European Journal of Clinical Nutrition (2009) 63, S5–S21
 0954-3007/09 $32.00
www.nature.com/ejcn

REVIEW

Health effects of *trans*-fatty acids: experimental and observational evidence

D Mozaffarian[1,2,5], A Aro[3,5] and WC Willett[2,4]

[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;* [3]*Department of Health and Functional Capacity, National Public Health Institute (KTL), Helsinki, Finland and* [4]*Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA*

Background/Objectives: Growing evidence indicates that *trans*-fatty acids (TFA) adversely affect cardiovascular health. As part of the World Health Organization (WHO) Scientific Update on TFA, we reviewed the evidence for effects of TFA consumption on coronary heart disease (CHD).
Subjects/Methods: We searched Medline publications examining TFA consumption and CHD risk factors or outcomes, emphasizing results of studies in humans. We evaluated and synthesized evidence from both controlled feeding trials evaluating risk factors and long-term observational studies evaluating risk factors or clinical outcomes, each of which have complementary strengths and limitations, to enable the most robust and reliable inferences of effects.
Results: The effects of TFA consumption on risk factors most consistently seen in both controlled trials and observational studies included adverse lipid effects (for example ↑low-density lipoprotein cholesterol, ↓high-density lipoprotein cholesterol (HDL-C), ↑total/HDL-C ratio), proinflammatory effects (for example ↑tumor necrosis factor-α activity, ↑interleukin-6, ↑C-reactive protein) and endothelial dysfunction. These effects were most prominent in comparison with *cis* unsaturated fats; adverse effects on total/HDL-C and endothelial function were also seen in comparison with saturated fatty acids (SFA). TFA may also worsen insulin sensitivity, particularly among individuals predisposed to insulin resistance; possible effects on weight gain and diabetes incidence require further confirmation. Five retrospective case–control studies and four prospective cohort studies demonstrated positive associations between TFA consumption and CHD events. A meta-analysis of prospective studies indicated 24, 20, 27 and 32% higher risk of myocardial infarction (MI) or CHD death for every 2% energy of TFA consumption isocalorically replacing carbohydrate, SFA, *cis* monounsaturated fatty acids and *cis* polyunsaturated fatty acids, respectively. The differential effects of specific TFA isomers may be important but are less well established. The available evidence indicates that *trans*-18:1 and particularly *trans*-18:2 isomers have stronger CHD effects than *trans*-16:1 isomers. The limited data suggest that the experimental effects of ruminant and industrial TFA are similar when consumed in similar quantities, but very few persons consume such high levels of ruminant TFA, and observational studies do not support adverse CHD effects of ruminant TFA in amounts actually consumed.
Conclusions: Controlled trials and observational studies provide concordant evidence that consumption of TFA from partially hydrogenated oils adversely affects multiple cardiovascular risk factors and contributes significantly to increased risk of CHD events. The public health implications of ruminant TFA consumption appear much more limited. The effects of specific TFA isomers require further investigation.
European Journal of Clinical Nutrition (2009) **63,** S5–S21; doi:10.1038/sj.ejcn.1602973

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

Introduction

Trans-fatty acids (TFA) are unsaturated fatty acids with at least one unsaturated, nonconjugated double bond in the *trans* (rather than the typical *cis*) configuration. As part of the World Health Organization Scientific Update on TFA, we review herein the evidence for health effects of TFA consumption in adults. The majority of evidence related to

Correspondence: Dr D Mozaffarian, 665 Huntington Avenue, Bldg 2-319, Boston, MA 02115, USA.
E-mail: dmozaffa@hsph.harvard.edu
[5]These authors contributed equally to this work.

risk of coronary heart disease (CHD), for which we evaluated and synthesized the findings of both controlled trials and observational studies.

Methods

All authors searched Medline for publications through January 2008 examining TFA consumption and changes in risk factors or associations with clinical outcomes, supplemented by hand searching of reference lists of relevant articles. The relevance of studies for inclusion and interpretation of findings were assessed and potential differences resolved by unanimous agreement. We emphasized the results of studies in humans, supplemented by animal experiments when insufficient evidence was available from human studies. The preponderance of available data related to cardiovascular risk and outcomes.

Nature of the evidence
We focused on evidence from both controlled trials and observational studies. Randomized controlled dietary trials minimize residual confounding (assuming randomization, blinding, compliance and follow-up remain intact), but often evaluate only limited dietary changes, shorter durations of exposure and generally healthier individuals. Also, owing to considerations of cost, practicality, compliance and ethics, controlled dietary trials often only assess effects on intermediary risk factors (such as serum lipoprotein concentrations) rather than effects on disease outcomes. Observational studies can evaluate a wider range of actual and habitual dietary exposures in more representative populations and can assess relationships of diet with disease outcomes. However, observational studies can be limited by errors (misclassification) in dietary assessment, which in prospective studies typically attenuates the true impact of diet, and by residual confounding due to unmeasured or imprecisely measured factors. Thus, both controlled dietary trials and observational studies have limitations, and neither is 'perfect' for assessing the true effects of diet. Fortunately, these different study designs provide highly complementary strengths, and each design overcomes some of the inherent limitations of the other design. Consequently, inferences about the effects of diet are most reliable when they are based on the totality of evidence from both controlled experiments and long-term observational studies, and the conclusions are most robust when both research paradigms provide concordant findings.

Results

Effects of TFA on cardiovascular risk factors

Serum lipoproteins: experimental evidence. Mensink and Katan (1990) compared the effects on blood lipids of TFA, 10.9% of total calories consumed (10.9%E), with similar amounts of *cis* monounsaturated fatty acids (MUFA) and saturated fatty acids (SFA) in 59 healthy volunteers. Compared with the MUFA diet, TFA increased serum low-density lipoprotein cholesterol (LDL-C) and decreased high-density lipoprotein cholesterol (HDL-C) levels. As concluded in a review of 10 studies by the European Food Safety Authority (2004), these findings were confirmed by subsequent clinical trials. In three trials (Mensink and Katan, 1990; Zock and Katan, 1992; Aro *et al*. 1997), partially hydrogenated high-oleic-acid sunflower oil (not often used in commercially available food products) was the main source of TFA, in which 18:1 $n-9$ ($\Delta 9$) elaidic acid was the most common TFA isomer. Partially hydrogenated soybean oil was used in three other trials (Almendingen *et al*., 1995; Muller *et al*., 1998a; Lichtenstein *et al*., 1999), and in four trials the source of TFA was not specified (Judd *et al*., 1994, 1998, 2002; Lovejoy *et al*., 2002).

Because each of these individual trials included small numbers of subjects, their statistical power and precision are limited. In a meta-analysis of eight controlled trials of TFA consumption (Mensink *et al*., 2003), it was estimated that when TFA replaces 1%E of carbohydrates, serum LDL-C increases by 0.04 mmol/l but HDL-C levels remain unchanged. In this respect, TFA are unique among the fatty acids, since all other fatty acid classes (including SFA, MUFA and *cis* polyunsaturated fatty acids (PUFA)) increase serum HDL-C in comparison with carbohydrates. In one trial (Lichtenstein *et al*., 1999) comparing different levels of TFA (from 0.6 to 6.7%E) from partially hydrogenated soybean oil consumed for 5 weeks each, increasing TFA consumption linearly increased LDL-C levels, but HDL-C levels remained constant at lower TFA intakes (between 0.9 and 4.2%E), being lowered by the highest level of TFA (6.7%E) only. Conversely, lower levels of habitual TFA intake are associated with differences in HDL-C levels (see section, Serum lipoproteins: observational evidence). In the meta-analysis of eight trials (Mensink *et al*., 2003), replacing 1%E of 18:1 TFA isoenergetically with a 1:1:1 mix of carbohydrates, MUFA and PUFA was estimated to decrease the ratio of serum total/HDL-C by 0.044, similar to the estimated effect of a 2%E reduction in SFA intake. Reducing TFA intake by 2%E, corresponding to the estimated average intake of industrial TFA in the USA in 1990, and replacing with a 1:1 mix of MUFA and PUFA, would reduce the total/HDL-C ratio by 0.11. In a more recent meta-analysis including 12 controlled trials of TFA consumption (Mozaffarian *et al*., 2006), the consumption of 1%E from TFA in place of other fats was estimated to increase the serum total/HDL-C ratio by 0.022 when replacing SFA, 0.051 when replacing MUFA and 0.057 when replacing PUFA. Similar to the prior meta-analysis, it was estimated that replacing 2%E of TFA intake isoenergetically with a 1:1 mix of MUFA and PUFA would reduce the serum total/HDL-C ratio by 0.11.

A new meta-analysis evaluated the effects of TFA consumption on serum lipids and lipoproteins in 13 randomized controlled trials, adjusting for differences in age, gender,

weight, duration of diets and consumption of other types of fat, dietary cholesterol, protein intake and total energy (Mozaffarian and Clarke, 2009) (Figure 1). Compared with equivalent calories from other fats, TFA consumption significantly increased the total/HDL-C ratio and apoprotein (Apo)B levels, particularly versus MUFA or PUFA as well as versus SFA; lowered HDL-C and ApoA-I levels; and raised Lp(a) levels. The effects on ApoB and ApoA-I were only partly attenuated (~50%) by adjustment for changes in the total/HDL-C ratio, suggesting that TFA consumption independently affects both blood lipid concentrations and apolipoprotein levels (Mozaffarian and Clarke, 2009). These data do not allow the estimation of possible different effects of ruminant TFA (reviewed later).

TFA consumption adversely affects blood lipids and lipoproteins beyond changes in LDL-C and HDL-C. Compared with MUFA or PUFA, TFA raises fasting triglyceride levels (Mozaffarian and Clarke, 2009). In one trial (Mauger *et al*., 2003), the consumption of partially hydrogenated soybean oil in the form of semiliquid margarines containing 0.6–26/100 g of TFA decreased LDL particle size, a possible independent CHD risk factor, in a dose-dependent manner. However, in another study, the consumption of corn oil-based margarine providing 4.2%E from TFA did not significantly affect LDL particle size (Cuchel *et al*., 1996). Lp(a) particles (consisting of LDL that includes a glycoprotein called ApoA) are also associated with increased CHD risk. TFA consumption increased Lp(a) levels in six studies (Mensink *et al*., 1992; Almendingen *et al*., 1995; Aro *et al*., 1997; Clevidence *et al*., 1997; Judd *et al*., 1998; Lichtenstein *et al*., 2003) but not significantly in one study (Muller *et al*., 1998a). A meta-analysis of these trials indicated that TFA significantly raises Lp(a) in comparison to SFA, MUFA, or PUFA (Mozaffarian and Clarke, 2009). Interestingly, other dietary factors (including other dietary fats) are not well-known to affect Lp(a) levels; nevertheless, the effect of TFA of Lp(a) may be modest compared with estimated genetic influences (Katan *et al*., 1995). In two studies (Mensink *et al*., 1992; Clevidence *et al*., 1997), the increase in Lp(a) with TFA consumption showed a positive correlation with initial Lp(a) levels.

