Effect of Animal and Industrial Trans Fatty Acids on HDL and LDL Cholesterol Levels in Humans – A Quantitative Review

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Abstract

Background: Trans fatty acids are produced either by industrial hydrogenation or by biohydrogenation in the rumens of cows and sheep. Industrial trans fatty acids lower HDL cholesterol, raise LDL cholesterol, and increase the risk of coronary heart disease. The effects of conjugated linoleic acid and trans fatty acids from ruminant animals are less clear. We reviewed the literature, estimated the effects trans fatty acids from ruminant sources and of conjugated trans linoleic acid (CLA) on blood lipoproteins, and compared these with industrial trans fatty acids.

Methodology/Principal Findings: We searched Medline and scanned reference lists for intervention trials that reported effects of industrial trans fatty acids, ruminant trans fatty acids or conjugated linoleic acid on LDL and HDL cholesterol in humans. The 39 studies that met our criteria provided results of 29 treatments with industrial trans fatty acids, 6 with ruminant trans fatty acids and 17 with CLA. Control treatments differed between studies; to enable comparison between studies we recalculated for each study what the effect of trans fatty acids on lipoprotein would be if they isocalorically replaced cis mono unsaturated fatty acids. In linear regression analysis the plasma LDL to HDL cholesterol ratio increased by 0.055 (95%CI 0.044–0.066) for each % of dietary energy from industrial trans fatty acids replacing cis monounsaturated fatty acids. The increase in the LDL to HDL ratio for each % of energy was 0.038 (95%CI 0.012–0.065) for ruminant trans fatty acids, and 0.043 (95% CI 0.012–0.074) for conjugated linoleic acid (p = 0.99 for difference between CLA and industrial trans fatty acids; p = 0.37 for ruminant versus industrial trans fatty acids).

Conclusions/Significance: Published data suggest that all fatty acids with a double bond in the trans configuration raise the ratio of plasma LDL to HDL cholesterol.

Introduction

Trans fatty acids arise either from industrial hydrogenation, or from biohydrogenation in ruminant animals. Artificial trans fatty acids are produced by partial hydrogenation of vegetable or fish oils with hydrogen gas and a metal catalyst. Consumption of such industrial trans fatty acids raises the total to HDL cholesterol ratio in blood and the risk of coronary heart disease [1–5]. Natural trans fatty acids are produced in the rumens of cows and sheep. They arise through partial hydrogenation and/or isomerization of cis-unsaturated fatty acids from the feed by hydrogen produced during oxidation of substrates, with bacterial enzymes as catalysts. As a result the fat in milk, butter, cheese and beef contains 2–9% trans fatty acids [6–8]. Because of the steep reduction in the production and intake of industrial trans fatty acids, ruminant fats are now the major source of trans fatty acids in most European countries [9] and will likely become so in the USA [10].

The effects of ruminant trans fatty acids on lipoproteins and heart disease are unclear. Some epidemiological studies showed no association [1,3,11] between ruminant trans fatty acid intake and heart disease risk, one showed a non-significant inverse association [2] and one a non-significant positive association [4]. Data on the effects of ruminant trans fatty acids on plasma lipoproteins in humans are limited. One study found adverse effects of high intakes, but not of low intakes of ruminant trans fatty acids [12]. Another study suggested that ruminant trans fatty acids produce higher LDL and HDL cholesterol levels than industrial trans fatty acids in women, but not in men [13].

Industrial and ruminant fats contain similar species of trans fatty acids, but in different proportions (figure 1). Industrial trans fatty acids come in two kinds: partially hardened vegetable oils mainly contain trans isomers of oleic acid (figure 1a), the major one being C18:1 trans-9 or elaidic acid (figure 1d) and C18: 1 trans-10. Partially hydrogenated fish oils mainly contain trans isomers of C20:1, 20:2, 22:1 and 22:2 (figure 1f). Partially hydrogenated vegetable oils also contain smaller amounts of C18: 1 trans-6, and C18:1 trans-11 or vaccenic acid (figure 1b), and trans isomers of alpha-linolenic acid may arise during deep-fat frying. All these

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industrial trans fatty acids raise the LDL to HDL cholesterol ratio [5,14–16].

