−/−mice	0.5% (wt:wt) LA, c9,t11-CLA, t10,c12, or 1:1 CLA mixture; 12 wk	Compared to LA, CLA supplementation had no effect on lesion area in en face preparations of aorta or in aortic root cross-sections. Plasma TC and TG were higher in t10,c12 group vs. other groups.	Dietary supplementation with CLA did not affect atherosclerosis in apoE−/− mice on a high cholesterol diet.
Arbones-Mainar et al. (75)	ApoE−/−mice	1% wt:wt c9,t11-CLA, t10,c12-CLA, or LA; 12 wk	c9,t11-CLA decreased aortic lesions. c9,t11-CLA decreased plasma TC, FFA, glucose/insulin, and aortic lesions; increased apoA-I. t10,c12-CLA had a profound proatherogenic effect; opposite effects of c9,t11-CLA.	c9,t11-CLA reduced atherosclerosis development; t10,c12-CLA had a proatherogenic effect.
Toomey et al. (76)	ApoE−/−mice	1% c9,t11-CLA or COX-1 inhibitor; 16 wk	c9,t11-CLA decreased atherosclerotic lesion development and induced aortic lesion regression. c9,t11-CLA increased PPAR- γ expression at aortic root.	c9,t11-CLA benefits atherosclerosis as PPARγ agonist, not COX-1 inhibitor.
Nestel et al. (78)	ApoE−/−mice	0.9% wt:wt (90% pure c9,t11-CLA); CLA oil was mixed in with standard powder chow; 20 wk	In diabetic apoE −/− mice, CLA resulted in lower TG and higher HDL-C. No differences in aortic arch atherosclerosis between c9,t11-CLA group and control.	c9,t11-CLA improved plasma risk markers for atherosclerosis but did not reduce the severity of aortic atherosclerosis.
Wang et al. 2008 (64)	Lean and obese JCR:LA-cp rats	1.5% VA (wt:wt) or control; 3 wk	VA led to 40% decrease in TG in obese rats vs. obese controls. VA decreased IL-10 in lean and obese rats.	VA had significant hypo-triglyceridemic effects in dyslipidemic obese rats; neutral effect under normolipidemic conditions.
Wang et al.2009 (66)	Obese JCR:LA-cp rats	1% VA or control (typical Western diet); 16 wk	VA decreased fasting plasma TC, LDL-C, TG, and hepatic fatty acid synthesis. VA decreased postprandial TG and apoB.	Chronic VA supplementation significantly improved dyslipidemia in both the fasted and postprandial state in JCR:LA-cp rats.

(Continued)

Table 5. (*Continued*)

Reference	Animal model	Intervention	Results[2]	Summary
Jacome-Sosa et al. (67)	JCR:LA-cp rats	VA+CLA (1% wt:wt of each), c9,t11-CLA (1% wt:wt), and control (typical Western diet); 16 wk	TC, LDL-C, and TG were significantly lower in JCR:LA-cp rats fed either VA+CLA diet or CLA diet compared with control diet. VA+CLA diet resulted in LDL-C and TG comparable to that of normolipidemic lean rats.	VA+CLA had beneficial effects on lipids and lipoproteins in JCR:LA-cp rats.
Dupasquier et al. (83)	LDL receptor-deficient mice	Fed 1 of 8 experimental diets for 14 wk: regular fat, elaidic shortening, regular butter, VA butter, 2% cholesterol, 2% cholesterol + elaidic shortening, 2% cholesterol + regular butter, 2% cholesterol + VA butter	VA butter decreased serum cholesterol and TG vs. EA and regular butter diet. VA butter was more effective when the diet contained higher cholesterol.	EA shortening diet was atherogenic, while VA butter diet was not atherogenic.
Cancer				
Mammary cancer				
Banni et al. (114)	Healthy rats, rats with MNU-induced mammary tumors	Study 1: healthy rats fed diets containing 0, 1, 2, 3% wt:wt VA (purified); 3 wk. Study 2: rats with MNU-induced mammary tumors fed diets containing 2% wt:wt VA or 1% wt:wt c9,t11-CLA; 6 wk	Study 1: VA increased c9,t11-CLA in mammary gland and liver; maximum CLA production at 2% wt:wt Study 2: VA decreased total number of premalignant lesions by ~50%.	Anticarcinogenic properties of VA was mediated by its conversion to CLA.
Corl et al. (118)	Rats with MNU-induced mammary tumors	0.13–1.6% wt:wt VA, 0.05–0.37% wt:wt c9,t11-CLA (from butter); 24 wk	Increased tissue CLA content with increased dietary VA. Decreased tumor formation in mammary gland.	Dose-dependent effect of VA + CLA on carcinogenesis.
Ip et al. 1999 (113)	Rats with MNU-induced mammary tumors	Control butter (0.1% CLA), high-CLA butter (0.8% CLA), c9,t11-CLA (0.8% CLA), or CLA mixture (0.8% CLA); 4 wk	All CLA diets decreased tumor cell number and proliferation. CLA mixture & CLA butter diet showed similar efficacy. Selective uptake of c9,t11-CLA over t10, c12 isomer.	Milk fat CLA reduced risk of mammary cancer. c9,t11-CLA were as potent as mixture. High bioactivity in CLA butter was due to additional endogenous CLA produced from VA.
Lock et al. 2004 (115)	Rats with MNU-induced mammary tumors	0.13% VA, 0.4% VA, 1.6% VA, 1.6% VA + Δ9-desaturase inhibitor (sterculic oil); 6 wk	1.6% VA increased CLA in tissues and reduced tumor growth. 1.6% VA + Δ9-desaturase inhibitor increased CLA concentrations in liver and plasma vs. low VA groups. Inhibitor reversed effects of VA.	The anticarcinogenic effect of VA was predominantly mediated via conversion to c9,t11-CLA via Δ9-desaturase.
Ip et al. 2002 (125)	Rats with MNU-induced mammary tumors	0.5% wt:wt of purified c9,t11-CLA or t10,c12 CLA isomers; 6 wk	Both isomers reduced premalignant lesions; no difference between groups. Tissue concentrations of c9,t11-CLA in mammary fat pad was much higher than t10,c12-CLA.	Anticancer effects of c9,t11-CLA and t10,c12 isomers were similar.
Lavillonniere et al. (126)	Rats with MNU-induced mammary tumors	1.0% wt:wt sunflower oil, 1.0% wt:wt c9,t11-CLA, purified, or 1.0% CLA mixture; 20 wk	c9,t11-CLA and mixture decreased tumor mass. Incidence and latency were unaffected by diet.	c9,t11-CLA had anticarcinogenic properties in experimental mammary carcinogenesis.

