European Journal of Clinical Nutrition (2009) 63, S22–S33
 0954-3007/09 $32.00
www.nature.com/ejcn

ORIGINAL ARTICLE

Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils

D Mozaffarian[1,2] and R Clarke[3]

[1]*Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA;* [2]*Departments of Epidemiology and Nutrition, Harvard School of Public Health, Boston, MA, USA and* [3]*Clinical Trial Service Unit, University of Oxford, Oxford, England*

Background/Objectives: Reduced consumption of *trans*-fatty acids (TFA) is desirable to lower coronary heart disease (CHD) risk. In practice, partially hydrogenated vegetable oils (PHVO) that contain both TFAs and other fatty acids are the unit of replacement and could be replaced with diverse alternative fats and oils. We performed quantitative estimates of CHD effects if a person's PHVO consumption were to be replaced with alternative fats and oils based on (1) randomized dietary trials and (2) prospective observational studies.

Subjects/Methods: We performed meta-analyses of (1) the effects of TFAs on blood lipids and lipoproteins in controlled dietary trials and (2) associations of habitual TFA consumption with CHD outcomes in prospective cohort studies. On the basis of these results and corresponding findings for saturated fatty acids (SFA), *cis*-monounsaturated fatty acids (MUFA) and *cis*-polyunsaturated fatty acids (PUFA), we calculated the effects on CHD risk for replacing 7.5% of energy from three different PHVO formulations (containing 20, 35 or 45% TFAs) with butter, lard, palm or vegetable oils.

Results: In controlled trials, each 1% energy replacement of TFAs with SFAs, MUFAs or PUFAs, respectively, decreased the total cholesterol (TC)/high-density lipoprotein cholesterol (HDL-C) ratio by 0.31, 0.54 and 0.67; the apolipoprotein (Apo)-B/ApoAI ratio by 0.007, 0.010 and 0.011; and lipoprotein (Lp)(a) by 3.76, 1.39 and 1.11 mg/l ($P<0.05$ for each). We also included possible effects on C-reactive protein (CRP) of TFAs vs other fats from one trial. On the basis of these risk factor changes in controlled trials, CHD risk would be variably decreased by different fats and oils replacing 7.5% of energy from 20% TFA PHVO (CHD risk reduction: −2.7% (butter) to −9.9% (canola)); 35% TFA PHVO (−11.9% (butter) to −16.0% (canola)); or 45% TFA PHVO (−17.6% (butter) to −19.8% (canola)). In prospective cohort studies, each 2% energy replacement of TFAs with SFAs, MUFAs or PUFAs would lower CHD risk by 17% (95% confidence interval (CI) = 7–25%), 21% (95% CI = 12–30%) or 24% (95% CI = 15–33%), respectively. On the basis of these associations in observational studies, CHD risk would be variably decreased by different fats and oils replacing 7.5% of energy from 20% TFA PHVO (CHD risk reduction: +0.5% (butter) to −21.8% (soybean)); 35% TFA PHVO (−14.4% (butter) to −33.4% (soybean)); or 45% TFA PHVO (−22.4% (butter) to −39.6% (soybean)). The demonstrated effects on TC/HDL-C, ApoB/ApoAI, Lp(a), and CRP in randomized feeding trials together accounted for ~65–80% and ~50% of the estimated risk reduction for replacing PHVO with animal fats and vegetable oils, respectively, that would be calculated from prospective cohort studies.

Conclusions: Effects on CHD risk of removing PHVO from a person's diet vary depending on the TFA content of the PHVO and the fatty acid composition of the replacement fat or oil, with direct implications for reformulation of individual food products. Accounting for the summed effects of TFAs on multiple CHD risk factors provides more accurate estimates of potential risk reduction than considering each risk factor in isolation, and approaches the estimated risk reduction derived from prospective cohort studies.

European Journal of Clinical Nutrition (2009) **63**, S22–S33; doi:10.1038/sj.ejcn.1602976

Keywords: *trans*-fatty acids; coronary heart disease; meta-analysis; randomized controlled trials; epidemiology

Introduction

Dietary consumption of *trans*-fatty acids (TFA) from partially hydrogenated vegetable oils (PHVO) adversely affects multiple

Correspondence: Dr D Mozaffarian, 665 Huntington Ave, Bldg 2-319, Boston, MA 02115, USA.
E-mail: dmozaffa@hsph.harvard.edu

cardiovascular risk factors and is associated with higher risk of coronary heart disease (CHD) (Mozaffarian *et al*., 2009). On the basis of changes in blood lipids (for example, the ratio of total cholesterol (TC)/high-density lipoprotein cholesterol (HDL-C)) in short-term randomized controlled feeding trials and of associations of habitual TFA consumption with clinical endpoints in prospective cohort studies, the estimated effects on CHD risk of replacing TFAs with equivalent calories from carbohydrate or *cis*-unsaturated fats has been estimated (Mozaffarian *et al*., 2006). However, in practice, TFAs in foods cannot be simply replaced on a 1:1 basis with other specific fatty acids. Rather, the unit of replacement is the PHVO, which is composed of various fatty acids, including TFAs, saturated fatty acids (SFA), *cis*-polyunsaturated fatty acids (PUFA) and *cis*-monounsaturated fatty acids (MUFA). PHVO removed from an individual's diet (by reformulation of specific food items or cooking processes) could be replaced by several alternative fats and oils (Eckel *et al*., 2007), each comprising different combinations of SFAs, PUFAs and MUFAs. For example, PHVO in a person's diet might be replaced by vegetable oils, tropical oils, lard or butter, each of which may have a different health impact. Additionally, different PHVO formulations can contain varying amounts of TFAs, for example, ranging from 20% to 60% of total fatty acids. Thus, the potential effects on an individual's CHD risk would depend both on the average TFA content of the PHVO being replaced and the type of fat and oil being used for replacement. To address these important considerations, we calculated the effects on CHD risk of reducing TFAs in an individual's diet by replacing PHVO with other fats and oils, taking into account both different formulations of PHVO being replaced and different types of fats and oils being used for replacement. We also hypothesized that some of the quantitative differences between prior estimates based on changes in the TC/HDL-C ratio in short-term dietary trials vs observed CHD outcomes in cohort studies (Mozaffarian *et al*., 2006) may relate to the effects of TFA consumption on cardiovascular risk factors besides the TC/HDL-C ratio, such as apolipoproteins, triglycerides, lipoprotein (Lp)(a), inflammation, endothelial dysfunction, insulin resistance and weight gain (Mozaffarian *et al*., 2009). Thus, we calculated the effects of reducing TFAs in foods by replacing PHVO with other fats and oils based on (1) data from controlled trials of changes in multiple CHD risk factors in response to consumption of various dietary fats and (2) evidence from prospective cohort studies for associations between habitual consumption of different dietary fats and CHD clinical outcomes.

Methods

We calculated the effects on CHD risk of replacing three different PHVO formulations (containing 20, 35 or 45% TFA) with other specific fats or oils likely to be used for replacement, including palm oil, butter, lard, cottonseed oil, high oleic sunflower oil, soybean oil and canola oil, on the basis of the content of TFAs, SFAs, MUFAs and PUFAs in each fat and oil. Estimates were based on isocaloric replacement of 7.5% of energy from PHVO in an individual's diet, although in some countries the average PHVO consumption may be 12.5% of energy or higher (Mozaffarian *et al*., 2007). For simplicity, calculations did not differentiate alpha-linolenic acid (18:3 $n-3$) from other PUFAs (that is, all *cis*-PUFAs in vegetable oils were considered to have similar effects) or between different SFAs (that is, SFAs from C12:0 to C18:0 were considered to have similar effects). On the basis of the fatty acid contents of each PHVO and replacement fat or oil, the effects on CHD risk were calculated based on two lines of evidence:

(1) *Risk factors in trials*. The effects in randomized controlled trials of consumption of TFAs, SFAs, MUFAs or PUFAs on risk factors, including the TC/HDL-C ratio, apolipoprotein (Apo)-B/ApoAI ratio Lp(a), and CRP, together with the relationships of these risk factors with incidence of CHD.

(2) *Disease outcomes in cohorts*. The associations in prospective cohort studies of habitual consumption of TFAs, SFAs, MUFAs or PUFAs with incidence of CHD events, after adjustment for other cardiovascular risk factors and lifestyle habits.