In milk and meat C18:1 trans-11 (vaccenic acid) is the predominant trans fatty acid. In addition, ruminant fats contain small amounts of cis-9, trans-11 18:2 (conjugated linoleic acid, abbreviated to CLA in this paper unless otherwise mentioned; figure 1c). cis-9, trans-11 18:2 CLA is also formed from ingested vaccenic acid in animals and in humans [17]. CLA is also widely sold as a supplement in the form of capsules. Most CLA capsules contain a mix of cis-9, trans-11 CLA and another CLA isomer trans-10, cis-12 CLA. These CLA-preparations are promoted for weight loss, although studies in humans have been inconclusive on this aspect [18,19].

Countries such as Denmark that have banned the use of trans fatty acid in foods have excluded ruminant trans fatty acids. The
US Food and Drug Administration includes ruminant trans fats in its labeling rules for trans fatty acids, but exempts CLA.

As the effects of the natural trans fatty acids are unclear we here review effects of different trans fatty acids on lipoprotein levels in human intervention trials.

Methods

We reviewed randomized intervention trials that investigated effects of either industrial trans fatty acids, or conjugated linoleic acid, or other ruminant trans fatty acids on the LDL to HDL cholesterol ratio, and on LDL and HDL cholesterol concentrations.

Selection of Studies

We searched Medline for all relevant original-research papers published in English between January 1990 and January 2010 using as search terms: “(trans fat OR trans fatty acids, OR CLA) AND LDL”. We limited our search to human studies. We also scanned reference lists to ensure completeness.

We selected dietary trials that reported effects of industrial trans fatty acids, CLA or other ruminant trans fatty acids on LDL and HDL cholesterol levels. We also included studies that reported effects of CLA supplements. Such supplements usually contain a mix of cis-9, trans-11 CLA, the same conjugated linoleic acid as in ruminant fats, and trans-12 CLA.

Studies had to have a parallel, crossover, or Latin-square design. We excluded before-and-after (sequential) designs that lacked a control or comparison group or period. Treatment periods had to be at least 13 days as that is the minimum period to achieve a new steady-state concentration of plasma lipoproteins [20,21]. Trials in which subjects lost or gained significant amounts of weight were excluded [22,23] as this has an effect on blood lipoproteins independent of dietary composition [24,25].

Statistical Analysis

Some studies compared trans fatty acids with saturated fatty acids, or compared two sources of trans fatty acids only. We recalculated these results to effects relative to isocaloric amounts of cis mono-unsaturated fatty acids according to the equations of Mensink et al. [26]. To maintain uniformity, we recalculated the ratio of LDL to HDL cholesterol from mean LDL and HDL levels for all studies, even where ratios had been reported. Data from different studies were combined using linear regression analysis with intake of trans fatty acids as independent and change in the plasma LDL to HDL cholesterol ratio, LDL cholesterol and HDL cholesterol as dependent variables. We did not weigh studies by number of subjects or standard error of the estimate because the LDL to HDL ratios are calculated on the basis of mean values of treatment differences within studies without an estimate of variation. Regression lines were forced through the origin because a zero change in diet should produce a zero change in blood lipids. We also tested logarithmic models in order to check whether our assumption of a linear dose-response relation was appropriate.

All studies of CLA except one [27] used CLA supplements on top of uncontrolled ad-lib diets. Doses of CLA in these studies were reported as grams per day. In order to recalculate these to percent of energy we assumed an energy intake of 2250 kcal/day, because a usual energy intake is 2500 kcal per day for men and 2000 kcal per day for women, and most studies enrolled approximately equal numbers of men and women.

