European Journal of Clinical Nutrition (2013) **67,** 541–547

www.nature.com/ejcn

REVIEW

Trans fatty acids and cardiovascular health: research completed?

IA Brouwer[1], AJ Wanders[2] and MB Katan[1]

This review asks the question if further research on *trans* fatty acids and cardiovascular health is needed. We therefore review the evidence from human studies on *trans* fatty acids and cardiovascular health, and provide a quantitative review of effects of *trans* fatty acid intake on lipoproteins. The results show that the effect of industrially produced *trans* fatty acids on heart health seen in observational studies is larger than predicted from changes in lipoprotein concentrations. There is debate on the effect of ruminant *trans* fatty acids and cardiovascular disease. Of special interest is conjugated linoleic acid (CLA), which is produced industrially for sale as supplements. Observational studies do not show higher risks of cardiovascular disease with higher intakes of ruminant *trans* fatty acids. However, CLA, industrial and ruminant *trans* fatty acids all raise plasma low-density lipoprotein and the total to high-density lipoprotein ratio. Gram for gram, all *trans* fatty acids have largely the same effect on blood lipoproteins. In conclusion, the detrimental effects of industrial *trans* fatty acids on heart health are beyond dispute. The exact size of effect will remain hard to determine. Further research is warranted on the effects of ruminant *trans* fatty acids and CLA on cardiovascular disease and its risk factors.

European Journal of Clinical Nutrition (2013) **67,** 541–547; doi:10.1038/ejcn.2013.43; published online 27 March 2013

Keywords: trans fatty acids; CLA; ruminant; cardiovascular disease

BACKGROUND

Cardiovascular disease is an important cause of death. In Western countries approximately one in three people die of cardiovascular disease. Many of these deaths occur before the age of 65 years.[1] A high intake of *trans* fatty acids increases risk factors for cardiovascular diseases and is associated with increased risk incidence of such diseases.[2–4]

Trans fatty acids are unsaturated fatty acids with at least one double bond in the *trans* configuration, which results in a straighter shape than a double bond in a *cis* configuration. *Trans* fatty acids can be divided in two groups: artificial *trans* fatty acids (industrial) and natural *trans* fatty acids (ruminant). Although *trans* fatty acids may be formed from *n*-3 and *n*-6 fatty acids, they do not serve any vital functions and are not essential fatty acids. Industrial *trans* fatty acids are formed by partial hydrogenation of vegetable and fish oils, a process that transforms oils into semi-solid fats, which are easier to process into foods. Ruminant *trans* fatty acids are produced in the rumens of cows and sheep and are present in dairy and meat. Finally, conjugated linoleic acid (CLA) is a *trans* fat first discovered in milk, which is now produced industrially as an aid in weight loss.

In this overview we summarize the evidence on *trans* fatty acids and cardiovascular health. Differences and similarities between industrial and ruminant *trans* fatty acids are discussed; only evidence from human studies is taken into account. We will first briefly discuss the acknowledged detrimental effects of industrial *trans* fatty acids on cardiovascular health and then focus on *trans* fatty acids from ruminant sources and more specifically on CLA. Finally, we will examine whether there are still important questions in this field to be solved, or whether research on *trans* fatty acids and cardiovascular health can be considered completed.

Sources, structure, nomenclature and intake of *trans* fatty acids

Industrial *trans* fatty acids are formed during partial hydrogenation of vegetable or fish oils with hydrogen gas and metal catalysts. Ruminant *trans* fatty acids are produced in the rumens of cows and sheep. They arise through biohydrogenation and/or isomerization of *cis*-unsaturated fatty acids from the feed by hydrogen produced during oxidation of substrates with bacterial enzymes as catalysts.[5,6] In both cases, a range of *trans* fatty acid isomers are formed that differ in the position of the double bond along the fatty acid molecule. The notation for such positional isomers is exemplified by vaccenic acid, C18:1 Δ11*t*. It has 18 C atoms and 1 double bond in the *(trans)* configuration at C-atom position number 11.

Industrial and ruminant *trans* fatty acids consist of the same positional *trans* isomers, but in different proportions. The isomer profile depends on conditions of hydrogenation, such as catalysts used and temperature of hydrogenation for industrial *trans* fatty acids and rumen pH, and the composition of oils in the diet for ruminant *trans* fatty acids. C18:1Δ10t and Δ9t (elaidic acid) are typically the main isomers of industrial *trans* fat, and vaccenic acid (C18:1 Δ11*t*) is usually the main component of ruminant *trans* fat. However, industrial *trans* fats also contain a considerable amount of vaccenic acid.[7]

CLA is another *trans* fatty acid. CLA has two double bonds, one in the *trans* and one in the *cis* configuration. The double bonds in CLA are conjugated, which means that CLA has only one carbon atom between the two double bonds instead of the usual two. The two main isomers of CLA are *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA. *Cis*-9, *trans*-11 CLA is naturally present in small amounts in ruminant fat. Supplements are a much larger dietary source of CLA. CLA in these supplements is manufactured from vegetable oils[8,9] and contains both *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA.

[1]Department of Health Sciences and the EMGO Institute for Health Care Research, Faculty of Earth and Life Sciences, VU University, Amsterdam, The Netherlands and [2]Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands. Correspondence: Dr IA Brouwer, Department of Health Sciences and the EMGO Institute for Health Care Research, Faculty of Earth and Life Sciences, VU University, De Boelelaan 1085, Amsterdam 1081 HV, The Netherlands.
E-mail: Ingeborg.Brouwer@vu.nl
Received 2 November 2012; accepted 14 January 2013; published online 27 March 2013

In this review, we follow the chemical nomenclature and designate all fatty acids with at least one double bond in the *trans* configuration as *trans* fatty acids or *trans* fats for short. However, the Codex Alimentarius standard and some countries do not define animal trans fats and *trans* fats with conjugated double bonds as *trans* fats in a legal sense, because they are thought to lack the harmful effects of 'real' *trans* fatty acids. Thus, in those countries CLA does not have to be labeled as *trans* fat on a food product. The government of Denmark has put a limit on *trans* fat in foods, but exempts ruminant *trans* fats.