(Continued)

Table 5. (*Continued*)

Reference	Animal model	Intervention	Results[2]	Summary
Ip et al. 2007 (131)	Transgenic mice overexpressing erbB2 in mammary epithelium	0.5% wt:wt purified c9,t11-CLA or t10,c12-CLA	t10,c12-CLA increased mammary tumor development and metastasis, while c9,t11-CLA did not.	t10,c12-CLA stimulated mammary tumorigenesis in this model.
Hubbard et al. 2007 (130)	Mice transplanted with metastatic tumor cells in mammary fat pad	0, 0.1, 0.5, 1.0% wt:wt CLA mixture; 3 wk	Increased expression of matrix metalloproteinase (MMP) inhibitor, TIMP-1&2	Suppression of MMP activity may be a pathway through which CLA reduces tumor invasion and spread
Hubbard et al. 2003 (132)	Mice transplanted with metastatic tumor cells in mammary fat pad	Transplantation occurred after 3 wk of diet. Fed additional 4 wk: no CLA, low c9,t11-CLA (0.1%), high c9,t11-CLA (0.25%), low t10, c12-CLA (0.1%), high t10,c12-CLA (0.25%), or CLA mixture (0.125% each).	No effect on latency or growth of primary tumor line by separate isomers or mixture. Reduced tumor burden and metastasis by separate isomers and mixture in a dose-dependent manner	Both CLA isomers have similar antitumor efficacy. Possible similar mechanisms for decreasing tumor burden in this model; no additive effects of c9,t11-CLA and t10,c12 isomers.
Ip et al. 2001 (128)	Healthy rats	Study 1: basal diet or 1% CLA mixture; 4 wk. Study 2: healthy rats fed control butter (0.1% wt:wt) or CLA-butter (0.8% wt:wt); 4 wk. Study 3: healthy rats fed basal diet or 1% purified c9,t11-CLA; 2, 4, 6, 8 wk.	CLA groups led to reduced proliferative activity, cyclin D1, and cyclin A. Highly proliferative cells are more responsive to CLA intervention.	CLA mixture and c9,t11-CLA from butter were equally effective. Responsiveness to CLA dependent on proliferative status.
Gastrointestinal cancer				
Mandir et al. (146)	*Apc*$^{min/+}$mice	Control diet, 1% c9,t11-CLA, 1% t10,c12-CLA, or 0.5% c9,t11-CLA +0.5% t10,c12-CLA; 6 wk	Reduced polyp number. Reduced colonic weight and crypt fission length only by c9,t11-CLA. Increased polyp diameter and intestine weight by t10,c12-CLA.	c9,t11-CLA was more protective than other isomers.
Soel et al. (147)	BALB/c mice injected with tumor cells	Diet without CLA or 0.1% c9,t11-CLA or t10,c12; 4 wk. SW480 colon cancer cells treated with no CLA, c9,t11-CLA, or t10c12-CLA at 1, 2, or 4 μmol/L for 24 h.	Both CLA isomers reduced pulmonary nodule number in vivo. Only RA inhibited cell migration and MMP-9 activity in vitro.	c9,t11-CLA was more effective in inhibiting colon cancer metastasis. c9,t11-CLA and t10,c12 isomers may act via different mechanisms.
Rajakangas et al. (148)	Min mice	Control diet, 1% purified c9,t11-CLA, or t10,c12-CLA; 8 wk	No effect on adenoma number, but t10, c12-CLA increased adenoma size in distal intestine, lipid peroxidation, and cyclin D1 expression; decreased nuclear NFκB.	t10,c12-CLA can act as a cancer promoter in colon carcinogenesis. c9,t11-CLA showed no adverse effects.
Park et al. (149)	Male Sprague-Dawley rats	I.m. injected with 15 mg/kg of DMH twice per week for 6 wk and fed a control diet, 1% t10, c12 or 1% c9,t11-CLA for 30 wk	Tumor numbers were decreased and apoptotic index increased in colonic mucosa of c9,t11-CLA and t10,c12 groups vs. control group. Bcl-2 and cyclooxygenase-2 were decreased in both isomer groups; Bax was increased. Thromboxane B2 in colonic mucosa was lower in both isomer groups.	c9,t11-CLA decreased colon tumor number in rats. No significant differences between isomers.

[1] FA, fatty acids; VLDL-C, VLDL cholesterol; MCFA, medium-chain fatty acids; MMP, matrix metalloproteinase; TIMP, tissue inhibitors of MMP; DMH, dimethylhydrazine.
[2] Effects are considered significant at $P < 0.05$.

higher concentration of urinary 8-iso-$PGF_2\alpha$, a biomarker of oxidative stress, compared to baseline and the control group (51). There were no differences in biomarkers of cyclooxygenase-mediated inflammatory response or oxidative DNA damage. In this study, foods were provided to participants during the last week of the intervention and dietary guidance was given to limit TFA and CLA intake as part of the diet; however, participants' basal diets were not controlled and compliance with dietary recommendations was not evaluated during the intervention period. Another limitation of this study was that it had a small sample size for a parallel design study (n = 12/group).