For the first calculation (risk factors in trials), we performed meta-analyses of the effects of TFA consumption on blood lipids and lipoproteins in randomized controlled trials. By means of MEDLINE searches together with hand-searching of references of prior meta-analyses or reviews, we identified crossover or parallel design randomized trials of controlled dietary ('metabolic ward') interventions of TFA consumption published through January 2008 in which the food intake was controlled and described, blood lipids were measured at the end of each dietary intervention, and each dietary period lasted at least 2 weeks. We used previously described methods (Clarke *et al*., 1997) to perform multivariable regression analysis of changes in the risk factors in the trials against age, weight, duration of dietary intervention, and intakes of TFAs, SFAs, MUFAs, PUFAs, protein, dietary cholesterol and total energy, stratified by gender and inverse weighted by the number of individuals in each trial. Coefficients from these analyses were used to assess the effects of isocaloric replacement of TFAs for SFAs, MUFAs or PUFAs while also taking into account the consumption of each of the other dietary fats. Effects on apolipoproteins were calculated with and without additional adjustment for changes in the TC/HDL-C ratio in each trial to assess the effects on ApoB and ApoAI above and beyond changes in TC/HDL-C. The effects on blood lipid concentrations of isocaloric changes between consumption of SFAs, MUFAs and PUFAs were calculated from a prior meta-analysis of randomized controlled dietary trials (Mensink *et al*., 2003). TFA consumption may also affect other risk factors, such as inflammation, endothelial dysfunction, insulin resistance and weight gain (Mozaffarian *et al*., 2009), but insufficient data were

available to perform a meta-analysis for these non-lipid risk factors. Hypothesis-generating analyses were performed for one non-lipid risk factor, C-reactive protein (CRP), using findings from one randomized controlled trial (Baer *et al.*, 2004).

On the basis of these effects, the changes in each risk factor were determined for replacement of a given PHVO (with its specific combination of fats) with a particular nonhydrogenated fat or oil (with its specific combination of fats). For each risk factor, the following changes in CHD risk were used on the basis of prior studies relating differences in the risk factors with incidence of CHD. For a 1.33 decrease in the TC/HDL-C ratio, the relative risk (RR) of CHD (weighted across age groups) was 0.61 (95% confidence interval (CI) = 0.55–0.69) after correction for regression dilution bias (Prospective Studies Collaboration, 2007). The CHD risk estimates associated with differences in the levels of other risk factors were obtained from estimates not corrected for regression dilution bias (and hence may underestimate the true difference in risk associated with unit changes in these risk factors). For a 0.68 higher ApoB/ApoAI ratio, the RR was 3.25 (95% CI = 2.81–3.76) (Yusuf *et al.*, 2004). For an estimated 50 mg/l higher Lp(a), the RR was 1.7 (95% CI = 1.4–1.9) (Danesh *et al.*, 2000). For an estimated 1.2 mg/l higher CRP, the RR was 1.58 (95% CI = 1.48–1.68) (Danesh *et al.*, 2004).

For the second estimate of risk reduction (disease outcomes in cohorts), we carried out a meta-analysis of the published prospective cohort studies evaluating the multivariable adjusted RR of CHD (nonfatal myocardial infarction or CHD death) associated with habitual TFA consumption. Data were extracted on the adjusted RR (95% CI) per 2% higher energy from TFAs, the number of participants and CHD cases, and the covariates adjusted for in the model. Summary estimates were obtained using random effects meta-analysis weighted by the inverse variance log RRs of the individual studies (DerSimonian and Laird, 1986). These analyses estimated the RR of CHD for isocaloric replacement of carbohydrate with TFA while also taking into account the consumption of each of the other dietary fats. The RR of CHD for isocaloric replacement of SFA, MUFA or PUFA with carbohydrate was assessed on the basis of pooled analyses of the two largest prospective cohort studies, the Nurses Health Study (including 1705 CHD events among 77 395 women followed for 20 years) and the Health Professionals Follow-up Study (including 1702 CHD events among 38 461 men followed for 14 years). Dietary habits were assessed using cumulative updating over time by means of serial questionnaires, and risk estimates were multivariable adjusted for other cardiovascular risk factors and lifestyle habits, including age, time period of follow-up, smoking, major cardiovascular risk factors (diabetes, hypertension and hypercholesterolemia, adjusted for using covariates or by restriction), physical activity, body mass index, aspirin use, alcohol use and dietary consumption of TFAs, SFAs, PUFAs, MUFAs, protein, fibre and total energy; analyses in women were also adjusted for family history of early myocardial infarction, multivitamin use, postmenopausal status and postmenopausal hormone use. The effects of isocaloric replacement between the different dietary fats was determined by subtraction of the summary coefficients for TFAs, SFAs, MUFAs and PUFAs derived from the meta-analysis and pooled analysis, and the effect on CHD risk was calculated for replacement of a given PHVO (with its specific combination of fats) with a particular nonhydrogenated fat or oil (with its specific combination of fats).

Results

Table 1 shows the fatty acid contents of the different PHVO formulations and replacement fats and oils considered. The percent TFA in each PHVO increased largely at the expense of PUFAs. The highest SFA content was present in butter, followed by palm oil and lard; the latter two also contained comparable amounts of MUFAs. Cottonseed oil and soybean oil contained the highest amounts of PUFAs, followed by canola oil. High oleic sunflower oil contained the highest amounts of MUFAs.

Effects of TFA replacement: risk factors

The 13 randomized trials in the meta-analysis of blood lipid and lipoprotein effects of TFA consumption (Table 2)

Table 1 Fatty acid composition of different fats and oils

	Fatty acid composition (% of total fatty acids)									
	PHVO no. 1	*PHVO no. 2*	*PHVO no. 3*	*Palm oil*	*Butter*	*Lard*	*Cotton seed oil*	*High oleic sunflower oil*	*Soybean oil*	*Canola oil*
TFA	20	35	45	<1	6	4	<1	<1	<1	<1
SFA	21	23	23	49	68	43	27	11	15	6
MUFA	34	31	27	40	25	42	18	84	24	57
PUFA	25	11	5	10	3	11	55	5	60	35

Abbreviations: MUFA, *cis*-monounsaturated fatty acids; PHVO, partially hydrogenated vegetable oils; PUFA, *cis*-polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, *trans*-fatty acids.
Compositions of PHVO based on data for PHVO used for both cooking and baking (Mozaffarian *et al.*, 2007) and in margarines (Ratnayake *et al.*, 2007). Compositions of other fats and oils are based on Eckel *et al.*, 2007 and Skeaff, 2009. Numbers may not sum to 100 due to rounding.

Table 2 Randomized controlled dietary trials assessing the effects of TFA consumption, relative to other fats, on blood lipids and apolipoproteins

Trial	*Design*	n	*Mean age (years)*	*Duration of each diet (days)*	*Amounts of TFA in the tested diets (%E)*	*Lipid outcomes*		
						Total/HDL-C	*ApoB, ApoAI*	*Lp(a)*
Laine *et al.*, 1982	Cross-over	24	25	70	0, 5	+		
Mensink and Katan, 1990	Cross-over	59	25	21	0, 0.8, 11	+	+	+
Zock and Katan, 1992	Crossover	56	25	21	0.1, 0.3, 7.7	+	+	
Judd *et al.*, 1994	Crossover	58	43	42	0.7, 3.7, 6.4	+	+	+
Almendingen *et al.*, 1995	Crossover	31	28	20	0.9, 8, 8.5	+	+	+
Aro *et al.*, 1997	Parallel	80	29	35	0.4, 0.8, 8.7	+	+	+
Judd *et al.*, 1998	Crossover	46	47	35	2.4, 2.7, 3.9	+	+	+
Muller *et al.*, 1998	Crossover	27	27	17	0.1, 0.2, 7	+	+	+
Lichtenstein *et al.*, 1999	Crossover	36	63	35	0.6, 0.9, 1.3, 3.3, 4.2, 6.7	+	+	+
de Roos *et al.*, 2001	Crossover	32	30	28	0.3, 9.3	+		
Judd *et al.*, 2002	Crossover	50	42	35	0.1, 0.2, 0.3, 4.2, 8.3	+		
Lovejoy *et al.*, 2002	Crossover	25	28	28	0, 7.3	+		
Sundram *et al.*, 2007	Crossover	30	30	28	0, 3.2	+		

