Update on trans fatty acids and health

Position statement by the Scientific Advisory Committee on Nutrition

2007

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Preface

The purpose of this position statement is to consider recent evidence regarding the health effects of *trans* fatty acids (*trans* FA) in order to determine whether current population dietary advice on *trans* FA should be revised. The review arose from a request from the Secretary of State for Health to the Food Standards Agency for advice on the evidence in relation to the health effects of *trans* FA.

In its report on the *Nutritional Aspects of Cardiovascular Disease* (1994) the Committee on the Medical Aspects of Food and Nutrition Policy (COMA) concluded that there was sufficient evidence for an association between *trans* FA intakes and coronary heart disease (CHD) and for adverse effects on circulating lipoprotein concentrations, to recommend that the average population intake of *trans* FA should not exceed 2% food energy. This recommendation is the basis for current dietary advice on *trans* FA intake.

During the 1990s, reports from prospective cohort studies provided further evidence for an association between *trans* FA intakes and CHD. A number of well controlled dietary intervention studies also consistently confirmed *trans* FA to have adverse effects on serum lipoprotein profiles. This evidence, as well as that relating to potential associations between *trans* FA intakes and other disease states, were reviewed in the early 2000s (WHO & FAO, 2003; European Food Safety Authority, 2004) and in its Expert Consultation report, the WHO recommended that population goals should be to achieve *trans* FA intake levels less than 1% energy.

At a horizon scanning meeting in 2003 SACN considered the need for an updated risk assessment on the health effects of *trans* FA and agreed that the original risk assessments made by COMA in 1994 remained appropriate at that time.

The SACN *Framework for the Evaluation of Evidence* (SACN 2002) was used as the basis to identify and assess evidence published on CHD since the EFSA report (2004) and WHO/FAO Expert Consultation report (2003) and to review evidence for the other main diseases considered here (cancer, obesity, diabetes). The evidence base was mainly restricted to retrospective and prospective epidemiology and randomised, controlled trials in humans. In the epidemiology, measures of exposure included both direct measures of dietary *trans* FA intakes, as well as levels of *trans* FA in blood and tissues, which are taken to provide surrogate biomarkers of *trans* FA intakes. In drawing conclusions and making recommendations this report also takes into account recent re-estimates of *trans* FA intakes in the UK based upon reported consumption data from 2000/01 (Henderson *et al*, 2003) and new food composition data recently provided by industry.

The review concludes that there is sufficient evidence upon which to base a risk estimate for *trans* FA and CHD, but not for other diseases. It endorses the 1994 COMA recommendation, that the average *trans* FA intake should not exceed 2% of food energy, since there is currently no firm scientific basis for its revision. The review also concludes that there are inadequate data to demonstrate that *trans* FA from different dietary sources have differential effects upon CHD risk or lipoprotein profiles.

I would like to note that, as a result of the deadline given by the Minister, this review of the health effects of *trans* FA was carried out in an extraordinarily short period. Therefore, while the SACN *Framework for the Evaluation of Evidence* (SACN 2002) was used as the basis for identifying and assessing evidence included in the review the final report has not been subject to public consultation. Consultation is an important part of the process by which SACN normally carries out its work in an open and transparent fashion and it should not be omitted other than in exceptional circumstances. Furthermore, the speed of the report's preparation has severely limited the opportunity for mature reflection and deep cogitation.

It is also noteworthy that this is the first time a SACN report has been prepared by experts outside the SACN Secretariat and I, along with the Secretariat and other members of the Committee, will reflect upon the process and consider how this approach might be used usefully in future. The review upon which this position statement is based was conducted by Professor Christine Williams and her colleagues Dr Anne Marie Minihane, Dr Abby Thompson and Dr Danielle Shaw at the University of Reading. I would like to thank them for their work as, in spite of the constraints placed upon them they have carried out a comprehensive and rigorous review which enables the Committee to be confident in the advice on the health effects of *trans* FA it now presents to the Food Standards Agency.

Professor Alan Jackson
Chair of the Scientific Advisory Committee on Nutrition
December 2007

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1. Summary

Background

1. In its report *Nutritional Aspects of Cardiovascular Disease* (Department of Health, 1994), the Committee on Medical Aspects of Food and Nutrition Policy (COMA) concluded there was sufficient evidence for an association between *trans* FA intakes and coronary heart disease (CHD) and for adverse effects on circulating lipoprotein concentrations to recommend average population intakes of *trans* fatty acids (*trans* FA) should not exceed 2% food energy. In October 2007, the Food Standards Agency (FSA) in response to a request from the Secretary of State for Health asked the Scientific Advisory Committee on Nutrition (SACN) to review recent data on the health effects of *trans* FA, and to advise whether there is sufficient evidence to make a recommendation that all individuals in the population should consume less than 1% food energy as *trans* FA. The review was also to consider whether, on the basis of present evidence, it is possible to distinguish the health effects of *trans* FA from vegetable oil versus animal origin.

Methodology

2. The SACN *Framework for the Evaluation of Evidence* (SACN 2002) was used as the basis to identify and assess evidence published on the health effects of *trans* FA. The evidence base was mainly restricted to human epidemiological evidence and data from randomised controlled trials (RCTs), with limited animal and cell studies evidence included as appropriate. In the epidemiology, measures of exposures included direct measures of dietary *trans* FA intakes, as well as levels of *trans* FA in blood and tissues, which are taken to provide surrogate biomarkers of *trans* FA intakes.

3. The main focus was an examination of the evidence for an association between *trans* FA and CHD published since the World Health Organization/Food and Agriculture Organisation (WHO/FAO) Expert Consultation Report and the European Food Safety Authority (EFSA) Report in 2003 and 2004, respectively. The evidence relating *trans* FA intake to cancer, obesity, diabetes and other health concerns was also evaluated.

Trans FA in the UK food chain

4. *Trans* FA are unsaturated fatty acids (FA) found in the food chain, with one or more of their double bonds in the '*trans*' orientation rather than the common 'cis' configuration. This altered double bond configuration has an impact on both the physiochemical and functional properties of the FA, with consequences for their metabolism *in vivo*.

5. *Trans* FA are naturally occurring at low levels in dairy products and meats from ruminant animals. They may also be produced by the industrial hydrogenation of vegetable oils to produce the semi-solid and solid fats that are widely used in food manufacture (e.g. margarines, biscuits) and catering outlets.

6. The average adult (19-64 years) intake of *trans* FA in the UK was reported to be 1.2% food energy in the 2000/01 National Diet and Nutrition Survey (NDNS) (Henderson *et al*, 2003). A 2007 estimation of intake, using dietary data from 2000/01 NDNS (Henderson *et al*, 2003) and new *trans* FA composition data provided by industry, has given an estimated average value of 1.00% food energy for current *trans* FA intake in the UK adult population (FSA, 2007).

Trans FA and health

Coronary Heart Disease

7. The prospective epidemiological studies reviewed here provide consistent evidence of a moderate increase in risk of CHD over the range of *trans* FA intakes similar to, or slightly higher than, those seen in the UK population. Well-controlled RCTs have demonstrated adverse effects of *trans* FA on low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) and total:HDL-C ratio, providing a plausible biochemical mechanism to explain the pathophysiology underlying the prospective epidemiological findings. Studies that have evaluated the impact of *trans* FA on other CHD biomarkers (blood pressure, C-reactive protein (CRP), postprandial lipemia, lipid oxidation, haemostasis, endothelial function and vascular inflammation), have generally reported neutral effects, with inconsistency amongst the small number of studies reported to date.

8. The ability to quantify the increased risk of CHD attributable to that percentage of the general population currently consuming > 1% food energy as *trans* FA is limited by: i) estimates of risk from epidemiology for quintiles of intake in the region 1-2% food energy do not differ significantly from 1.0; ii) a lack of evidence for a linear relationship between *trans* FA intake and CHD risk over the range of 1-2% dietary energy; and iii) RCTs that have evaluated the impact of varying doses of *trans* FA on lipoproteins have not compared levels of intake between 1-2% food energy.

9. Extrapolation of the effect of *trans* FA on CHD risk using the pooled variance-weighted risk from a meta-analysis of studies suggests that a 1% decrease in energy from *trans* FA would be associated with a 12.5% decrease in risk of CHD (Oomen *et al*, 2001). To achieve a situation in which all individuals in the UK consumed < 1% food energy as *trans* FA, against a current average intake of 1.0-1.2% food energy from *trans* fatty acids, would require an average reduction in intake of 0.6% energy as *trans* FA. If this target were achieved there would be an

estimated reduction in risk of CHD of approximately 5.0-7.5%. These estimates of risk reduction require cautious interpretation as they are based on extrapolation from prospective epidemiology, which have generally observed higher *trans* FA intakes than the current UK levels and are based on the assumption of a linear dose-response between *trans* FA intake and CHD risk. Evidence presented in this report demonstrates a non-linear distribution of risk estimates at intake levels relevant to the UK population (0.5- 2.5% food energy). This suggests that the actual reduction in risk is likely to be less than 5.0-7.5%.

Cancer

10. There is weak and inconsistent evidence for a relationship between *trans* FA and breast or colorectal cancer. Evidence for an association between *trans* FA and prostate cancer is limited, but a recent large case-control study has shown a strong interaction between risk and *trans* FA intake for a particular genotype that makes up ~35% of the population. This potential association requires further investigation. The single study on non-Hodgkin's lymphoma reported a strong positive association, but only used a single assessment of dietary *trans* FA made in 1980.

Weight gain and obesity

11. The evidence for a relationship between *trans* FA intake on risk of obesity or increased weight gain is limited. A small number of studies have reported positive associations between *trans* FA intake and gain in weight or waist circumference over time. However, the effects observed are small, corresponding to an 8-year weight gain of approximately 0.5-1.0 kg for an increase of 1% energy from *trans* FA.

Diabetes

12. The limited number of prospective cohort studies evaluating the effect of *trans* FA intake on risk of diabetes have reported inconsistent results, with only one study retaining statistical significance after adjustment. RCTs and meal studies in healthy individuals have shown no effect of moderately high (2-5% energy) levels of *trans* FA on insulin sensitivity or glucose tolerance. However, consumption of unphysiological levels of dietary *trans* FA (20% energy) has been reported to induce postprandial hyperinsulinemia in obese subjects with type 2 diabetes. An acute meal study found a significantly higher insulin response following *trans*- rather than *cis* FA-containing meals (10% dietary energy), and a small number of *in vitro* studies using isolated pancreatic islets have observed a potentially negative impact of *trans* FA on glucose-stimulated insulin secretion (GSIS).