The intake of *trans* fatty acids has decreased considerably over the past two decades because the food industry has largely eliminated industrial *trans* fatty acids from foods. This has resulted in average intakes in European countries of 1–2 energy percent[10] and less than 1 energy percent in the United Kingdom, with a major proportion coming from dairy and meat.[11] Intakes in the USA are coming down as well, but not as much as in Europe.[12] In Europe and the USA the average intake of *trans* fatty acids from ruminant sources is around 0.5 energy percent.[10,12]

INDUSTRIAL *TRANS* FATTY ACIDS AND CARDIOVASCULAR HEALTH

In the early 1990s metabolic studies showed that consumption of industrial *trans* fatty acids raised low-density lipoprotein (LDL) and lowered high-density lipoprotein (HDL) cholesterol levels in the blood.[13,14] Furthermore, observational studies showed that a higher intake of *trans* fatty acids was associated with a higher risk of coronary heart disease.[15] In 2006, results of observational studies were pooled and that resulted in a multivariable adjusted relative risk of 1.23 (95% confidence interval 1.11–1.37) for coronary heart disease for isocaloric replacement of 2 energy percent of carbohydrate by *trans* fatty acids.[16]

Thus, both observational studies on the incidence of cardiovascular disease and metabolic intervention studies on lipoproteins indicate that industrial trans fatty acids have detrimental effects on cardiovascular health.[4,15–17] Based on these results, industries and governments have taken actions to limit the intake of *trans* fatty acids from industrial sources.[18,19] There is still debate about the size of the effect of industrial *trans* fatty acids on cardiovascular disease and about effects of industrial *trans* fatty acids on metabolic pathways other than lipoproteins that may lead to cardiovascular disease. Still, the detrimental effects on cardiovascular disease are scientifically established, and it is unlikely that industrial *trans* fatty acids will be brought back into the food chain. At present, the debate has shifted towards the effects of ruminant *trans* fatty acids and CLA.

RUMINANT *TRANS* FATTY ACIDS, AND CLA AND CARDIOVASCULAR HEALTH

Effects on cardiovascular disease

Investigating effects of ruminant *trans* fatty acids on disease outcomes and comparing these effects with industrial *trans* fatty acids is challenging because ruminant *trans* fatty acids are only present in small amounts in the diet. Until the mid 1990s partially hydrogenated vegetable oils containing up to 50% *trans* fatty acids were widely used in the food industry. In contrast, milk fat naturally contains only some 5% *trans* fatty acids. As a result, observational studies have lacked the power to pick up an effect of this small amount of ruminant *trans* fatty acids, if any, amid so many other factors that affect the risk of cardiovascular disease. Many observational studies have investigated the associations between *trans* fatty acid concentrations in blood or adipose tissue and the risk of cardiovascular disease, but when blood or adipose tissues are studied it is not possible to distinguish which *trans* fatty acid is coming from which source. Only very few observational studies have investigated separate effects of dietary ruminant *trans* fatty acids. A meta-analysis of four prospective cohort studies did not show a significant association between intake of ruminant *trans* fatty acids and coronary heart disease (relative risk = 0.92; 95% confidence interval 0.76–1.11). Intake of total *trans* fatty acids in these studies ranged from 2.8–10 g/day.[20]

One could argue that the question whether ruminant *trans* fatty acids cause cardiovascular disease is irrelevant, because their intake is so low.[21] This holds even more so for CLA from natural sources, because CLA constitutes a tiny fraction of ruminant *trans* fatty acids. However, intake of CLA in the form of supplements can be appreciable, recommended dosages are up to 6 g/day.

Effects on lipoproteins: updated review

In 2010, we reviewed available metabolic studies on effects of ruminant and industrial *trans* fatty acids on HDL and LDL cholesterol level in humans.[4] We now updated our review and added data on 4 more studies on ruminant *trans* fatty acids and 15 more studies on CLA. Other differences with our previous review are that we now weighed the studies for study size, we compared the effect of the treatment with the original control treatment of the study, instead of recalculating all studies to *cis* monounsaturated fatty acids, and we report the results in grams of fatty acids instead of energy percentages.

We combined data from different studies using linear regression, with intake of *trans* fatty acids as the independent variables and change in LDL cholesterol, HDL cholesterol, the plasma LDL/HDL cholesterol ratio and the total/HDL cholesterol ratio as dependent variables. We calculated these ratios on the basis of mean values of treatment differences within studies, therefore, we lacked an estimate of variation for these ratios. To maintain uniformity, we recalculated the ratios of LDL/HDL cholesterol and the ratios of total to HDL cholesterol from the mean LDL, HDL and total cholesterol levels for all studies, even when ratios had been reported. We weighed the studies for size using the inverse of the square root of the number of participants. We used comparisons with the original control treatments in all cases.

The regression lines calculated with the conventional least square methods showed a positive intercept with the y-axis. This may be an artefact because conventional linear regression algorithms presuppose that *x*-values have zero error.[22] However, not every meal or capsule consumed in a CLA trial is precisely analyzed for CLA, and therefore reported CLA intakes contain random errors. As a result the slope calculated with conventional regression software is biased downwards, and the *y*-intercept goes up. Correction of this error requires knowledge of the size of the error in the intake variable, but that is unknown. Forcing the regression line through the origin is a suitable alternative because a zero change in diet should produce a zero change in blood lipids. Treatment with a zero dose of CLA as such does not necessarily produce a zero change, because enrolling subjects in a trial may affect their lipid levels. However, all data points analyzed represent the effect of a treatment with a certain amount of CLA minus the effect of a concurrent control treatment that contained a different amount of CLA. If the test and control treatment both contain 0 g of CLA, then logic allows no other outcome for the change in a lipid value than 0 plus or minus a random error term, and a regression line that does not contain the (0,0) point contradicts logic.