Results

Figure 2 shows a flow diagram of the selection of studies for this review. Table 1 provides an overview of the studies and their outcomes for the LDL to HDL cholesterol ratio. For industrial trans fatty acids we started out with the data of Ascherio et al. [28] and extended these with results of studies on industrial or ruminant trans fatty acids published since [10,12-15,27,29-41]. There were 23 studies with controlled diets in which the fat in the diet was provided by the investigators, so-called dietary controlled studies on industrial trans fatty acids [12,13,29,38,39], and 1 CLA study with dietary control [27] and 12 studies on CLA supplements in which the diet was not controlled [50-61]. We excluded two studies in which the subjects lost significant amounts of weight [22,23] as this is known to influence lipoprotein levels. We also excluded one study which used a sequential design [62] and one small Malaysian study which showed a discordant, extremely strong adverse effect, of industrial trans fatty acids [63].

The 23 trials provided 28 data points on the effect of industrial trans fatty acids. Linear regression showed that the plasma LDL to HDL ratio increased by 0.055 (95% CI 0.044–0.066) for every % of dietary energy provided by these artificial trans fatty acids in the place of cis-mono unsaturated fatty acids (figure 3a). LDL increased by 0.048 mmol/L (95% CI 0.037 to 0.058; figure 4) and HDL decreased by −0.01 mmol/L (95% CI −0.013 to −0.007) (figure 5) for each % of energy from industrial trans fatty acids replacing cis-mono unsaturates.

The 5 trials provided 6 data points on the effect of ruminant trans fatty acids from milk. Linear regression showed that the plasma LDL to HDL ratio increased by 0.038 (95% CI 0.012–0.065) when one % of dietary energy as cis-mono unsaturated fat is replaced by these natural trans fatty acids (figure 3b). LDL increased by 0.045 mmol/L (95% CI −0.02 to 0.093; figure 4)
<table>
<thead>
<tr>
<th>Trial</th>
<th>Design</th>
<th>Tested fat</th>
<th>N (men/women)</th>
<th>Duration of treatment</th>
<th>Diet / Supplement</th>
<th>Randomisation and stratification</th>
<th>Drop out Rate (%)</th>
<th>Difference in LDL/HDL</th>
<th>Mensink and Katan, 1990 Cross-over Industrial</th>
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<th>21 d Diet Random order stratified by sex and OC use</th>
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<td>Diet Control period fixed / interventions random order</td>
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<td>Duration of treatment</td>
<td>Diet / Supplement</td>
<td>Randomisation and stratification</td>
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<td>0.24</td>
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<tr>
<td>Naumann et al. 2006</td>
<td>Parallel</td>
<td>t10,c12CLA</td>
<td>92 (51/41)</td>
<td>13 wk</td>
<td>Supplement</td>
<td>Random</td>
<td>5.4</td>
<td>1.2</td>
<td>0.16</td>
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<tr>
<td>Lambert et al. 2007</td>
<td>Parallel</td>
<td>CLA 50:50</td>
<td>64 (26/38)</td>
<td>12 wk</td>
<td>Supplement</td>
<td>Random</td>
<td>3.1</td>
<td>1.6</td>
<td>0.14</td>
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<tr>
<td>Steck et al. 2007</td>
<td>Parallel</td>
<td>CLA 50:50</td>
<td>48 (13/35)</td>
<td>12 wk</td>
<td>Supplement</td>
<td>Random</td>
<td>12.7</td>
<td>1.3</td>
<td>0.08</td>
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<tr>
<td>Steck et al. 2007</td>
<td>Parallel</td>
<td>CLA 50:50</td>
<td>48 (13/35)</td>
<td>12 wk</td>
<td>Supplement</td>
<td>Random</td>
<td>12.7</td>
<td>2.6</td>
<td>0.15</td>
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<tr>
<td>Iwata et al. 2007</td>
<td>Parallel</td>
<td>CLA 50:50</td>
<td>60 (60/0)</td>
<td>12 wk</td>
<td>Supplement</td>
<td>Random</td>
<td>0</td>
<td>1.4</td>
<td>~0.07</td>
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<tr>
<td>Iwata et al. 2007</td>
<td>Parallel</td>
<td>CLA 50:50</td>
<td>60 (60/0)</td>
<td>12 wk</td>
<td>Supplement</td>
<td>Random</td>
<td>0</td>
<td>2.7</td>
<td>0.11</td>
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<tr>
<td>Gaullier et al. 2007</td>
<td>Parallel</td>
<td>CLA 50:50</td>
<td>105 (21/84)</td>
<td>6 mo</td>
<td>Supplement</td>
<td>Random</td>
<td>21.2</td>
<td>1.4</td>
<td>~0.05</td>
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<tr>
<td>Slijjs et al. 2010</td>
<td>Parallel</td>
<td>CLA 80:20</td>
<td>401 (167/234)</td>
<td>6 mo</td>
<td>Supplement</td>
<td>Random</td>
<td>13.7</td>
<td>1.6</td>
<td>~0.01</td>
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<tr>
<td>Wanders et al. 2010</td>
<td>Cross-over</td>
<td>CLA 80:20</td>
<td>61 (25/36)</td>
<td>3 wk</td>
<td>Diet</td>
<td>Random order</td>
<td>3.2</td>
<td>8.9</td>
<td>0.29</td>
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* industrial = industrial trans fatty acid mixture; ruminant = natural trans fatty acids from milk fat; 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.