Other health concerns

13. There is inadequate information from well-designed studies to support an adverse effect of *trans* FA on early development. It is reported that the level of

trans FA in plasma and tissue lipids is inversely proportional to the levels of long-chain ω-6 polyunsaturated fatty acids (PUFA). This raises the possibility that, indirectly, *trans* FA may interfere with the metabolism of essential fatty acids (EFA), which are important in growth and development. The data require cautious interpretation, as the use of FA compositional data from observational studies as the basis for proposing effects of *trans* FA on long-chain ω-6 PUFA metabolism lacks rigour. This area requires further investigation by well-designed studies.

14. *Trans* FA intake has been considered as a potential risk factor for a number of additional health issues. A single study found a significant positive relationship between *trans* FA and gallstone formation. Positive trends have been reported for *trans* FA and Alzheimer's disease, cognitive decline and ovulatory infertility, but these failed to reach statistical significance. Further research is needed before any conclusions can be made regarding the effect of *trans* FA intake on these diseases.

Trans FA of animal and vegetable oil origin

15. A small number of early prospective studies provided some evidence to suggest a more significant association between risk of CHD and dietary *trans* FA from vegetable oil than from animal origin. However, more recent reports from the same studies but with longer follow-up periods, as well as additional studies in different cohorts, have mostly not reported separate associations for the dietary *trans* FA from vegetable or animal origin. It is therefore concluded that at present there is inadequate information for determining whether *trans* FA from different sources have differential effects on disease risk.

16. It has been proposed that the levels of *trans* 18:2 and *trans* 16:1 in tissues and blood may provide an indication of *trans* FA consumption from vegetable oil or animal origin, respectively. The evidence regarding the robustness of this approach is limited, and systematic validation is required before these biomarkers can be used with confidence as a marker for intake of *trans* FA from different sources.

17. Foods of animal origin that naturally contain *trans* FA (dairy products, beef, lamb) are valuable sources of other nutrients, particularly protein, calcium and iron. The proportion of *trans* FA derived from these food products is approximately 40-50%, and this proportion is increasing as the *trans* FA levels in manufactured foods fall. Any recommendation for further reductions in *trans* FA intakes should consider the beneficial contribution these animal products make to overall diet of the UK population.

Trans FA and saturated fatty acids (SFA)

18. To contextualise the impact of changes in *trans* FA on the diet as a whole, the Committee have also briefly considered the current intake levels of SFA. Despite the public health recommendations, SFA intakes remain higher than target levels. There are some indications that industry efforts to reformulate fat compositions to reduce *trans* FA levels may have resulted in increased SFA levels. This is of concern given the priority for reducing SFA as a population measure for reducing CHD risk. It is therefore important to monitor the lipid composition of the diet (*trans* FA, SFA, monounsaturated fatty acids (MUFA), PUFA) and evaluate any potential adverse impact of the overall dietary lipid profile by regular measurement of CHD risk markers (LDL-C and HDL-C).

Conclusions

19. Since the report of COMA (DH, 1994), the epidemiological evidence from prospective studies with up to 20 years of follow-up, consistently supports an adverse effect of *trans* FA on CHD risk, although the estimated size of the effect has reduced. Evidence from RCTs provides strong support for adverse effects of *trans* FA on LDL-C (increases) and HDL-C (decreases). In addition, the evidence for cardioprotective effects of HDL-C has strengthened, increasing the recognition of the potential hazards of *trans* FA due to their unique properties in reducing HDL-C compared with other FA classes.

20. In most cases, the dietary data from prospective studies are for ranges of *trans* FA intakes slightly higher than those of current UK intakes. Using the NDNS 2000/2001 (Henderson *et al*, 2003) estimate of average *trans* FA intake (1.2% food energy) and a target for the entire population of < 1% food energy as *trans* FA, provides an estimated reduction in CHD risk of approximately 7.5%. However, recent Food Standards Agency (FSA) estimates for average *trans* FA intakes of 1.0% food energy would correspond to a reduction in CHD risk in the region of 5%.

21. Data on the potential impact of *trans* FA on some types of cancers (colon, prostate, non-Hodgkin's lymphoma), diabetes, body weight and body fat accumulation is limited, and further research is required before any association between *trans* FA intake and any of these diseases can be confirmed and subsequently quantified. There is no evidence of any association between *trans* FA consumption and breast cancer.

22. The average *trans* FA intake for the UK population has more than halved in the last 20 years. Recommendations for further reductions in *trans* FA may have adverse consequences for attempts to reduce dietary SFA, for the overall lipid profile of the diet, and on the consumption of animal products.

Recommendations

23. There is consistent evidence to support a moderate effect of *trans* FA on increasing the risk of CHD. The primary mechanism for this effect appears to be via changes in the serum lipoprotein profile. Findings for effects of *trans* FA on inflammatory responses and endothelial function remain inconclusive.

24. The evidence relating *trans* FA intakes to risk of diseases other than CHD is limited, and no reliable risk assessments can be made. However, future reports on these associations should be monitored, particularly the effect of *trans* FA on insulin sensitivity and diabetes, and the *trans* FA-genotype interaction with risk of prostate cancer.

25. This review endorses the current recommendation set by COMA (1994), that the average *trans* FA intake should not exceed 2% of food energy, as there is currently no firm scientific basis for its revision.

26. The current data provide insufficient evidence to justify the differentiation of *trans* FA from vegetable oil and animal sources based on the isomeric forms of the *trans* FA. There are also inadequate data to demonstrate that *trans* FA from different dietary sources have differential effects on CHD risk or lipoprotein profiles.

27. The impact of the reformulation of fats within the diet should be monitored to ensure there are no unintended adverse consequences for dietary lipid profiles and related CHD risk factors.

2. Introduction

28. In 1994 the Committee on Medical Aspects of Food and Nutrition Policy (COMA) published its report on *Nutritional Aspects of Cardiovascular Disease*, which recommended that average intakes of *trans* fatty acids (*trans* FA) should not exceed 2% of food energy. Later reviews (WHO & FAO, 2003; European Food Safety Authority, 2004), confirmed the association between high intake of *trans* FA and CHD. The WHO/FAO Expert Consultations report, *Diet, Nutrition and Chronic Disease* (2003) advised that the population goal should be less than 1% energy from *trans* FA. In 2003, the Danish Government introduced legislation to require that industrially produced *trans* FA should be limited to 2% of the total amount of fat or oil in a food. During 2006, voluntary guidelines regarding the *trans* FA content of foods sold in retail outlets and catering establishments were established in Canada.

29. In October 2007, the Food Standards Agency received a request from the Secretary of State for Health to consider whether, because of concerns about the health impacts of *trans* FA consumption, there is sufficient evidence to make a recommendation that all individuals in the population should consume less than 1% food energy as *trans* FA. As a result, the Agency asked the Scientific Advisory Committee on Nutrition (SACN) to review the current evidence on the health effects of *trans* FA.

Terms of reference

30. The present review was established with the following terms of reference to:

- Consider the evidence regarding health effects of *trans* FA on CHD since the EFSA (2004) and the WHO/FAO Expert Consultation reports (2003);
- Consider evidence relating to other health effects of *trans* FA, particularly cancer, obesity and diabetes;
- Determine whether there is sufficient data available on which to revise current UK recommendations that the average *trans* FA intake of the population should not exceed 2% of food energy. In particular, whether any revised recommendations should state that intakes be reduced to 1% or less of food energy;
- Determine on the basis of present evidence whether it is possible to distinguish the health effects of *trans* FA from vegetable oil versus animal origin.

Background

31. In its report *Nutritional Aspects of Cardiovascular Disease* (Department of Health, 1994), COMA concluded there was sufficient evidence for an association between *trans* FA intakes and CHD and for adverse effects on circulating lipoprotein concentrations, to recommend population intakes of *trans* FA should not exceed 2% food energy. The report considered evidence for adverse effects of saturated fatty acids (SFA) to be strong and given the high levels of SFA in the UK population at that time (>17% food energy), recommended major efforts to change population food choices and reduce levels of SFA in the UK food chain.

32. During the 1990s, reports from prospective cohort studies provided further evidence for an association between *trans* FA intakes and CHD. A number of well-controlled dietary intervention studies also consistently confirmed *trans* FA to have adverse effects on serum LDL-C, HDL-C and triacylglycerol (TAG). This evidence, as well as that relating to potential associations between *trans* FA intakes and other disease states, was reviewed in the early 2000s (WHO & FAO, 2003; EFSA 2004). In its Expert Consultation report, the WHO/FAO recommended population goals should be to achieve *trans* FA intake levels less than 1% energy.

33. At a horizon-scanning meeting in 2003, SACN considered the need for an updated risk assessment on the health effects of *trans* FA. The Committee agreed that the original risk assessments made by COMA in 1994 remained appropriate. Although reductions in SFA intakes in the UK diet had been achieved since 1994, they remained above the target set by COMA (1994) and were considered to pose a greater risk to health than *trans* FA.

Methodology

Selection of evidence

34. The SACN *Framework for the Evaluation of Evidence* (SACN 2002) was used as the basis to identify and assess evidence published on CHD since the EFSA (2004) and WHO/FAO Expert Consultation (2003) report, and to review published evidence for the other main diseases considered here (cancer, obesity, diabetes). The evidence base for this report is mainly restricted to retrospective and prospective epidemiology and RCTs in humans. In the epidemiology, measures of exposure include both direct measures of dietary *trans* FA intakes, as well as levels of *trans* FA in blood and tissues, which are taken to provide surrogate biomarkers of *trans* FA intakes. In this review, evidence from animal and cell studies is also considered, in particular to assess whether there are plausible biological mechanisms that can support associations observed in epidemiological studies.

35. In examining the epidemiological evidence, consideration has been given to the limitations of each type of study. Ecological (cross cultural) studies examine putative relationships between ranges of average dietary intakes, within or between populations, and mortality and morbidity rates for diseases of interest. However, ecological studies do not enable causal relationships to be established, since they are subject to considerable confounding by unmeasured variables. Even when such variables can be measured, they may be co-related with both the dietary exposure and the disease outcomes of interest. Retrospective case-control studies allow hypotheses generated from cross-cultural studies to be examined in more detail. However, case-control studies are known to be subject to recruitment and reporting bias, since retrospective recall of diet has been shown to differ in cases compared with control subjects. Prospective cohort or nested case-control studies are less subject to recruitment and recall bias. However, because of the large number of subjects involved in prospective studies, dietary data is usually based on assessment of food frequency questionnaires (FFQ), which are subject to significant measurement error. Some prospective studies reported in this review have undertaken only a single measure of diet at baseline, which may lead to misclassification of diet in long term follow up, due to changes in subjects' habitual diets over time, as well as changes in food formulations. The latter is a particular issue with respect to the assessment of relationships between *trans* FA intakes and disease risk, because of the efforts made by food manufacturers to replace *trans* FA in oils and spreads which took place during the 1990s.