Abbreviations: Apo, apolipoprotein; %E, percent of total energy; HDL-C, high-density lipoprotein cholesterol; Lp, lipoprotein; TFA, *trans*-fatty acids.

included 66 dietary interventions ('diets') among men and women involving a total of 518 individuals (Laine *et al.*, 1982; Mensink and Katan, 1990; Zock and Katan, 1992; Judd *et al.*, 1994, 1998, 2002; Almendingen *et al.*, 1995; Aro *et al.*, 1997; Muller *et al.*, 1998; Lichtenstein *et al.*, 1999; de Roos *et al.*, 2001; Lovejoy *et al.*, 2002; Sundram *et al.*, 2007). Overall, the mean (s.d.) age was 32 (14) years; weight, 71 (11) kg and duration of each diet, 34 (12) days. These studies typically involved generally young and healthy adults without obesity, diabetes, or dyslipidemia. Overall, the mean values of blood lipids reflected the population mean values for this age group: mean (s.d.) concentrations of TC, low-density lipoprotein cholesterol and HDL-C were 5.02 (0.66), 3.21 (0.63) and 1.34 (0.20) mmol/l, respectively. The overall mean (s.d.) ratio of TC/HDL-C was 3.94 and of ApoB/ApoAI, 0.74 (0.20). Average Lp(a) levels varied considerably between the trials, with an overall mean (s.d.) of 165 (88) mg/l.

The effects of isocaloric changes in consumption of different dietary fats on blood lipids, apolipoproteins and Lp(a) are shown in Table 3. Each 1% energy replacement of TFA with SFA, MUFA or PUFA decreased the TC/HDL-C ratio by 0.31, 0.54 and 0.67, respectively. This was mirrored by changes in apolipoprotein levels and the ApoB/ApoAI ratio. The inverse of each of these values indicated the effects of the converse replacement of SFA, MUFA or PUFA with TFA. For example, Lp(a) levels were increased by replacement of any of the dietary fats with TFAs.

Insufficient data were available to perform a meta-analysis of potential effects of TFA consumption on other non-lipid risk factors such as inflammation, endothelial dysfunction, insulin resistance or weight gain (Mozaffarian *et al.*, 2009). For example, CRP was evaluated in only two randomized dietary trials. In one trial ($n=36$), significant effects on CRP were not seen (Lichtenstein *et al.*, 2003). In the second trial ($n=50$) (Baer *et al.*, 2004), mean CRP levels were 1.27 mg/l following TFA consumption (8% of energy for 5 weeks), compared to 1.07 ($P<0.05$), 1.05 ($P<0.05$) and 1.14 ($P=$NS) mg/l following carbohydrate, MUFA or SFA consumption, respectively. This potential CRP-raising effect of TFA is consistent with observational studies of habitual TFA consumption (Mozaffarian, 2006). In hypothesis generating analyses, we used the results of this randomized trial to estimate the potential effects of TFA consumption on CRP, recognizing the relatively large uncertainty of this quantitative estimate.

On the basis of effects of TFA, SFA, MUFA and PUFA consumption on risk factors in randomized trials, we calculated the changes in risk factors if 7.5% of energy in a person's diet from three different PHVO formulations (20, 35 or 45% TFA) were replaced with alternative fats or oils. Results are shown for the TC/HDL-C ratio (Figure 1). For PHVO with 20% TFA, replacement with butter slightly increased (+0.04) the TC/HDL-C ratio, while replacement with palm oil or lard only slightly decreased (−0.02) it; replacement with vegetable oils produced larger reductions (−0.09 to −0.12). For PHVO with 35% TFA, the TC/HDL-C ratio was slightly decreased by replacement with butter (−0.03); decreased further by replacement with palm oil (−0.10) or lard (−0.09); and decreased the most by replacement with vegetable oils (−0.17 to −0.20). Decreases in the TC/HDL-C ratio were largest when PHVO with 45% TFA was replaced, including modest decreases by replacement with butter (−0.08), palm oil (−0.14) or lard (−0.14) and larger decreases by replacement with vegetable oils (−0.21 to −0.25).

Using similar methods, we calculated the impact on ApoB/ApoAI, Lp(a) and CRP if 7.5% of energy from PHVO were replaced with alternative fats or oils (data not shown). For ApoB/ApoAI, we used the estimates adjusted for TC/HDL-C to assess the (smaller) effects on ApoB/ApoAI above and beyond changes in blood lipid concentrations. Because the effects of isocaloric exchanges between SFA, MUFA and PUFA on ApoB/ApoAI, after first adjusting for changes in TC/HDL-C, were not previously assessed (Table 3), we used

Table 3 Effects of dietary fat consumption (each 1% energy of isocaloric replacement) on blood lipids and lipoproteins in meta-analyses of randomized controlled dietary trials

	Δ Total/HDL-C	*Δ Total Cholesterol (mmol/l)*	*Δ LDL-C (mmol/l)*	*Δ HDL-C (mmol/l)*	*Δ Triglycerides (mmol/l)*	*Δ ApoB (mg/l)*	*Δ ApoAI (mg/l)*	*Δ ApoB/ ApoAI*	*Δ Lp(a) (mg/l)*	*Further adjusted for Δ Total/HDL-C*		
										Δ ApoB (mg/l)	*Δ ApoAI (mg/l)*	*Δ ApoB/ApoAI*
No. of contributing trials/diets[a]	13/41	13/41	13/41	13/41	13/41	8/28	8/28	8/28	8/25	8/28	8/28	8/28
TFA→SFA	−0.031* (0.007)	0.006 (0.006)	−0.008 (0.005)	0.013* (0.002)	−0.004 (0.002)	−3.5* (1.1)	7.0* (0.9)	−0.007* (0.002)	−3.76* (0.42)	−1.0 (1.4)	5.9* (1.1)	−0.004 (0.002)
TFA→MUFA	−0.054* (0.009)	−0.032* (0.008)	−0.038* (0.007)	0.010* (0.003)	−0.014* (0.003)	−10.0* (1.8)	5.3* (1.5)	−0.010* (0.003)	−1.39* (0.46)	−5.0 (2.5)	3.3 (2.0)	−0.005 (0.004)
TFA→PUFA	−0.067* (0.009)	−0.047* (0.008)	−0.051* (0.007)	0.013* (0.003)	−0.015* (0.003)	−10.9* (1.6)	5.3* (1.3)	−0.011* (0.002)	−1.11* (0.40)	−5.9* (2.4)	2.8 (2.0)	−0.004 (0.003)
No. of contributing trials/diets[b]	35/91	35/90	35/90	35/90	35/91	25/66	25/65	NA	NA	NA	NA	NA
SFA→MUFA	−0.029* (0.007)	−0.042* (0.005)	−0.041* (0.005)	−0.002* (0.002)	0.002 (0.004)	−7.4* (2.6)	−0.5 (2.3)	NA	NA	NA	NA	NA
SFA→PUFA	−0.035* (0.007)	−0.057* (0.005)	−0.051* (0.005)	−0.004* (0.002)	−0.005 (0.004)	−10.3* (2.7)	−3.5 (2.3)	NA	NA	NA	NA	NA
MUFA→PUFA	−0.006 (0.007)	−0.015* (0.004)	−0.010* (0.004)	−00.002 (0.002)	−0.007 (0.004)	−2.9 (2.5)	−3.0 (2.2)	NA	NA	NA	NA	NA

Abbreviations: Apo, apolipoprotein; Lp, lipoprotein; MUFA, *cis*-monounsaturated fatty acids; NA, Not assessed in the prior meta-analysis; PUFA, *cis*-polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, *trans*-fatty acids.
Values are change (SE). The inverse of each of these values indicates the effects of the reverse replacement (e.g. SFA→TFA).
*Indicates corresponding *P*-value is <0.05.
[a]Calculated from our meta-analysis of randomized controlled dietary trials (see Methods), adjusted for the effects of the other types of fat, dietary cholesterol, protein intake, duration of diet, total calories, weight and age. Diets among men and women were considered separately to increase precision, including 66 total diets for blood lipids; 40 for apolipoproteins and 34 for Lp(a). Effects on ApoB/ApoAI were calculated with and without additional adjustment for changes in total/HDL-C.
[b]Derived from a prior meta-analysis of randomized controlled dietary trials (Mensink *et al.*, 2003).

subtraction of the coefficients from the TFA meta-analysis, which suggested small effects (SFA→MUFA: −0.001, SFA→PUFA: 0.000, MUFA→PUFA: 0.001). Subtraction of Lp(a) coefficients from the TFA meta-analysis suggested that isocaloric exchanges between SFA, MUFA and PUFA might affect Lp(a) (SFA→MUFA: +2.37 mg/l, SFA→PUFA: +2.65 mg/l, MUFA→PUFA: +0.28 mg/l). This potential Lp(a) lowering effect of SFA has been seen previously (Ginsberg *et al.*, 1998; Muller *et al.*, 2003). However, because the trials in the meta-analysis were designed to assess isocaloric exchanges of TFAs for other fats, rather than effects of exchanges between SFAs, MUFAs and PUFAs on Lp(a), we conservatively assumed that isocaloric exchanges between SFAs, MUFAs and PUFAs produced no net changes in Lp(a).