Ruminant trans fatty acids. We identified a total of eight published and one unpublished study on effects of ruminant *trans* fatty acids on lipoproteins; these include 11 comparisons and 672 subjects (Table 1). The unpublished results are from a study[23] that has not yet been published in a peer reviewed journal (September 2012). Therefore, we used the values as presented

Table 1. Randomized trials assessing the effect of ruminant *trans* fatty acids or CLA, relative to control fats, on lipoproteins

Trial	*Design*	N	*Duration of treatment*	*Diet/ supplement*	*Tested fat*	*Amount (in grams of fat)*	*Weight stable*[a]
Desroches *et al.* [26]	Cross-over	16	4 weeks	Diet	Ruminant	6.0	Yes
Tholstrup *et al.* [27]	Parallel	42	5 weeks	Diet	Ruminant	3.7	Yes
Tricon *et al.* [29]	Cross-over	32	6 weeks	Diet	Ruminant	6.9	Yes
Chardigny *et al.* [24]	Cross-over	40	3 weeks	Diet	Ruminant	11.0	Yes
Motard–Belanger *et al.* [32]	Cross-over	38	4 weeks	Diet	Ruminant	8.0	Yes
	Cross-over	38	4 weeks	Diet	Ruminant	2.0	Yes
Lacroix *et al.* [33]	Cross-over	61	4 weeks	Diet	Ruminant	2.8	Yes
Brown *et al.* [34]	Parallel	18	8 weeks	Diet	Ruminant	0.82	Yes
Venkatraman *et al.* [35]	Cross-over	15	8 weeks	Diet	Ruminant	1.3	Yes
Baer *et al.* (unpublished)	Cross-over	105	3 weeks	Diet	Ruminant	7.5	Yes
	Cross-over	105	3 weeks	Diet	Ruminant	2.5	Yes
Aryaeian *et al.*[37]	Parallel	44	12 weeks	Supplement	CLA 50:50	2.0	Yes
	Parallel	43	12 weeks	Supplement	CLA 50:50 + vitamin E	2.0	Yes
Berven *et al.* [38]	Parallel	47	12 weeks	Supplement	CLA 50:50	3.4	Yes
Benito *et al.* [39]	Parallel	17	9 weeks	Supplement	CLA 50:50	3.9	Yes
Blankson *et al.* [40]	Parallel	22	12 weeks	Supplement	CLA 50:50	1.7	No
	Parallel	18	12 weeks	Supplement	CLA 50:50	3.4	No
	Parallel	21	12 weeks	Supplement	CLA 50:50	5.1	No
	Parallel	21	12 weeks	Supplement	CLA 50:50	6.8	No
Diaz *et al.* [41]	Parallel	35	12 weeks	Supplement	CLA 50:50 + chromium	1.8	No
Pfeuffer *et al.* [42]	Parallel	42	4 weeks	Supplement	CLA 50:50	4.5	No
Gaullier *et al.* [43]	Parallel	105	6 months	Supplement	CLA 50:50	3.4	Yes
Gaullier *et al.* [44]	Parallel	157	12 months	Supplement	FFA CLA 50:50	3.6	No
	Parallel	157	12 months	Supplement	TAG CLA 50:50	3.4	No
Iwata *et al.* [45]	Parallel	40	12 weeks	Supplement	CLA 50:50	3.4	Yes
	Parallel	40	12 weeks	Supplement	CLA 50:50	6.8	Yes
Joseph *et al.* [46]	Cross-over	27	8 weeks	Diet	CLA 50:50	2.8	Yes
	Cross-over	27	8 weeks	Diet	CLA 90:10	2.7	Yes
Kamphuis *et al.* [47]	Parallel	27	13 weeks	Supplement	CLA 50:50	1.8	No
	Parallel	27	13 weeks	Supplement	CLA 50:50	3.6	No
Lambert *et al.* [48]	Parallel	62	12 weeks	Supplement	CLA 50:50	3.9	Yes
Laso *et al.* [49]	Parallel	21	12 weeks	Diet	CLA 50:50	3.0	Yes
	Parallel	23	12 weeks	Diet	CLA 50:50	3.0	Yes
Moloney *et al.* [50]	Parallel	32	8 weeks	Supplement	CLA 50:50	3.0	Yes
Mougios *et al.* [51]	Parallel	22	8 weeks	Supplement	CLA 50:50	1.0	Yes
Nazare *et al.* [52]	Parallel	44	14 weeks	Diet	TAG CLA 50:50	3.76	Yes
Noone *et al.* [53]	Parallel	51	8 weeks	Supplement	CLA 80:20	1.76	No
	Parallel	51	8 weeks	Supplement	CLA 50:50	2.02	No
Raff *et al.* [54]	Parallel	38	5 weeks	Diet	CLA 50:50	4.3	Yes
Riserus *et al.* [56]	Parallel	24	4 weeks	Supplement	CLA 50:50	4.2	Yes
Riserus *et al.* [55]	Parallel	38	12 weeks	Supplement	CLA 50:50	3.4	Yes
	Parallel	38	12 weeks	Supplement	CLA 90:10	3.4	No
Riserus *et al.* [57]	Parallel	25	3 months	Supplement	CLA 90:10	3.0	No
Smedman and Vessby [58]	Parallel	50	12 weeks	Supplement	CLA 50:50	4.2	Yes
Steck *et al.* [59]	Parallel	48	12 weeks	Supplement	CLA 50:50	3.2	Yes
	Parallel	48	12 weeks	Supplement	CLA 50:50	6.4	Yes
Taylor *et al.* [60]	Parallel	40	12 weeks	Supplement	CLA 50:50	4.5	Yes
Tholstrup *et al.* [61]	Parallel	51	16 weeks	Supplement	CLA 50:50	4.6	Yes
Venkatraman *et al.* [35]	Cross-over	15	8 weeks	Diet	CLA 50:50	1.3	No
Watras *et al.* [62]	Parallel	40	6 months	Supplement	CLA 50:50	3.2	No
Attar–Bashi *et al.* [63]	Parallel	16	8 weeks	Supplement	CLA 50:50	3.2	Yes
Michishita *et al.* [64]	Parallel	22	12 weeks	Supplement	CLA 50:50	1.6	Yes
Petridou *et al.* [65] (average)	Cross-over	16	45 days	Supplement	CLA 50:50	2.1	Yes
Naumann *et al.* [66]	Parallel	92	13 weeks	Supplement	CLA 80:20 c9t11 CLA	3.0	Yes
	Parallel	92	13 weeks	Supplement	CLA 20:80 t10c12 CLA	3.0	Yes
Sluijs *et al.* [67]	Parallel	346	6 months	Supplement	CLA 80:20	3.2	No
Tholstrup *et al.* [61]	Parallel	50	16 weeks	Supplement	CLA 90:10	5.1	Yes
Wanders *et al.* [36]	Cross-over	61	3 weeks	Diet	CLA 80:20	20.0	Yes