36. Measurements of blood and tissue levels of FA have been shown to provide reliable surrogate markers of dietary intakes, at least for some dietary FA. Tissue biomarkers of *trans* FA levels have been used in a number of retrospective and nested case-control studies included in this review. These measures avoid the inaccuracies and recall bias associated with collecting dietary information by questionnaires, and for this reason it is suggested that greater reliance may be placed on findings from case-control evidence where status has been derived from measurement of tissue FA levels. However, some disease states result in changes in FA metabolism that are reflected in altered tissue compositions, which could confound retrospective case-control comparisons. In addition, because FA compositional data are expressed as % of total FA, any increase or decrease in one FA will result in reciprocal changes in one or more of the other FA present. A further limitation is that because it is not possible to estimate dietary *trans* FA intakes from biomarker *trans* FA levels, the findings from these studies cannot be used to develop public health recommendations for dietary intake levels associated with reduced risk of disease.

Report contents

37. The report considers the chemistry of *trans* FA, the different isomeric forms of *trans* FA and their origin and occurrence in foods. Dietary intakes of *trans* FA and

changes in intakes in recent years are reported. The relationship between *trans* FA and CHD, cancer, diabetes, obesity and insulin resistance, are considered. A detailed review of the literature relating to the association between *trans* FA and CHD outcomes, as well as risk biomarkers for CHD, was undertaken by EFSA in 2004. These data have been considered in detail and a summary of the findings presented in the section on CHD. Additional literature published since 2004 on the association between *trans* FA intakes and CHD, as well as RCTs undertaken to study the effects of *trans* FA on risk biomarkers for CHD, has been reviewed in the present report. Data are presented here in a similar format to the EFSA report. Epidemiological, RCT and animal data published in the literature for effects of *trans* FA on cancer, diabetes, insulin resistance and obesity have been reviewed in the present report. Brief consideration of the relationship between *trans* FA intake and fetal development is also provided.

38. It should be noted that this report has not undertaken a detailed review of the association between conjugated linoleic acid (CLA) and health; brief consideration to the recent literature in this area is given in section 4.

Methods used for quantification of dietary exposures of trans FA

39. As stated above, studies included in the report have quantified variation in *trans* FA exposure levels using both dietary assessment and measurement of levels in tissues. For FA which largely originate from the diet, and which cannot be synthesised *de novo* in the body, such as the EFA, linoleic acid (18:2, n-6) and linolenic acid (C18:3, n-3), the long chain n-3 PUFA (only limited synthetic capacity) and *trans* FA, their measurement in tissue lipid pools is considered to be a more reliable assessment of dietary exposure than dietary questionnaires. This may be particularly important for the relative quantification of *trans* FA exposure, as the inability of food composition databases to keep pace with rapidly changing compositions of foods, which include partially hydrogenated vegetable oil sources, may result in significant inaccuracies in estimations of dietary *trans* FA intake levels. Levels of *trans* FA in plasma lipid pools such as cholesterol esters and phospholipids, and in platelets, red blood cells, and adipose tissue, may be used as indices of integrated exposure over the previous few days, few months and 1-2 years respectively. For many of the more recent reports from epidemiological studies FA status has been determined by quantification of erythrocyte and adipose tissue FA levels.

Presentation of statistical data relating to the association between dietary intake and risk of disease

40. Throughout the text and tables included in the present report, the odds ratio (OR) and relative risk (RR) stated are those which have been fully adjusted for potential confounding factors. When a significant difference in an associated risk was reported, the specific factors that were adjusted for in the model have been

shown within the tables. The p for trend values refer to the level of difference between the highest and lowest tertile/ quartile/ quintile of intake.

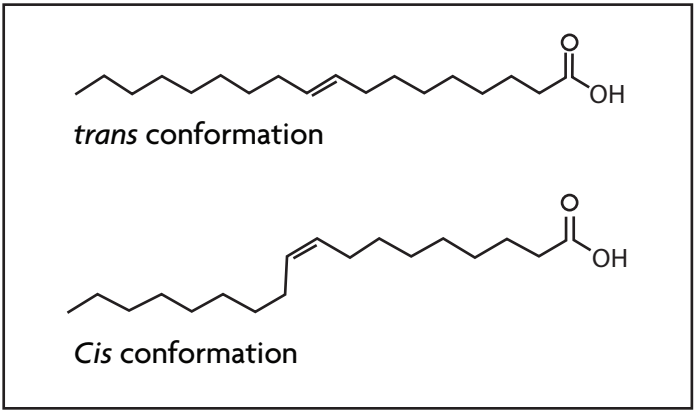
41. Dietary *trans* FA intakes positively correlate with dietary SFA. Therefore, it is essential that epidemiological studies designed to assess the association between dietary *trans* FA intake and CHD risk fully adjust for potential confounding by this class of FA. When this has not occurred, this limitation is stated in the text.

Trans FA in the food chain

Structure of trans FA and their origins in the diet

42. Most unsaturated FA in foods have double bonds in the *cis* (bent) configuration. However, unsaturated FA with *trans* (straight) bond configurations are also found (Figure 1). *Trans* FA are naturally occurring in dairy products and meats from ruminant animals; the rumen contains bacteria that produce isomerases capable of converting the *cis* double bonds of polyunsaturated fats in the animals' diets to a *trans* position. Since the early 20th century, industrial hydrogenation of vegetable oils, which results in the conversion of a proportion of *cis* double bonds to the *trans* configuration, has been used to produce semi-solid and solid fats that are now widely used in food manufacture (e.g. margarines, biscuits) and catering outlets. *Trans* FA are also formed during high temperature treatment of oils and during deodorisation of unsaturated oils to remove unstable by-products of oxidation.

Figure 1. *Cis* and *trans* FA configuration



43. In Europe and Canada, hydrogenation was mainly used to produce hydrogenated fish oils, whereas in North America hydrogenation of vegetable oils became common after the late 1920s. Hydrogenated fish oils are no longer used in food manufacture, and the long chain (C20, C22) *trans* isomers typical of hydrogenated fish oils are no longer found in food products. More recent modifications in the conditions of the partial hydrogenation process have yielded different products that have been used for deep-frying, margarines, and shortenings for baking which have better organoleptic properties. Light processing (brush hydrogenation) is also used primarily to produce a liquid oil with greater shelf life.

UK intakes of trans FA in adults and children

44. As detailed in Annex 3, the most recent published estimates of UK adult *trans* FA consumption from the National Diet and Nutrition Survey (NDNS) of Adults, aged 19-64 years, 2000/1 (Henderson *et al*, 2003) indicate an average intake of 1.2% of food energy for both adult males and females (Annex 3, Table 1), which equates to 2.91g and 2.04g per day, respectively. These intakes have declined significantly in recent years, with intakes of 2.2% of food energy reported in 1986/87 (Gregory *et al*, 1990). This decline has been attributed to a number of factors, including reformulation by manufacturers to reduce the use of partially hydrogenated oils; a general fall in consumption of foods which included these oils; and the use of more up-to-date food composition tables in the later surveys.

45. As part of the background to this review, an estimate of current *trans* FA intake was undertaken by the FSA using information provided by industry on the maximum or average level of *trans* FA in a number of processed food categories. The estimate used included industry values[1] for the following categories: the maximum level of *trans* FA in biscuits, cakes and pastries (excluding products made with butter), the maximum level of *trans* FA in fat spreads and the average *trans* FA level in ice cream. *Trans* FA intake values published in the NDNS 2000/01 for adults (Henderson *et al*, 2003) and 2003/05 Low Income Diet and Nutrition Survey (Nelson *et al*, 2007) (1.2% and 1.3% of food energy respectively) were based on earlier data for these categories: *trans* FA data for biscuits, cakes and pastries were based on analysis mainly in the early 1990s, data for ice-cream were based on analysis in 1998 and data for fat spreads were based on estimates from manufacturers data collected prior to the 2000/01 NDNS. Recalculation of mean intake using consumption data from the 2000/01 NDNS (Henderson *et al*, 2002) with the new industry figures gave an estimated value of 1.00% food energy for the mean intake of *trans* FA in the British adult population (19-64 years) (FSA 2007; Annex 3, para 10-12). This figure is likely to be an overestimate of actual intake as it was not possible in the time available to take account of all the reductions in *trans* FA levels in the model.

1 Provided by members of the Biscuit, Cake, Chocolate and Confectionary Association (BCCCA); Margarine and Spreads Assocation (MSA); and the Food and Drink Federation (FDF).

46. Comparison of intakes of the 2000/01 general adult population (Henderson *et al*, 2003) with those from the more recent 2003/05 LIDNS (Nelson *et al*, 2007), which recorded the diets of the lowest 15% of the population with respect to material deprivation, indicate comparable intakes between the two survey groups, with mean intakes of 1.3% of food energy from *trans* FA for males and 1.2% energy for females reported in the 2003/05 LIDNS (Nelson *et al*, 2007; Annex 3). As discussed in paragraph 45, it is likely that mean intakes will now be lower than these earlier estimates, due to reformulation of fats used in food manufacture.

47. It has been estimated that only 3% of the general adult population consume more than 2% food energy as *trans* FA (Henderson *et al*, 2003), with the figure rising to 9% of males and 6% of females in the low income cohort (Nelson *et al*, 2007) (Annex 3, para 3). It should be noted that this higher proportion of individuals in the low income groups consuming at these higher intake levels appears to be in large part attributable to a greater proportion of total *trans* FA derived from fat spreads with relatively high *trans* FA contents in this subgroup (Annex 3). As discussed in paragraph 44, it should be noted that the level of *trans* FA within such sources has been reduced due to action taken by the food industry since these estimates of intakes in LIDNS (Nelson *et al*, 2007) were made.

48. According to the most recent available data, children have modestly higher total *trans* FA intakes relative to adults, with mean intakes of 1.3-1.4% food energy in the various age groups (4-18 years) (Gregory *et al*, 2000). However, this may simply be reflective of the earlier survey date (1997) compared to the recent adult data (Henderson *et al*, 2003), since the food composition data used in 1997 were largely based on analyses made during the 1980s and early 1990s.

Sources and distribution of trans FA isomers in UK diet

49. Partially hydrogenated oils contain many different isomers of unsaturated fatty acids (usually oleic or linoleic acid), with *trans* isomers comprising 10-40% of the total fatty acids. Large numbers of different isomers are also found in ruminant meat and milk where the *trans* FA concentration typically ranges from 3-8% of total fat; levels of *trans* FA tend to be higher in lamb and mutton than beef fat.