The effects on an individual's CHD risk for the changes in TC/HDL-C, ApoB/ApoAI, Lp(a) and CRP if 7.5% of energy from PHVO were replaced with alternative fats and oils were calculated using established relationships between these risk factors and the incidence of CHD (Danesh *et al.*, 2000, 2004; Yusuf *et al.*, 2004; Prospective Studies Collaboration, 2007). Results are shown in Figure 2. We assumed additivity of the calculated risk differences; multiplicative models were not appreciably different (typically <1% total difference). For PHVO with 20% TFA, replacement with butter would result in a very small net decrease (2.7%) in CHD risk, while replacement with palm oil or lard would more modestly (7.6 and 6.0%) decrease risk. Conversely, replacement with soybean, canola or high oleic sunflower oils would produce the largest (8.8–9.9%) CHD risk reductions. For PHVO with 35% TFA, risk reductions for replacement fats and oils ranged from 11.9 to 16.0%, with the largest predicted declines in CHD risk for replacement with vegetable oils. Predicted risk reductions were greatest for replacement of PHVO with 45% TFA, including risk reductions of 18.7 and 19.8% for replacement with soybean and canola oil, respectively.

Effects of TFA replacement: clinical outcomes based on cohort studies

Four prospective cohort studies reporting on the association of habitual dietary consumption of TFAs with incidence of CHD events were identified (Figure 3) (Pietinen *et al.*, 1997; Oomen *et al.*, 2001; Oh *et al.*, 2005; Mozaffarian *et al.*, 2006). These consisted of two North American cohorts (Nurses Health Study and Health Professionals Follow-up Study) and two European cohorts (Finnish ATBC study and Zutphen Elderly Study) and included 4965 CHD cases prospectively ascertained among 139 836 participants. Stronger relationships with CHD risk were seen in two studies that utilized prospective updating of dietary habits by means of serial questionnaires over time (Oh *et al.*, 2005; Mozaffarian *et al.*, 2006), compared with the study in which dietary habits were assessed only at baseline (Pietinen *et al.*, 1997). The meta-analysis of these studies demonstrated that a 2% higher energy intake from TFAs, as an isocaloric

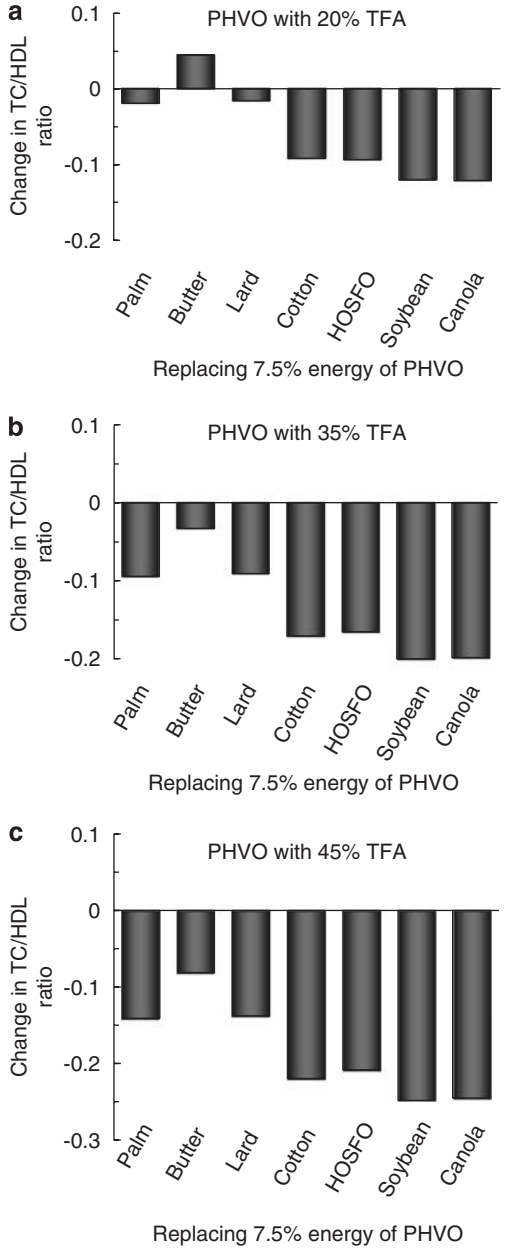

Figure 1 (**a**–**c**) Change in the TC/HDL-C ratio when 7.5% of energy from PHVO is replaced with alternative fats or oils, based on effects of different fats in controlled dietary studies. Effects are shown for PHVO containing 20% (**a**), 35% (**b**), or 45% (**c**) TFA. PHVO, partially hydrogenated vegetable oils; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol.

replacement for carbohydrate, was associated with a multivariable adjusted RR of 1.23 (95% CI = 1.11–1.37). By means of a pooled analysis of the two largest cohorts (see Methods), we also determined the multivariable adjusted RR of CHD for isocaloric replacement of 2% energy from carbohydrate with SFAs, MUFAs or PUFAs. By subtraction of these multivariable adjusted coefficients, we assessed the effects of replacing 2%

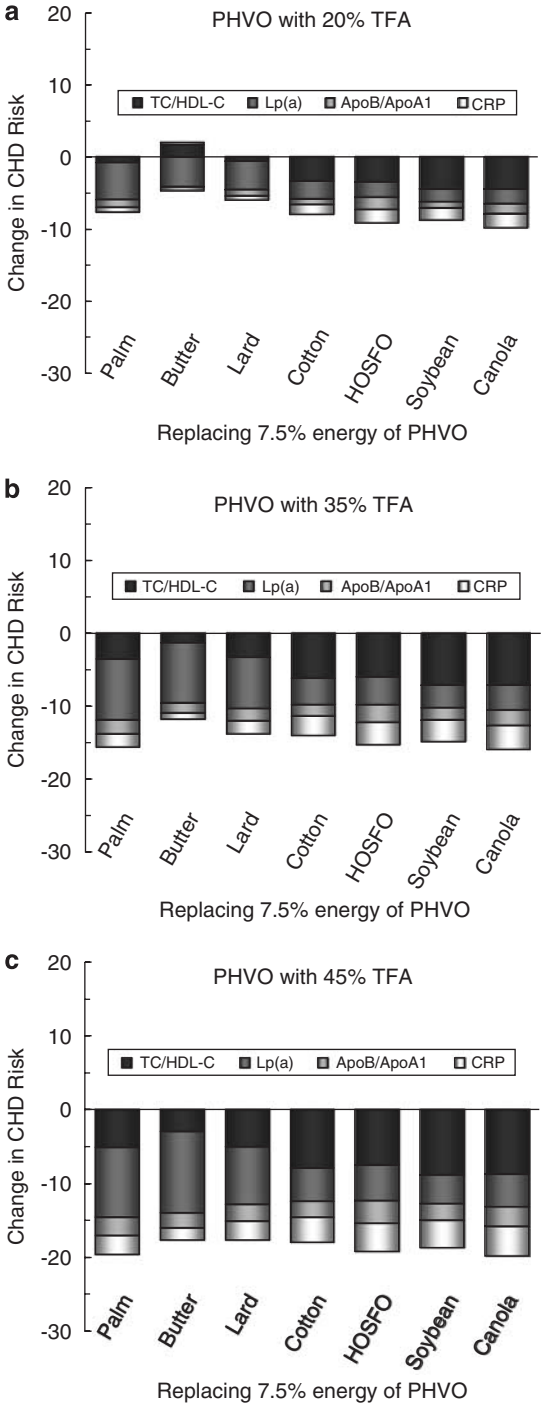

Figure 2 (**a**–**c**) Change in CHD risk when 7.5% of energy from PHVO is replaced with alternative fats or oils, based on effects of different fats on the TC/HDL-C, ApoB, ApoAI, Lp(a) and CRP in controlled dietary studies and the relations of these risk factors with incidence of CHD. Effects are shown for PHVO containing 20% (**a**), 35% (**b**), or 45% (**c**) TFA. Apo, apolipoprotein; CHD, coronary heart disease; CRP, C-reactive protein; Lp, lipoprotein; PHVO, partially hydrogenated vegetable oils; TC/HDL-C, total cholesterol/high-density lipoprotein cholesterol.