In a recent crossover design study, the effects of CLA [80:20 mixture of c9,t11-CLA and *trans*-10, *cis*-12 CLA (t10,c12-CLA)], iTFA, and oleic acid (OA; control), each providing ~7% energy, were compared in healthy men and women (n = 61) (52). The CLA mixture significantly increased LDL-C and decreased HDL-C compared with the control diet. Compared to the CLA diet, the iTFA diet resulted in significantly higher LDL-C, TG, and apoB, whereas there were no differences between the diets for the ratios of TC:HDL-C and LDL-C:HDL-C. Although this study was adequately powered, the amount of CLA that was provided in the CLA diet was higher than what is typically consumed; therefore, the effect of CLA at usual intakes remains unclear. Furthermore, the CLA diet did not contain pure c9,t11-CLA but rather a mixture of c9,t11-CLA (19.6 g/d, 6.9% of energy) and t10,c12-CLA isomers (4.4 g/d, 1.5% of energy). Some clinical data have suggested that t10,c12-CLA may have adverse effects on lipids and lipoproteins compared with c9,t11-CLA. In a study in healthy men (n = 49), the plasma TG concentration and the ratios of LDL-C:HDL-C and TC:HDL-C were higher after supplementation (~2% energy) with t10,c12-CLA compared with c9,t11-CLA (53). In contrast, in a study in overweight individuals with LDL phenotype B, there were no differences in lipids and lipoproteins following consumption of a drinkable dairy product enriched with c9,t11-CLA (3 g, n = 34), t10, c12-CLA (3 g, n = 19), or placebo without CLA (n = 34) for 13 wk (54). In a study in obese men (n = 25), supplementation with 3 g of a CLA preparation (83% c9,t11-CLA) for 3 mo did not affect lipids or lipoproteins compared to olive oil; however, insulin resistance and lipid peroxidation increased (55). In a recent study, overweight and obese individuals (n = 401) were randomized to receive either 4 g/d of CLA (2.5 g c9,t11-CLA, 0.6 g t10,c12-CLA) or placebo (56). After 6 mo, there was no effect of CLA supplementation on lipids and lipoproteins. In addition, there was no effect on C-reactive protein, blood pressure, or aortic stiffness as measured by pulse wave velocity. In a study in overweight or obese children (n = 62; ages 6–10 y) aimed to determine the effect of CLA (80% CLA, 50:50 mixture c9,t11-CLA:t10,c12-CLA) on body fat accretion, LDL-C did not differ and there was a greater decrease in HDL-C in the CLA group (-5.1 ± 7.3 mg/dL) compared with the placebo group (-0.7 ± 8 mg/dL) (P = 0.05).

A recent review of human intervention trials concluded that all TFA, regardless of the source (iTFA, rTFA, CLA), increased the ratio of LDL-C:HDL-C (57). However, it has been criticized that the conclusions from this review do not fully support the data at hand (58) and are not consistent with those from the recent WHO scientific update on TFA, which reported that clinical data are inconclusive from the small number of trials that have compared rTFA and iTFA, and observational studies generally do not support an adverse effect of rTFA on CHD risk in the relatively low amounts typically consumed (59). Because low amounts are typically consumed (<1.5% of energy), it may not be critical to investigate whether very high intake of rTFA adversely affects risk of CHD.

Review of animal and cell culture studies.

Vaccenic acid. At present, there are only a handful of research groups who have explored the effect of purified VA preparation on CVD risk, due largely to its limited availability (**Table 5**). Meijer et al. (60) fed hamsters a diet with VA, elaidic acid (EA), OA, palmitic acid, or a combination of medium-chain SFA for 4 wk (10% of energy). The effect of VA on blood cholesterol profile and 2 lipid metabolizing enzymes (cholesterylester transfer protein and phospholipid transfer protein) did not differ from OA but were lower than SFA. More recently, a 2.0% wt:wt supplementation of purified VA, EA, and PHVO was compared to control (typical Western diet) in hamsters to assess their impact on CVD risk factors (61). After 4 wk of treatment, PHVO increased the ratio of TC:HDL-C, whereas VA and EA decreased the ratio compared to controls. Plasma TG was not affected by any diet. The findings suggest that there may be other bioactive components in PHVO other than EA or VA that are responsible for hypercholesterolemic effects.

Studies of the effects of rTFA also have been conducted in guinea pigs, which is a useful model, because they metabolize fatty acids similarly to humans (62). In a recent study, male Hartley guinea pigs were fed a hypercholesterolemic diet containing PHVO (iTFA), butter oil (rTFA), coconut oil, or soybean oil [9% by weight for each; same fats fed in (48)] for 8 or 12 wk (63). Compared with the soybean oil group, the rTFA and iTFA groups had higher plasma TC, LDL-C, and HDL-C. When comparing rTFA and iTFA, TC, LDL-C, HDL-C, and TG did not differ. However, total and small HDL particles were higher in the rTFA group than in the iTFA group (P < 0.01). The results from this study demonstrate that at higher doses, iTFA and rTFA may have similar effects on traditional risk factors of CVD. Interestingly, rTFA led to a plasma HDL particle profile that has been previously hypothesized to be associated with a lower risk of CHD. These findings warrant further research.

VA has been shown to have beneficial effects on lipids and lipoproteins in the JCR:LA-cp rat, a rodent model of metabolic syndrome. In obese rats fed a diet enriched with purified VA (1.5% wt:wt) for 3 wk, TG decreased by 40% (P < 0.05) compared to obese rats fed a control diet designed to resemble a typical Western diet (64). Furthermore, VA was shown to decrease the proinflammatory markers IL-2 and TNFα (65) in this rat strain. There was no effect of VA on these parameters in normal rats, suggesting that the efficacy of VA to

benefit CVD might be more profound under disease conditions. In a subsequent study, JCR:LA-cp rats fed VA (1% wt:wt) for 16 wk had lower TC ($P < 0.001$), LDL-C ($P < 0.01$), and TG ($P < 0.001$) compared with obese rats fed a control diet designed to resemble a typical Western diet (66). Interestingly, LDL-C did not differ between obese rats fed the VA diet and lean rats fed the control diet. In a more recent study using this same rat model, the effect of a diet enriched with c9,t11-CLA in combination with VA (VA+CLA, 1% wt:wt of each) was compared to a diet enriched with c9,t11-CLA (1% wt:wt) and a control diet designed to resemble a typical Western diet (67). After 16 wk, concentrations of TC ($P < 0.001$), LDL-C ($P < 0.001$), and TG ($P < 0.05$) were significantly reduced in JCR:LA-cp rats that were fed either the VA+CLA diet or the CLA diet compared with the rats fed the control diet. The VA +CLA diet resulted in concentrations of LDL-C and TG comparable to that of the normolipidemic lean rats. In addition, the VA+CLA diet further decreased nonesterified fatty acids (NEFA) compared to the CLA diet alone. Interestingly, rats fed the VA+CLA diet had a higher food intake but lower body weight than the CLA-fed group ($P < 0.05$).