Abbreviations: CLA, conjugated linoleic acid; CLA 50:50, 50:50 mixture of c9,t11 CLA and t10,c12 CLA; CLA 80:20, 80:20 mixture of c9,t11 CLA and t10,c12 CLA; CLA 90:10, 90:10 mixture of c9,t11 CLA and t10,c12 CLA; FFA, free fatty acid; ruminant, natural *trans* fatty acids from milk fat; TAG, triacylglycerol. [a]Weight stable = studies not intended for weight loss and no more than 1.5 kg weight change during the study.

at the International Dairy Conference in November 2010 (http://www.wds2010.com/delegates/presentations/10wed/06-Session0_2-David%20Baer.pdf). Linear regression showed that replacement of 1 g of control fat by ruminant *trans* fat increased the plasma LDL/HDL ratio and increased LDL cholesterol (Table 2; Figure 1). Results were similar to our earlier review except for the effect on HDL, for which we do not show a significant effect now. This apparent discrepancy is explained by addition of new studies and

Table 2. Results of randomized studies of the effects of diets high in ruminant *trans* fatty acids and supplemental CLA compared with control treatment on the ratio of LDL/HDL cholesterol, LDL cholesterol, HDL cholesterol and the ratio of total cholesterol/HDL cholesterol[a]

	LDL/HDL	*LDL*	*HDL*	*TC/HDL*
Ruminant trans fatty acids compared with control fat				
Effect of substituting ruminant fat by control fat/g/day	0.014	0.017	0.000	0.019
95% confidence interval	0.004–0.024	0.005–0.029	− 0.004–0.004	0.003–0.035
P-value	0.016	0.016	0.897	0.033
CLA compared with control fat (all studies)				
Effect of substituting CLA by control fat/g/day	0.018	0.014	− 0.006	0.027
95% confidence interval	0.006–0.030	0.004–0.024	− 0.012– − 0.0001	0.011–0.043
P-value	0.006	0.005	0.020	0.002
CLA compared with control fat (studies with weigh-stable subjects[b]*)*				
Effect of substituting CLA by control fat/g/day	0.022	0.015	− 0.006	0.025
95% confidence interval	0.009–0.035	0.005–0.025	− 0.012–0.000	0.008–0.043
P-value	0.003	0.011	0.066	0.007

Abbreviations: CLA, conjugated linoleic acid; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol. [a]To maintain uniformity, we calculated the ratio of LDL/HDL cholesterol from mean LDL and HDL levels, even where ratios had been reported. Results are based on linear regression analysis and the values are based on replacement of 1 g control fat by either ruminant *trans* fat or CLA. Studies are weighed by study size by taking the square root of the number of study subjects into account. [b]Weight stable = studies not intended for weight loss and no more than 1.5 kg weight change during the study.

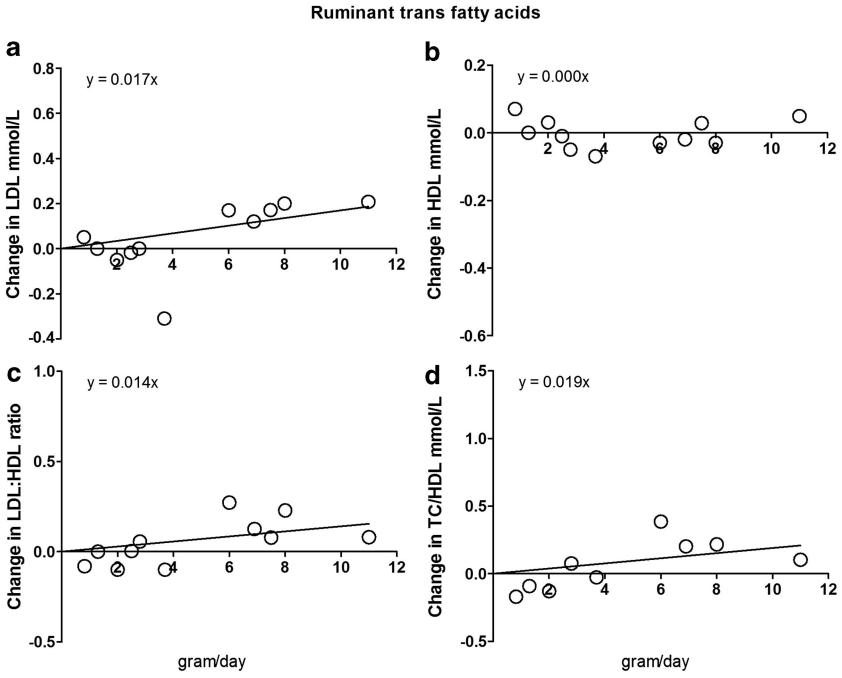

Figure 1. Results of randomized studies of the effects of diets high in ruminant *trans* fatty acids compared with control fatty acids on lipoproteins. To maintain uniformity, we calculated the ratio of LDL/HDL cholesterol from mean LDL and HDL levels, even where ratios had been reported. Studies are weighed for the square root of the number of subjects of each study.

by the use of results of the original control treatment instead of recalculating effects to *cis* monounsaturated fatty acids. Specifically, one study that used industrial *trans* as control treatment and provided a raise of HDL on ruminant trans fatty acids of 0.049 mmol/l/g in the current review,[24] whereas comparison with *cis* monounsaturated fatty acids would lead to a decrease in HDL of − 0.06 mmol/l HDL/g.