Most trials have evaluated 18:1 TFA, and the effects of 18:2, 18:3 or C20-22 *trans*-isomers on serum lipoproteins are less well established. In one trial in Europe (Vermunt *et al*., 2001), 88 healthy men were randomized to 1.4 g/day (0.5%E on a 2500 kcal/day diet) of *trans*-isomers of α-linolenic acid (18:3 *n*−3) versus a control diet for 6 weeks. The 18:3 TFA diet increased LDL-C levels but did not significantly affect HDL-C; the LDL:HDL-C ratio was increased by 8.1%, suggesting that the effects on serum lipoproteins of 18:3 TFA may be similar to those of 18:1 TFA. The *trans*-isomers of linoleic acid (18:2 *n*−6) have not been studied separately in

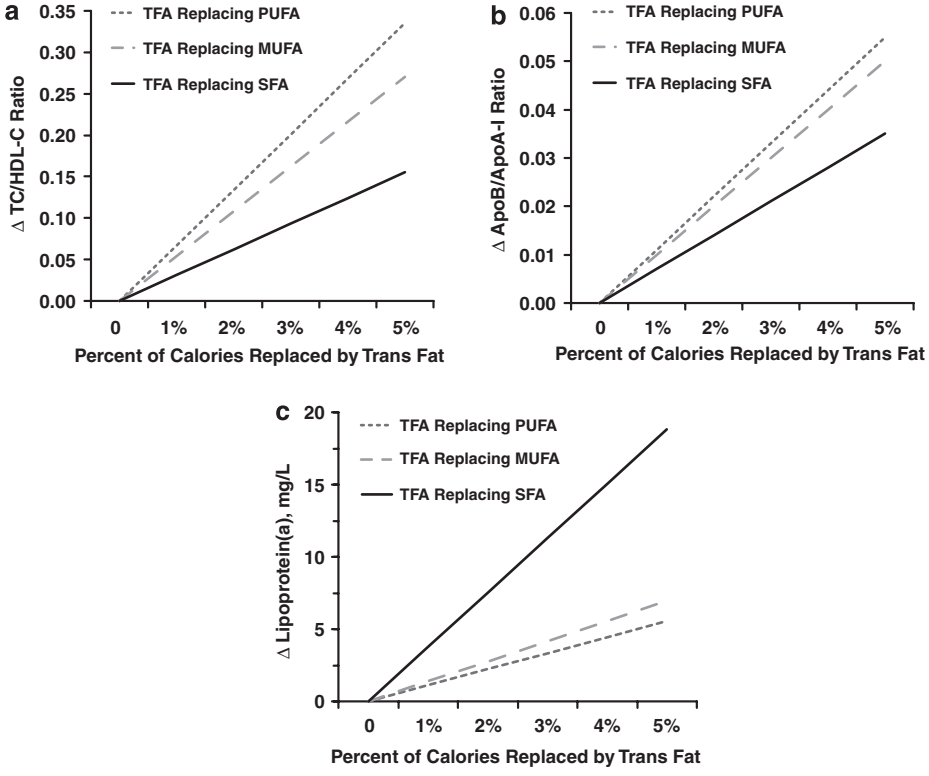

Figure 1 Effects of TFA consumption on serum lipids and lipoproteins, as an isocaloric replacement for PUFA, MUFA or SFA, in a meta-analysis of 13 randomized controlled trials ($P<0.05$ for each effect) (Mozaffarian and Clarke, 2009). (**a**) TC HDL-C ratio, (**b**) ApoB/ApoA-I ratio, (**c**) lipoprotein(a). MUFA, *cis*-monounsaturated fatty acid; PUFA, *cis*-polyunsaturated fatty acid; SFA, saturated fatty acid; TFA, *trans*-fatty acid.

controlled trials. The effects of partially hydrogenated fish oil, which contain long-chain C20-22 *trans*-isomers, have been assessed in two clinical trials in Norway. In one study (Almendingen *et al*., 1995), TFA from partially hydrogenated fish oil (8.0%E) were compared with TFA from partially hydrogenated soybean oil (8.5%E) and with butter. In comparison with both partially hydrogenated soybean oil and butter, partially hydrogenated fish oil increased LDL-C and reduced HDL-C levels, suggesting that the effects of partial hydrogenation of C20-22 fatty acids on these blood lipids were more harmful than those of the alternatives. In another trial, the consumption of a margarine providing 7.8%E TFA from partially hydrogenated fish oil and 9.2% SFA increased the LDL:HDL-C ratio compared with margarine providing 1.1%E TFA and 13.3%E SFA (Muller *et al*., 1998b). It is possible that the strong effects of partially hydrogenated fish oil on lipoproteins depend on the long-chain C20-22 *trans*-isomers present in fish oil. However, like other sources of TFA, partially hydrogenated fish oil also contains 18:1 *trans*-isomers, particularly elaidic acid, making it difficult to assess the specific effects of the C20-22 *trans*-isomers from these data. On the basis of findings of the Norwegian studies, partially hydrogenated fish oils have been considered particularly harmful, and the use of these TFA has been rapidly reduced or abandoned by the food industry in industrialized countries (Johansson *et al*., 2006), although this may not be the case in other regions of the world (Valenzuela and Uauy, 1999).

A review of potential molecular–cellular mechanisms of effects of TFA consumption is beyond the scope of this paper and is available elsewhere (Mozaffarian *et al*., 2006). Effects on both production and metabolism of lipoproteins appear to occur; an increase in the activity of cholesterol ester transfer protein (Abbey *et al*., 1994; van Tol *et al*., 1995) may also contribute to a rise in LDL-C and a reduction in HDL-C with TFA consumption. Additional effects of TFA consumption on adipocyte, liver, endothelial and monocyte–macrophage tissues have been demonstrated in both human and animal experimental studies (Mozaffarian *et al*., 2006). Potential differential mechanisms for effects of specific TFA isomers have not been studied in any detail.

Serum lipoproteins: observational evidence. Given the robust clinical trial evidence for adverse effects of TFA consumption on serum lipoprotein concentrations, few observational studies have formally evaluated the relationships between TFA consumption and serum lipid concentrations. Nevertheless, because clinical trials often evaluate effects of higher doses of TFA over shorter durations of intake and in generally healthier individuals, the evidence for effects of habitual TFA consumption on serum lipids in observational studies is important. The relationships of erythrocyte TFA levels, a biomarker of dietary intake, and serum lipids were evaluated in 327 US women selected as controls in a nested prospective study of CHD events (Sun *et al*., 2007). After adjustment for age, smoking, body mass index (BMI), physical activity, alcohol intake, family history of myocardial infarction (MI) and fasting status at the time of blood draw, higher TFA levels (corresponding to a range of habitual TFA intake from 2.5 to 3.6 g/day) were associated with significantly higher LDL-C, lower HDL-C and a higher LDL-C:HDL-C ratio (each $P<0.01$). Thus, the observed directions of effect were very similar to those seen in randomized controlled trials.

Diabetes and insulin sensitivity: experimental evidence. The effects of TFA on insulin sensitivity have been examined in relatively few experimental studies in humans, each small ($n=14$–25) and of brief duration (ranging from a single meal to up to 6 weeks). Among 16 obese subjects with type II diabetes (Christiansen *et al*., 1997), diets with 30%E total fat, including either 20%E from SFA, MUFA or TFA, were provided for 6 weeks in a randomized crossover design. Postprandial insulin levels were 59% higher and plasma C-peptide concentrations 32% higher on the TFA compared with the MUFA diet. No significant differences were seen between the SFA and TFA diets. Vega-Lopez *et al*. (2006) compared the effects of 20%E as palm, soybean, canola or partially hydrogenated soybean oil (4.2%E TFA) for 5 weeks each in 15 moderately hyperlipidemic subjects (mean BMI 26.0 kg/m^2). Both fasting insulin and insulin resistance assessed by homeostatic model assessment were significantly higher on the palm oil and partially hydrogenated soybean oil diets, compared with the canola or soybean oil diets; homeostatic model assessment was highest, although not significantly so, on the partially hydrogenated soybean oil versus palm oil diet. Lefevre *et al*. (2005) evaluated the acute effects of two single 50%E fat meals, including 10%E from either TFA or MUFA, fed at 1-week intervals to 22 moderately overweight men and women who carried at least one copy of a fatty acid-binding protein-2 gene variant. Higher postprandial insulin and C-peptide concentrations were observed after the TFA meal, compared with the MUFA meal.

These results were not seen in two trials of healthy volunteers. Lovejoy *et al*. (2002) compared 9%E from SFA, MUFA or TFA, each over 4-week periods, in a randomized crossover study of 25 volunteers. Overall, no significant effects on insulin sensitivity were seen; in the seven overweight subjects, insulin sensitivity was decreased 24% on the SFA diet and 11% on the TFA diet, compared with the MUFA diet, but these differences were not statistically significant. Louheranta *et al*. (1999) studied 14 healthy young women and compared a diet of 5.1% energy from TFA versus 5.2% energy from MUFA, replacing SFA, for 4 weeks. No statistically significant differences were observed in fasting glucose levels or measures of insulin sensitivity between the diets.

Together, these few clinical trials suggest that TFA worsens insulin resistance in overweight or diabetic individuals (that is, those predisposed to insulin sensitivity) but may have smaller or little effect in young lean subjects. Because of the relatively few numbers and small sizes of these trials, more

investigation is needed, particularly in overweight or obese subjects with impaired glucose tolerance, the metabolic syndrome or diabetes. Additionally, each study was relatively short term; a controlled trial in nonhuman primates (reviewed below) suggests that longer durations of consumption (months to years) may be important to assess fully the effects of TFA on insulin resistance.

Experimental studies in animals also suggest that TFA consumption induces insulin resistance. Axen *et al*. (2003) fed rats a high-fat (60%E) diet with 17%E TFA or a low-fat (12%E) diet with no TFA and found that the high-fat, high-TFA diet increased visceral fat mass and liver lipid content and impaired glucose tolerance and insulin sensitivity. Ibrahim *et al*. (2005) demonstrated that rats fed a diet with 3%E TFA had increased fasting insulin levels compared with control and that this increase was larger than for a diet with 10%E from SFA. Natarajan *et al*. (2005) found that rats consuming 10%E TFA had decreased insulin-stimulated glucose transport compared with a diet with 10%E from SFA. Thus, each of these studies suggests that TFA induce insulin resistance, even compared to SFA. Perhaps most relevant to humans, a 6-year randomized controlled trial in nonhuman primates evaluated diets containing 8%E from either TFA or MUFA (Kavanagh *et al*., 2007). Monkeys consuming the TFA diet exhibited significantly elevated fructosamine (a circulating marker of hyperglycemia), markedly reduced insulin sensitivity in muscle (as assessed by insulin-induced phosphorylation of Akt), and trends toward reduced insulin sensitivity in adipose tissue, not accounted for by changes in weight.

Diabetes and insulin sensitivity: observational evidence. Observational studies have not evaluated associations between TFA consumption and insulin resistance. However, three prospective cohort studies have investigated the relationship of TFA consumption with risk of incident diabetes. TFA intake was not significantly associated with incidence of diabetes in one US study of men (van Dam *et al*., 2002), but the TFA consumption in this population of health professionals was low (median overall intake = 1.3%E, and median intake in the highest quintile = 2.0%E). TFA intake was also not significantly associated with diabetes risk in a cohort of women in Iowa (Meyer *et al*., 2001). In this study, the diagnosis of diabetes was based on self-report and was found to be incorrect in more than one-third of cases in a validation study (Meyer *et al*., 2001), and diet was assessed only at baseline and may have changed over time; thus, misclassification of both exposure and outcome may have been substantial, each of which would attenuate any true effect toward the null. In a US study of 84 941 women followed for 16 years, in whom diabetes diagnoses were validated and information on dietary habits was updated over time, TFA consumption was significantly associated with diabetes incidence (Hu *et al*., 2001b). After adjustment for other risk factors, the risk of diabetes was 40% higher in the top quintile of TFA intake, compared with the lowest ($P<0.001$). The association of TFA consumption with higher diabetes risk was most evident among individuals who were overweight or obese or who had lower levels of physical activity (Salmeron *et al*., 2001).