*patients with type 2 diabetes.

doi:10.1371/journal.pone.0009434.t001
and HDL decreased by $-0.009 \text{ mmol/L} \ (-0.025 \text{ to } 0.007)$ (figure 5) for each % of energy from animal trans fatty acids replacing cis-monounsaturates.

The 13 trials provided 17 data points on the effect of CLA supplements. Linear regression showed that the plasma LDL to HDL ratio increased by $0.043 \ (0.012 \text{ to } 0.074)$ for every % of dietary energy as CLA replacing cis-monounsaturated fat (figure 3c). LDL increased by $0.038 \text{ mmol/L} \ (95\% \ CI \ 0.005 \text{ to } 0.071$; figure 4) and HDL decreased by $-0.008 \text{ mmol/L} \ (-0.023 \text{ to } 0.007)$ (figure 5) for each % of energy from CLA replacing cis-monounsaturates. We excluded the study of Tricon et al. [62] because of its sequential design. If we included this study using the baseline values as control the slope of the regression line for LDL to HDL changed from $0.043$ to $0.041$.

The effect of CLA on the LDL to HDL ratio increased to $0.064$ per % of energy if we excluded our own study [27] from the regression analysis. This was the only controlled dietary study on CLA, but it used a much higher dose of CLA.
approximately 20 grams/day as opposed to doses between 1.8 and 6.8 grams/day in the CLA supplement studies. Most studies used supplements with a 50:50 ratio of cis-9, trans-11 and trans-10, cis-12 CLAs. If we excluded the study that investigated these CLA isomers separately [58] and the two interventions that used CLA with an 80:20 ratio of the cis-9, trans-11 and trans-10, cis-12 isomers [27,52], the effect of CLA on the LDL to HDL ratio became 0.056.