Conjugated linoleic acid. Several animal studies, in multiple models, have investigated the effects of CLA intake on atherosclerotic lesions and risk factors of CVD (Table 5). Most of these studies have used mixtures of CLA isomers, whereas fewer studies have investigated the effects of specific isomers. In a study in rabbits with an atherogenic diet, CLA (c9,t11-CLA, t10,c12 isomer, or mixture) led to a significant reduction of severity of atheromatous lesions, with similar reductions in all 3 CLA-fed groups (68). In hamsters, some studies have suggested that less cholesterol accumulates in the aortic arch and fewer fatty streak lesions develop when supplemented with c9,t11-CLA (69–71), although significance was not reached in 2 of these studies (69,70). The hamster is a useful model, because unlike most rodents, when fed a high-fat, high-cholesterol diet, it carries the additional plasma cholesterol in the VLDL and LDL fraction rather than HDL, similarly to that typically found in humans (72). In a study by Valeille et al. (71), hyperlipidemic hamsters were fed diets containing 20% (wt:wt) butter fat or the same diet augmented with either 1% (wt:wt) c9,t11-CLA-rich oil or 1% (wt:wt) fish oil. After 12 wk, there was significantly less aortic lipid deposition in the c9,t11-CLA group compared to the other groups. Furthermore, c9,t11-CLA led to a decrease in the ratio of non-HDL-C:HDL-C ($P < 0.05$). In a more recent study, hyperlipidemic hamsters were fed a semipurified diet supplemented with either 1% c9,t11-CLA, 1% t10,c12-CLA, or a mixture of isomers (73). After 12 wk, there was no formation of fatty streaks in any of the groups. Furthermore, concentrations of plasma TC, LDL-C, and HDL-C were significantly lower in the c9, t11-CLA group relative to the control group, whereas there was no effect of t10,c12-CLA. Although the cholesterol-fed hamster is a useful model because of similarities to human lipid metabolism, the apoE-knockout mouse is a useful model to study the progression of fatty lesions due to its genetic susceptibility to atherosclerosis (74). When fed c9, t11-CLA (1% wt:wt) for 12 wk, the development of atherosclerosis in apoE$^{-/-}$ mice was significantly reduced and further associated with reductions in plasma cholesterol and FFA as well as increased concentrations of apoA-I. In addition, there was significantly less cross-sectional lesion area in the aortic root of treated apoE$^{-/-}$ mice (75). In contrast, t10,c12-CLA increased lesion area compared with control apoE$^{-/-}$ mice, suggesting that there may be differential effects of CLA isomers on the development of atherosclerosis. In addition to reduced progression, the regression of preestablished atherosclerotic lesions also has been reported in the aorta of CLA-fed apoE$^{-/-}$ mice (76). In a study by Toomey et al. (77), apoE$^{-/-}$ mice were fed 1% cholesterol for 8 wk and were then randomized to 1 of 3 groups: 1% CLA isomer blend (80:20 c9,t11-CLA/t10,c12-CLA), 1% SFA (control), or continued with 1% cholesterol. After 8 wk, the CLA blend completely abolished atherosclerosis compared to control. In diabetic apoE$^{-/-}$ mice, TG were significantly lower and HDL-C was significantly higher following 20 wk of CLA supplementation (0.9% wt:wt, 90% c9,t11-CLA); however, there was no effect on aortic atherosclerosis (78).

The effects of CLA isomers on CVD risk factors, specifically lipids and lipoproteins, have been studied in numerous animal studies. Many studies have used blends of CLA isomers. Results have been mixed in distinguishing whether c9,t11-CLA or t10,c12-CLA is the active isomer affecting lipids and lipoproteins. Some studies suggest that t10,c12-CLA may be the active isomer (79,80). In contrast, in several animal models, c9,t11-CLA has been reported to improve blood lipid profiles at an average dose of 1.0% wt:wt (equivalent to 3–5% of energy) (70,71,73,81). In hamsters, plasma TC, LDL-C (especially small dense LDL), and TG concentrations were shown to be lower when fed 0.5–1% c9,t11-CLA for 12 wk (70,73). Similar changes have been observed in a rat model of metabolic syndrome that spontaneously develops cardiovascular complications (67). In apoE$^{-/-}$ mice fed a high-cholesterol diet, CLA supplementation at a concentration of 0.5% wt:wt (c9,t11-CLA, t10,c12 isomer, or a 1:1 mixture) for 12 wk did not affect aortic lesions (82). Supplementation with t10,c12-CLA resulted in adverse changes in adipocyte function and plasma and liver lipid metabolism, which were partially ameliorated by the inclusion of c9,t11-CLA. It is important to note that although the estimated CLA intake in humans varies considerably, it is well below the high doses (1.0% wt:wt on average, 3–5% energy) typically used in animal and cell culture studies.

Dairy fat enriched with VA and CLA. Due to the limited availability of purified rTFA isomers, many studies have used dairy fat enriched in rTFA. Using enriched dairy fat allows for the investigation of the potential synergistic bioactivity of VA and c9,t11-CLA. Most studies that have explored the bioactivity of rTFA-enriched dairy fat on CVD risk have been in rodents. In a study in hamsters, feeding 20% butter fat led to increased reverse cholesterol transport potential as

well as decreased aortic cholesterol-ester deposition, LDL-peroxidability index, and IL-1 mRNA abundance in aorta (71). In a study in LDL receptor-deficient mice, TFA from EA-rich hydrogenated vegetable shortening for 14 wk led to greater atherosclerosis than a butter rich in VA (83). In addition, when a VA-/CLA-enriched butter was fed to rodents, serum cholesterol and TG (84) and the extent of atherosclerosis (83) were reduced compared to regular butter. Two studies using a rabbit model compared the effects of a VA-/CLA-enriched dairy diet to either a t10-C18:1 diet, a major TFA in PHVO, or a control diet low in these fatty acids. These studies reported a primarily neutral effect of VA/CLA butter on risk factors of atherogenesis, as well as less aorta fatty streak development (85,86); however, the ratio of atherogenic:antiatherogenic lipoproteins, VLDL+LDL to HDL, was significantly lower in the VA/CLA butter group compared to the t10-C18:1 group (86).