Thus, controlled trials have evaluated biomarkers of insulin resistance, while observational studies have assessed incidence of clinical diabetes. Although results are not as consistent as for effects of TFA on serum lipoproteins, the findings suggest that TFA consumption reduces insulin sensitivity and increases diabetes risk. This effect appears to be greatest among individuals who are more predisposed to insulin resistance, such as those with preexisting insulin resistance, greater adiposity or lower physical activity levels. Further research is needed to confirm the magnitude and dose–response of this effect and the populations that may be at highest risk.

Systemic inflammation: experimental evidence. In a study of hypercholesterolemic patients, the production of interleukin (IL)-6 and tumor necrosis factor-α (TNF-α), but not IL-1β, by cultured mononuclear cells was increased after 1 month of TFA intake (6.7%E) from margarine containing partially hydrogenated soybean oil, compared with nonhydrogenated soybean oil (Han *et al*., 2002). Effects of the TFA margarine on these inflammatory markers were not significantly different from those of butter. Among 36 volunteers with hypercholesterolemia, C-reactive protein (CRP) levels were not significantly different following 5 weeks of different diets providing 20%E from different fats and oils with variable amounts of TFA (Lichtenstein *et al*., 2003). In contrast, in a trial among 50 generally healthy men (Baer *et al*., 2004), 5 weeks of a TFA diet (8%E) increased IL-6 levels by 16%, compared with equivalent calories from MUFA (but not stearic acid, shorter-chain SFA or carbohydrate). The TFA diet also significantly increased CRP levels by 21, 19 and 21%, respectively, compared with equivalent calories from MUFA, carbohydrate or a 1:1 combination (4%E each) of TFA + stearic acid, and tended to raise CRP levels (+11–12%) compared with equivalent calories from stearic acid or shorter-chain (12:0–16:0) SFA, although these latter differences were not statistically significant. Overall, these trials demonstrate that TFA are proinflammatory compared with *cis* unsaturated fats or carbohydrate; comparisons with SFA require further study.

Systemic inflammation: observational evidence. In a cross-sectional study among 823 generally healthy women, greater TFA consumption was associated with significantly higher levels of soluble TNF-α receptors (circulating biomarkers of TNF-α system activity) after adjustment for other risk factors that might influence inflammation, including age, smoking, physical activity, medication use, alcohol consumption and other dietary habits (Mozaffarian *et al*., 2004a). Adjustment for blood lipid concentrations only partly attenuated (~25%) these associations, suggesting that they were not predominantly mediated by the effects of TFA on lipids. In

these healthy women, TFA consumption was not associated with levels of IL-6 or CRP overall; however, a significant interaction was seen with BMI in that TFA intake was associated with higher IL-6 and CRP levels in women with higher BMI (Mozaffarian *et al*., 2004a). In a cross-sectional study of 730 mostly overweight women (mean BMI = 26.3 kg/m^2), greater TFA consumption was associated with higher levels of soluble TNF-α receptors and also IL-6 and CRP (Lopez-Garcia *et al*., 2005). Compared to individuals in the lowest quintile of TFA consumption (0.9% E), those in the highest quintile (2.1%E) had 73% higher CRP levels (absolute difference = 0.8 mg/l). Together, these two studies suggest that TFA consumption increases TNF-α activity and, among individuals with greater adiposity, IL-6 and CRP. Given the strong link between systemic inflammation, particularly as reflected by IL-6 and CRP levels, and insulin resistance, the latter findings provide some support to the possibility suggested by controlled trials that adverse effects of TFA intake on insulin sensitivity may be greater in individuals more predisposed to insulin resistance. This further suggests that controlled trials testing the effects of TFA consumption on inflammation or insulin resistance in young or lean subjects may underestimate the effects, and that future trials should target individuals with visceral adiposity or other more direct measures of reduced insulin sensitivity. Strong positive relationships between erythrocyte TFA levels (a biomarker of dietary intake) and systemic inflammatory markers were also seen among individuals with established heart disease, including substantially higher levels of IL-6, TNF-α, soluble TNF receptors and monocyte chemoattractant protein (Mozaffarian *et al*., 2004b). Thus, both observational studies and controlled trials indicate that TFA consumption is proinflammatory.

Endothelial function: experimental evidence. TFA induce apoptosis in human endothelial cells *in vitro* (Zapolska-Downar *et al*., 2005). In generally healthy men, five weeks of TFA consumption (8%E) increased levels of E-selectin, a circulating biomarker of endothelial dysfunction, by 10, 14, 6 and 6% compared with equivalent calories from carbohydrate, MUFA, stearic acid or shorter-chain (12:0–16:0) SFA, respectively (Baer *et al*., 2004). Consumption of a single meal enriched with TFA or SFA showed similar acute adverse effects on postprandial flow-mediated vasodilatation of the brachial artery, a functional measure of endothelial health (de Roos *et al*., 2002). A longer duration (4 weeks) of TFA intake (9.2%E) significantly lowered HDL-C (21% reduction) and impaired brachial artery flow-mediated vasodilatation (29% reduction) compared with equivalent calories from SFA (de Roos *et al*., 2001). Thus, these trials indicate that TFA impair endothelial function, even when TFA are compared to SFA.

Endothelial function: observational evidence. In a cross-sectional study among women, greater TFA consumption was associated with higher levels of several circulating markers of endothelial dysfunction, including soluble intercellular adhesion molecule-1, soluble vascular cell adhesion molecule-1 and E-selectin (Lopez-Garcia *et al*., 2005). After adjustment for age, the levels of intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin were 10, 10 and 20% higher, respectively, across extreme quintiles of TFA intake. Further adjustment for other risk factors, including BMI, physical activity, smoking, alcohol intake, hormone replacement therapy and intakes of total MUFA, PUFA and SFA intake, had little effect on these results. These results, including the magnitudes of the observed differences in E-selectin levels, are consistent with the experimental evidence that TFA consumption adversely affects endothelial function.

Adiposity and weight gain: experimental evidence. The long-term effects of TFA consumption on adiposity and weight gain have not been evaluated in controlled trials in humans. Because of the ethical limitations of assessing long-term effects of TFA consumption in human trials, controlled trials in nonhuman primates may be the best alternative to assess the effects of TFA on weight gain in a clinical trial. As described previously, a randomized controlled trial fed green monkeys diets containing either TFA or MUFA (8%E) for 6 years (Kavanagh *et al*., 2007); body weight was monitored every 8 weeks or less and calories were adjusted to meet maintenance energy requirements without weight gain. Nevertheless, primates on the TFA diet gained 7.2% of their body weight during the trial, compared with 1.8% weight gain on the MUFA diet. Body fat distribution, assessed by computed tomography scan, demonstrated that the differential weight gain was due to increased visceral adiposity, reflected by a significantly higher ratio of intra-abdominal (visceral) to subcutaneous fat on the TFA diet.

Adiposity and weight gain: observational evidence. In a cohort of more than 16 000 men who provided two measurements of abdominal circumference over 9 years, each 2%E increase in TFA consumption (versus equivalent calories from PUFA) was associated with a 2.7 cm increase in abdominal circumference ($P < 0.001$) after adjustment for measurement error and other risk factors (Koh-Banerjee *et al*., 2003). In a second study among more than 41 000 women who provided two measurements of weight over 8 years, increases in TFA consumption were robustly associated with increases in body weight in both cross-sectional and longitudinal analyses, after adjustment for other risk factors (Field *et al*., 2007). In both of these studies, changes in consumption of other fats, including total fat, SFA, MUFA and PUFA, were much less strongly associated with adiposity/weight gain, consistent with prior findings that neither total dietary fat nor most fat subtypes are major determinants of body fat or weight gain (Willett and Leibel, 2002). Thus, findings in a long-term controlled trial in nonhuman primates and two long-term prospective observational studies suggest that TFA consumption promotes weight gain, particularly

accumulation of abdominal fat. Additional studies are needed to confirm these findings, although the concordance of these results, as well as their consistency with studies indicating adverse effects of TFA intake on insulin resistance and systemic inflammation, is notable.

Hemostatic and blood pressure: experimental evidence. Randomized trials have not documented consistent evidence of an effect of TFA on markers of hemostatic function. Almendingen *et al*. (1996) found an unfavorable antifibrinolytic effect of partially hydrogenated soybean oil compared with partially hydrogenated fish oil, and butter increased fibrinogen levels compared with partially hydrogenated fish oil. In contrast, Mutanen and Aro (1997) compared TFA with stearic acid and found similar effects on markers of coagulation and fibrinolysis, and Louheranta *et al*. (1999) reported similar effects on coagulation factors of TFA versus MUFA. Armstrong *et al*. (2000) found no effects on platelet aggregation or blood coagulation by *trans*-isomers of α-linolenic acid. No effect on hemostatic risk markers by high vaccenic acid milk fat was observed by Tholstrup *et al*. (2006). The effects of TFA on blood pressure have been measured in few studies. In these studies in healthy normotensive subjects, no significant effects of TFA were found, compared with SFA, MUFA or PUFA (EFSA, 2004).

Hemostatic and blood pressure: observational evidence. The independent effect, if any, of TFA consumption on coagulation factors or blood pressure has not been evaluated in observational studies.

Effects of TFA on clinical outcomes

Experimental evidence. The effects of TFA consumption on CHD events or other disease outcomes have not been evaluated in randomized controlled trials in humans. Such trials are unlikely to be performed, given the considerations of cost, practicality, compliance and, perhaps most importantly, the ethical limitations of randomizing individuals to an intervention with strong evidence for significant harm. Thus, much like evidence for other harmful exposure such as smoking, lead paint, mercury or arsenic, the evidence for effects of TFA consumption on clinical outcomes in humans is derived from observational studies.

Observational evidence. Observational evidence for the effects of TFA on disease outcomes can be derived from descriptive (ecologic) studies of disease rates across populations and over time, retrospective case–control studies, and prospective cohort studies. Both dietary estimates of TFA intake and biomarkers of TFA intake have been used to assess relationships with disease risk. Each of these approaches has advantages and disadvantages. Ecologic studies allow evaluation of broad geographic and temporal differences in dietary habits and disease risk but have limited ability to adjust for potential confounding from other factors. Case–control and prospective cohort studies allow individual-level multivariable adjustment for other risk factors and lifestyle characteristics that can substantially reduce the magnitude of confounding, although some level of residual confounding by unmeasured factors may remain. Retrospective case–control studies often exclude fatal events and may be prone to recall and selection bias, although the use of objective biomarkers to assess TFA consumption (for example adipose tissue concentrations) can reduce recall bias. Prospective cohort studies minimize bias due to case selection, recall or control selection, but typically utilize dietary estimates of TFA consumption that may attenuate findings due to errors in the dietary estimation and changes in dietary intake over time. Given these differing strengths and weaknesses, evidence for the causality and magnitude of an exposure–disease relationship can be considered strongest when each type of study demonstrates consistent results.

Descriptive studies. In the first half of the 20th century, various forms of partially hydrogenated vegetable oils replaced a substantial proportion of the lard and butter used in homes and commercial applications in many industrialized nations. By the early 1990s, TFA consumption was approximately 2.6%E in the US (about 7.4% of all dietary fat) (Allison *et al*., 1999). Similar intakes during this time were reported in the UK, Sweden and Germany (Akesson *et al*., 1981).While this increase in TFA consumption correlated with increased rates of MI (Neubauer *et al*., 2006), the many other changes in diet and lifestyle that occurred during this period preclude conclusions from these time-trends regarding the specific effects of TFA.