50. Based on NDNS 2000/01 (Henderson *et al*, 2003), it is estimated that approximately 55-65% of total *trans* intake is derived from vegetable oil sources, with the remainder of animal origin. During the intervening years between 1986/87 and 2000/01, there was a considerable decline in the proportion of total *trans* FA derived from fat spreads, with an associated rise in the proportion from milk and milk products and meat and meat products (Annex 3, Figure 3).

51. Based on the analysis of 1995 Total Diet Study samples (MAFF, 1997), the *trans* 18:1 isomers comprise approximately 65% of total *trans* FA intakes. In the UK, the *trans* 18:2 isomers represents the next most prevalent dietary *trans* FA, comprising approximately 12% of total intake.

52. The nomenclature of the *trans* 18:1 and *trans* 18:2 isomers and their typical compositions in animal and vegetable oil sources are given in Table 1 (EFSA 2004). These data show that the *trans* isomer patterns of vegetable oil and animal sources show considerable overlap, with many isomers common to both food sources. In milk fat or ruminant meat the predominant (30-60%) *trans* FA is vaccenic acid (*trans* 18:1, n-7) with other *trans* MUFA such as 14:1 and 16:1, as well as 18:2 and 18:3 isomers, also present. The *trans* 16:1, n-7 isomer has been suggested as a possible biomarker of animal *trans* FA, as it is not present in partially hydrogenated oils, with the exception of fish oils. In the EU and Canada, partially hydrogenated fish oils products are no longer used by food processors; therefore intakes from this source have fallen dramatically worldwide. In partially hydrogenated vegetable oils, the main isomer is elaidic acid (*trans* 18:1, n-9), typically contributing up to 30% of the total *trans* FA in these oils. *Trans* 18:1, n-7 typically constitutes 10-20% of total *trans* FA from these sources. The long chain polyunsaturated *trans* FA (C20, C22) are no longer found in foods since the use of hydrogenated fish oils was discontinued.

Biomarkers of vegetable oil versus animal *trans* FA intakes

53. Some of the larger prospective studies which have shown an association between *trans* FA intakes and CHD have demonstrated the association to apply only to *trans* FA originating from industrial hydrogenation of vegetable oils, with some evidence that animal sources of *trans* FA (mainly *trans* 18:1, n-7) do not share the adverse effects of isomers of vegetable oil origin (mainly *trans* 18:1, n-9). It has also been proposed that further distinction between *trans* FA of animal versus vegetable oil origin can be drawn from measurement of different isomers in foods, and in human tissues, and that these measurements might have the potential to act as surrogate markers of intake from the two sources.

54. However, with the potential exception of *trans* 16:1, n-7, most *trans* FA are present in both sources, and therefore use of specific isomers as markers of *trans* source is likely to be associated with a large amount of inherent error. In particular, the predominant *trans* 18:1 isomers do not lend themselves well as markers of animal versus vegetable oil sources, because even though there is variation in the isomer proportions in the two sources, the isomers are present in both animal and non-animal sources. Although *trans* 18:2 are largely derived from hydrogenated vegetable oil sources, small amounts are also present in animal fats, and also cannot be reliably used as a marker of source. In addition, it should be noted that *trans* 16:1 is also found in hydrogenated fish oils, thereby affecting the interpretation of data from some of the earlier studies conducted at a time when hydrogenated fish oils remained within the food chain. This report has therefore been cautious in drawing conclusions on the origin of specific isomers and their use as markers of *trans* FA of animal or vegetable oil origin.

Table 1: Nomenclature of FA and typical proportions of positional *trans* 18:1 isomers and total *trans* 18:2 from animal and vegetable oil sources

Trans isomer	Delta position of double bond	Milk fat, cow (%)	Industrially hydrogenated fats (%)
18:1, n-2	16	6-8	1
18:1, n-3	15	4-6	2
18:1, n-4	14	8	*
18:1, n-5	13	6-7	9-12*
18:1, n-6	12	6-10	8-13
18:1, n-7 (vaccenic acid)	11	30-50	10-20
18:1, n-8	10	6-13	10-20
18:1, n-9 (elaidic acid)	9	5-10	20-30
18:1, n10 to n12	6-8	2-9	14-18
18:1, n-13	5	<1	2
18:1, n-14	4	<1	1
18:2 total (linolelaidic acid)	-	0.6	2-4

* combined n-4 and n-5 isomers
(Precht & Molkentin, 1997; Wolff *et al*, 2000; Precht *et al*., 2001; European Food Safety Authority, 2004)

3. *Trans* FA and Health

Trans FA and coronary heart disease

55. A large body of literature on the relationships between *trans* FA and CHD has previously been reviewed (DH, 1994; WHO & FAO, 2003; EFSA, 2004). The current report has been commissioned to consider evidence made available since the publication of these three key reports. The earlier reports are consistent in their conclusions that *trans* FA: i) increase the risk of CHD, ii) raise LDL-C and reduce HDL-C, and iii) on an isoenergetic basis, the lipoprotein profile of plasma is more atherogenic following consumption of *trans* FA than SFA.

56. A review of the literature relating to the association between *trans* FA and CHD outcomes, as well as risk biomarkers for CHD, was undertaken by EFSA in 2004. The present review has largely focused on epidemiological evidence published since 2004, as well as on RCTs undertaken to study the effects of *trans* FA on risk biomarkers for CHD. However, some studies published prior to 2004 are also described here, in the text and in data presented in the tables, where: i) they are considered to provide information that has not previously been reviewed in depth; ii) the data may be helpful in drawing conclusions regarding the impact of specific isomers; or iii) they provide information on risk associated with levels of intake relevant to the UK diet. A large prospective study, the Nurses' Health Study, has published long-term follow up data since 2004. Therefore all data from this study, including that published prior to 2004, are considered as part of the present review to enable a full evaluation of its findings.

Epidemiological studies and RCTs on *trans* FA and CHD

Summary of findings from the EFSA review (2004)

57. EFSA reviewed 7 case-control and 5 cohort studies published between 1990-2004. As case-control studies are considered more sensitive to information and selection bias than prospective studies, EFSA placed greater weight on findings from the prospective data in drawing its conclusions; the same approach has been applied here. Of the 7 case-control studies published between 1990-2004 (Siguel & Lerman, 1993; Ascherio *et al*, 1994; Aro *et al*, 1995; Roberts *et al*, 1995; Van de Vijver *et al*, 1996; Baylin *et al*, 2003; Clifton *et al*, 2004), 2 measured *trans* FA exposure by FFQ, the remainder measured *trans* FA levels by the use of biomarkers (plasma phospholipids or adipose tissue). In the case of the diet studies, one showed no significant association with CHD (Clifton *et al*, 2004) and the other (Ascherio *et al*, 1994), a strong positive association (OR 2.03, 95% CI, 0.98-4.22; p for trend 0.0001) with total *trans* FA in the diet. In the latter study, a strong association was observed for *trans* FA of vegetable oil origin, but no association was shown for *trans* FA of animal origin. One study (in Costa Rica)

showed a strong positive association for total *trans* FA in adipose tissue in first myocardial infarction (MI) patients (Baylin *et al*, 2003). Another biomarker study showed higher levels of total *trans* FA, *trans* 18:2 and *trans* 16:1, n-7 in adipose tissue in cases than controls (Siguel & Lerman, 1993). The other case-control studies which used biomarkers to assess *trans* FA reported no significant association between *trans* FA levels and CHD (Aro *et al*, 1995; Roberts *et al*, 1995; Van de Vijver *et al*, 1996).

58. Consistent findings were observed for the 5 prospective studies (Willet *et al*, 1993; Ascherio *et al*, 1996; Hu *et al*, 1997; Pietinen *et al*, 1997; Oomen *et al*, 2001) reviewed by EFSA (2004). All 5 studies showed positive associations between total *trans* FA intakes and CHD risk and, with the exception of one study (Ascherio *et al*, 1996), the p for trend reached statistical significance. Three of the studies evaluated relationships between *trans* FA of vegetable oil (hydrogenated vegetable fat) and animal origin, and CHD risk. One study reported no distinction in the association between *trans* FA from animal and vegetable oil sources and risk of CHD (Oomen *et al*, 2001). The other two studies showed positive associations only for dietary assessed *trans* FA from vegetable oil sources (Willet *et al*, 1993; Pietinen *et al*, 1997). Both reported an inverse association between animal *trans* FA intake and CHD risk, which reached significance in the report by Pietinen *et al* (1997).

Case-control studies – CHD outcomes

59. Three of the larger case-control studies considered by EFSA are presented here along with 2 other studies published since 2004 (Table 1A, Annex 2).

60. The EURAMIC study (Aro *et al*, 1995) assessed the relationship between adipose tissue total *trans* 18:1 and risk of acute MI in European countries. There was no difference in *trans* 18:1 content in adipose tissue between controls and cases for the whole cohort. However, in Norway and Finland, higher adipose tissue *trans* 18:1 were associated with increased MI risk, with reported ORs of 5.4 (95% CI, 1.5-3.1; p for trend 0.01) and 5.0 (95% CI, 1.3-19.6; p for trend 0.02) for highest versus lowest quartiles of adipose tissue trans. In contrast, inverse associations were reported for Russia and Spain (p for trend 0.01 for both).

61. In a Costa Rican MI case-control study (Baylin *et al*, 2003), adipose tissue total *trans* FA were associated with increased risk of MI (p for trend 0.004) when adjustment for multiple factors was included. For individual FA, *trans* 18:2 and *trans* 16:1 were also significantly associated with increased risk of MI. Adipose tissue *trans* 18:1 was not associated with MI risk.

62. A case-control study of *trans* FA and risk of MI carried out by Clifton *et al* (2004) in an Australian population (1995-1997) included assessment of both adipose tissue *trans* FA and dietary *trans* FA (FFQ). In 1996, mid-way into the study, *trans*

FA were removed from margarine in Australia (January-March 1996). In 1995 and 1996, there was a positive association between dietary *trans* 18:1 intake from margarine and adipose tissue *trans* 18:1 levels, but this was not evident in 1997. Dietary intake data showed cases consumed 0.51 g/day more *trans* FA than controls (p =0.002), but *trans* FA intakes as % dietary energy did not differ between the groups. Subjects in the highest quintile of *trans* FA intake (g/d) had an OR for first MI of 2.25 (95% CI, 1.16-4.32; p for trend 0.01), but this was not independent of SFA intake. The proportion of adipose tissue total *trans, trans* 16:1, *trans* 18:1, *trans* 18:1, n-7 and n-9 were significantly higher in cases compared with controls before 1996 but not after this time. Logistic regression showed adipose tissue *trans* 18:1, n-7 was an independent predictor of first MI (p =0.03) when blood lipids, dietary intake and other adipose tissue FA were controlled for.