CLA. We found 32 papers that investigated the effect of CLA on lipoproteins (Table 1); these included 47 comparisons and 2048 subjects. Replacing 1 g of control fat by CLA in the linear regression model increased LDL cholesterol, decreased HDL cholesterol, increased plasma LDL/HDL ratio and increased the total cholesterol/HDL ratio (Table 2). An intake of 3 g of CLA, as advised as minimal amount by most producers, would be expected to lead to an increase in LDL cholesterol of 0.045 mmol/l (Table 2).

Weight loss affects lipoprotein concentrations. Therefore, we also calculated the effect of CLA if we excluded studies that were intended for weight loss or where subjects had a change in body weight of more than 1.5 kg. This left 23 studies with 30 comparisons (Figure 2; Table 1). The linear regression model for

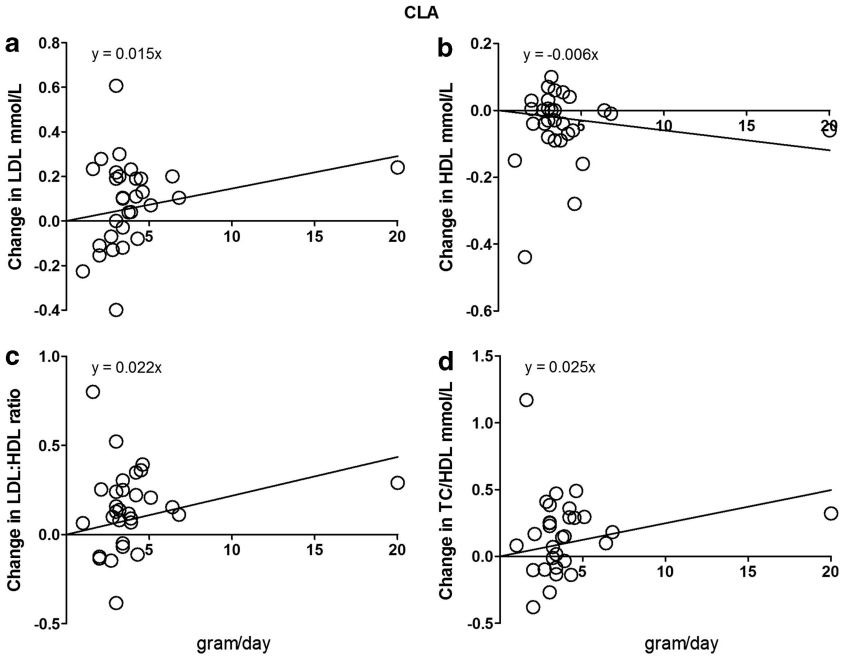

Figure 2. Results of randomized studies of the effects of CLA compared with control fatty acids on lipoproteins. Only studies in which subjects were weight stable were included. To maintain uniformity, we calculated the ratio of LDL/HDL cholesterol from mean LDL and HDL levels, even where ratios had been reported. Studies are weighed for the square root of the number of subjects of each study.

those studies again showed that CLA significantly increases LDL cholesterol, the plasma LDL/HDL ratio, and the total cholesterol/HDL ratio (Table 2).

Gram for gram, ruminant *trans* fatty acids, CLA and industrial *trans* fatty acids had largely the same effect on blood lipoproteins. We therefore suggest that fatty acids with a double bond in the *trans* configuration unfavorably affect blood lipids and lipoproteins, regardless of whether they have been produced in factories or in the rumens of cattle and sheep.

OTHER PATHWAYS THROUGH WHICH *TRANS* FATTY ACIDS MAY INFLUENCE CARDIOVASCULAR HEALTH

One of the unsolved questions in this research field is what the exact effect is of *trans* fatty acids on cardiovascular disease. The risk estimates from the observational studies are much larger than can be explained by the effect on lipoproteins only. This could be due to residual confounding, but there could also be additional adverse effects of *trans* fatty acids on cardiovascular disease through pathways other than lipoproteins.

Other suggested pathways are systemic inflammation, oxidative stress, endothelial function and insulin resistance or diabetes. Several studies, but not all, suggest that industrial trans fatty acids have unfavorable effects on inflammation.[25] For inflammatory effects of ruminant *trans* fatty acids and CLA there are not many indications that they have strong effects on inflammatory processes.[26–30] However, the available number of studies is small and the dosages of ruminant *trans* fatty acids used in these studies are low.

Effects on other pathways are much less clear. For example, future research has to show if *trans* fatty acids affect the glucose–insulin homeostasis or diabetes. Unfavorable effects on this pathway would also have consequences for the magnitude of the effect of *trans* fatty acids on cardiovascular disease. It could partly explain why the association between *trans* fatty acids and cardiovascular disease is stronger than predicted from changes in lipoproteins. However, until now, results of trials and observational studies on diabetes risk or on indicators of diabetes are equivocal.[25,30]

CONCLUSIONS

Summarizing resolved and unresolved questions: all *trans* fatty acids have detrimental effects on lipoproteins. That includes HDL when *trans* fatty acids are compared with *cis* fatty acids.[4] Effects on other pathways are less clear. Avoidance of *trans* fatty acids is likely to reduce the risk of cardiovascular disease. Changes in the formulation of foods, labeling of food products and actions from governments have resulted in a dramatic decrease in the intake of *trans* fatty acids from industrial sources.[18,19] The intake can be further decreased if industrial *trans* fats are avoided in the preparation of fried foods and bakery shortenings.[31]

The average intake of *trans* fatty acids from ruminant sources is only about 0.5 energy percent. The effect of animal *trans* fatty acids on cardiovascular disease risk is still unclear, but the impact if any must be minor and removal of these *trans* fatty acids from milk and meat is technically not feasible. However, consumers who follow the long-standing advice to choose low fat dairy products will automatically also reduce their intake of ruminant *trans* fatty acids.