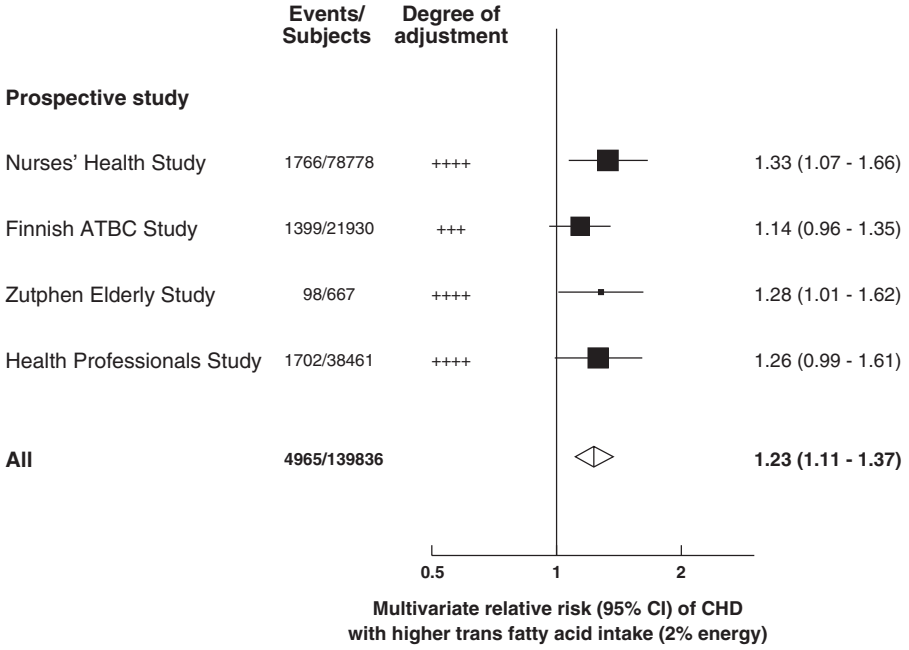

Figure 3 Meta-analysis of prospective cohort studies of habitual TFA consumption and CHD risk, including 5215 incident CHD events among 140 542 participants. The black squares and horizontal lines indicate the RR and 95% CI in each study; the size of the black squares is proportional to the inverse variance weight in the meta-analysis. The unshaded diamond indicates the combined RR and 95% CI. The degree of adjustment for confounders is denoted as + + + (adjusted for age, smoking, education, body mass index, blood pressure, physical activity, alcohol intake and consumption of fibre and total energy) and + + + + (further adjusted for consumption of other dietary fats and protein). CHD, coronary heart disease; CI, confidence interval; RR, relative risk; TFA, *trans*-fatty acids. Adapted from Mozaffarian *et al.* (2006).

of energy from TFAs with SFAs (RR = 0.83, 95% CI = 0.75–0.93), MUFAs (RR = 0.79, 95% CI = 0.70–0.88) or PUFAs (RR = 0.76, 95% CI = 0.67–0.85).

On the basis of these observed relationships between habitual consumption of dietary fats and incidence of CHD, the effects on a person's CHD risk of replacing 7.5% of energy from PHVO with alternative fats and oils were determined (Figure 4). For PHVO with 20% TFA, replacement with butter would have little net effect on CHD risk (0.5% higher risk), while replacement with palm oil or lard would modestly (9.1 and 7.3%, respectively) decrease risk. Replacement with high oleic sunflower oil would reduce risk by 15.9%, and replacement with cottonseed, soybean or canola oils would produce the largest reductions (19.0–21.8%) in CHD risk. For PHVO with 35% TFA, risk reductions for replacement fats and oils ranged from 14.4 to 33.4%, with the largest predicted declines in CHD risk for replacement with vegetable oils. Predicted risk reductions were greatest for replacement of PHVO with 45% TFA, including risk reductions of 39.6 and 38.6% for replacement with soybean and canola oil, respectively. A comparison of the calculated reductions in CHD risk based on (1) the effects of dietary fats on TC/HDL, ApoB/ApoAI, Lp(a) and CRP in randomized dietary trials vs (2) associations of habitual consumption of dietary fats with incident CHD events in prospective cohort studies is shown in Table 4.

Discussion

A growing number of food manufacturers, food services, restaurants and government agencies have completed or are considering voluntary, labelling or regulatory efforts to reduce the content of industrially produced TFAs in foods. A critical question is what should be used to replace TFAs. In practice, TFAs cannot be specifically targeted for replacement, but rather PHVO that contains both TFAs and other fatty acids must be removed and replaced with other fats or oils. We have calculated, for the first time, the predicted effects on an individual's CHD risk of replacing different PHVO formulations with alternative fats and oils likely to be used for replacement.

To provide the most robust and reliable estimates, we used two different lines of evidence, the first based on the effects of dietary fats (TFAs, SFAs, MUFAs and PUFAs) on blood lipids, lipoproteins and (possibly) CRP in randomized controlled trials, and the second based on the relationship of habitual consumption of dietary fats with CHD events in prospective observational studies. In the meta-analysis of randomized controlled dietary trials, notable effects of TFAs included raising of the TC/HDL-C ratio and ApoB levels, particularly vs MUFAs or PUFAs but also vs SFAs; lowering of HDL-C and ApoAI; and raising of Lp(a). Interestingly, the effects on ApoB and ApoAI were only partly attenuated (~50%) after adjustment for changes in the TC/HDL-C ratio,

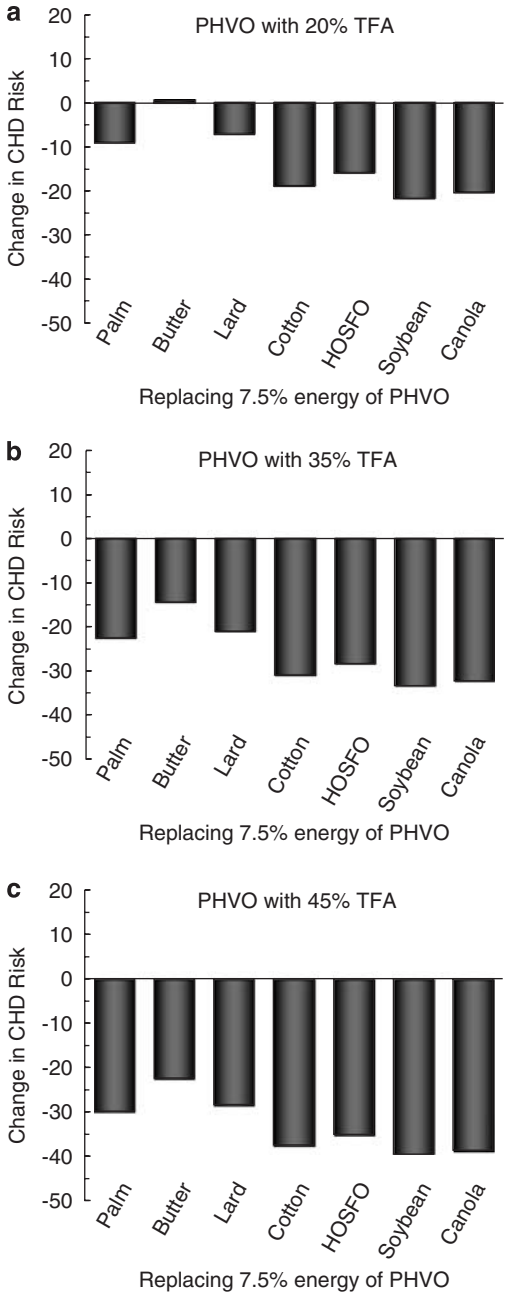

Figure 4 (**a**–**c**) Change in CHD risk when 7.5% of energy from PHVO is replaced with alternative fats or oils, based on relationships of habitual dietary consumption of different fats with disease outcomes in prospective cohort studies. Effects are shown for PHVO containing 20% (**a**), 35% (**b**), or 45% (**c**) TFA. CHD, coronary heart disease; PHVO, partially hydrogenated vegetable oils.