63. A further Costa Rican study (Colón-Ramos *et al*, 2006) investigated the relationship between *trans* FA status, as assessed by adipose tissue levels, and first case MI, both before (1994-1999, n =954) and after attempts to lower levels of industrial *trans* FA levels in foods (2000-2003, n =2638). The median values for quintiles of adipose tissue total *trans* FA, 18:1 *trans* FA and 18:2 *trans* FA were higher before 2000 than during the period between 2000 and 2003. Before reduction in *trans* FA, adipose tissue total *trans* FA were associated with MI with an OR of 3.28 (95% CI, 1.68-6.82; p for trend <0.001) with multiple adjustment, including dietary factors. This was not the case in the period 2000-2003, when the OR was 1.03 (95% CI, 0.75-1.42; p for trend 0.65). Similarly, adipose tissue *trans* 18:2 levels were associated with MI prior to the year 2000 with an OR of 4.76 (95% CI, 2.24-10.11; p for trend <0.001), but not over the time period 2000-2003 (p for trend 0.56). Adipose tissue *trans* 18:1 was not associated with MI before or after the year 2000, with or without multiple adjustments.

64. A Portuguese case-control study of risk of MI (Lopes *et al*, 2007) also included assessment of both adipose tissue *trans* FA and dietary *trans* FA. Findings from this study showed adipose tissue *trans* FA did not correlate with dietary intake as assessed by FFQ (r =-0.02). Using FFQ data, there was no significant difference in dietary intakes of total *trans* FA (% of total fat intake) between cases and controls (p =0.089); total *trans* FA intakes were not associated with risk of acute MI, either with crude or adjusted analysis, with an adjusted OR of 0.81 (95% CI, 0.48-1.137; p for trend 0.341). It should be noted that dietary SFA intake was not controlled for in this study. In contrast, adipose tissue *trans* FA levels were significantly lower in cases compared to controls (p <0.001) and showed a significant inverse association with risk of MI across tertiles, in both crude and adjusted analysis, with an adjusted OR of 0.04 (95% CI, 0.006-0.32; p for trend =0.001).

Prospective studies – CHD outcomes

65. Prospective data from the Nurses' Health Study and subsequent follow-ups, as well as the Cardiovascular Health Study (CHS) are considered in this section (Table 2A, Annex 2).

66. Since 1980, the relationship between *trans* FA intake and CHD incidence has been reported several times during the follow-up of the Nurses' Health Study, a cohort of ~85,000 females recruited without diagnosed CVD, diabetes or elevated cholesterol levels at baseline. Willett *et al* (1993) first reported findings after an 8 year follow up with 431 new CHD incidences recorded (non fatal MI or death from CHD). Dietary total *trans* FA (% energy) was positively associated with risk of CHD. The trend was influenced by adjustment for multiple CHD risk factors and dietary fat intake, with an adjusted OR of 1.67 (95% CI, 1.05-2.66; p for trend 0.002). This study showed the CHD risk attributed to dietary *trans* FA was entirely accounted for by isomers from vegetable fat (RR 1.78; 95% CI, 1.12-2.83; p for trend 0.009) rather than isomers from animal fat (RR 0.59; 95% CI, 0.30-1.17; p for trend 0.230).

67. After 14 year follow up of the Nurses' Health Study (n =80,082) with 939 incident CHD cases reported (non fatal MI or fatal CHD), the association between dietary *trans* FA (FFQ) and CHD risk, remained. With multiple adjustment, the OR associated with the highest quintile of intake (2.9% energy as *trans* FA) was 1.53 (95% CI, 1.16-2.02; p for trend 0.002) (Hu *et al*, 1997).

68. In the most recent analysis of the Nurses' Health Study (n =78,778), based on a 20 year follow up with 1766 incident CHD cases reported, dietary *trans* FA were once again associated with increased CHD risk (Oh *et al*, 2005). The OR for the highest quintile versus the lowest quintile of intake (a difference of 1.5% dietary energy as *trans* FA), with multiple adjustment for factors including dietary fat intakes, was 1.33 (95% CI, 1.07-1.66; p for trend 0.01). Additional sub-group analysis, with multiple adjustment, showed the association was stronger in women less than 65 years compared to older women (OR 1.50, 95%CI, 1.13-2.00; p for trend 0.01) and in women with a body mass index (BMI) < 25 kg/m^2 (OR 1.53, 95%CI, 1.09-2.15; p for trend 0.02). However, in a subset of women diagnosed with type 2 diabetes mellitus since taking part in the Nurses' Health Study (n = 5672), *trans* FA intake did not show a positive relationship with CHD risk (non fatal MI and fatal CHD) following age or multivariate adjustment (Tanasescu *et al*, 2004).

69. A case-control study nested within the CHS (Lemaitre *et al*, 2006) identified cases that experienced fatal ischemic heart disease (IHD) between June 1992 and June 1998. Plasma phospholipids were determined in fasting blood samples collected approximately 3 y before the event. Total plasma *trans* FA and plasma *trans* 16:1 were not associated with IHD in multivariate analysis, with adjusted ORs of 0.94 (95% CI, 0.65-1.34) and 0.95 (95% CI, 0.64-1.42), respectively. *Trans* 18:1 was associated with a reduced risk of IHD with an adjusted OR of 0.38 (95% CI, 0.17-0.86). Levels of *trans* 18:2 were associated with increased risk of IHD (OR 4.54; 95%

CI, 1.83-11.20) when adjustment for *trans* 18:1 was included. In an analysis restricted to the 95 cases with sudden cardiac death, higher *trans* 18:2 was associated with a greater than 2 fold increased risk (OR 2.34, 95% CI, 1.27-4.31). Higher *trans* 18:1 was associated with lower risk of sudden cardiac death (OR for levels in highest quintile 0.18, 95%CI, 1.01-2.37).

70. Sun *et al* (2007a) evaluated the relationship between *trans* FA intake, as assessed by levels of *trans* FA in erythrocytes and plasma, and CHD risk (non fatal MI and fatal IHD) in a case-control study nested in the Nurses' Health Study (cases of incident IHD n =166, controls n =327). Total *trans* FA content in erythrocytes were significantly correlated with dietary *trans* FA intake, as assessed by FFQ, with multiple adjustment (r=0.44, p <0.01) (Sun *et al*, 2007a). Total erythrocyte *trans* FA and a number of specific isomers were significantly greater in cases compared to controls (p <0.05). After adjustment for known risk factors for CHD and for long chain n-3 and total n-6 FA, the RR for total *trans* FA erythrocyte content, erythrocyte total *trans* 18:1 and erythrocyte total *trans* 18:2 remained significantly associated with risk of CHD. These were 3.3 (95% CI, 1.5-7.2; p for trend <0.01), 3.1 (95% CI, 1.5-6.7; p for trend <0.01) and 2.8 (95% CI, 1.2-6.3; p for trend <0.01), respectively. These risk ratios were reported to be attenuated with the addition of the LDL-C to HDL-C ratio to the multivariate models. The RR attributed to total erythrocyte *trans* FA was reduced to 2.2 (95% CI, 0.9-5.4; p for trend =0.07) and was no longer statistically significant. The RR for erythrocyte *trans* 18:1 was also reduced to 2.2 (95% CI, 1.0-5.2; p for trend 0.02) but retained statistical significance. The data indicate the effects of *trans* FA are mediated to some extent via their well established actions on plasma lipoproteins. However, at least for some of the isomers, other pathophysiological effects appear to be involved which are independent of the lipoprotein effects.

71. In a further analysis of this nested control study, Sun *et al* (2007b) reported the relationship between erythrocyte and plasma *trans* 16:1, n-7 FA intake and CHD risk (non fatal MI and fatal IHD). Analysis of control samples showed *trans* 16:1, n-7 in erythrocyte membranes and plasma correlated positively with average dairy fat intake (1986-1990), as assessed by semi quantitative FFQ, when adjustments for various factors including age, BMI and menopausal status were made (r= 0.32, p <0.01 and r =30, p <0.01, respectively). There were no differences in erythrocyte or plasma *trans* 16:1, n-7 between cases and controls. The RRs for *trans* 16:1, n-7 in plasma and erythrocytes, with adjustment for various risk factors for IHD, were not statistically significant.

72. Data from these case-control and prospective cohort studies which evaluated dietary *trans* FA intake are summarised in Figure 2. Data which evaluated the effects of *trans* FA from different dietary sources are summarised in Figure 3. Data which evaluated the CHD risk associated with different adipose tissue *trans* isomers is summarised in Figure 4.

Figure 2. Risk of CHD from case-control and prospective epidemiological studies that evaluated dietary intake of *trans* FA.

Trans FA intake in the UK was 1.2% of food energy or 2.04 g/day for women (white arrow) and 1.2% of food energy or 2.91 g/day for men (black arrow) in NDNS 2000/01 (Henderson *et al*, 2003) and in 2007 is estimated to be, for all adults 19-64 years, 1% of total energy or 2.07 g/day (grey arrow). Risk of CHD is plotted as the RR (fully adjusted for confounding factors), with bars showing ± 95% confidence interval for intake ranges above reference (RR = 1) in each study. Case-control studies are shown as white symbols. Data from Hu *et al* (1997) and Oh *et al* (2005) were converted from % of total energy to g/day based on an energy intake of 1700 kcal/day (both studies only included women). Oomen *et al* (2001) was excluded from the below graph due to the much higher *trans* FA intakes of the cohort (middle tertile = 3.99 % or 10.6 g/day, RR 1.34; upper tertile = 4.86% or 13 g/day, RR = 2.00).