Intake of CLA can be substantial because CLA is sold as supplements. We conclude that CLA has unfavorable effects on lipoprotein levels similar to those of other *trans* fatty acids. It is unclear what the effects of CLA are on markers of diabetes. The Food Standards Australia and New Zealand bi-national government agency decided on this basis to ban foods enriched with CLA from the market (http://www.austlii.edu.au/cgi-bin/sinodisp/au/cases/cth/AATA/2012/551.html?stem=0&synonyms=0&query=title(Food%20Standards%20)). The Decision of the Tribunal that upheld this policy gives a good overview of the potential adverse effects of taking CLA supplements on a regular basis.

Industrial *trans* fatty acids have been shown to have detrimental health effects. Therefore, future research should continue to find alternatives for these fats that have similar baking properties, but not the adverse health effects. This would specifically be helpful

for the bakery industry. Thus, further research on industrial *trans* fatty acids and cardiovascular health is not a top priority.

The proportion of ruminant *trans* fatty acids in foods is low and will further decrease if consumers follow the advice to decrease intake of saturated fatty acids. Therefore, ruminant *trans* fatty acids are not an urgent research topic either. Even though there are gaps in our knowledge of their effects. Future research is warranted to provide more precise insights into effects of CLA on both lipoprotein concentrations and on inflammatory processes, and insulin and diabetes related factors.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

Our studies on trans fatty acids and lipoproteins were supported by the Netherlands Heart Foundation (Grant No. 2006B176) and the Foundation for Nutrition and Health Research. MBK was supported by an Academy Professorship of the Royal Netherlands Academy of Arts and Sciences. AJW was partially supported by Food Standards Australia New Zealand for updating statistical analyses. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