In 1958 and 1964, several potential risk factors for CHD were measured by Ancel Keys and co-workers in the Seven Countries Study among 12 763 middle-aged men in 16 cohorts in seven countries. In 1987 and 1988, equivalent food composites representing the average food intake of each cohort at baseline were collected locally and analyzed in a central laboratory (Kromhout *et al*., 1995). Deaths attributed to CHD were documented during 25 years of follow-up, and correlation coefficients were calculated between the estimated intake of 18:1 TFA and the cohort mortality rates. Positive correlations were observed between 25-year CHD death rates and average intake of the four major SFA, lauric, myristic, palmitic and stearic acid ($r>0.8$, $P<0.001$); 18:1 TFA ($r=0.78$, $P<0.001$) and dietary cholesterol ($r=0.55$, $P<0.05$). This ecological study provided support for a relation between TFA intake and CHD, but the ability to draw strong conclusions was limited by the measurement of only 18:1 TFA (which represented both industrial elaidic acid and the Δ10-isomer, and ruminant vaccenic acid) and by difficulty in such ecologic analyses to control for confounding variables.

Retrospective case–control studies. Retrospective case–control studies of dietary and lifestyle factors can be limited by potential biases due to the survival of the cases (often only

nonfatal cases are included), selection of control subjects, self-selection by participation and differential recall of previous diet and other lifestyle habits. The latter limitation can be addressed by the use of biomarkers of TFA intake, such as adipose tissue concentrations that reflect long-term diet and are unlikely to be affected by a recent cardiac event or recent changes in diet. One retrospective case–control study has evaluated the estimated dietary intake of TFA and CHD risk, five have evaluated adipose measures of TFA and CHD risk, and one has evaluated erythrocyte TFA levels and risk of sudden death.

UK Study of Fatal CHD. This retrospective study measured post-mortem adipose TFA levels in 136 persons who had died from CHD and 95 persons who had died from other causes (Thomas *et al*., 1983a, b). The cases had a higher concentration of total TFA and also *trans*-16:1 and *trans*-18:1 isomers. No association was seen with 20- and 22-carbon *trans*-isomers, which comprised approximately half of the total TFA isomers, reflecting substantial use of partially hydrogenated fish oils (as opposed to vegetable oils) in this population. Owing to the small numbers of cases, the use of autopsy controls (which were unlikely to represent the true control population from which the cases arose), and lack of adjustment for most other cardiac risk factors, only limited conclusions can be drawn from these data.

Boston Area Health study. This case–control study of nonfatal MI in six hospitals in the Boston area included 239 cases and 282 population controls (Ascherio *et al*., 1994). TFA consumption was estimated using a validated food frequency questionnaire. After adjustment for age, sex and total energy intake, the intake of TFA was directly related to the risk of MI (extreme quintile relative risk (RR) = 2.44; 95% confidence interval (CI) = 1.42–4.19; P for trend <0.001), and this relationship remained significant after adjustment for other established coronary risk factors, multivitamin use and other dietary factors.

UK Study of Sudden Cardiac Death. Adipose TFA levels, including levels of *trans*-18:1 and *trans*-18:2 (but not *trans*-16:1), were measured in 66 cases of sudden cardiac death and 286 healthy controls (Roberts *et al*., 1995). In multivariable analyses, neither *trans*-18:1 nor *trans*-18:2 isomers were significantly associated with risk of sudden death. Comparing the highest versus lowest quintiles, the RR for *trans*-18:1 was 0.59 (95% CI = 0.19–1.83; P for trend = 0.67) and that for *trans*-18:2 was 0.99 (95% CI = 0.35–2.84; P for trend = 0.94). The small size of this study, reflected in the wide CI, limits strong conclusions.

The EURAMIC Study. In this international multicenter study in eight European countries and Israel, *trans*-18:1 $n-9$ levels (but not other TFA isomers or total TFA) were measured in adipose tissue samples obtained from 671 men with acute nonfatal MI in 1991–1992 and 717 men without a history of MI (controls) (Aro *et al*., 1995). In overall analyses, adipose *trans*-18:1 levels were not significantly associated with risk of MI: the multivariable-adjusted odds ratio for the highest versus lowest quartile was 0.97 (95% CI = 0.56–1.67). However, the investigators found major differences between countries in average levels of adipose *trans*-18:1. This was particularly evident in Spain in which mean TFA levels were extremely low in both cases and controls (consistent with the use of olive oil rather than partially hydrogenated oils as a primary source of dietary fat), so that nearly all the Spanish subjects (cases and controls) had values in the lowest quartile of the northern European distribution. When the investigators excluded the Spanish centers from the analysis, the multivariable-adjusted odds ratio for the highest versus lowest quartile of *trans*-18:1 was 1.44 (95% CI = 0.94–2.20). This trend was not statistically significant, but the magnitude of the higher risk was consistent with the findings of subsequent studies.

Costa Rican case–control study. In a population-based case–control study in Costa Rica (Baylin *et al*., 2003), 482 people with first nonfatal MI were matched with the same number of control participants. After adjustment for established risk factors and other confounders, the RRs across increasing quintiles of total TFA in adipose tissue were 1.00, 1.34, 2.05, 2.22 and 2.94 (P for trend <0.01). Although in most populations, *trans*-18:1 isomers comprise the great majority of total TFA consumed, in this study nearly half of the total TFA were *trans*-18:2 isomers due to the high content of *trans*-18:2 in the partially hydrogenated soybean oils consumed in Costa Rica. Higher CHD risk was mainly attributable to differences in *trans*-18:2 levels, which were abundant in the partially hydrogenated soybean oil, margarines and baked products consumed by this population. Across increasing quintiles of *trans*-18:2, the RRs were 1.00, 0.96, 2.09, 3.51 and 5.05 (P for trend <0.001). Adipose tissue *trans*-16:1 were also associated with MI, with RRs across increasing quintiles of 1.00, 1.57, 1.39, 1.34 and 2.58 (P for trend <0.05) adjusted for other risk factors, including saturated fat intake. *Trans*-18:1 isomers were not significantly associated with risk (multivariate OR across extreme quintiles = 0.75, 95% CI = 0.34–1.65). Because 18:2 isomers can come from multiple sources, the association with these isomers and CHD risk does not clearly distinguish between industrial versus ruminant TFA intake, but it is consistent with an overall harmful effect of these specific 18:2 isomers.

In follow-up measurements conducted after TFA consumption in Costa Rica were greatly reduced, adipose TFA levels were lower, and no association was seen with risk of MI (Colon-Ramos *et al*., 2006). The lack of association with TFA levels in this follow-up evaluation could be due to a substantial reduction of excess risk resulting from lower TFA intake, but could also partly result from misclassification because the recently reduced levels would no longer accurately reflect each person's habitual long-term (prior) TFA intake.

Australian case–control study. In this Australian case–control study of first nonfatal MI (Clifton *et al*., 2004), TFA were measured in adipose tissue among 209 cases and 179 controls between 1995 and 1997. During the study period, TFA were eliminated from margarines (the major TFA source in the food supply) in Australia and the adipose TFA levels dropped. Cases enrolled before mid-1996 had significantly higher levels of *trans*-18:1 $n-9$ (32% higher, $P<0.03$) and *trans*-18:1 $n-11$ (23% higher, $P<0.001$) compared with corresponding controls. As seen in the Costa Rican study, when TFA were eliminated from margarines after June 1996, there were no longer significant differences between cases and controls in any adipose tissue TFA measured. Similar to that study, the lack of association between TFA levels and MI risk following this relatively acute change in TFA in the food supply could be due to a substantial reduction of excess risk resulting from lower TFA intake or misclassification because the recently reduced levels would no longer accurately reflect each person's habitual long-term (prior) TFA consumption.

Cardiac Arrest Blood Study. In this population-based case–control study (Lemaitre *et al*., 2002), 179 cases of out-of-hospital cardiac arrest (sudden death) were matched to 285 community controls. TFA levels were measured in red blood cells collected at the time of arrest. In multivariable-adjusted analyses, total TFA levels were associated with higher risk of sudden death (RR = 1.47 for the interquintile range, 95% CI = 1.01–2.13). In multivariable analyses, the higher risk seen with TFA levels was entirely due to *trans*-18:2 isomers. After adjusting for other cardiac risk factors and levels of omega-3 fatty acids, the RR for the interquintile range was 1.19 (95% CI = 0.80–1.77) for *trans*-18:1 isomers and 2.66 (95% CI = 1.58–4.46) for *trans*-18:2 isomers. *Trans*-16:1 isomers were not associated with risk.

Prospective cohort studies. In prospective cohort studies, dietary intake is assessed before the diagnosis of cardiovascular disease; and both fatal and nonfatal disease events can be included. These strengths greatly reduce methodologic biases due to differential recall, selection, and survival. Although residual confounding by unmeasured factors cannot be totally excluded, multivariable adjustment can minimize confounding by other cardiovascular risk factors and lifestyle habits. Dietary consumption of TFA and other nutrients is typically estimated from questionnaires, introducing random error (misclassification) due to inaccuracies in the questionnaire as well as changes in consumption over time. Misclassification errors tend to diminish the magnitude of the associations, causing underestimation of the true effects. Misclassification may be minimized by the use of biomarkers of TFA intake, although inaccuracies due to changes in consumption over time would persist and continue to attenuate the magnitude of the associations to some degree.

The Nurses' Health Study. The relation between TFA intake and CHD has been evaluated several times during the follow-up of this cohort. In the first report, TFA intake was calculated from dietary questionnaires completed by 85 095 women without diagnosed CHD, stroke, diabetes or hypercholesterolemia in 1980. During 8 years of follow-up, 431 cases of new CHD (nonfatal MI or CHD death) were documented. In multivariable analysis, intake of TFA was directly related to risk of CHD (extreme quintile RR = 1.50, 95% CI = 1.12–2.00, P for trend = 0.001). The association was stronger for the 69 181 women whose margarine consumption over the previous 10 years had been stable (RR = 1.67, 95% CI = 1.05–2.66, P for trend = 0.002). Intakes of foods that were major sources of *trans*-isomers (margarine, cookies, cake and white bread) were each significantly associated with higher risks of CHD (Willett *et al*., 1993). After 14 years of follow-up, during which time TFA consumption was updated using serial dietary questionnaires and a total of 939 cases of CHD had been diagnosed, TFA consumption was again found to be associated with higher risk (Hu *et al*., 1997). The most recent analysis was performed after 20 years of follow-up, including 1766 incident cases of CHD; comparing the highest to lowest quintiles of TFA consumption (a difference of 1.5%E), the RR was 1.33 (95% CI = 1.07–1.66) and was particularly strong among younger women (Oh *et al*., 2005).

Also in the Nurses' Health Study, a nested case–control study was conducted using measurement of TFA levels in plasma and red blood cells (Sun *et al*., 2007). The blood samples were collected in 1989–1990 while the women were generally healthy. During 6 years of follow-up, 166 incident cases of CHD were ascertained and matched with 327 controls. In this study, estimated dietary TFA consumption was more strongly correlated with erythrocyte TFA levels than with plasma TFA levels. After multivariable adjustment, higher total erythrocyte TFA content was associated with higher CHD risk; compared to the lowest quartile, the multivariate RRs (95% CI) of CHD in the second to fourth quartiles of erythrocyte TFA levels were 1.6 (0.7–3.6), 1.6 (0.7–3.4) and 3.3 (1.5–7.2) (P for trend <0.01), respectively. The corresponding RRs were 1.1, 1.3 and 3.1 (P for trend <0.01) for *trans*-18:1 isomers and 1.5, 2.5 and 2.8 (P for trend <0.01) for *trans*-18:2 isomers. In contrast, the levels of *trans*-16:1 $n-7$ were not associated with higher CHD risk. The stronger results using measured TFA levels (Sun *et al*., 2007), compared with estimated TFA intake from dietary questionnaires (Willett *et al*., 1993; Hu *et al*., 1997; Oh *et al*., 2005) in the same cohort, suggest that inaccuracies (random error) in dietary questionnaire estimation may significantly attenuate the true association with CHD risk and/or that TFA in cell membranes may be a more proximate measure of their ultimate biologic effects than consumed TFA.