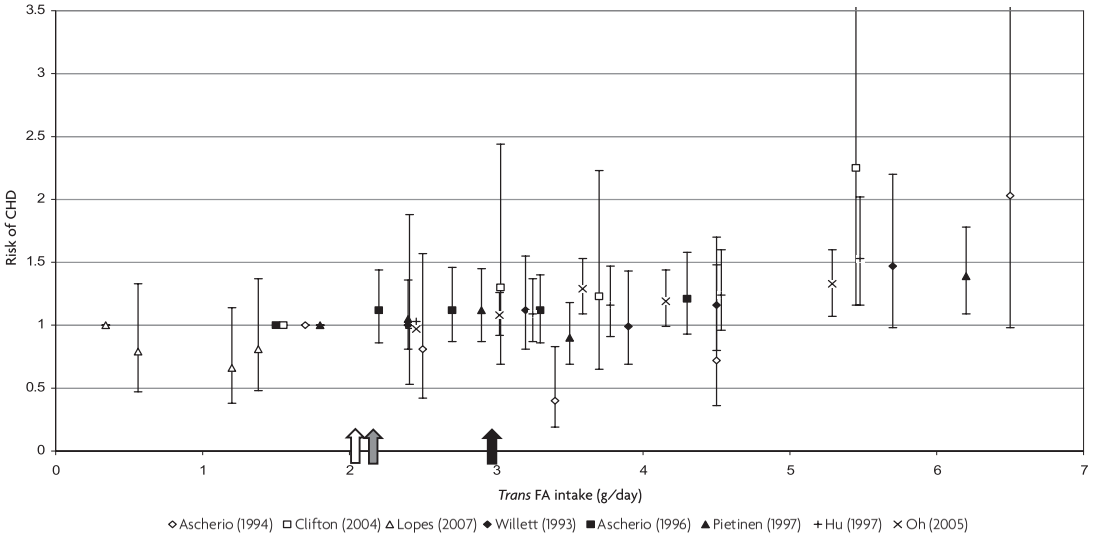


Figure 3. Risk of CHD from prospective epidemiological studies that evaluated dietary sources of *trans* FA

Trans FA intake in the UK was 1.2% of food energy or 2.04 g/day for women (white arrow) and 1.2% of food energy or 2.91 g/day for men (black arrow) in NDNS 2000/01 (Henderson *et al*, 2003) and in 2007 is estimated to be, for all adults 19-64 years, 1% of food energy or 2.07 g/day (grey arrow). *Trans* FA isomers from animal sources are shown as black symbols and *trans* FA isomers from vegetable oil sources as white symbols. Risk of CHD is plotted as the RR (fully adjusted for confounding factors), with bars showing ± 95% confidence interval for intake ranges above reference (RR = 1) in each study.

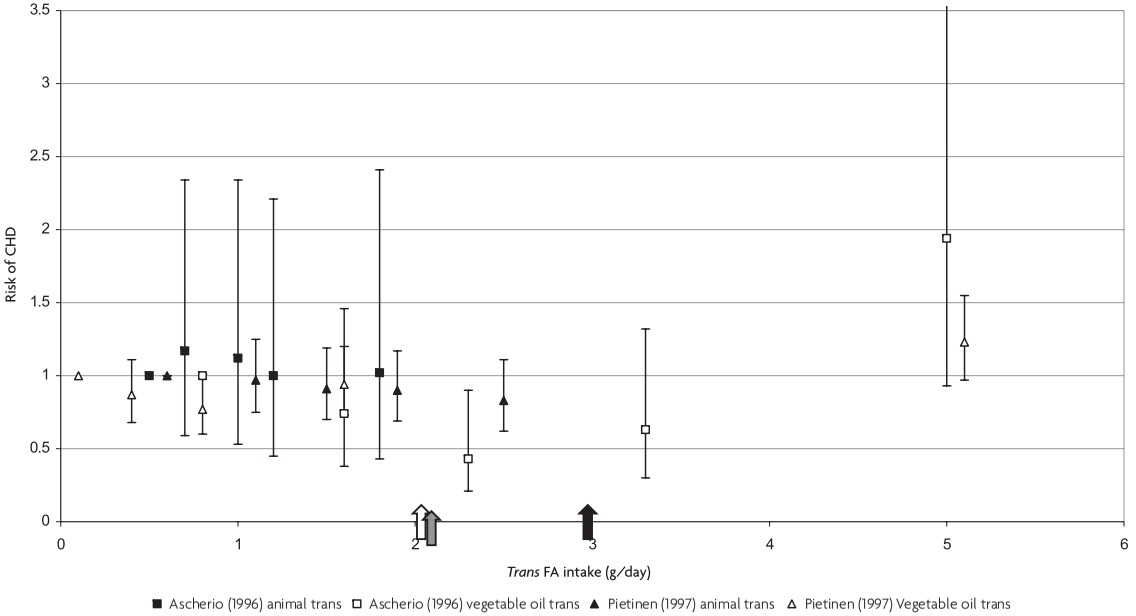
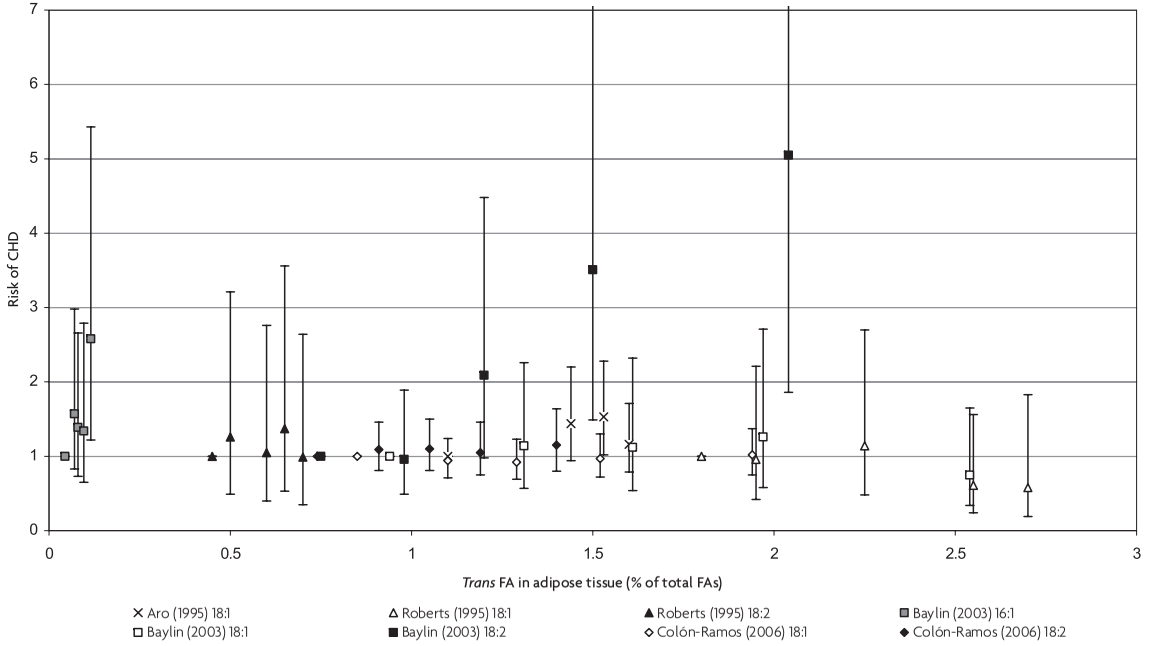


Figure 4. Risk of CHD from case-control studies that evaluated the level of *trans* FA isomers in adipose tissue

Trans 16:1 isomers are shown as grey symbols, *trans* 18:1 isomers are shown as white symbols and *trans* 18:2 isomers as black symbols. The data shown from Colón-Ramos *et al* (2006) was from their collection period 2000-2003. Risk of CHD is plotted as the RR (fully adjusted for confounding factors), with bars showing ± 95% confidence interval for intake ranges above reference (RR = 1) in each study.



Summary – epidemiology of trans FA and CHD risk

73. Of 9 case-control studies, 3 have assessed associations between dietary *trans* FA levels ranging from ~0.34-6.5 g/d and risk of CHD (Ascherio *et al*, 1994; Clifton *et al*, 2004; Lopes *et al*, 2007). Two showed positive associations (Ascherio *et al*, 1994; Clifton *et al*, 2004) but in one (Clifton *et al*, 2004) this did not remain when the data were adjusted for SFA intakes. Seven of the studies have measured biomarker levels of *trans* FA (Siguel & Lerman, 1993; Aro *et al*, 1995; Roberts *et al*, 1995; Van de Vijver *et al*, 1996; Baylin *et al*, 2003; Colón-Ramos *et al*, 2006; Lopes *et al*, 2007). Three of these provide evidence of higher risk associated with increased *trans* FA levels (Siguel & Lerman, 1993; Baylin *et al*, 2003; Colón-Ramos *et al*, 2006). In one case (Colón-Ramos *et al*, 2006) this was no longer observed when *trans* FA from vegetable oil sources were removed from the population diet. Three showed no association (Aro *et al*, 1995; Roberts *et al*, 1995; Van de Vijver *et al*, 1996) and one showed an inverse association between adipose tissue *trans* FA levels and CHD risk (Lopes *et al*, 2007).

74. Of the 10 prospective studies which have reported since 1993, 6 reports are based on the Nurses' Health Study. Three of the 10 reports have not observed a significant relationship between *trans* FA and CHD (Ascherio *et al*, 1996; Tanasescu *et al*., 2004; Sun *et al*, 2007b). Five studies have reported significant positive associations between dietary *trans* FA and CHD (Willet *et al*, 1993; Hu *et al*, 1997; Pietinen *et al*, 1997; Oomen *et al*, 2001; Oh *et al*, 2005) and 2 studies have reported positive associations between biomarker *trans* 18:2 levels and risk of CHD (Lemaitre *et al*, 2006; Sun *et al*, 2007a). One of these studies reported an inverse association between *trans* 18:1 and CHD risk (Lemaitre *et al*, 2006) and the other reported a positive association (Sun *et al*, 2007a). Of the 5 studies which have reported significant associations for dietary *trans* FA and CHD, 3 are outputs from the Nurses' Health Study (Willet *et al*, 1993; Hu *et al*, 1997; Oh *et al*, 2005) where repeat dietary assessments and updated food composition data make it unlikely that the findings reflect misclassification of diet due to changes in *trans* FA levels in the food chain which have occurred since the baseline measurements were made. The most recent output from the Nurses' Health Study (Oh *et al*, 2005) reported an RR of 1.33 (95% CI, 1.07-1.66; p for trend 0.01) with quintiles of intake ranging from 1.3- 2.8 % dietary energy. This range of intakes is slightly higher than, but compares reasonably well with, the current range of UK intakes (0.5-2.1% dietary energy in men, 0.4-2.1% dietary energy in women), the data for which are based on the 2001/1 NDNS dietary surveys and updated food composition figures (Henderson *et al*, 2003). The study of Oomen *et al* (2001) only reported for tertiles of intake (<3.11, 3.11-4.86, > 4.86 % dietary energy) and is less valuable for assessing likelihood of risk in a UK population. The other dietary study (Pietinen *et al*, 1997) which reported positive associations between total *trans* FA intake and CHD risk (OR 1.39, 95% CI, 1.09-1.79; p for trend 0.004) reported intakes as g/d with the range 1.8-6.2 g/d. This compares reasonably well with current average intakes for the UK (2.91 g/d in men, 2.04 g/d in women).

75. There have not been any prospective studies published since the assessment made by EFSA in 2004. However, the updated output from the main Nurses' Health Study cohort has confirmed a significant association between *trans* FA intakes and CHD (Oh *et al*, 2005) and this relationship is supported by reports from 2 prospective studies which demonstrate positive associations between biomarker *trans* FA levels and risk of CHD (Lemaitre *et al*, 2006; Sun *et al*, 2007a). The prospective epidemiology therefore continues to provide consistent evidence for a moderate impact of dietary *trans* FA on risk of CHD.