REFERENCES

1 Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB *et al.* Heart disease and stroke statistics—2012 update: a report from the American Heart Association. *Circulation* 2012; **125**: e2–e220.
2 Mensink RP, Zock PL, Kester AD, Katan MB. Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* 2003; **77**: 1146–1155.
3 Ascherio A. Trans fatty acids and blood lipids. *Atheroscler Suppl* 2006; **7**: 25–27.
4 Brouwer IA, Wanders AJ, Katan MB. Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans—a quantitative review. *PloS One* 2010; **5**: e9434.
5 Parodi PW. Distribution of isomeric octadecenoic fatty acids in milk fat. *J Dairy Sci* 1976; **59**: 1870–1873.
6 Precht D, Molkentin J. Trans fatty acids: implications for health, analytical methods, incidence in edible fats and intake (a review). *Nahrung* 1995; **39**: 343–374.
7 Hulshof KF, van Erp-Baart MA, Anttolainen M, Becker W, Church SM, Couet C *et al.* Intake of fatty acids in western Europe with emphasis on trans fatty acids: the TRANSFAIR Study. *Eur J Clin Nutr* 1999; **53**: 143–157.
8 Ma DW, Wierzbicki AA, Field CJ, Clandinin MT. Conjugated linoleic acid in canadian dairy and beef products. *J Agric Food Chem* 1999; **47**: 1956–1960.
9 Yang TS, Liu TT. Optimization of production of conjugated linoleic acid from soybean oil. *J Agric Food Chem* 2004; **52**: 5079–5084.
10 Craig-Schmidt MC. World-wide consumption of trans fatty acids. *Atheroscler Suppl* 2006; **7**: 1–4.
11 Pot GK, Prynne CJ, Roberts C, Olson A, Nicholson SK, Whitton C *et al.* National Diet and Nutrition Survey: fat and fatty acid intake from the first year of the rolling programme and comparison with previous surveys. *Br J Nutr* 2012; **107**: 405–415.
12 Kris-Etherton PM, Lefevre M, Mensink RP, Petersen B, Fleming J, Flickinger BD. Trans fatty acid intakes and food sources in the US. Population: NHANES 1999–2002. *Lipids* 2012; **47**: 931–940.
13 Katan MB, Zock PL, Mensink RP. Trans fatty acids and their effects on lipoproteins in humans. *Annu Rev Nutr* 1995; **15**: 473–493.
14 Mensink RP, Zock PL, Katan MB, Hornstra G. Effect of dietary cis and trans fatty acids on serum lipoprotein[a] levels in humans. *J Lipid Res* 1992; **33**: 1493–1501.
15 Willett WC, Ascherio A. Trans fatty acids: are the effects only marginal? *Am J Public Health* 1994; **84**: 722–724.
16 Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. *N Engl J Med* 2006; **354**: 1601–1613.
17 Ascherio A, Katan MB, Zock PL, Stampfer MJ, Willett WC. Trans fatty acids and coronary heart disease. *N Engl J Med* 1999; **340**: 1994–1998.
18 Korver O, Katan MB. The elimination of trans fats from spreads: how science helped to turn an industry around. *Nutr Rev* 2006; **64**: 275–279.
19 L'Abbe MRS, Skeaff S, Ghafoorunissa M, Tavella M. Approaches to removing trans fats from the food supply in industrialized and developing countries. *Eur J Clin Nutr* 2009; **63**: S50–S67.
20 Bendsen NT, Christensen R, Bartels EM, Astrup A. 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* 2011; **65**: 773–783.
21 Willett W, Mozaffarian D. Ruminant or industrial sources of trans fatty acids: public health issue or food label skirmish? *Am J Clin Nutr* 2008; **87**: 515–516.
22 Snedecor GW, Cochran WG. *Statistical methods*. 8th edn. Iowa State University Press: Ames, Iowa, 1989.
23 Gebauer SK, Destaillats F, Mouloungui Z, Candy L, Bezelgues JB, Dionisi F *et al.* Effect of trans fatty acid isomers from ruminant sources on risk factors of cardiovascular disease: study design and rationale. *Contemp Clin Trials* 2011; **32**: 569–576.
24 Chardigny JM, Destaillats F, Malpuech-Brugere C, Moulin J, Bauman DE, Lock AL *et al.* 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* 2008; **87**: 558–566.
25 Wallace SK, Mozaffarian D. Trans-fatty acids and nonlipid risk factors. *Curr Atheroscler Rep* 2009; **11**: 423–433.
26 Desroches S, Chouinard PY, Galibois I, Corneau L, Delisle J, Lamarche B *et al.* Lack of effect of dietary conjugated linoleic acids naturally incorporated into butter on the lipid profile and body composition of overweight and obese men. *Am J Clin Nutr* 2005; **82**: 309–319.
27 Tholstrup T, Raff M, Basu S, Nonboe P, Sejrsen K, Straarup EM. Effects of butter high in ruminant trans and monounsaturated fatty acids on lipoproteins, incorporation of fatty acids into lipid classes, plasma C-reactive protein, oxidative stress, hemostatic variables, and insulin in healthy young men. *Am J Clin Nutr* 2006; **83**: 237–243.
28 Gagliardi AC, Maranhao RC, de Sousa HP, Schaefer EJ, Santos RD. Effects of margarines and butter consumption on lipid profiles, inflammation markers and lipid transfer to HDL particles in free-living subjects with the metabolic syndrome. *Eur J Clin Nutr* 2010; **64**: 1141–1149.
29 Tricon S, Burdge GC, Jones EL, Russell JJ, El-Khazen S, Moretti E *et al.* Effects of dairy products naturally enriched with cis-9,trans-11 conjugated linoleic acid on the blood lipid profile in healthy middle-aged men. *Am J Clin Nutr* 2006; **83**: 744–753.
30 McCrorie TA, Keaveney EM, Wallace JM, Binns N, Livingstone MB. Human health effects of conjugated linoleic acid from milk and supplements. *Nutr Res Rev* 2011; **24**: 206–227.
31 Katan MB. Regulation of trans fats: the gap, the Polder, and McDonald's French fries. *Atheroscler Suppl* 2006; **7**: 63–66.
32 Motard-Belanger A, Charest A, Grenier G, Paquin P, Chouinard Y, Lemieux S *et al.* Study of the effect of trans fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. *Am J Clin Nutr* 2008; **87**: 593–599.
33 Lacroix E, Charest A, Cyr A, Baril-Gravel L, Lebeuf Y, Paquin P *et al.* 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* 2012; **95**: 318–325.
34 Brown AW, Trenkle AH, Beitz DC. Diets high in conjugated linoleic acid from pasture-fed cattle did not alter markers of health in young women. *Nutr Res* 2011; **31**: 33–41.
35 Venkatramanan S, Joseph SV, Chouinard PY, Jacques H, Farnworth ER, Jones PJ. Milk enriched with conjugated linoleic acid fails to alter blood lipids or body composition in moderately overweight, borderline hyperlipidemic individuals. *J Am Coll Nutr* 2010; **29**: 152–159.
36 Wanders AJ, Brouwer IA, Siebelink E, Katan MB. Effect of a high intake of conjugated linoleic acid on lipoprotein levels in healthy human subjects. *PloS One* 2010; **5**: e9000.
37 Aryaeian N, Shahram F, Djalali M, Eshragian MR, Djazayeri A, Sarrafnejad A *et al.* Effect of conjugated linoleic acids, vitamin E and their combination on the clinical outcome of Iranian adults with active rheumatoid arthritis. *Int J Rheum Dis* 2009; **12**: 20–28.
38 Berven G. Safety of conjugated linoleic acid (CLA) in overweight or obese human volunteers. *Eur J Lipid Sci Technol* 2000; **102**: 455–462.
39 Benito P, Nelson GJ, Kelley DS, Bartolini G, Schmidt PC, Simon V. The effect of conjugated linoleic acid on plasma lipoproteins and tissue fatty acid composition in humans. *Lipids* 2001; **36**: 229–236.
40 Blankson H, Stakkestad JA, Fagertun H, Thom E, Wadstein J, Gudmundsen O. Conjugated linoleic acid reduces body fat mass in overweight and obese humans. *J Nutr* 2000; **130**: 2943–2948.
41 Diaz ML, Watkins BA, Li Y, Anderson RA, Campbell WW. Chromium picolinate and conjugated linoleic acid do not synergistically influence diet- and exercise-induced changes in body composition and health indexes in overweight women. *J Nutr Biochem* 2008; **19**: 61–68.
42 Pfeuffer M, Fielitz K, Laue C, Winkler P, Rubin D, Helwig U *et al.* 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* 2011; **30**: 19–28.