Health Professionals Follow-up Study. In the first report from this study (Ascherio *et al*., 1996), 734 acute MI or CHD deaths were documented during 6 years of follow-up among

43 757 health professionals aged 40–75 years and free of diagnosed cardiovascular disease or diabetes at baseline. Estimated TFA consumption was associated with a moderately higher risk of CHD, but this was not statistically significant in multivariable analysis. In updated analyses including 14 years of follow-up, 1702 acute MI or CHD deaths were documented among 38 461 individuals; using cumulative updating of dietary habits by means of serial questionnaires and adjusting for age, time period of follow-up, smoking, physical activity, BMI, aspirin use, history of hypertension or hypercholesterolemia, alcohol use, and consumption of dietary SFA, *n*−3 PUFA, *n*−6 PUFA, MUFA, protein, fiber and total energy, the multivariable RR associated with a 2%E increment in TFA intake was 1.26 (95% CI = 0.98–1.60) (Mozaffarian *et al*., 2006).

ATBC Study. The relation of intakes of specific dietary fats and risk of CHD was examined in a cohort of 21 930 Finnish men who were initially free of diagnosed cardiovascular disease and participating in a trial of beta-carotene and vitamin E (Pietinen *et al*., 1997). The participants completed a validated dietary questionnaire at baseline, and during 6.1 years of follow-up 1399 CHD events (nonfatal MI or CHD death) were documented. For men in the top quintile of TFA intake (median 6.2 g/day), the multivariate RR of total CHD events was 1.14 (95% CI = 0.96–1.35; *P* for trend = 0.16) and that of CHD death was 1.39 (95% CI = 1.09–1.78; *P* for trend = 0.004), compared with men in the lowest quintile of intake (median 1.3 g/day).

Zutphen Heart Study. In this Dutch population (Oomen *et al*., 2001), 667 men aged 64–84 years and free of CHD at baseline were followed for up to 10 years, during which time 98 cases of fatal and nonfatal CHD were documented. After adjustment for potential confounders, TFA intake at baseline was positively associated with the 10-year risk of CHD. The multivariate RR for a difference of 2%E in TFA consumption was 1.28 (95% CI = 1.01–1.61).

Cardiovascular Health Study. This prospective nested case–control study (Lemaitre *et al*., 2006) was performed in a population-based study of older US adults. A total of 214 cases of fatal CHD were identified between 1992 and 1998 and matched to controls, and TFA exposure was assessed using plasma phospholipid levels, a biomarker of TFA consumption, in stored samples at baseline. After adjustment for other risk factors, total TFA levels were not significantly associated with risk (RR for interquintile range = 0.94, 95% CI = 0.65–1.34). However, *trans*-18:2 levels were positively associated with CHD risk (RR for interquintile range = 1.31, 95% CI = 0.99–1.72), while *trans*-18:1 levels were inversely associated with CHD risk (RR for interquintile range = 0.53, 95% CI = 0.31–0.90). Simultaneous adjustment for both *trans*-18:1 and *trans*-18:2 levels further strengthened these associations. *Trans*-16:1 levels were not associated with CHD risk (RR for interquintile range = 0.95, 95% CI = 0.64–1.42).

Meta-analysis of prospective studies of TFA and CHD. On the basis of a meta-analysis of prospective cohort studies evaluating the habitual dietary consumption of TFA and incidence of CHD (Mozaffarian *et al*., 2006), the pooled multivariable-adjusted RR for 2%E of TFA, as an isocaloric replacement for carbohydrate, was 1.23 (95% CI = 1.11–1.37) (Figure 2). The inclusion of results for several retrospective studies that utilized biomarkers of TFA consumption strengthened this pooled estimate: RR = 1.29, 95% CI = 1.11–1.49, $P < 0.001$ (Mozaffarian *et al*., 2006). In practice, TFA would be interchanged with other dietary fats, not carbohydrate. Based on meta-analyses of prospective cohort studies (Mozaffarian and Clarke, 2009), the pooled multivariable-adjusted RR of CHD for 2%E of TFA replacing SFA was 1.20 (95% CI = 1.07–1.34); of TFA replacing MUFA was 1.27 (95% CI = 1.14–1.42), and of TFA replacing PUFA was 1.32 (95% CI = 1.17–1.49) (Mozaffarian and Clarke, 2009).

TFA consumption and other clinical outcomes. The relation of TFA intake to other disease outcomes has been examined less extensively than for CHD. As described above, the Iowa Women's Health Study (Meyer *et al*., 2001) and Health Professionals Follow-up Study (van Dam *et al*., 2002) detected no significant associations of TFA consumption with incidence of type II diabetes. However, in the Nurses' Health Study, which included a longer follow-up, repeated measures of intake, and a larger number of validated cases of diabetes, a significant positive intake of TFA with incidence of diabetes was seen (Salmeron *et al*., 2001; Hu *et al*., 2001a). Few studies have evaluated relationships between TFA consumption and cancer incidence, and significant associations have generally not been observed (World Cancer Research Fund, 2007), although in the 20-year follow-up of the Nurses' Health Study, TFA intake during the premenopausal years was associated with incidence of postmenopausal breast cancer (Kim *et al*., 2006). TFA was associated with higher risk of dementia in one prospective study (Doney *et al*., 2004), suggestive of a potentially adverse effect on cerebrovascular disease. However, in a prospective study of male health professionals (He *et al*., 2003), TFA intake was not significantly associated with incidence of clinical stroke. Higher TFA intake has been associated with incidence of cholelithiasis (Tsai *et al*., 2005) and anovulatory infertility (Chavarro *et al*., 2007), both conditions associated with insulin resistance. Additional investigation is required to elucidate further the potential effects of TFA consumption on each of these disease outcomes.

Specific TFA isomers

Experimental evidence. The specific *trans*-isomers present in foods may vary considerably depending on the types of

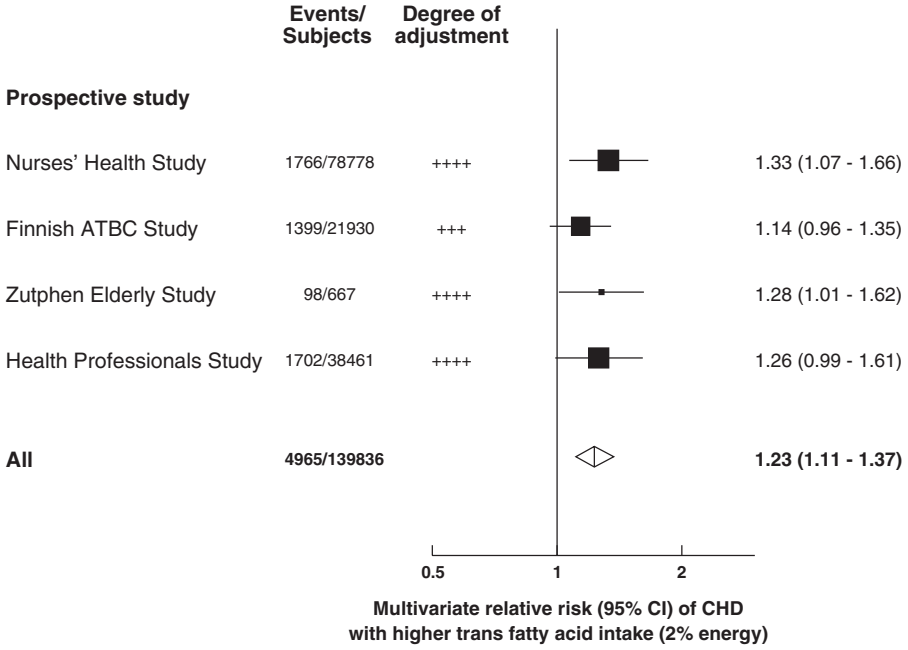

Figure 2 Meta-analysis of prospective cohort studies of habitual TFA consumption and CHD risk, including 5215 incident CHD events among 140 542 participants. The black squares and horizontal lines indicate the RR and 95% CI in each study; the size of the black squares is proportional to the inverse-variance 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, BMI, blood pressure, physical activity, alcohol intake and consumption of fiber and total energy) and + + + + (further adjusted for consumption of other dietary fats and protein). Reproduced with permission from (Mozaffarian and Clarke, 2009). BMI, body mass index; CI, confidence interval; CHD, coronary heart disease; RR, relative risk; TFA, *trans*-fatty acid. Adapted from Mozaffarian *et al*. (2006).

oils/fats used and the methods of processing, such as partial hydrogenation, deodorization, and so on. In one meta-analysis of controlled trials (Mensink *et al*., 2003), the estimates of TFA effects on serum lipoproteins were based on calculations of changes in 18:1 TFA intake, the predominant isomer in partially hydrogenated vegetable oils. It is less well established how other TFA isomers such as 16:1 or 18:2 TFA may affect serum lipoproteins, other risk factors or clinical outcomes. In one trial, TFA trienes (*trans*-18:3; 1.4 g/day) increased the LDL:HDL-C ratio (Vermunt *et al*., 2001), suggesting similar effects as *trans*-18:1 on serum lipoproteins. As reviewed, one trial indicates that partially hydrogenated fish oil containing C20-22 *trans*-isomers (but also other TFA isomers) may increase the LDL:HDL-C ratio compared with partially hydrogenated soybean oil (Almendingen *et al*., 1995). Controlled feeding trials have not included information on 16:1 or 18:2 TFA isomers, precluding separate analysis of their specific effects. Performing experimental studies on specific effects of 18:2 or 18:3 TFA isomers is difficult because these isomers are usually present in relatively small amounts (<5% of fatty acids) and, even within each isomer class, several different positional isomers are found.

Observational evidence. Insights on potentially different effects of different TFA isomers on cardiovascular risk can be gained from studies that have estimated dietary consumption of different TFA isomers or, even better, measured specific TFA isomers in blood or adipose tissue. Several different TFA isomers can be measured as biomarkers of consumption. *Trans*-18:1 $n-7/\Delta 11$ (vaccenic acid) is the primary *trans*-isomer in ruminant fat, and *trans*-18:1 $n-9/\Delta 9$ (elaidic acid) is the primary *trans*-isomer in partially hydrogenated vegetable oils. However, both of these isomers are present in both sources, so their levels cannot unequivocably distinguish between ruminant versus industrial sources. The levels of *trans*-18:2 isomers tend to be higher in partially hydrogenated oils than ruminant fats, but small amounts are also present in ruminant TFA and in oils that have undergone deodorization. The 16-carbon *trans*-isomers (primarily *trans*-16:1 $n-7$ [$\Delta 9$]) represent ruminant sources of TFA and are not found in partially hydrogenated vegetable oils. The levels of *trans*-16:1 isomers will typically be correlated with the intake of saturated fat from these same foods, and thus saturated fat intake should be adjusted for when evaluating associations of *trans*-16:1 levels with CHD risk. *Trans*-isomers of 20- and 22-carbon fatty acids can be used as a specific biomarker of partially hydrogenated fish oils, but few studies have been conducted where the intake of *trans*-isomers from this source is common.