indicating that TFA consumption independently affects both blood lipid concentrations and apolipoprotein levels. Because these randomized dietary trials were generally performed in healthy individuals and only assessed brief (usually 4 to 5 weeks) TFA consumption, these results might underestimate the effects of longer-term TFA consumption in higher risk individuals. Thus, we also considered evidence from the observed relationships between habitual TFA consumption and incidence of CHD, after adjustment for other risk factors and lifestyle behaviours, in prospective cohort studies. A meta-analysis of these studies demonstrated that a 2% higher energy intake from TFAs, as an isocaloric replacement for carbohydrate, was associated with 23% higher CHD risk.

On the basis of the effects of TFAs and other dietary fats on lipids and lipoproteins in randomized trials, we calculated the predicted changes in CHD risk if 7.5% of energy from PHVO were replaced with other fats and oils. We also considered the possible effects on CRP levels, although these estimates should be considered as hypothesis generating because of the limited data available. The results generally indicated that replacement of PHVO with any alternative fat and oil would lower CHD risk. However, the magnitude of the expected benefits varied. For a 20% TFA PHVO, replacement with butter would have minimal effects on CHD risk, while replacement with vegetable oils would lower risk by $\sim$10%. For PHVO with 35 or 45% TFAs, any of the alternative fats and oil, including butter, lard, palm oil or vegetable oils, would lower risk by 12–20%, with the largest benefits coming from tropical or vegetable oils.

These results indicate that for comparing the relative effects of the alternative fats and oils, a key consideration is the concentration of TFAs in the PHVO. For any given PHVO replaced in a person's diet (or in a particular food product), changing the total quantity of PHVO replaced would also, of course, proportionally increase or decrease the absolute expected changes in risk, but this would not alter the relative effects of the different alternative fats and oils. For example, our findings suggest that for a 20% TFA PHVO, replacement with butter would be relatively neutral and replacement with vegetable oils would be beneficial; this would be independent of the total energy of PHVO (or TFA) replaced. For a 35% TFA or 45% TFA PHVO, replacement with any alternative, including butter, lard or tropical oils based on these data, would lower risk, again independent of the percent of total energy of PHVO (or TFA) replaced. The relative effects of these different alternative fats and oils have direct implications for food companies' and policy makers' decisions regarding replacement of PHVO in food products and the food supply. The absolute expected changes in risk can be scaled upward or downward for larger or smaller absolute changes in PHVO consumed or replaced.

Bakery shortenings and other hard PHVO, the predominant sources of TFAs in the US (Federal Citizen Information Center, 2007), typically contain 30–50% TFA. Similar amounts of TFAs are seen in PHVO stick margarines (mean 39% TFA (range 36–43%)) whereas PHVO soft (tub) margarines generally contain lower amounts of TFAs (mean 20% (range 17–33%)) (Ratnayake *et al*., 2007). Particular concerns may exist for developing countries in which PHVO represent inexpensive and stable sources of dietary fat. In Iran, for example, government-subsidized PHVO are the

Table 4 Calculated reductions in CHD risk (%) for replacement of partially hydrogenated vegetable oils (7.5% of energy) with alternative fats/oils

	Based on effects of TFA, SFA, MUFA and PUFA consumption on total cholesterol/HDL, ApoB/ApoAI, Lp(a) and CRP in randomized controlled trials[a]			*Based on relationships of TFA, SFA, MUFA and PUFA consumption with CHD events in prospective observational studies*[b]		
	Partially hydrogenated oil			*Partially hydrogenated oil*		
	20% TFA	*35% TFA*	*45% TFA*	*20% TFA*	*35% TFA*	*45% TFA*
Replacement fat/oil						
Palm oil	−7.6	−15.7	−19.7	−9.1	−22.5	−29.9
Butter	−2.7	−11.9	−17.6	0.5	−14.4	−22.4
Lard	−6.0	−13.8	−17.7	−7.3	−21.0	−28.4
Cottonseed oil	−8.0	−14.0	−18.0	−19.0	−31.0	−37.5
High oleic sunflower oil	−9.2	−15.3	−19.2	−15.9	−28.3	−35.1
Soybean oil	−8.8	−14.9	−18.7	−21.8	−33.4	−39.6
Canola oil	−9.9	−16.0	−19.8	−20.4	−32.3	−38.6

Abbreviations: Apo, apolipoprotein; CHD, coronary heart disease; MUFA, *cis*-monounsaturated fatty acids; PHVO, partially hydrogenated vegetable oils; PUFA, *cis*-polyunsaturated fatty acids; SFA, saturated fatty acids; TFA, *trans*-fatty acids.
[a]Derived from a meta-analysis of 13 randomized controlled dietary trials, except for effects on CRP that were derived from one randomized controlled dietary trial, together with the prior established relationships between these risk factors and CHD risk.
[b]Derived from a meta-analysis of four prospective cohort studies evaluating habitual dietary consumption and incidence of CHD events, including 4965 incident cases among 139 836 participants.

most common fats used for cooking in the home, accounting for 12.5% of total calories; the top two PHVO contain 34–36% TFA (Mozaffarian *et al.*, 2007). In India, the fat vanaspati is commonly used for home cooking; as a substitute for ghee; in the bakery industry and in preparation of commercially fried, processed, frozen, ready-to-eat and street foods; vanaspati is typically blended from PHVO and other oils and contains an average of 18–43% TFA (L'Abbe *et al.*, 2009). Thus, the formulations of PHVO and the total amounts consumed may vary between different countries. Notably, not simply the overall average consumption in any country but particularly the right 'tail' of the population distribution should be considered, as certain subgroups of the population will consume much higher amounts of PHVO than others (L'Abbé *et al.*, 2009).

A comparison of the calculations based on changes in risk factors in randomized controlled trials, with the estimates based on observed associations between dietary habits and CHD risk in cohort studies, raises several interesting points. First, for replacement of PHVO with butter, lard or palm oil, the predicted CHD effects based on risk factor changes in trials were both qualitatively and quantitatively similar to those based on observed associations with outcomes in cohort studies. Indeed, for these fats, the predicted changes in risk based on four risk factors (TC/HDL-C, ApoB/ApoAI, Lp(a) and CRP) accounted for ~65–80% of the differences in CHD risk expected from the observational studies. The concordance between these two estimates, derived using very different methods and assumptions, is notable. These findings also suggest that the observed effects on CHD risk of exchanging PHVO with butter, lard or palm oil may largely relate to effects on these particular risk factors.

For replacement of PHVO with vegetable oils, the predicted CHD effects based on changes in the selected risk factors in trials were qualitatively similar to those derived from associations with outcomes in cohort studies, but the magnitude of former estimates were only ~50% of the latter. Whether this is due to an underestimation of benefits based on risk factor changes in trials or an overestimation of benefits based on CHD incidence in cohort studies cannot be discriminated from the present analysis. Interestingly, the calculations based on risk factor changes in controlled trials indicated that replacing typical (35 or 45% TFA) PHVO with either animal fats or vegetable oils would provide similar CHD benefits, whereas the calculations based on observed outcomes in cohort studies indicated that replacing PHVO with vegetable oils would provide approximately twice the benefit of replacing PHVO with animal fats (Table 4). Because vegetable oils are generally considered healthier alternatives than animal fats, this suggests that the estimates based on cohort studies may be closer to the true effects. In addition to effects on TC/HDL-C, ApoB/ApoAI, Lp(a) and CRP, *cis*-unsaturated fats in vegetable oils may have beneficial effects on other pathways related to cardiovascular risk, such as insulin sensitivity (Summers *et al.*, 2002; Paniagua *et al.*, 2007) and endothelial function (Perez-Jimenez *et al.*, 1999; Nicholls *et al.*, 2006), in comparison with animal fats. Some vegetable oils (for example, soybean oil) also contain omega-3 fatty acids (alpha-linolenic acid), which may further contribute to the lowering of CHD risk independently of effects on blood lipids, lipoproteins or CRP (Mozaffarian, 2005). Thus, the calculated CHD benefits of replacing PHVO with vegetable oils based only on changes in TC/HDL-C, ApoB/ApoAI, Lp(a) and CRP in short-term trials may underestimate the full benefits, and the magnitude of

benefits calculated from the cohort studies may be closer to the true effects.