Potential mechanisms of action of VA and CLA. The potential underlying mechanisms of the cardiovascular effects of c9,t11-CLA have been investigated in some studies. A proposed mechanism through which CLA elicits pleiotropic effects is via activation of PPAR (71,87–92). PPAR are ligand-activated nuclear receptors that regulate the expression of genes involved in lipid and glucose homeostasis. PPARα regulates the expression of genes involved in fatty acid oxidation and energy homeostasis [reviewed in (93)]. Studies with pure isomers suggest that c9,t11-CLA may be a more potent activator of PPARα than t10,c12-CLA (90). However, in a study in PPARα null mice, feeding a mixture of CLA resulted in lower plasma TG, suggesting that the lipid-lowering effects may be independent of PPARα (94). Upregulation of PPARγ has been shown to normalize insulin sensitivity, exhibit anti-inflammatory effects, improve lipid metabolism and clearance of lipoproteins, and restore vascular contractility and endothelial function (93). Immunohistochemical staining of aortic vessels has shown an increased expression of PPARγ in c9,t11-CLA–supplemented animals compared to controls (76). Collectively, these changes may explain the reduced accumulation of cholesterol in arterial vessels and attenuated progression of atherosclerosis demonstrated in some studies (95,96). Treatment with c9,t11-CLA results in augmented acceptor-dependent cholesterol efflux from RAW264.7 macrophage-derived foam cells and increased mRNA expression of CD36, ABCA1, LXR-α, NPC1, and NPC2 (97). CLA also causes suppression of proinflammatory cytokine production (i.e. PGE_2 and TNFα) and IFN-γ induced COX-2 activity in mouse and human macrophages, both of which are considered characteristic changes of a PPARγ agonist treatment (87,98,99). In vascular smooth muscle cells (SMC), treatment with c9,t11-CLA or t10,c12-CLA inhibited collagen production in a PPARγ-dependent manner (100). Collagen production by SMC is considered a hallmark of atherogenesis, because it contributes to intima thickening and plaque formation (100). As reviewed by Eder et al. (101), many other beneficial effects of CLA and metabolites of CLA have been reported in SMC and vascular endothelial cells that may explain in part the antiatherogenic effects of CLA observed in experimental studies, including reduction in secretion of inflammatory and thrombogenic mediators, inhibition of mononuclear cell adhesion, and upregulation of antioxidant defense mechanisms.

CLA also has been reported to increase the expression of the hepatic receptors (apoB100/E-receptor) responsible for the clearance of cholesterol-rich lipoproteins, including LDL and remnant lipoproteins. Upregulation of the hepatic apoB100/E receptor and the inhibition of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase are the primary mechanisms targeted by lipid-lowering therapies, such as statins (102). Valeille et al. (81) reported that an increased mass of LDL-receptor could be detected in the livers of hamsters supplemented with either c9,t11-CLA alone or mixed isomeric CLA preparations. However, the increase in LDL-receptor mass was independent of the effects on HMG-CoA reductase in reducing cholesterol synthesis, suggesting that CLA may have direct regulatory effects on the molecular signaling/production pathways of LDL-receptor expression, such as the insulin-signaling cascade involving phosphatidylinositol 3-kinase (PI3-kinase) and/or sterol regulatory element-binding protein (SREBP-1) pathways.

Some studies have demonstrated divergent mechanisms of c9,t11-CLA and t10,c12-CLA (75,103). In a study in $apoE^{-/-}$ mice, feeding t10,c12-CLA for 12 wk led to higher concentrations of plasma TG, NEFA, and glucose compared with control (LA), whereas c9,t11-CLA led to lower concentrations of plasma TG, NEFA, glucose, and insulin (103). Proteomics analysis suggested that the treatment effect of t10,c12-CLA was explained by upregulation of key enzymes in pathways related to gluconeogenesis, β-oxidation, and ketogenesis. In contrast, c9,t11-CLA induced expression of the antiinflammatory heat shock protein 70 kD protein and decreased expression of the proinflammatory macrophage migration inhibitory factor (103).

Currently, there are few studies that have investigated the mechanistic effects of purified VA on CVD risk factors, due largely to its limited availability. The hypolipidemic effects of VA have been shown to occur independently of the in vivo conversion to CLA. It has been suggested that VA may modulate eicosanoid production to regulate a variety of downstream metabolic pathways involved in atherogenesis, including lipid metabolism, immune response, vascular function, and platelet aggregation (88).

Cancer

Review of epidemiological studies. There are very few epidemiological studies that have investigated the association of intake of VA (104–108) and CLA (104,105,109,110) and risk of cancer (Table 3). Of the 4 case-control studies that have been conducted, 3 studies have reported a direct association with VA concentrations in serum or erythrocytes and risk of breast (106,108) or prostate cancer (107), whereas one study reported an inverse association of serum VA and breast cancer among postmenopausal women (104). In the Netherlands Cohort Study, energy-adjusted intake of VA was associated with an

increased risk of breast cancer after multivariate adjustment (RR = 1.34; 95% CI = 0.98–1.82; *P*-trend = 0.006); however, after multivariate adjustment, there was no relationship between breast cancer incidence and animal fat intake (RR = 1.05; 95% CI = 0.79–1.40, *P*-trend = 0.87) or intake of milk/milk products and meat from ruminants ($P > 0.05$), the major dietary sources of VA (105).