Observational studies have generally not evaluated the effects of specific *trans*-isomers on serum lipoproteins.

However, the data from three cohort studies suggest that 18:1 and 18:2 TFA have stronger effects on both systemic inflammation and endothelial dysfunction than 16:1 TFA. In two studies among generally healthy women (Mozaffarian *et al*., 2004a; Lopez-Garcia *et al*., 2005), the estimated dietary consumption of both 18:1 and 18:2 TFA, but not 16:1 TFA, was positively associated with inflammatory markers, including soluble TNF receptors, IL-6 and CRP, and circulating markers of endothelial dysfunction, including endothelin-1, intercellular adhesion molecule-1 and vascular cell adhesion molecule-1; in fact, the estimated dietary intake of *trans*-16:1 was associated with lower inflammatory and endothelial dysfunction markers in one study (Lopez-Garcia *et al*., 2005). The relationship between specific *trans*-isomers and inflammation was evaluated in 86 patients with established heart disease using erythrocyte TFA levels, an objective biomarker of dietary consumption (Mozaffarian *et al*., 2004b). After adjustment for other risk factors, both 18:1 and 18:2 TFA, but not 16:1 TFA, were strongly positively associated with markers of inflammation, including IL-6 and TNF-α.

Epidemiologic studies also provide some distinction among isomers of TFA, directly measured in tissues, and risk of clinical outcomes (Table 1). The details of each of these individual studies were reviewed above (see section, Effects on clinical outcomes). Only one study evaluated the long-chain TFA isomers from partially hydrogenated fish oil (C20-22), detecting no signification association. Two studies found higher risk with 16:1 TFA, but three found no significant association. Four studies found higher risk with 18:1 TFA, but three found no significant association, and one found lower risk. Four studies found higher risk with 18:2 TFA (and in two studies the higher risk was notably greater than that of other TFA isomers), and only one found no significant association. These studies varied in design (retrospective versus prospective), clinical outcome (nonfatal MI versus CHD death versus SCD), statistical power (from 66 to 671 cases) and biomarker source for TFA measurement (adipose versus erythrocyte versus phospholipids). Nevertheless, overall, these findings indicate that 18:2 TFA, which are generally present in much smaller concentrations than 16:1 or 18:1 TFA isomers, are most consistently associated with higher cardiovascular risk. Findings for 18:1 TFA are more equivocal, possibly because both *trans*-18:1 $n-7$ ($\Delta 11$) (vaccenic acid, the primary *trans*-isomer in ruminant fat) and *trans*-18:1 $n-9$ (elaidic acid, the primary *trans*-isomer in many partially hydrogenated vegetable oils) are captured, and these positional isomers may have different effects on risk. Higher risk with 16:1 TFA would suggest a contribution of ruminant fat intake to risk of CHD, but three of five studies observed no significant association, including the two prospective studies that would be least limited by potential selection bias.

Experimental evidence. Vaccenic acid is the most common TFA isomer in ruminant fats, representing 30–50% of the *trans*-isomers. In humans, vaccenic acid can be metabolized to 9c, 11t conjugated linoleic acid (CLA), a *trans*-fat with two double bonds separated by only one single bond (that is conjugated). Tricon *et al*. (2006) evaluated, in 32 healthy men, the effects of 6 weeks of dairy products enriched with vaccenic acid and CLA (produced by feeding cows a mixture of fish oil and sunflower oil), supplying 2.1%E *trans*-18:1, mostly (75%) in the form of vaccenic acid, and 0.5%E CLA. An increase in LDL:HDL-C ratio was found (0.11 increase versus 0.10 decrease on the control diet), as well as trends toward higher triglycerides ($P=0.09$) and increased LDL density ($P=0.07$); no significant effects were seen on susceptibility to oxidation of LDL. Tholstrup *et al*. (1998) compared control dairy fat to dairy fat enriched (by changes in cow feed) with oleic acid, stearic acid and TFA (4.3%E), mainly replacing C12-16 SFA, in 18 men in two 4-week crossover periods. Total cholesterol and VLDL-C were significantly higher on the TFA diet; the LDL:HDL ratio also tended to be higher ($+0.09$), although this difference was not statistically significant. In a second study (Tholstrup *et al*., 2006), 42 young healthy men were randomized to a 5-week diet with either control butter or butter higher in vaccenic acid (1.1%E) and oleic acid (14.9 versus 9.6%E). The intervention butter lowered total cholesterol by 6% and lowered HDL-C by 9%; the total/HDL-C ratio was not significantly affected.

Table 1 Relationships between specific TFA isomers and cardiovascular outcomes

Study	*Design*	*Outcome (no. of cases)*	*Measure of TFA*	*TFA isomer*			
				16:1	*18:1*	*18:2*	*C20-22*
UK Study of Fatal CHD	Retrospective case–control	CHD death ($n=136$)	Post-mortem adipose	↑Risk	↑Risk		↔
UK Study of SCD	Retrospective case–control	SCD ($n=66$)	Post-mortem adipose		↔	↔	
EURAMIC	Retrospective case–control	Nonfatal MI ($n=671$)	Adipose		↑Risk[a]		
Costa Rica Study	Retrospective case–control	Nonfatal MI ($n=482$)	Adipose	↑Risk	↔	↑↑Risk	
Australia Study	Retrospective case–control	Nonfatal MI ($n=209$)	Adipose		↑Risk		
Cardiac Arrest Blood Study	Retrospective case–control	SCD ($n=179$)	Erythrocytes	↔	↔	↑Risk	
Cardiovascular Health Study	Prospective cohort	CHD Death ($n=214$)	Phospholipids	↔	Risk	↑Risk	
Nurses Health Study	Prospective cohort	MI or CHD death ($n=166$)	Erythrocytes	↔	↑Risk	↑↑Risk	

Abbreviations: CHD, coronary heart disease; MI, myocardial infarction; NA, not assessed; SCD, sudden cardiac death.
[a]After exclusion of Spanish centers in which TFA levels were extremely low.

Recently, TFA (5%E) from modified milk fat versus partially hydrogenated vegetable oil were compared in a crossover study of 46 healthy subjects (Chardigny *et al*., 2008). The diets contained similar total SFA; the milk fat contained more short-chain SFA, including lauric (12:0) and myristic (14:0) acids, and the industrial fat more palmitic (16:0) and stearic (18:0) acids. Compared with the partially hydrogenated oil diet, the milk fat diet raised both HDL-C and LDL-C levels in women but not in men. No difference was found between the diets in the total/HDL-C ratio. Moloney *et al*. (2004) found that a chemically produced blend of CLA isomers increased serum HDL-C and reduced the LDL:HDL-C ratio in 32 patients with type II diabetes, but it also impaired blood glucose control and reduced insulin sensitivity in accordance with other studies (Riserus *et al*., 2002, 2004).

Each of these studies reflects the difficulty in separately evaluating the effects of TFA versus vaccenic acid versus other fats in ruminant products. Overall the findings do not present strong evidence for different effects of ruminant versus industrial TFA on serum lipoprotein lipids or other risk factors. Performing studies on individual TFA isomers in humans is difficult due to the lack of sufficient availability of purified specific *trans*-isomers. In one study in hamsters (Meijer *et al*., 2001), the effects of elaidic acid (33%E), the main 18:1 *trans*-isomer in many partially hydrogenated vegetable oils versus vaccenic acid (33%E), the main TFA isomer in ruminant fat, were compared over 4 weeks. Compared with elaidic acid, vaccenic acid increased the serum LDL:HDL-C ratio. Thus, overall, these few small studies provide insufficient evidence to conclude that the experimental effects of ruminant TFA or CLA are either similar or different from industrial TFA.

Observational evidence. The effects of estimated dietary consumption of industrial versus ruminant TFA on CHD outcomes has been examined in several observational studies.

Boston Area Health Study. In this retrospective case–control study of nonfatal MI (239 cases, 282 population controls) conducted in the Boston area (Ascherio *et al*., 1994), the estimated intake of TFA was partitioned into industrial and ruminant sources. After adjustment for other risk factors, the overall positive association for total TFA intake was entirely accounted for by industrial TFA (RR for top versus bottom quintile = 1.94, P for trend = 0.001), rather than ruminant TFA (RR for top versus bottom quintile = 1.02, P for trend = 0.57).

Nurses' Health Study. In the first report on TFA from the Nurses' Health Study (Willett *et al*., 1993), when total intake of TFA was partitioned into industrial and ruminant sources, the positive association with CHD risk was entirely explained by industrial TFA. For industrial TFA, the multivariable-adjusted RRs for increasing quintiles were 1.0, 1.43, 1.11, 1.39 and 1.79 (P for trend = 0.009), whereas for ruminant fat the corresponding multivariable-adjusted RRs were 1.0, 0.76, 0.69, 0.55 and 0.59 (P for trend = 0.23).

ATBC Study. In this prospective cohort study of 21 930 Finnish men (Pietinen *et al*., 1997), total TFA consumption was strongly correlated with intake of margarine but not with intake of butter. Estimated consumption of industrial TFA was positively associated with risk of CHD (multivariate RR for highest versus lowest quintile = 1.23, 95% CI = 0.97–1.78; P for trend = 0.004). In contrast, estimated intake of TFA from ruminant sources was inversely related to risk (RR for highest versus lowest quintile = 0.83, 95% CI = 0.62–1.11, P for trend = 0.04).

Zutphen Heart Study. In this prospective cohort study among 667 Dutch men (Oomen *et al*., 2001), total TFA intake was associated with higher CHD risk. The small size and few numbers of cases in this population did not allow reliable distinction of effects of different sources of TFA. For an increment of 0.5% energy of TFA, the RR was 1.17 (95% CI = 0.69–1.98) for ruminant TFA, 1.05 (0.94–1.17) for *trans*-18:1 isomers and 1.07 (95% CI = 0.99–1.15) for all other TFA.

Danish Cohort Studies. In four prospective cohort studies in Denmark (Jakobsen *et al*., 2007), dietary habits were assessed in 3686 adults enrolled between 1974 and 1993 and followed for a median of 18 years. After adjustment for other risk factors, no significant associations were found between ruminant TFA consumption and risk of CHD. These negative findings are noteworthy given the relatively high consumption of ruminant TFA from dairy products in Denmark, the intake of ruminant TFA was relatively high (median 1.1%E in the highest quintile of intake).

Overall, the evidence from observational studies suggests that higher CHD risk is related to consumption of industrially produced TFA rather than ruminant TFA. Because ruminant fat contains low levels of TFA (usually $<6\%$ of fatty acids), the quantities of ruminant TFA consumed were low in most of the populations studied (generally $<1.0\%$E). Thus, even when total ruminant fat intake is relatively high, the potential amount of TFA from this source is still quite modest. These data do not discount the possibility that much higher amounts of ruminant fat could have adverse effects, but in the amounts consumed in actual diets ruminant TFA do not appear to be major contributors to CHD risk. Conversely, people consume a mix of TFA, and moderate intake of some *trans*-isomers in ruminant fat (for example *trans*-18:2) may be of importance when consumed on top of amounts derived from industrial sources. This issue has implications for policy, for example whether TFA labeling and regulation should include only industrial TFA

or total TFA. At amounts currently consumed, ruminant TFA do not have detectable adverse relationships with disease risk, but further investigation is warranted. At the present time, both sources of TFA, and especially specific TFA isomers, should be considered when assessing effects on disease risk.