Figure 4. Results of randomized studies of the effects of diets high in industrial trans fatty acids (---○---) or ruminant trans fatty acids (-----▲-----) or CLA (→→) compared with cis-unsaturated fatty acids on LDL cholesterol. doi:10.1371/journal.pone.0009434.g004

Figure 5. Results of randomized studies of the effects of diets high in industrial trans fatty acids (---○---) or ruminant trans fatty acids (-----▲-----) or CLA (→→) compared with cis-unsaturated fatty acids on HDL cholesterol. doi:10.1371/journal.pone.0009434.g005
The slope of the regression line for the LDL to HDL ratio was steeper for industrial trans fatty acids than for ruminant trans fatty acids or CLA, but the differences between the regression coefficients did not reach any limit of statistical significance. The p-value was p = 0.37 for ruminant versus industrial, p = 0.99 for CLA versus industrial and p = 0.64 for CLA versus ruminant, or p = 0.36 if we excluded our own study [27]. For ruminant trans fatty acids the explained variance (R²) of effects on the LDL to HDL ratio among the studies included in the regression analysis was 74%. A logarithmic model showed a better fit for the data, with an explained variance of 89%. However, this model did not make biological sense because it predicted that a zero intake of ruminant trans fatty acids would cause an infinitely large decrease in the LDL to HDL ratio.

**Discussion**

This review provides the first quantitative comparison of the effect of ruminant trans fatty acids and CLA with that of industrial trans fatty acids on blood lipoproteins in humans. Our analysis shows that all three classes of trans fatty acids raise the ratio of LDL to HDL, and therefore, presumably, the risk of coronary heart disease. The effect of ruminant trans fatty acids and CLA on the LDL to HDL ratio was less than that of industrial trans fatty acids although the difference was not significant. Further studies will be needed to decide whether this difference is real or due to chance.

**Strengths and Limitations**

Our search strategy ensured that we included all important studies. We cannot completely exclude the possibility of publication bias. In theory, interests of the sponsoring industry could have prevented publication of studies that showed an unfavorable increase in LDL and/or a decrease in HDL-cholesterol. We have no indications that results have not been published, but if they exist then the adverse effects of ruminant trans fatty acids and CLA on blood lipids may have been larger than shown here.

All supplement trials included were performed double-blind, except for one which was single blind [51]. In the dietary studies masking was attempted but was probably incomplete. However, we consider it unlikely that this influenced cholesterol concentrations.

In our calculations we did not take differences in size between the studies into account. This would require standard errors of treatment differences within studies, which generally were not given. We do not think this will affect our estimations much, because almost all studies had between 50 and 100 participants. Thus, weighing for size is not expected to change the regression line much.

Comparison of the effect of the two CLA isomers – the 9,11 isomers found in milk and supplements, and the 10,12 isomer found only in supplements - is difficult as most studies only investigated a 50:50 mixture of cis-9, trans-11 and trans-10, cis-12 CLA. The studies that investigated either the 80:20 mixture or pure cis-9, trans-11 CLA found less of an effect. We cannot exclude that trans-10, cis-12 CLA raises the LDL to HDL ratio more than cis-9, trans-11 CLA. However, it is clear that all CLA compounds raise the LDL to HDL ratio.

**Validity of the Models**

The first study on industrial trans fatty acids and blood lipoproteins in humans [47] reported an adverse effect of trans fatty acids, but a high dose not found in regular diets. At the time it was suggested that there was a threshold for this effect and that lower intakes of trans fatty acids as found in regular foods had no effect. Later studies showed that this was not the case and that the effect was proportional with the dosage up to very high intakes (figure 3). Mensink et al. [26] showed that effects of saturated and cis- and trans- unsaturated fatty acids on lipoproteins are also linear with dosage. Figures 3b and c suggest that the same holds true for conjugated linoleic acid and ruminant trans fatty acids, and that there is no threshold level below which these trans fatty acids fail to raise the LDL to HDL ratio.

Figure 3b depicts the dietary studies on ruminant trans fatty acids. In this figure only studies on total ruminant trans fatty acids are included because there are no dietary studies on isolated vaccenic acid or pure cis-9, trans-11 CLA. For total ruminant trans fatty acids the explained variance (R²) was 74%. A logarithmic model showed a better fit with an R² of 89%. However, calculations of explained variance are of limited value because intakes of ruminant trans fatty acids did not follow a normal distribution (figure 3b). Besides, this model does not make biological sense because a zero intake would cause an infinitely large decrease in the ratio of LDL to HDL. Furthermore, the logarithmic model was completely driven by a single study that found a decrease in the ratio of LDL to HDL with a small increase in the intake of ruminant trans fat. That study was underpowered to convincingly show an effect of this low dose [12]. Therefore, we consider the linear regression line the most parsimonious model for these studies. However, it is based on a limited number of data points and the coefficient may change as more data become available.