There have been 4 case-control studies that have investigated CLA intake and cancer. Of these, one study has reported an inverse association with dietary intake of CLA and risk of colorectal cancer (110), and one study found significantly lower dietary intake and serum concentrations of CLA in individuals with breast cancer compared to those without breast cancer among postmenopausal women (104). In the colorectal cancer study, women who consumed ≥4 servings/d of high-fat dairy foods had a relative risk of 0.59 compared to women who consumed <1 serving/d after multivariate adjustment (95% CI = 0.44–0.79). In women in the highest quartile of CLA intake, there was a 29% reduction in the risk of colorectal cancer compared to those in the lowest quartile of intake (RR = 0.71; 95% CI = 0.55–0.91; *P*-trend = 0.004) (110). In the other 2 case-control studies, there was no significant association of CLA [either as dietary intake (111) or concentration of CLA incorporated into adipose tissue (109)] and the risk of breast cancer. However, in one of the studies, there was a reduced risk of having an estrogen receptor (ER)-negative tumor, in premenopausal women, when comparing the highest quartile of CLA intake and the lowest (OR = 0.40; 95% CI = 0.16–1.01) (111). In a prospective cohort study, intake of CLA was weakly associated with breast cancer incidence when comparing the highest and lowest quintiles of intake (RR = 1.24; 95% CI = 0.91–1.69; *P*-trend = 0.02) (105). To our knowledge, there has been only one study that investigated the association of CLA and metastasis in individuals with initially localized breast cancer (112). There was no significant association between the concentration of CLA in breast adipose tissue at the time of diagnosis and the risk of either metastasis or death. However, the authors did note that the concentration of CLA in breast adipose tissue may have been below the limit of detection for the sample conditions (112).

Review of clinical studies. To our knowledge, there are no clinical trials to date that have investigated the effects of rTFA on cancer risk or tumor growth. There are 2 studies that are registered with clinicaltrials.gov. One study is designed to determine whether CLA consumption suppresses expression of lipogenesis markers in breast cancer tissue in newly diagnosed breast cancer patients (NCT00908791). The other study will investigate whether oral CLA blocks metabolism of lipids in patients with advanced cancers (NCT00951158).

Review of animal and cell culture studies.

Vaccenic acid. Some studies in cancer cell lines and rodent tumor models have demonstrated that VA may reduce cell growth and/or tumor metabolism (113–118), whereas others have not (119). Treatment of VA on HT-29 human colonic adenocarcinoma cells showed inhibitory effects on tumor growth, whereas EA had no effect (116). Similar effects of reduced cell growth were observed in other cell lines (MCF-7 breast cancer cells, SW480 colon cancer cells) following 4 d of treatment with pure VA (20 μg/mL) (117). SW480 cells exhibited increased DNA fragmentation and reduced cytosolic glutathione, suggesting that cell death was the result of increased apoptosis (117). The eicosanoid profile was altered with VA treatment similarly to that observed during c9,t11-CLA treatment.

Studies investigating the effects of pure VA on carcinogenesis in animal models are limited (Table 5), largely due to the high cost of pure VA oil. In a mouse hepatoma model, in situ perfusion with purified VA for 2.5 h led to inhibition of fatty acid uptake by the tumor, as well as decreased cAMP and extracellular signal-regulated kinase p44/p42 phosphorylation (120). These results suggest that VA may have an acute inhibitory effect on tumor growth. In a short-term feeding study, Banni et al. (114) fed methylnitrosourea (MNU)-injected female rats with 2% VA-supplemented basal diet. After 6 wk, treated rats had almost 50% fewer premalignant lesions compared to control rats. Some studies have shown that the anticarcinogenic effects of VA may be due to its conversion to c9,t11-CLA (115,118).

Conjugated linoleic acid. CLA has been reported to affect initiation, promotion, and metastasis of mammary/breast, prostate, and gastrointestinal cancers in experimental animal models, either carcinogen-induced or genetically modified (121–123). Several isomers of CLA have been shown to exhibit antitumorigenic properties; however, in this review, we will focus on c9,t11-CLA (Table 5).

CLA and mammary cancer. Young female Sprague Dawley rats injected with carcinogens, such as MNU, dimethylbenz[a]anthracene, and benzo[a]pyrene, are a class of well-developed carcinogenesis models with detectable premalignant lesions in mammary gland within a few weeks after carcinogen administration (124). Dietary c9,t11-CLA (0.5–1%) treatment has been shown to effectively reduce the number of premalignant lesions, proliferation rate of epithelial cells, and apoptosis of preneoplastic lesions in these induced cancer models (114,125–127). Inhibition of proliferation by CLA has been accompanied by reduced size and density of terminal end bud structures, the site of tumor formation in both rats and humans, likely due to modulation of cell cycle regulators such as cyclin A and cyclin D1 (128,129). In a transplantable mouse model with mammary tumor and spontaneous lung metastasis, CLA [mixture of isomers: c9,t11-CLA (32.5 g/100 g), t10,c12-CLA (33.5 g/100 g), 18:1 (18 g/100 g), and other isomers (16 g/100 g)] reduced the metastasis rate, total tumor burden, and the survival of metastatic cells, in a dose-dependent manner, without affecting primary tumor growth (130). However, several other studies have demonstrated no effect of c9,t11-CLA, particularly on latency in mice bearing mammary tumors (130–133). Data from cell

culture studies have demonstrated antiproliferative effects of CLA in MCF-7 breast cancer cells (134,135), possibly due to effects on PPARγ and E-cadherin/β-catenin pathways (136). Some studies have shown a strong inhibitory effect of c9, t11-CLA in MCF-7 cells, an ER-positive breast cancer model (137), but not in ER-negative MDA-MB-231 cells (138), suggesting that ER may play a vital role in mediating the effect of CLA on mammary tumor. Miller et al. (139) attributed the growth-suppressing effect of CLA to changes in arachidonic acid distribution among cellular lipids and an altered prostaglandin profile. In contrast, Tanmahasamut et al. (138) reported that c9,t11-CLA was the least potent in inhibiting MCF-7 cell growth among 5 commercially available CLA isomers. More recently, the inhibition of cell growth in MCF-7 cells and human breast epithelial and stromal cells was greater with t10,c12-CLA than with c9,t11-CLA (140,141). Other investigators have observed similar weak effects of c9,t11-CLA when studying metabolic pathways (142–145).