Conclusions

TFA consumption induces pleiotropic effects. For cardiovascular risk factors, the effects of TFA consumption that are most consistently seen in both controlled trials and observational studies include adverse lipid effects, including increased LDL-C, reduced HDL-C and an increased total/HDL-C ratio; proinflammatory effects, including higher TNF-α system activity, IL-6 levels and CRP levels; and endothelial dysfunction, as assessed by both circulating markers and functional measures. Each of these effects are most prominent in comparison with *cis* unsaturated fats (MUFA or PUFA); the adverse effects on the total/HDL-C ratio and endothelial function have also been documented in comparison with SFA. Controlled studies and observational studies also suggest that TFA worsen insulin resistance, particularly among predisposed individuals, such as those with preexisting insulin resistance, visceral adiposity or lower physical activity; further study is needed to confirm apparent effects on weight gain and diabetes incidence in humans. Together, these findings suggest that among dietary fats, TFA consumption produces a unique cardiometabolic imprint (Mozaffarian and Willett, 2007), aggravating multiple related pathways linked to the insulin resistance/metabolic syndrome. Additional effects of TFA consumption to increase triglycerides and possibly the prevalence of smaller, denser LDL particles are also consistent with this imprint.

The long-term effects of habitual TFA consumption on clinical outcomes have not been assessed in controlled trials in humans; ethical limitations make it unlikely that such trials could ever be performed. Among observational studies, one small postmortem study and a European retrospective case–control study did not observe significant associations between TFA consumption and CHD risk (although in the latter study consistent trends toward higher risk were evident after excluding two centers with extremely low TFA intake). Conversely, five other retrospective case–control studies and four prospective cohort studies have demonstrated significant positive associations between TFA consumption and CHD events. A pooled meta-analysis of the prospective studies indicates 24, 20, 27 and 32% higher risk of MI or CHD death for every 2%E higher TFA consumption as an isocaloric replacement for carbohydrate, SFA, *cis* MUFA and *cis* PUFA, respectively.

The differential effects of specific TFA isomers are less well established. Too few studies have evaluated partially hydrogenated fish oils to draw strong conclusions about the relation of 20- and 22-carbon TFA isomers with the risk of CHD, although effects on blood lipids would suggest significant harm. Performing controlled trials on 18:2 or 18:3 TFA isomers is limited by the relatively lower concentrations of these isomers in partially hydrogenated oils. In observational studies using biomarkers of TFA consumption, both 18:1 and 18:2 isomers appear to contribute to the risk of CHD; most studies did not detect an effect of 16:1 TFA. The available data also suggest that *trans*-18:2 isomers may be more strongly associated with CHD risk than *trans*-18:1 isomers, but the current evidence is relatively limited and precludes definitive conclusions that only one of these is responsible for the association of partially hydrogenated oils with risk. This distinction has potential implications because some processes, such as light hydrogenation or deodorization, may create proportionally more 18:2 *trans*-isomers and thus could have greater effects than would be expected on the basis of content of total TFA alone.

Experimental studies of ruminant fats are limited by the difficulty in distinguishing the effects of changes in TFA from changes in other fats in ruminant products; the few small trials reported to date provide inconclusive evidence that experimental effects of ruminant TFA or CLA are different from industrial TFA at similar doses. Evidence from observational studies, in which estimated TFA consumption from industrial and ruminant sources of TFA have been distinguished, and from studies in which specific TFA have been measured using biomarkers generally do not support an adverse effect of ruminant TFA (in contrast to industrial TFA) on risk of CHD. Whether or not very high intakes of ruminant TFA could affect CHD risk is unresolved, but very few persons habitually consume such high levels. The potential effects of specific TFA isomers in ruminant fat (many of which are shared with partially hydrogenated oils) require further investigation.

In summary, controlled trials and observational studies provide concordant evidence that the consumption of TFA from partially hydrogenated oils adversely affects several cardiovascular risk factors and contributes significantly to increased risk of CHD event. Ruminant TFA cannot be removed entirely from the diet, but their intake is already low in most populations and not significantly associated with CHD risk in several studies. Because TFA produced by partial hydrogenation are industrial additives to food with no health benefits, their use should be avoided by restaurants and food manufacturers and their consumption avoided by consumers. Indeed, as reviewed elsewhere in this Supplement (L'Abbé *et al.*, 2009), experiences in Denmark, the Netherlands and New York City indicate that it is possible to largely eliminate industrial TFA in foods. Attention should also be given to possible health effects of TFA created during other industrial processes, such as deodorization and prolonged deep frying. On the basis of effects on cardiovascular risk factors and associations with disease outcomes, the removal of partially hydrogenated vegetable oils from foods would result in substantial health benefits, with greatest health benefits being obtained when

replacement oils are rich in MUFA and PUFA (Mozaffarian and Clarke, 2009).

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 Antti Aro: Consultancy with the Scientific Advisory Board, Valio Ltd (current).

Professor Walter Willet: None declared.

Peer-reviewers

Dr Robert Clarke: None declared.

Dr Richard Deckelbaum: None declared.

Dr Wolfgang Gelbman: None declared.

Dr Ghafoorunissa: Chairperson of the Edible Oils and Fats Subcommittee PFA, Central Committee of Foods Standards (Ministry of Health & FW, Government of India) (current); Member of the Nutrition Advisory Council for the Malaysian Palm Oil Promotion Council (2004–2005); Consultant in the area of "heart health" for Hindustan Unilever Limited (2006–2007); Life Member of the Nutrition Society of India (current).

Dr Leng Heng: None declared.

Dr Martijn B Katan: None declared.

Professor Jim Mann: None declared.

Dr Ulf Risérus: None declared.

Dr Pilar Rodríguez Iglesias: None declared.

Dr Thomas Sanders: Chair of Nutritional Advisory Board of Cadbury PLC (current); Consultant with Seven Seas Ltd (current).

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

Dr Silvia Valtueña Martinez: None declared.

Dr Bengt Vessby: Economic support (from Karlshamn AB/Unilever Best Foods/Karlshamn Mejeri/Nestle Sweden) for Adjunct Professorship at Uppsala University (ceased 2005); Research support (grant) from Swedish Dairies (ceased 2007).

Acknowledgements

We thank Dr Robert Clarke, Dr Richard Deckelbaum, Dr Ghafoorunissa, Dr Martijn B Katan, Professor Jim Mann, Dr Ulf Risérus, Dr Thomas Sanders, Professor Ricardo Uauy and Dr Bengt Vessby for their helpful comments during the preparation of this paper. We also acknowledge the helpful comments received from Panel on Dietetic Products, Nutrition and Allergies (NDA), European Food Safety Authority (EFSA), in particular, Dr Silvia Valtueña Martinez, Dr Pilar Rodríguez Iglesias, Dr Wolfgang Gelbman and Dr Leng Heng. 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

Abbey M, Noakes M, Belling GB, Nestel PJ (1994). Partial replacement of saturated fatty acids with almonds or walnuts lowers total plasma cholesterol and low-density-lipoprotein cholesterol. *Am J Clin Nutr* **59**, 995–999.

Akesson B, Johansson BM, Svensson M, Ockerman PA (1981). Content of *trans*-octadecenoic acid in vegetarian and normal diets in Sweden, analyzed by the duplicate portion technique. *Am J Clin Nutr* **34**, 2517–2520.

Allison DB, Egan SK, Barraj LM, Caughman C, Infante M, Heimbach JT (1999). Estimated intakes of *trans* fatty and other fatty acids in the US population. *J Am Diet Assoc* **99**, 166–174.

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.

Almendingen K, Seljeflot I, Sandstad B, Pedersen JI (1996). Effects of partially hydrogenated fish oil, partially hydrogenated soybean oil, and butter on hemostatic variables in men. *Arterioscler Thromb Vasc Biol* **16**, 375–380.

Armstrong RA, Chardigny JM, Beaufrere B, Bretillon L, Vermunt SH, Mensink RP *et al.* (2000). No effect of dietary *trans* isomers of alpha-linolenic acid on platelet aggregation and haemostatic factors in European healthy men. The TRANSLinE study. *Thromb Res* **100**, 133–141.

Aro A, Kardinaal AF, Salminen I, Kark JD, Riemersma RA, Delgado-Rodriguez M *et al.* (1995). Adipose tissue isomeric *trans* fatty acids and risk of myocardial infarction in nine countries: the EURAMIC study. *Lancet* **345**, 273–278.

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.

Ascherio A, Hennekens CH, Buring JE, Master C, Stampfer MJ, Willett WC (1994). *Trans* fatty acids intake and risk of myocardial infarction. *Circulation* **89**, 94–101.

Ascherio A, Rimm EB, Giovannucci EL, Spiegelman D, Stampfer MJ, Willett WC (1996). Dietary fat and risk of coronary heart disease in men: cohort follow-up study in the United States. *BMJ* **313**, 84–90.

Axen KV, Dikeakos A, Sclafani A (2003). High dietary fat promotes syndrome X in nonobese rats. *J Nutr* **133**, 2244–2249.

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.

Baylin A, Kabagambe EK, Ascherio A, Spiegelman D, Campos H (2003). Adipose tissue alpha-linolenic acid and nonfatal acute myocardial infarction in Costa Rica. *Circulation* **107**, 1586–1591.

Chardigny JM, Destaillats F, Malpuech-Brugère C, Moulin J, Bauman DE, Lock AL *et al.* (2008). Do industrially-produced and natural *trans* fatty acid sources have the same impact on cardiovascular diseases risk factors in healthy subjects? Results of the TRANSFACT Study. *Am J Clin Nutr* **87**, 558–566.

Chavarro JE, Rich-Edwards JW, Rosner BA, Willett WC (2007). Dietary fatty acid intakes and the risk of ovulatory infertility. *Am J Clin Nutr* **85**, 231–237.

Christiansen E, Schnider S, Palmvig B, Tauber-Lassen E, Pedersen O (1997). Intake of a diet high in *trans* monounsaturated fatty acids or saturated fatty acids. Effects on postprandial insulinemia and glycemia in obese patients with NIDDM. *Diabetes Care* **20**, 881–887.

Clevidence BA, Judd JT, Schaefer EJ, Jenner JL, Lichtenstein AH, Muesing RA *et al.* (1997). Plasma lipoprotein (a) levels in men and women consuming diets enriched in saturated, *cis*-, or *trans*-monounsaturated fatty acids. *Arterioscler Thromb Vasc Biol* **17**, 1657–1661.

Clifton PM, Keogh JB, Noakes M (2004). *Trans* fatty acids in adipose tissue and the food supply are associated with myocardial infarction. *J Nutr* **134**, 874–879.

Colon-Ramos U, Baylin A, Campos H (2006). The relation between *trans* fatty acid levels and increased risk of myocardial infarction does not hold at lower levels of *trans* fatty acids in the Costa Rican food supply. *J Nutr* **136**, 2887–2892.

Cuchel M, Schwab US, Jones PJ, Vogel S, Lammi-Keefe C, Li Z *et al.* (1996). Impact of hydrogenated fat consumption on endogenous cholesterol synthesis and susceptibility of low-density lipoprotein to oxidation in moderately hypercholesterolemic individuals. *Metabolism* **45**, 241–247.

de Roos NM, Bots ML, Katan MB (2001). Replacement of dietary saturated fatty acids by *trans* fatty acids lowers serum HDL cholesterol and impairs endothelial function in healthy men and women. *Arterioscler Thromb Vasc Biol* **21**, 1233–1237.

de Roos NM, Siebelink E, Bots ML, van Tol A, Schouten EG, Katan MB (2002). *Trans* monounsaturated fatty acids and saturated fatty acids have similar effects on postprandial flow-mediated vasodilation. *Eur J Clin Nutr* **56**, 674–679.