For the CLA studies the linear model also seems the most appropriate model. Figure 3c again gives no indication for a threshold below which CLA has no effect.

It was earlier shown that various trans 18:1 isomers [fig 1a; [47,48]), trans isomers of alpha-linolenic acid (fig 1e; [16]) and trans C20 and C22 isomers (fig 1f; [14,15]) raise the LDL to HDL cholesterol ratio. Our present review adds ruminant trans fatty acids and CLA. This suggests that all trans fatty acids, including cis-9, trans-11 CLA, share the same qualitative effect on the LDL to HDL cholesterol ratio in humans (Figure 3a). Although the slopes of the regression lines in figure 3a were not significantly different, we cannot exclude the possibility that small differences in effect exist between trans fatty acids from various sources.

A stringent of our conclusion will be provided by an ongoing study at the United States Department of Agriculture [http://clinicaltrials.gov/ct2/show/NCT00942656]. It examines pure vaccenic acid, a trans fatty acid not included in our analyses. We predict that 3% of energy from vaccenic acid will significantly raise the LDL to HDL cholesterol ratio by about 0.11 compared to cis-monounsaturates. The study also examines 1% of energy from cis-9, trans-11 conjugated linoleic acid. This should raise the LDL to HDL ratio, but not sufficiently to reach significance. A Swiss study [http://clinicaltrials.gov/ct2/show/NCT00933322] will examine the effect of 2% of energy from trans fatty acids in butter. This should raise the LDL to HDL cholesterol ratio by 0.076, but the study may lack the statistical power to pick up this effect.

**Public Health Implications**

Most of the trans fatty acids in milk and meat consist of vaccenic acid and other trans-mono-unsaturated fatty acids similar to those found in partially hydrogenated vegetable oils (figure 1). Our results suggest that all such fatty acids with a double bond in the trans configuration raise LDL and lower HDL cholesterol. Removing all such ruminant trans fatty acids from the diet would lower the total trans fatty acid intake in the United States and
Europe by about 0.5% of energy [9,64] and might therefore reduce cardiovascular disease risk by 1.5 to 6% [5]. Such a specific removal of ruminant trans fatty acids from milk and meat is, however, technically not feasible. Our results do reinforce the widespread advice to reduce intake of ruminant fats, because these are also the major source of saturated fatty acids in affluent diets. Recent changes in dairy cattle feeding have led to milk with a lower content of saturated fatty acids and a higher content of cis-9, trans-11 CLA and other dairy trans fatty acids [65]. Our data suggest that the effect of these changes on heart disease risk in consumers of milk and meat fat are at the very least equivocal.

CLA is a minor animal trans fatty acid. The effect of dietary CLA on cholesterol will be negligible if we assume that our model is correct. However, intakes from supplements can easily reach 3 grams of CLA a day. This should increase the LDL to HDL cholesterol ratio by 0.050, which would correspond with a 3 to 12% increase in the risk of cardiovascular disease [5].

Conclusion

Based on this overview we speculate that all fatty acids with one or more bonds in the trans configuration raise the ratio of LDL to HDL cholesterol irrespective of their origin or structure. Thus, our results provide an additional argument besides the high content of saturated fatty acids to lower the intake of ruminant animal fats.

Acknowledgments

We are indebted to Dr Alberto Ascherio, for providing data for figure 3 [28].

Author Contributions

Conceived and designed the experiments: IAB AJW MBK. Analyzed the data: IAB AJW MBK. Wrote the paper: IAB AJW MBK.

References