Prior studies have demonstrated a discrepancy in calculated effects of TFA consumption on CHD risk based on effects on the TC/HDL ratio in short-term trials vs observed outcomes in cohort studies (Mozaffarian *et al.*, 2006). Some of this difference may relate to differences in populations evaluated, durations of TFA consumption, random errors and potential biases in randomized controlled trials vs observational studies. Our findings suggest that this quantitative difference is also partly related to effects of TFAs on other cardiovascular risk factors besides the TC/HDL-C ratio and that accounting for such effects, as established from randomized controlled dietary trials, produces calculated effects that are more similar to those predicted by cohort studies.

Our analysis has several strengths. The focus on PHVO as the unit of replacement, rather than TFA, has direct implications for practice in that food manufacturers, restaurants, and those who cook with PHVO at home must reduce TFAs by replacing a given amount of PHVO with an alternative fat and oil. We performed new meta-analyses of randomized controlled dietary trials and prospective cohort studies of TFA consumption, increasing the precision and power to quantify effects. The calculated benefits based on risk factors reflect physiologic effects of dietary fats in controlled trials. Thus, these estimates are free of residual confounding from other lifestyle or dietary behaviours. We considered the effects on several risk factors, more completely accounting for the full impact of dietary fats on risk. The calculated benefits based on cohort studies reflect actual observed relationships of habitual consumption of dietary fats with CHD events and thus may even more completely account for total effects. Also, these calculations are derived from completely different data sources, with different strengths, limitations, assumptions and potential biases, compared with results from risk factor changes in dietary trials, increasing the robustness and reliability of the findings.

Potential limitations should also be considered. Estimates were based on isocaloric replacement of 7.5% of energy from PHVO, but PHVO consumption may be higher or lower in different populations or specific subgroups. However, this would not alter the relative effects of the different alternative fats and oils, and the absolute expected changes in risk can be scaled upward or downward for larger or smaller absolute amounts of PHVO replaced. For simplicity, calculations considered $n-3$ and $n-6$ PUFAs as having similar effects and did not account for potential additional benefits of $n-3$ PUFAs in some replacement fats and oils. The effects of different SFAs (for example, C12:0–18:0) were also considered together, and it is possible that SFAs of different chain lengths (or in different positions on the glycerol backbone, for example, comparing some tropical oils vs animal fats) may have different effects on cardiovascular risk. The TC/HDL-C ratio and ApoB/ApoAI ratio are both reliable indicators of CHD risk and generally superior to other lipid measures, but the impact of diet-induced changes in blood lipid and lipoprotein levels on CHD outcomes has not been conclusively proven in randomized clinical trials. In our calculations of effects based on changes in risk factors, we did not include other demonstrated or putative effects of TFA consumption, for example, on triglyceride levels, other inflammatory mediators besides CRP, endothelial function, insulin resistance or weight gain (Mozaffarian *et al.*, 2009). Also, the relationships with CHD risk of ApoB/ApoAI, Lp(a) and CRP levels were not corrected for regression dilution bias. Thus, these calculations may underestimate the full impact. The calculations based on cohort studies are subject to residual confounding from other lifestyle factors and to measurement error in assessment of dietary consumption from questionnaires. The former may cause an overestimation of effects and the latter would cause its underestimation. For example, the use of objective biomarkers of TFA consumption (for example, erythrocyte membrane levels) (Sun *et al.*, 2007) reveals stronger associations between TFA intake and CHD risk than the use of dietary questionnaires (Willett *et al.*, 1993; Hu *et al.*, 1997; Oh *et al.*, 2005).

Our findings indicate that the replacement of PHVO with alternative fats and oils would substantially lower CHD risk and that the discrepancies between estimates from controlled dietary trials vs prospective cohort studies can be at least partly explained by considering the effects of TFA on multiple risk factors. On the basis of the amounts and formulations of PHVO being replaced and the alternative fats and oils used, the total predicted benefit for a given individual, population or food product could be determined. The calculated effects, derived from either randomized trials or observational studies, indicate that benefits would be greatest for replacement of PHVO with vegetable oils but that even replacement with tropical oils or animal fats would result in benefits, particularly for replacement of PHVO having higher (35–45%) levels of TFAs. These data do not lend support to concerns that replacement of PHVO with tropical oils or animal fats would be neutral or harmful. However, these results also indicate that food manufacturers, food services and restaurants should take advantage of the expense and effort involved in food reformulations to not only reduce the TFA content but also maximize the overall health benefit by using replacement fats and oils with higher content of *cis*-unsaturated fats.

Conflict of interest

During the preparation and peer review of this paper in 2007, the authors and peer reviewers declared the following interests.

Authors
Dr Dariush Mozaffarian: None declared.
Dr Robert Clarke: None declared.

Peer-reviewers
Dr Mary L'Abbé: None declared.

Professor Murray Skeaff: Led a research project that tested the effects of a plant-sterol enriched fat spread on blood cholesterol concentrations; costs of research partially funded by Unilever Research and Development (2003–2004); participated in a subcontract to conduct a randomized controlled trial of a milk product enriched with an antioxidant extract from vegetables, which was partially funded by Fonterra, a milk company in New Zealand (2005–2007). All industry supported research projects were organized and administered through the University of Otago Research and Enterprise Unit.

Professor Steen Stender: Shares in Novozymes (enzymes for various processes, including interesterification).

Professor Ricardo Uauy: Scientific Advisor to Unilever and Wyeth (*ad hoc* basis); Scientific Advisor to Knowles and Bolton, Danone, DSM and Kellogg's (*ad hoc* basis).

Acknowledgements

We thank Dr Jemma Hopewell for statistical expertise and acknowledge Dr Mary L'Abbé, Professor Murray Skeaff, Professor Steen Stender, and Professor Ricardo Uauy for their helpful comments during the preparation of this paper. Dr Mozaffarian was supported by a grant from the National Heart, Lung, and Blood Institute, National Institutes of Health (K08-HL-075628). We thank Jemma Hopewell and Paul Sherliker for their assistance in statistical analyses and figure production.

References

Almendingen K, Jordal O, Kierulf P, Sandstad B, Pedersen JI (1995). Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on serum lipoproteins and Lp(a) in men. *J Lipid Res* **36**, 1370–1384.

Aro A, Jauhiainen M, Partanen R, Salminen I, Mutanen M (1997). Stearic acid, *trans* fatty acids, and dairy fat: effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein(a), and lipid transfer proteins in healthy subjects. *Am J Clin Nutr* **65**, 1419–1426.

Baer DJ, Judd JT, Clevidence BA, Tracy RP (2004). Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. *Am J Clin Nutr* **79**, 969–973.

Clarke R, Frost C, Collins R, Appleby P, Peto R (1997). Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. *Brit Med J* **314**, 112–117.

Danesh J, Collins R, Peto R (2000). Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. *Circulation* **102**, 1082–1085.

Danesh J, Wheeler JG, Hirschfield GM, Eda S, Eiriksdottir G, Rumley A *et al*. (2004). C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *N Engl J Med* **350**, 1387–1397.

de Roos N, Schouten E, Katan M (2001). Consumption of a solid fat rich in lauric acid results in a more favorable serum lipid profile in healthy men and women than consumption of a solid fat rich in *trans*-fatty acids. *J Nutr* **131**, 242–245.

DerSimonian R, Laird N (1986). Meta-analysis in clinical trials. *Control Clin Trials* **7**, 177–188.

Eckel RH, Borra S, Lichtenstein AH, Yin-Piazza SY (2007). Understanding the complexity of *trans* fatty acid reduction in the American diet: American Heart Association Trans Fat Conference 2006: Report of the Trans Fat Conference Planning Group. *Circulation* **115**, 2231–2246.