43 Gaullier JM, Halse J, Hoivik HO, Hoye K, Syvertsen C, Nurminiemi M *et al.* Six months supplementation with conjugated linoleic acid induces regional-specific fat mass decreases in overweight and obese. *Br J Nutr* 2007; **97**: 550–560.
44 Gaullier JM, Halse J, Hoye K, Kristiansen K, Fagertun H, Vik H *et al.* Conjugated linoleic acid supplementation for 1 y reduces body fat mass in healthy overweight humans. *Am J Clin Nutr* 2004; **79**: 1118–1125.
45 Iwata T, Kamegai T, Yamauchi-Sato Y, Ogawa A, Kasai M, Aoyama T *et al.* Safety of dietary conjugated linoleic acid (CLA) in a 12-weeks trial in healthy overweight Japanese male volunteers. *J Oleo Sci* 2007; **56**: 517–525.
46 Joseph SV, Jacques H, Plourde M, Mitchell PL, McLeod RS, Jones PJ. Conjugated linoleic acid supplementation for 8 weeks does not affect body composition, lipid profile, or safety biomarkers in overweight, hyperlipidemic men. *J Nutr* 2011; **141**: 1286–1291.
47 Kamphuis MM, Lejeune MP, Saris WH, Westerterp-Plantenga MS. Effect of conjugated linoleic acid supplementation after weight loss on appetite and food intake in overweight subjects. *Eur J Clin Nutr* 2003; **57**: 1268–1274.
48 Lambert EV, Goedecke JH, Bluett K, Heggie K, Claassen A, Rae DE *et al.* Conjugated linoleic acid versus high-oleic acid sunflower oil: effects on energy metabolism, glucose tolerance, blood lipids, appetite and body composition in regularly exercising individuals. *Br J Nutr* 2007; **97**: 1001–1011.
49 Laso N, Brugue E, Vidal J, Ros E, Arnaiz JA, Carne X *et al.* Effects of milk supplementation with conjugated linoleic acid (isomers cis-9, trans-11 and trans-10, cis-12) on body composition and metabolic syndrome components. *Br J Nutr* 2007; **98**: 860–867.
50 Moloney F, Yeow TP, Mullen A, Nolan JJ, Roche HM. Conjugated linoleic acid supplementation, insulin sensitivity, and lipoprotein metabolism in patients with type 2 diabetes mellitus. *Am J Clin Nutr* 2004; **80**: 887–895.
51 Mougios V, Matsakas A, Petridou A, Ring S, Sagredos A, Melissopoulou A *et al.* Effect of supplementation with conjugated linoleic acid on human serum lipids and body fat. *J Nutr Biochem* 2001; **12**: 585–594.
52 Nazare JA, de la Perriere AB, Bonnet F, Desage M, Peyrat J, Maitrepierre C *et al.* Daily intake of conjugated linoleic acid-enriched yoghurts: effects on energy metabolism and adipose tissue gene expression in healthy subjects. *Br J Nutr* 2007; **97**: 273–280.
53 Noone EJ, Roche HM, Nugent AP, Gibney MJ. The effect of dietary supplementation using isomeric blends of conjugated linoleic acid on lipid metabolism in healthy human subjects. *Br J Nutr* 2002; **88**: 243–251.
54 Raff M, Tholstrup T, Basu S, Nonboe P, Sorensen MT, Straarup EM. A diet rich in conjugated linoleic acid and butter increases lipid peroxidation but does not affect atherosclerotic, inflammatory, or diabetic risk markers in healthy young men. *J Nutr* 2008; **138**: 509–514.
55 Riserus U, Basu S, Jovinge S, Fredrikson GN, Arnlov J, Vessby B. 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* 2002; **106**: 1925–1929.
56 Riserus U, Berglund L, Vessby B. Conjugated linoleic acid (CLA) reduced abdominal adipose tissue in obese middle-aged men with signs of the metabolic syndrome: a randomised controlled trial. *Int J Obes Relat Metab Disord* 2001; **25**: 1129–1135.
57 Riserus U, Smedman A, Basu S, Vessby B. Metabolic effects of conjugated linoleic acid in humans: the Swedish experience. *Am J Clin Nutr* 2004; **79**(Suppl 6): 1146S–1148SS.
58 Smedman A, Vessby B. Conjugated linoleic acid supplementation in humans--metabolic effects. *Lipids* 2001; **36**: 773–781.
59 Steck SE, Chalecki AM, Miller P, Conway J, Austin GL, Hardin JW *et al.* Conjugated linoleic acid supplementation for twelve weeks increases lean body mass in obese humans. *J Nutr* 2007; **137**: 1188–1193.
60 Taylor JS, Williams SR, Rhys R, James P, Frenneaux MP. Conjugated linoleic acid impairs endothelial function. *Arterioscler Thromb Vasc Biol* 2006; **26**: 307–312.
61 Tholstrup T, Raff M, Straarup EM, Lund P, Basu S, Bruun JM. An oil mixture with trans-10, cis-12 conjugated linoleic acid increases markers of inflammation and in vivo lipid peroxidation compared with cis-9, trans-11 conjugated linoleic acid in postmenopausal women. *J Nutr* 2008; **138**: 1445–1451.
62 Watras AC, Buchholz AC, Close RN, Zhang Z, Schoeller DA. The role of conjugated linoleic acid in reducing body fat and preventing holiday weight gain. *Int J Obes* 2007; **31**: 481–487.
63 Attar-Bashi NM, Weisinger RS, Begg DP, Li D, Sinclair AJ. Failure of conjugated linoleic acid supplementation to enhance biosynthesis of docosahexaenoic acid from alpha-linolenic acid in healthy human volunteers. *Prostaglandins Leukot Essent Fatty Acids* 2007; **76**: 121–130.
64 Michishita T, Kobayashi S, Katsuya T, Ogihara T, Kawabuchi K. Evaluation of the antiobesity effects of an amino acid mixture and conjugated linoleic acid on exercising healthy overweight humans: a randomized, double-blind, placebo-controlled trial. *J Int Med Res* 2010; **38**: 844–859.
65 Petridou A, Mougios V, Sagredos A. Supplementation with CLA: isomer incorporation into serum lipids and effect on body fat of women. *Lipids* 2003; **38**: 805–811.
66 Naumann E, Carpentier YA, Saebo A, Lassel TS, Chardigny JM, Sebedio JL *et al.* Cis-9, trans- 11 and trans-10, cis-12 conjugated linoleic acid (CLA) do not affect the plasma lipoprotein profile in moderately overweight subjects with LDL phenotype B. *Atherosclerosis* 2006; **188**: 167–174.
67 Sluijs I, Plantinga Y, de Roos B, Mennen LI, Bots ML. Dietary supplementation with cis-9, trans-11 conjugated linoleic acid and aortic stiffness in overweight and obese adults. *Am J Clin Nutr* 2010; **91**: 175–183.