CLA and gastrointestinal cancer. In the *APCMIN/+* mouse, a transgenic model, purified c9,t11-CLA preparations have been shown to decrease colonic polyp number without increasing diameter (146). Similarly, Soel et al. (147) have reported that at low dietary concentrations (0.1% wt:wt), c9, t11-CLA effectively inhibits metastatic migration of mouse colon cancer cells when injected into BALB/c mice. However, in other studies using a similar model, there was no effect of c9, t11-CLA on the number of adenomas, nor any change in mucosal NF-κB or cyclin D1 protein mass (148). Moreover, it was suggested that t10,c12-CLA had a profound inhibitory impact on tumor progression and regulators of cell cycle. However, in a study in the Min mouse model of intestinal carcinogenesis, 1% t10,c12-CLA stimulated adenoma growth and increased urinary 8-isoPGF$_{2\alpha}$ compared with controls, whereas the 1% c9,t11-CLA group was not significantly different from controls in any variables measured (148). In addition, t10,c12-CLA increased cyclin D1, suggesting activation of NF-κB as a potential mechanism. In a study by Park et al. (149), Sprague-Dawley rats were i.m. injected with 15 mg/kg of 1,2-dimethylhydrazine, twice per week for 6 wk, and fed a control diet, 1% t10,c12-CLA, or 1% c9,t11-CLA. After 30 wk of feeding, the tumor numbers in the colonic mucosa were decreased and the apoptotic index was increased in both CLA groups, compared with the control group. There were no significant differences between c9,t11-CLA and t10,c12-CLA (149). Recent studies have investigated the effects of CLA on cancer cachexia (150, 151), due to the possible antiproliferative and antiinflammatory effects of CLA. In a recent study, c9,t11-CLA–rich oil (6:1 c9,t11-CLA/t10,c12-CLA) did not ameliorate wasting in late-stage cancer cachexia in a mouse model (150); instead, it resulted in more severe adipose atrophy and increased expression of inflammatory makers in the muscle.

Several in vitro studies have shown that t10,c12-CLA, and not c9,t11-CLA, inhibits cell proliferation and induces apoptosis in human colonic adenocarcinoma cells (such as HT-29, MIP-101, and Caco-2) (152–155). Other studies suggest a similar weak effect of c9,t11-CLA on cell proliferation and apoptosis compared to other isomers (156–158). Similar conclusions were made from other studies focusing on individual cell culture models in various cell lines (colorectal, breast, and prostate cancers), with different treatment times and dosages (144,159–161). Nevertheless, some studies demonstrate inhibitory effects of c9,t11-CLA on tumor progression. In one study, the proliferation and differentiation of both HT-29 and Caco-2 cells were significantly inhibited by c9, t11-CLA in a dose-dependent manner ranging from 10 to 200 μmol/L (119). Other studies have shown that concentrations of c9,t11-CLA ranging from 25 to 200 μmol/L induced apoptosis in gastric adenocarcinoma cell line SGC-7901 and inhibited cell growth and proliferation (162,163).

CLA and prostate cancer. There have been few animal and cell culture studies that have examined the effects of CLA and prostate cancer. Animal studies that have shown protective effects of CLA on prostate cancer have mainly used a mixture of c9,t11-CLA and t10,c12-CLA rather than individual preparations. CLA mixtures have been reported to decrease PhIP-induced mutagenesis in the prostate of transgenic rats (164). Earlier studies that used DU145 human prostate carcinoma cells transplanted into immunodeficient mice showed antitumorigenic effects of CLA mixtures similar to those observed with breast cancer (165). In contrast, CLA did not inhibit growth of prostate tumor cells in a prostatic adenocarcinoma rat model inoculated with R-3327-AT-1 tumor cells; instead, CLA significantly increased tumor volume compared with controls (166). In vitro studies using purified c9,t11-CLA and t10,c12-CLA have reported antiproliferative effects of both isomers in PC-3 prostatic carcinoma cells, with t10,c12-CLA acting as the more potent isomer (159,167), although studies of isomer-specific effects are limited. The isomers were found to affect different pathways; c9,t11-CLA modulated arachidonic metabolism and eicosanoid production via 5-lipoxygenase and cyclooxygenase expression, whereas t10,c12-CLA modulated apoptosis and cell cycle control via increased expression of p21 and decreased expression of bcl-2 (167). One study found that treatment of DU145 cells with t10,c12-CLA (2.5–10 μmol/L) resulted in a dose-dependent reduction in the number of viable cells, whereas c9,t11-CLA (5 μmol/L) slightly increased the number of viable cells (168). Other studies also have found isomer-specific mechanisms of action of CLA (169,170). In a study in LNCaP human prostate cancer cells, c9,t11-CLA, but not t10,c12-CLA, increased apoptosis by 59%, which correlated with a decrease in NF-κB activation ($P < 0.05$) (170).

Dairy fat enriched with VA and CLA. Numerous studies in rats with MNU-induced tumors have demonstrated that butter enriched with VA and CLA led to the development of fewer mammary tumors, with a dose-dependent reduction in premalignant lesions, tumor growth, and cell proliferation (113–115,118). In MCF-7 cells, CLA-containing milk fat suppressed cell growth and increased lipid peroxidation in a dose-dependent manner (134). Mixtures containing isomers of CLA and LA at similar concentrations to the milk fat samples were as effective at inhibiting growth and stimulating

peroxidation as the milk fatty acids; c9,t11-CLA and a mixture of CLA isomers were significantly more effective than t10,c12-CLA. In another study, CLA-enriched milk fat inhibited cell growth in MCF-7 and SW480 cells (171).

Potential mechanisms of action of VA and CLA. Results from animal and cell studies with CLA and cancer suggest that several potential mechanisms are likely involved, including effects on PPAR, alteration of arachidonic acid metabolism (COX-2, 5-LOX), changes in eicosanoid production, induction of apoptosis (bcl-2, caspase activity), and modulation of cell cycle control (cyclin A1, cyclin D) and cell proliferation (c-myc, c-jun) (119,158,163,167). Evidence suggests that c9,t11-CLA may inhibit the initiation and post-initiation/promotion stages of carcinogenesis, whereas very limited data are available regarding the effect of CLA on the progression stage of carcinogenesis. Overall, data from both animal and in vitro studies suggest that VA may exhibit anticancer properties, although some studies suggest that VA per se does not exhibit anticarcinogenic properties but rather plays a role as the precursor to endogenous synthesis of c9,t11-CLA (114,115,118,172). In contrast, others have proposed several additional mechanisms besides conversion to CLA that may play a role in the anticarcinogenic effects of VA, including effects on phosphatidylinositol hydrolysis, reduced proliferation, inhibition of fatty acid uptake, and increased apoptosis (116,117,120).