76. Regarding effects of specific dietary sources of *trans* FA, 3 of the prospective studies have addressed this issue with respect to possible differences between effects of *trans* FA of vegetable oil and animal origin (Willet *et al*, 1993; Pietinen *et al*, 1997; Oomen *et al*, 2001). Two studies showed positive associations for *trans* FA from vegetable oil sources and CHD risk (Willet *et al*, 1993; Pietinen *et al*, 1997). Both of these studies reported an inverse association between animal *trans* FA intake and CHD risk; Willet *et al* (1993) reported a non significant adjusted OR of 0.59 (95% CI, 0.30-1.17, p for trend 0.230), Pietinen *et al* (1997) reported a significant adjusted OR of 0.83 (95% CI, 0.62-1.11, p for trend 0.035). The other study reported no distinction in the association between *trans* FA from animal and vegetable oil sources and risk of CHD (Oomen *et al*, 2001).

77. Some additional information may be obtained from biomarker measurements of specific isomers in adipose tissue and blood, since it has been proposed that *trans* 18:2 levels may provide information on fats of vegetable oil origin, whilst those of 16:1 may provide an index of *trans* isomers of animal origin. The prospective studies which have evaluated biomarker levels of specific isomers (Lemaitre *et al*, 2006; Sun *et al*, 2007a; Sun *et al*, 2007b) demonstrated significant positive associations between *trans* 18:2 and risk of CHD. However, Lemaitre *et al* (2006) reported an inverse association between levels of *trans* 18:1 and CHD risk, whereas Sun *et al* (2007a) reported a positive association for this relationship. Both studies reported no association between *trans* 16:1 and CHD risk (Lemaitre *et al*, 2006; Sun *et al*, 2007b).

78. Biomarker data from case-control studies (which may be considered to be less subject to bias than dietary data) for specific *trans* isomers is inconsistent. Siguel and Lerman (1993) reported a positive association between *trans* 18:2 and MI risk, as did Colón-Ramos *et al* (2006) at a time before attempts were made to lower levels of industrial *trans* FA in foods. In contrast, Van de Vijver (1996) and Roberts *et al* (1995) reported no association between plasma *trans* 18:2 and CHD risk. Five case-control studies based on biomarker *trans* FA measurements reported no association between *trans* 18:1 and CHD risk (Siguel & Lerman, 1993; Aro *et al*, 1995; Roberts *et al*, 1995; Van de Vijver *et al*, 1996; Colón-Ramos *et al*, 2006). Two studies reported positive association between biomarker *trans* 16:1 and CHD risk (Siguel & Lerman, 1993; Baylin *et al*, 2003). In contrast, Van de Vijver (1996) reported no association between plasma *trans* 16:1 and CHD risk.

79. The epidemiological studies provide some evidence to suggest a more consistent positive association between biomarker levels of *trans* 18:2 and risk of CHD than for the other *trans* isomers. However, the data are not sufficiently robust to enable definitive conclusions to be drawn concerning the likelihood, or not, of more adverse effects of *trans* FA of vegetable oil rather than animal origin.

Randomised controlled trials of risk biomarkers for CHD

Possible mechanism for trans FA effects on CHD risk

80. The mechanism by which the major fatty acid classes (SFA, MUFA and PUFA) differentially influence circulating levels of LDL-C is now well established (Spady & Dietschy, 1985). The main regulatory site is the hepatic LDL receptor which controls the uptake of LDL-C from the circulation into the liver, where oxidation and removal of excess LDL-C takes place. SFA have been shown to reduce the number of LDL receptors expressed on the surface of hepatic cell membranes, thereby reducing the uptake and removal of LDL-C from the circulation. PUFA and MUFA have opposite effects so that when diets high in these fatty acids are fed, LDL receptor number is increased resulting in greater removal of LDL-C and a reduction in levels of LDL-C in the circulation. The molecular mechanisms which regulate this pathway are also well established, involving fatty dependent alteration in the cholesterol content of hepatic endoplasmic reticulum membranes, which results in activation or inhibition of Steroid Receptor Element Binding Protein 1c (SREBP1c). SREBP1c is a transcription factor that controls the promoter region of the LDL receptor causing increased or decreased expression of the protein in response to altered membrane cholesterol content. *Trans* FA appear to regulate the LDL receptor in a similar manner to that of the SFA (Hayashi *et al*, 1993), so that when diets containing *trans* FA are fed, membrane cholesterol content increases (Niu *et al*, 2005) and there is down regulation of the LDL receptor and increased circulating levels of LDL-C. The mechanism whereby *trans* FA lead to the reduction in HDL-C is less well established but appears to involve activation of a protein, cholesterol ester transfer protein (CETP) (van Tol *et al*, 1996; Khosla *et al*, 1997). CETP transfers cholesteryl esters from HDL to lipoproteins of lower density (LDL and very low density lipoprotein (VLDL)). When CETP activity is increased there may be excess transfer of cholesterol from HDL, leading to its catabolism and removal from the circulation. This process appears to be particularly active during the postprandial (fed) state, so that the overall fat content of a meal can determine the degree of activation of CETP (Gatto *et al*, 2003).

Fasting lipoprotein concentration and trans FA- data prior to 2004

81. There is conclusive evidence from epidemiological studies that raised circulating concentrations of LDL-C and lowered concentrations of HDL-C, as well as increased total to HDL-C ratio, are linked with increased risk of CHD; all 3 are regarded as robust markers of CHD risk. In addition, a large body of data exists

from RCTs in which the effects of *trans* FA (largely *trans* MUFA) have been compared with isoenergetic amounts of other fatty acid classes and of carbohydrate. These studies support the conclusion that *trans* FA have adverse effects on fasting lipoprotein concentrations. The LDL-C raising effects of *trans* FA are approximately equivalent to those of the SFA. However, the major difference between the SFA and *trans* FA are that the latter are the only fatty acid group which have been shown to reduce HDL-C concentrations. For this reason, *trans* FA have the most adverse effect on serum lipoproteins of all FA.

82. The RCT studies have been extensively reviewed in the literature (Katan *et al*, 1995; Mensink *et al*, 2003) as well as in recent expert reviews (WHO & FAO, 2003; EFSA 2004). The meta-analysis of Mensink *et al* (2003) concluded that there are slight differences in the LDL-C raising effects of SFA and *trans* FA, with potency in the order: lauric acid › myristic › *trans* FA= palmitic acid › stearic (neutral). In this comparison *cis*-MUFA were estimated to have LDL-C lowering effects which were slightly less than those of *cis* -PUFA. As a consequence of their reciprocal effects on LDL-C and HDL-C, *trans* FA increase the total cholesterol to HDL-C ratio. The data have been assessed and presented in detail in the review conducted by EFSA (2004) and will not be reconsidered here.

83. Although the first controlled intervention trial to evaluate the effects of *trans* FA on serum lipoproteins provided levels of 11% dietary energy, which is in excess of that found in habitual diets (Mensink & Katan, 1990), studies conducted since then have provided more modest levels of intake, ranging from approximately 1-10% dietary energy (Judd *et al*, 1994; Judd *et al*, 1998; Lichtenstein *et al*, 1999). The meta-analyses of the RCTs (Katan *et al*, 1995; Mensink *et al*, 2003) have demonstrated dose-dependent linear associations between levels of *trans* FA in the intervention diets and serum concentrations of LDL-C and HDL-C over the range 1- 10% dietary energy. There are few studies which have compared levels of intake at the lower end of this range (0.5-3.0% dietary energy), which is the reduced range of habitual intakes now observed in most countries. However, there is no reason to consider that the relationship between dose and impact on LDL-C or HDL-C are different at these lower levels of intake, so that efforts to reduce *trans* FA levels below 2% of food energy (the current UK recommendation) are likely to result in beneficial effects on the atherogenicity of the lipoprotein profile.

84. As well as adverse effects on LDL-C and HDL-C, studies evaluated by EFSA (2004) showed substitution of *trans* MUFA for other FA resulted in a raising of fasting triacylglycerol (TAG) levels. Since fasting TAG is positively associated with risk of CHD, this is an additional mechanism by which *trans* FA may increase the atherogenicity of the lipid and lipoprotein profile.

85. Lipoprotein (a) [Lp (a)] is an LDL particle with an additional apoprotein attached (apoprotein (a)); high concentrations of Lp(a), which are largely genetically determined, are linked with increased risk of CHD. EFSA (2004) evaluated the

evidence relating to effects of *trans* FA on Lp(a) and concluded that whilst there is some evidence to suggest *trans* FA increase Lp(a), the data are not consistent and effects may be confined to those individuals with initially high levels.

Fasting lipoprotein concentrations- randomised controlled trials conducted since 2004

86. In considering the RCT trials published since 2004, this review has focused on a number of key aspects of the data: i) the extent to which the findings are consistent with the body of evidence reviewed by EFSA (2004); ii) whether there are additional data for effects of *trans* FA within the range 0.5-3.0% dietary energy; and iii) whether there are any data which have directly compared effects of *trans* FA from vegetable oil versus animal origin (Table 3A, Annex 2).

87. In a double-blind, randomised cross-over study, Han *et al* (2002) compared the effects of 3 diets; a soybean oil diet (0.6% energy as *trans* FA), a soybean oil stick margarine diet (6.7% energy as *trans* FA) and a butter diet (1.3% energy as *trans* FA). Total cholesterol, VLDL-C, LDL-C and HDL-C levels were significantly different between all diet groups ($p < 0.05$ for all). The findings were broadly consistent with previous reports based on vegetable oils that have undergone hydrogenation, with higher levels for all cholesterol fractions except HDL after hydrogenation.

88. Lichtenstein *et al* (2003) reported the effects of 6 diets (20% energy as soybean oil, semi-liquid margarine, soft margarine, partially hydrogenated soybean oil as shortening or margarine sticks and butter), consumed for 5 weeks. Significant differences in TAG, LDL-C and total cholesterol concentrations were noted between diets; the findings were broadly consistent with what has previously been reported for effects of oils which have undergone varying degrees of hydrogenation. A further report from this study (Mauger *et al*, 2003) reported a dose-dependent effect of *trans* FA on LDL particle size, which was decreased with increasing *trans* FA intakes ($p < 0.001$).

89. Dyerberg *et al* (2004) completed an 8 week RCT that was designed to investigate the impact of *trans* FA and n-3 PUFA intake on markers of cardiovascular risk. Experimental fats were incorporated into bakery products and the effects of a control diet (0.9% energy as *trans* FA, 15.7% energy as SFA), a *trans* FA diet (6.8% energy from *trans* FA, 10.3% energy SFA) and a n-3 PUFA diet (0.9% energy as *trans* FA, 12.3% energy as SFA) were evaluated. The *trans* FA diet caused a significant reduction in HDL-C levels compared to control (-0.06 vs. 0.03 mmol/l, $p < 0.01$) but had no significant effects on total cholesterol, LDL-C or TAG.