Doney AS, Fischer B, Leese G, Morris AD, Palmer CN (2004). Cardiovascular risk in type 2 diabetes is associated with variation at the PPARG locus: a Go-DARTS study. *Arterioscler Thromb Vasc Biol* **24**, 2403–2407.

European Food Safety Authority (2004). Opinion of the scientific panel on dietetic products, nutrition and allergies on a request from the Commission related to the presence of *trans* fatty acids in foods and the effect on human health of the consumption of *trans* fatty acids. *EFSA J* **81**, 1–49.

Field AE, Willett WC, Lissner L, Colditz GA (2007). Dietary fat and weight gain among women in the Nurses' Health Study. *Obesity (Silver Spring)* **15**, 967–976.

Han SN, Leka LS, Lichtenstein AH, Ausman LM, Schaefer EJ, Meydani SN (2002). Effect of hydrogenated and saturated, relative to polyunsaturated, fat on immune and inflammatory responses of adults with moderate hypercholesterolaemia. *J Lipid Res* **43**, 445–452.

He K, Merchant A, Rimm EB, Rosner BA, Stampfer MJ, Willett WC *et al.* (2003). Dietary fat intake and risk of stroke in male US healthcare professionals: 14 year prospective cohort study. *BMJ* **327**, 777–782.

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.

Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG *et al.* (2001a). Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med* **345**, 790–797.

Hu FB, Manson JE, Stampfer MJ, Colditz G, Liu S, Solomon CG *et al.* (2001b). Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *N Engl J Med* **345**, 790–797.

Ibrahim A, Natrajan S, Ghafoorunissa R (2005). Dietary *trans*-fatty acids alter adipocyte plasma membrane fatty acid composition and insulin sensitivity in rats. *Metabolism* **54**, 240–246.

Jakobsen MU, Overvad K, Dyerberg J, Heitmann BL (2007). Intake of ruminant *trans* fatty acids and risk of coronary heart disease. *Int J Epidemiol* 12 December 2007 (e-pub ahead of print).

Johansson L, Borgejordet A, Pedersen JI (2006). [*Trans* fatty acids in the Norwegian diet]. *Tidsskr Nor Laegeforen* **126**, 760–763.

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.

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

Katan MB, Zock PL, Mensink RP (1995). *Trans* fatty acids and their effects on lipoproteins in humans. *Annu Rev Nutr* **15**, 473–493.

Kavanagh K, Jones KL, Sawyer J, Kelley K, Carr JJ, Wagner JD *et al.* (2007). *Trans* fat diet induces abdominal obesity and changes in insulin sensitivity in monkeys. *Obesity (Silver Spring)* **15**, 1675–1684.

Kim SH, Chunawala L, Linde R, Reaven GM (2006). Comparison of the 1997 and 2003 American diabetes association classification of impaired fasting glucose: impact on prevalence of impaired fasting glucose, coronary heart disease risk factors, and coronary heart disease in a community-based medical practice. *J Am Coll Cardiol* **48**, 293–297.

Koh-Banerjee P, Chu NF, Spiegelman D, Rosner B, Colditz G, Willett W *et al.* (2003). Prospective study of the association of changes in dietary intake, physical activity, alcohol consumption, and smoking with 9-y gain in waist circumference among 16 587 US men. *Am J Clin Nutr* **78**, 719–727.

Kromhout D, Feskens EJ, Bowles CH (1995). The protective effect of a small amount of fish on coronary heart disease mortality in an elderly population. *Int J Epidemiol* **24**, 340–345.

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

Lefevre M, Lovejoy JC, Smith SR, Delany JP, Champagne C, Most MM *et al.* (2005). Comparison of the acute response to meals enriched with cis- or *trans*-fatty acids on glucose and lipids in overweight individuals with differing FABP2 genotypes. *Metabolism* **54**, 1652–1658.

Lemaitre RN, King IB, Raghunathan TE, Pearce RM, Weinmann S, Knopp RH *et al.* (2002). Cell membrane *trans* fatty acids and the risk of primary cardiac arrest. *Circulation* **105**, 697–701.

Lemaitre RN, King IB, Mozaffarian D, Sotoodehnia N, Rea TD, Kuller LH *et al.* (2006). Plasma phospholipid *trans* fatty acids, fatal ischemic heart disease, and sudden cardiac death in older adults: the cardiovascular health study. *Circulation* **114**, 209–215.

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.

Lopez-Garcia E, Schulze MB, Meigs JB, Manson JE, Rifai N, Stampfer MJ *et al.* (2005). Consumption of *trans* fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. *J Nutr* **135**, 562–566.

Louheranta AM, Turpeinen AK, Vidgren HM, Schwab US, Uusitupa MI (1999). A high-*trans* fatty acid diet and insulin sensitivity in young healthy women. *Metabolism* **48**, 870–875.

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.

Mauger JF, Lichtenstein AH, Ausman LM, Jalbert SM, Jauhiainen M, Ehnholm C *et al.* (2003). Effect of different forms of dietary hydrogenated fats on LDL particle size. *Am J Clin Nutr* **78**, 370–375.

Meijer GW, van Tol A, van Berkel TJ, Weststrate JA (2001). Effect of dietary elaidic versus vaccenic acid on blood and liver lipids in the hamster. *Atherosclerosis* **157**, 31–40.

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, Katan MB, Hornstra G (1992). Effect of dietary *cis*- and *trans* fatty acids on serum lipoprotein (a) levels in humans. *J Lipid Res* **33**, 1493–1501.

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.

Meyer KA, Kushi LH, Jacobs Jr DR, Folsom AR (2001). Dietary fat and incidence of type 2 diabetes in older Iowa women. *Diabetes Care* **24**, 1528–1535.

Moloney F, Yeow TP, Mullen A, Nolan JJ, Roche HM (2004). Conjugated linoleic acid supplementation, insulin sensitivity, and lipoprotein metabolism in patients with type 2 diabetes mellitus. *Am J Clin Nutr* **80**, 887–895.

Mozaffarian D, Pischon T, Hankinson SE, Rifai N, Joshipura K, Willett WC *et al*. (2004a). Dietary intake of *trans* fatty acids and systemic inflammation in women. *Am J Clin Nutr* **79**, 606–612.

Mozaffarian D, Rimm EB, King IB, Lawler RL, McDonald GB, Levy WC (2004b). *Trans* fatty acids and systemic inflammation in heart failure. *Am J Clin Nutr* **80**, 1521–1525.

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

Mozaffarian D, Willett WC (2007). *Trans* fatty acids and cardiovascular risk: a unique cardiometabolic imprint? *Curr Atheroscler Rep* **9**, 486–493.

Mozaffarian D, Clarke R (2009). Quantitative effects on cardiovascular risk factors and coronary heart disease risk of replacing partially hydrogenated vegetable oils with other fats and oils. *Eur J Clin Nutr* **63**(Suppl 2), S22–S33.

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

Muller H, Jordal O, Seljeflot I, Kierulf P, Kirkhus B, Ledsaak O *et al*. (1998b). Effect on plasma lipids and lipoproteins of replacing partially hydrogenated fish oil with vegetable fat in margarine. *Br J Nutr* **80**, 243–251.

Mutanen M, Aro A (1997). Coagulation and fibrinolysis factors in healthy subjects consuming high stearic or *trans* fatty acid diets. *Thromb Haemost* **77**, 99–104.

Natarajan S, Ibrahim A, Ghafoorunissa (2005). Dietary *trans* fatty acids alter diaphragm phospholipid fatty acid composition, triacylglycerol content and glucose transport in rats. *Br J Nutr* **93**, 829–833.

Neubauer S, Welte R, Beiche A, Koenig HH, Buesch K, Leidl R (2006). Mortality, morbidity and costs attributable to smoking in Germany: update and a 10-year comparison. *Tob Control* **15**, 464–471.

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, Ocke 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.

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.

Riserus U, Basu S, Jovinge S, Fredrikson GN, Arnlov J, Vessby B (2002). Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin resistance. *Circulation* **106**, 1925–1929.

Riserus U, Vessby B, Arnlov J, Basu S (2004). Effects of cis-9, *trans*-11 conjugated linoleic acid supplementation on insulin sensitivity, lipid peroxidation, and proinflammatory markers in obese men. *Am J Clin Nutr* **80**, 279–283.

Roberts TL, Wood DA, Riemersma RA, Gallagher PJ, Lampe FC (1995). *Trans* isomers of oleic and linoleic acids in adipose tissue and sudden cardiac death. *Lancet* **345**, 278–282.

Salmeron J, Hu FB, Manson JE, Stampfer MJ, Colditz GA, Rimm EB *et al*. (2001). Dietary fat intake and risk of type 2 diabetes in women. *Am J Clin Nutr* **73**, 1019–1026.

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.

Tholstrup T, Sandstrom B, Hermansen JE, Holmer G (1998). Effect of modified dairy fat on postprandial and fasting plasma lipids and lipoproteins in healthy young men. *Lipids* **33**, 11–21.

Tholstrup T, Raff M, Basu S, Nonboe P, Sejrsen K, Straarup EM (2006). 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* **83**, 237–243.

Thomas LH, Winter JA, Scott RG (1983a). Concentration of 18:1 and 16:1 *trans*unsaturated fatty acids in the adipose body tissue of decedents dying of ischaemic heart disease compared with controls: analysis by gas liquid chromatography. *J Epidemiol Community Health* **37**, 16–21.

Thomas LH, Winter JA, Scott RG (1983b). Concentration of *trans*unsaturated fatty acids in the adipose body tissue of decedents dying of ischaemic heart disease compared with controls. *J Epidemiol Community Health* **37**, 22–24.

Tricon S, Burdge GC, Jones EL, Russell JJ, El-Khazen S, Moretti E *et al*. (2006). 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* **83**, 744–753.

Tsai CJ, Leitzmann MF, Willett WC, Giovannucci EL (2005). Long-term intake of *trans*-fatty acids and risk of gallstone disease in men. *Arch Intern Med* **165**, 1011–1015.

Valenzuela A, Uauy R (1999). Consumption pattern of dietary fats in Chile: $n-6$ and $n-3$ fatty acids. *Int J Food Sci Nutr* **50**, 127–133.

van Dam RM, Rimm EB, Willett WC, Stampfer MJ, Hu FB (2002). Dietary patterns and risk for type 2 diabetes mellitus in US men. *Ann Intern Med* **136**, 201–209.

van Tol A, Zock PL, van Gent T, Scheek LM, Katan MB (1995). Dietary *trans* fatty acids increase serum cholesterylester transfer protein activity in man. *Atherosclerosis* **115**, 129–134.

Vega-Lopez S, Ausman LM, Jalbert SM, Erkkila AT, Lichtenstein AH (2006). Palm and partially hydrogenated soybean oils adversely alter lipoprotein profiles compared with soybean and canola oils in moderately hyperlipidemic subjects. *Am J Clin Nutr* **84**, 54–62.

Vermunt SH, Beaufrere B, Riemersma RA, Sebedio JL, Chardigny JM, Mensink RP *et al*. (2001). Dietary *trans* alpha-linolenic acid from deodorised rapeseed oil and plasma lipids and lipoproteins in healthy men: the TRANSLinE Study. *Br J Nutr* **85**, 387–392.

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.

Willett WC, Leibel RL (2002). Dietary fat is not a major determinant of body fat. *Am J Med* **113** (Suppl 9B), S47–S59.

World Cancer Research Fund (2007). *Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective*. American Institute for Cancer Research: Washington DC.

Zapolska-Downar D, Kosmider A, Naruszewicz M (2005). *Trans* fatty acids induce apoptosis in human endothelial cells. *J Physiol Pharmacol* **56**, 611–625.

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