Conclusions

Although limited, feeding studies in rodent models, with either induced or spontaneous dyslipidemia, do not support adverse health effects of VA on markers of CVD risk. Furthermore, findings suggest that VA supplementation may improve dyslipidemia by lowering circulating TG and/or cholesterol and attenuate atherosclerotic progression. Most studies with CLA have used mixtures of c9,t11-CLA and t10,c12-CLA; however, more recent studies suggest that these isomers may have divergent physiological effects. Some studies in various animal models have shown a beneficial effect of c9,t11-CLA on atherosclerotic lesions and risk factors of CVD. Inconsistencies in these data may be the result of differences in experimental models and species, base diet (chow vs. semipurified), and dose and isomer of CLA. It is important to note that although the estimated intake of c9, t11-CLA in humans varies considerably, the doses used in animal and cell culture studies (1.0% wt:wt on average, 3–5% energy) are typically high relative to intakes reported in epidemiological studies ($\sim$0.1% energy).

Results from epidemiological studies generally have shown an inverse or no association between rTFA intake and CHD across multiple geographical locations. However, a trend for a direct association was reported in 2 studies (10,40). Inconsistencies in the data may be due in part to differences in the populations studied (i.e. gender) and in TFA intake. For example, in the Scottish Heart Health Study, intake of rTFA was relatively high (intake ranging from 1.2 to 4.9 g/d) compared with other studies (intake ranging up to 2.5 g/d). More epidemiologic data are needed to clarify the associations of rTFA intake and CHD risk. In particular, more information is needed to determine whether gender influences the cardiovascular effects of rTFA.

Results from clinical studies that have investigated the effects of VA and c9,t11-CLA on CVD risk factors remain unclear. There are multiple factors that may be contributing to the inconsistencies in the clinical data, including differences in dosage and isomer type, source of supplementation (capsules vs. diet), level of control of the overall diet, control diet used for comparison, duration of intervention, and study population (gender, age, and metabolic state of the participants, i.e. healthy vs. diseased). Some studies suggest that at lower doses, rTFA do not affect lipids and lipoproteins, but at higher doses, which are not attainable by diet, rTFA may have similar effects as iTFA.

Most of the studies that have investigated the effects of modified milk fat (resulting from dietary manipulation of dairy cows) have been conducted in animals. These studies have demonstrated a neutral or beneficial effect of VA/c9, t11-CLA on atherosclerosis and risk factors of CVD. It is important to note that modifying milk fat to increase VA/c9, t11-CLA also results in changes in other fatty acids, such as decreases in hypercholesterolemic SFA (lauric, myristic, and palmitic acids) and increases in neutral and hypocholesterolemic fatty acids (stearic acid, OA, and LA), thus making it difficult to distinguish which fatty acid modifications are responsible for any effects (i.e. decrease of SFA, increase of rTFA, increase of MUFA/PUFA, and/or increase of specific isomers of rTFA).

The anticarcinogenic properties of c9,t11-CLA have been studied in numerous experimental studies, various cell lines, and various animal models, both carcinogen induced and genetically modified. Animal studies generally show a benefit of c9,t11-CLA in mammary cancer and suggest that the anticarcinogenic effects of c9,t11-CLA may vary across species, with rats being more responsive than mice. Animal studies for gastrointestinal and prostate cancer are more limited and the data are inconclusive. Evidence from cell culture studies suggests that there may be isomer-specific effects of CLA, but results from different studies are very inconsistent. There are many factors that likely contribute to the discrepancies in the data from cell studies, including differences in tumor type, stage of development, treatment dose and isomer, and study duration. As mentioned above, it is important to keep in mind that the doses of CLA used in animal and cell culture studies are relatively high compared to the reported dietary intake in humans from epidemiological studies. There have been few animal studies with pure VA, largely due to high cost; however, the limited number of existing studies suggests that VA may inhibit tumor growth. Results from cell studies in human breast and colon adenocarcinoma cells generally show an inhibition of VA on cell growth. Although limited, the data suggest that dairy fat enriched with VA/c9,t11-CLA may reduce tumor development.

Epidemiological studies with VA and risk of cancer are very limited, although they generally do not support a

benefit of VA intake. There are limitations to assessing dietary intake of rTFA isomers in epidemiological studies, because they are relatively minor lipids in the diet and are found in low concentrations in serum. Dietary questionnaires and databases may not accurately depict intake and food composition of VA and c9,t11-CLA. In some of the epidemiological studies, comparisons were made between groups (i.e. cases vs. controls) with very small or no significant differences in VA intake. Although results from some in vitro and in vivo studies suggest an anticarcinogenic effect of c9,t11-CLA, to date, there have been no clinical trials conducted to study the effects of VA or c9,t11-CLA on markers of cancer risk in humans; thus, it is difficult to draw conclusions regarding VA and c9,t11-CLA and various cancers.

In conclusion, although data from experimental models suggest that rTFA may beneficially affect risk of CVD and cancer, further research is needed to determine the effects of VA and c9,t11-CLA in humans as well as clarify the isomer-specific effects of CLA. Data from existing human studies do not consistently support the findings from experimental studies. Many of the clinical studies that have investigated the effects of rTFA on markers of cardiovascular risk have not been adequately powered; thus, the lack of detection of treatment effects reported in some studies may be due to insufficient statistical power. In addition, many studies have used doses of rTFA that are not realistically attainable via diet; the effect of rTFA in amounts that are commonly consumed in the diet remains unclear. Further clinical studies are warranted due to the limited number of studies and inconsistencies in the available data; specifically, adequately powered, controlled-feeding studies are needed to determine the effects of VA and c9,t11-CLA on markers of risk of CVD and cancer.

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All authors wrote the paper and read and approved the final manuscript. S.K.G. and D.J.B. had primary responsibility for final content.

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