Federal Citizen Information Center (2007). *Revealing Trans Fats*. United States Food And Drug Administration: Pueblo At: http://pueblo.gsa.gov/cic_text/food/reveal-fats/reveal-fats.htm. Accessed 14 December 2007.

Ginsberg HN, Kris-Etherton P, Dennis B, Elmer PJ, Ershow A, Lefevre M *et al*. (1998). Effects of reducing dietary saturated fatty acids on plasma lipids and lipoproteins in healthy subjects: the DELTA Study, protocol 1. *Arterioscler Thromb Vasc Biol* **18**, 441–449.

Hu FB, Stampfer MJ, Manson JE, Rimm EB, Colditz GA, Rosner BA *et al*. (1997). Dietary fat intake and the risk of coronary heart disease in women. *N Eng J Med* **337**, 1491–1499.

Judd JT, Baer DJ, Clevidence BA, Kris-Etherton P, Muesing RA, Iwane M (2002). Dietary *cis* and *trans* monounsaturated and saturated fatty acids and plasma lipids and lipoproteins in men. *Lipids* **37**, 123–131.

Judd JT, Baer DJ, Clevidence BA, Muesing RA, Chen SC, Weststrate JA *et al*. (1998). Effects of margarine compared with those of butter on blood lipid profiles related to cardiovascular disease risk factors in normolipemic adults fed controlled diets. *Am J Clin Nutr* **68**, 768–777.

Judd JT, Clevidence BA, Muesing RA, Wittes J, Sunkin ME, Podczasy JJ (1994). Dietary *trans* fatty acids: effects on plasma lipids and lipoproteins of healthy men and women. *Am J Clin Nutr* **59**, 861–868.

L'Abbé MR, Stender S, Skeaff M, Ghafoorunissa, Tavella M (2009). Approaches to removing *trans* fats from the food supply in industrialized and developing countries. *Eur J Clin Nutr* **63**(Suppl 2), S50–S67.

Laine DC, Snodgrass CM, Dawson EA, Ener MA, Kuba K, Frantz Jr ID (1982). Lightly hydrogenated soy oil vs other vegetable oils as a lipid-lowering dietary constituent. *Am J Clin Nutr* **35**, 683–690.

Lichtenstein AH, Ausman LM, Jalbert SM, Schaefer EJ (1999). Effects of different forms of dietary hydrogenated fats on serum lipoprotein cholesterol levels. *N Engl J Med* **340**, 1933–1940.

Lichtenstein AH, Erkkila AT, Lamarche B, Schwab US, Jalbert SM, Ausman LM (2003). Influence of hydrogenated fat and butter on CVD risk factors: remnant-like particles, glucose and insulin, blood pressure and C-reactive protein. *Atherosclerosis* **171**, 97–107.

Lovejoy JC, Smith SR, Champagne CM, Most MM, Lefevre M, DeLany JP *et al*. (2002). Effects of diets enriched in saturated (palmitic), monounsaturated (oleic), or *trans* (elaidic) fatty acids on insulin sensitivity and substrate oxidation in healthy adults. *Diabetes Care* **25**, 1283–1288.

Mensink RP, Katan MB (1990). Effect of dietary *trans* fatty acids on high-density and low-density lipoprotein cholesterol levels in healthy subjects. *N Engl J Med* **323**, 439–445.

Mensink RP, Zock PL, Kester AD, Katan MB (2003). 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* **77**, 1146–1155.

Mozaffarian D (2005). Does alpha-linolenic acid intake reduce the risk of coronary heart disease? A review of the evidence. *Altern Ther Health Med* **11**, 24–30; quiz 31, 79.

Mozaffarian D (2006). *Trans* fatty acids—Effects on systemic inflammation and endothelial function. *Atheroscler Suppl* **7**, 29–32.

Mozaffarian D, Abdollahi M, Campos H, Houshiarrad A, Willett WC (2007). Consumption of trans fats and estimated effects on coronary heart disease in Iran. *Eur J Clin Nutr* **61**, 1004–1010.

Mozaffarian D, Aro A, Willett WC (2009). Health effects of *trans*-fatty acids: experimental and observational evidence. *Eur J Clin Nutr* **63**(Suppl 2), S5–S21.

Mozaffarian D, Katan MB, Ascherio A, Stampfer MJ, Willett WC (2006). *Trans* fatty acids and cardiovascular disease. *N Engl J Med* **354**, 1601–1613.

Muller H, Jordal O, Kierulf P, Kirkhus B, Pedersen JI (1998). Replacement of partially hydrogenated soybean oil by palm oil in margarine without unfavorable effects on serum lipoproteins. *Lipids* **33**, 879–887.

Muller H, Lindman AS, Blomfeldt A, Seljeflot I, Pedersen JI (2003). A diet rich in coconut oil reduces diurnal postprandial variations in circulating tissue plasminogen activator antigen and fasting lipoprotein (a) compared with a diet rich in unsaturated fat in women. *J Nutr* **133**, 3422–3427.

Nicholls SJ, Lundman P, Harmer JA, Cutri B, Griffiths KA, Rye KA *et al*. (2006). Consumption of saturated fat impairs the anti-inflammatory properties of high-density lipoproteins and endothelial function. *J Am Coll Cardiol* **48**, 715–720.

Oh K, Hu FB, Manson JE, Stampfer MJ, Willett WC (2005). Dietary fat intake and risk of coronary heart disease in women: 20 years of follow-up of the Nurses' Health Study. *Am J Epidemiol* **161**, 672–679.

Oomen CM, Ocké MC, Feskens EJ, van Erp-Baart MA, Kok FJ, Kromhout D (2001). Association between trans fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. *Lancet* **357**, 746–751.

Paniagua JA, de la Sacristana AG, Sanchez E, Romero I, Vidal-Puig A, Berral FJ *et al*. (2007). A MUFA-rich diet improves postprandial glucose, lipid and GLP-1 responses in insulin-resistant subjects. *J Am Coll Nutr* **26**, 434–444.

Perez-Jimenez F, Castro P, Lopez-Miranda J, Paz-Rojas E, Blanco A, Lopez-Segura F *et al*. (1999). Circulating levels of endothelial function are modulated by dietary monounsaturated fat. *Atherosclerosis* **145**, 351–358.

Pietinen P, Ascherio A, Korhonen P, Hartman AM, Willett WC, Albanes D *et al*. (1997). Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am J Epidemiol* **145**, 876–887.

Prospective Studies Collaboration (2007). Blood cholesterol and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55 000 vascular deaths. *Lancet* **370**, 1829–1839.

Ratnayake WMN, Gagnon C, Dumais L, Lillycrop W, Wong L, Meleta M *et al*. (2007). *Trans* fatty acid content of Canadian margarines prior to mandatory trans fat labelling. *J Am Oil Chem Soc* **84**, 817–825.

Skeaff CM (2009). Feasibility of recommending certain replacement or alternative fats. *Eur J Clin Nutr* **63**(Suppl 2), S34–S49.

Summers LK, Fielding BA, Bradshaw HA, Ilic V, Beysen C, Clark ML *et al*. (2002). Substituting dietary saturated fat with polyunsaturated fat changes abdominal fat distribution and improves insulin sensitivity. *Diabetologia* **45**, 369–377.

Sun Q, Ma J, Campos H, Hankinson SE, Manson JE, Stampfer MJ *et al*. (2007). A prospective study of *trans* fatty acids in erythrocytes and risk of coronary heart disease. *Circulation* **115**, 1858–1865.

Sundram K, Karupaiah T, Hayes KC (2007). Stearic acid-rich interesterified fat and trans-rich fat raise the LDL/HDL ratio and plasma glucose relative to palm olein in humans. *Nutr Metab (London)* **4**, 3.

Willett WC, Stampfer MJ, Manson JE, Colditz GA, Speizer FE, Rosner BA *et al*. (1993). Intake of *trans* fatty acids and risk of coronary heart disease among women. *Lancet* **341**, 581–585.

Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F *et al*. (2004). Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART Study): case–control study. *Lancet* **364**, 937–952.

Zock PL, Katan MB (1992). Hydrogenation alternatives: effects of *trans* fatty acids and stearic acid vs linoleic acid on serum lipids and lipoproteins in humans. *J Lipid Res* **33**, 399–410.