90. The reports described above do not provide information that enables conclusions regarding effects of small differences in *trans* FA intakes at total intake levels between 0.5-3.0% to be drawn.

91. A double blind, randomised parallel intervention trial (n =42 healthy men) carried out by Tholstrup *et al* (2006) compared 5 weeks consumption of a butter rich in *trans* FA (18:1, n-7 and n-9; ~2.2% energy as *trans* FA) with a butter containing lower levels of *trans* FA (~0.4% energy as *trans* FA). Total cholesterol and HDL-C were significantly reduced following the high *trans* FA diet compared to the control diet ($p = 0.05$ and $p = 0.002$, respectively), although the ratio between total:HDL-C or TAG levels did not differ significantly between diet groups. Although this study compared effects of *trans* FA at intake in the range of interest (0.5-3.0%), differences in FA levels other than *trans* FA in the experimental butters do not allow clear conclusions to be drawn.

92. Lichtenstein *et al* (2006) conducted an RCT comparing 5 different types of fats; soybean oil, low saturated soybean oil, high oleic acid soybean oil, low linolenic acid soybean oil and partially hydrogenated soybean oil (PHSO) (2.5% energy as *trans* FA). Total cholesterol was significantly higher following the PH soybean oil diet compared to all other diets investigated ($p < 0.05$) and LDL-C and total:HDL-C levels were highest with this oil, significantly so ($p < 0.05$) for all except the comparison with low α-linolenic acid soybean oil.

93. Vega-López *et al* (2006) compared the effects of 4 diets rich in soybean oil (0.55% energy as *trans* FA), palm oil (0.60% energy as *trans* FA), canola oil (0.98% energy as *trans* FA) and PHSO (4.15% energy as *trans* FA). Total cholesterol and LDL-C levels were significantly higher following the PHSO diet compared to the soybean oil and the canola oil diets ($p < 0.05$).

94. Sundram *et al* (2007) carried out an RCT which compared the effects of a palm olein diet (12% energy from palmitic acid), a PHSO diet (3.21% energy as *trans* FA) and a diet based on interesterified fat (12.5% energy from stearic acid), with each diet consumed for 4 weeks. HDL-C concentration was significantly lower, and LDL-C concentration was significant greater, following the *trans* FA diet compared with the palm olein diet ($p < 0.001$ and $p < 0.05$, respectively).

95. Although Lichtenstein *et al* (2006), Vega-López *et al* (2006) and Sundram *et al* (2007) compared effects of *trans* FA at intake in the range of interest (0.5-3.0%), differences in FA levels other than *trans* FA in the experimental diets do not allow clear conclusions to be drawn.

96. A randomised, double-blind cross-over study by Mensink (2007) compared the effects of two diets, comparable in physical characteristics, but with greater levels of stearic acid (+1.3% energy), *cis* 18:1 (+2.9% energy), α-linolenic acid (+0.1% energy) and *trans* FA (+0.5% energy) and lower levels of palmitic acid (-4.2% energy) and alcohol (-1.2% energy) in one diet compared with the other diet (all differences $p < 0.05$). Total cholesterol, LDL-C and HDL-C levels and total:HDL-C were significantly lower following the *trans* FA-containing diet compared to the palmitic-rich diet. Although effects of *trans* FA at levels relevant to the range of

UK intake were investigated in this study, differences in FA levels other than *trans* FA in the experimental diets do not allow clear conclusions to be drawn.

97. An evaluation of the relative effects of *trans* FA of vegetable oil origin (mainly *trans* 18:1, n-9) compared with *trans* FA of animal origin (mainly *trans* 18:1, n-7) has not previously been possible due to unavailability of sufficient amounts of *trans* 18:1, n-7 that can be fed in an intervention diet without introducing differences in other dietary FA. However, Chardigny *et al* (2007) have reported details of an RCT that has compared effects of 2 diets that provided ~4% energy as *trans* FA. One diet (IP) was rich in industrially produced vegetable oil (*trans* 18:1, n-9), the other diet (NatS) was rich in animal *trans* FA (*trans* 18:1, n-7). Gender specific effects in lipoprotein response to the test diets were reported for HDL-C, LDL-C and total cholesterol ($p < 0.05$ for all). In women, the NatS *trans* FA diet caused significant increases in total, HDL-C and LDL-C concentrations compared to the IP *trans* FA diet, whereas no differences were observed in men. Although no significant treatment by gender interaction was reported for TAG effects, levels were significantly higher following the NatS *trans* FA diet compared to the IP *trans* FA diet in women, but not in men. The data show *trans* FA sourced naturally and from industrial processing are both associated with risk factors for coronary heart disease. In the case of NatS *trans* FA there are relatively adverse effects on total, LDL-C and TAG compared with IP *trans* FA. However, in the case of IP *trans* FA there are relatively adverse effects on HDL-C compared with NatS *trans* FA. Why such effects should be evident in women but not men is not clear, but may need to be given further consideration in light of the fact that much of the epidemiological data is derived from a large prospective study in women (Nurses' Health Study).

Other circulating risk biomarkers for CHD

Postprandial TAG (lipemia) – randomised acute meal studies

98. Elevated postprandial TAG responses to fat containing meals has been shown to be associated with increased risk of CHD, and may be mediated via direct effects of TAG containing particles on the atherogenic process, or via indirect effects on other circulating lipoproteins (Williams, 1997). A number of studies have been conducted to assess the acute impacts of meals of varying *trans* FA levels on circulating postprandial TAG responses (Table 4A, Annex 2).

99. Sanders *et al* (2000) investigated the effects on postprandial lipemia of 5 high fat meals (enriched with medium chain FA, palmitic acid, stearic acid, *trans* 18:1, n-9 or *cis* 18:1, n-9) and a sixth low fat meal in 16 healthy subjects. Postprandial TAG at 3 hours following the *trans* FA meal was significantly greater than that recorded after the stearic acid, medium chain fatty acid and low fat meals (all, $p < 0.001$). The level of *trans* FA fed in the meal (~25% energy as *trans* FA) is markedly higher than might be found in typical mixed meals.

100. Tholstrup *et al* (2001) compared effects of 6 test meals high in stearic, palmitic, oleic and linoleic acids, *trans* FA 18:1 and palmitic plus myristic acids There were no significant differences reported between effects of the *trans* FA meal and the other 5 test meals.

101. Gatto *et al* (2003) compared the effects of two meals, which were identical except one contained 10% energy as *trans* FA 18:1, the other 10% energy as *cis* 18:1. There were no differences in postprandial lipid responses between meals. Rate of cholesteryl ester (CE) transfer between lipoproteins was increased on consumption of both meals, but this increase occurred to a significantly greater extent (28%) following the *trans* FA meal compared to the *cis* 18:1 meal ($p < 0.0001$).

102. Lefevre *et al* (2005) compared the acute effects of two test meals over a period of 16 days, during which background dietary composition was controlled. In the test meals 20% energy was provided in the form of MUFA, either all from *cis* 18:1, n-9 or else 10% energy from *cis* 18:1, n-9 as and 10% energy from *trans* 18:1, n-9. Although replacement of *cis* 18:1 for *trans* 18:1 tended to produce a lower postprandial TAG response, this did not reach statistical significance when baseline values were accounted for.

103. In a study carried out by Cantwell *et al* (2006), the acute effects of partially hydrogenated fish oil (23% energy as *trans* FA) on postprandial lipemia were compared with the effects of palm oil and lard in 8 healthy men. There were no significant differences in the postprandial lipid response between the three test meals.

104. The data provide no evidence that (at levels likely to be consumed in single meals) *trans* FA have adverse effects on postprandial lipemia. There may be effects of *trans* FA on transfer of lipid moieties between particles during the postprandial period, which may have implications for the composition and subsequent atherogenicity of some lipoprotein particles. However, there is insufficient information in the literature to draw any conclusions regarding this putative mechanism at the present time.

Oxidative stress

105. As reported by EFSA (2004), none of the studies which have assessed the *in vitro* susceptibility of LDL particles to undergo oxidation following consumption of high *trans* FA diets have revealed any adverse effects of *trans* FA on LDL oxidisibility. The relevance of *in vitro* findings for the *in vivo* situation is uncertain. Findings from 2 recent studies that have investigated the effects of *trans* FA on markers of oxidative stress are considered (Table 5A, Annex 2).

106. Kuhnt *et al* (2006) conducted a parallel trial in healthy subjects (n =24) which compared the effects of 6 weeks supplementation of a *trans* FA rich oil with a control, *trans* FA free, oil. The *trans* FA diet caused a significant increase in urinary

8-iso-PGF2· (an *in vivo* marker of free radical induced lipid peroxidation) over time which was also significantly higher than that reported in the control group. There were no effects of the diets on other markers of oxidative stress or DNA damage. However, plasma levels of α-tocopherol were significantly lower in the *trans* FA group compared to the control.

107. A double blind, randomised, parallel trial carried out by Tholstrup *et al* (2006) which compared the effects of two diets (2.2% energy as *trans* FA vs. 0.4% energy as *trans* FA) found no differences between the test diets in urinary 8-isoPGF2· levels ($p = 0.93$).

Haemostatic function

108. Seven studies that were not considered in the EFSA 2004 report have described effects of *trans* FA on haemostatic function (Table 6A, Annex 2).

109. Turpeinen *et al* (1998) conducted a randomised parallel study comparing the effects of 2 diets (~9% energy as stearic acid vs. ~9% energy as *trans* 18:1) in 80 healthy subjects. No significant differences in *in vivo* platelet aggregation, as assessed by β-thromboglobulin and 2,3-dinor-6-keto-PGF1· levels, or *in vitro* ADP-induced platelet aggregation or thromboxane B2 production were reported between diets. Collagen-induced platelet aggregation was significantly increased after the stearic acid diet ($p < 0.05$) compared to the *trans* FA diet.

110. In a randomised cross-over study carried out at 3 European centres in 88 male subjects, Armstrong *et al* (2000) reported no significant differences in collagen induced platelet aggregation, platelet thromboxane production, plasma fibrinogen levels, factor VII, activated factor VII or plasminogen activator inhibitor-1 (PAI-1) activity levels between a low *trans* 18:3 diet and a higher *trans* 18:3 (0-0.6% energy as *trans* 18:3).

111. In an RCT carried out by Sanders *et al* (2003), a diet composed of ~10% energy as *trans* 18:1 was investigated alongside 2 other diets for which *trans* 18:1 was replaced by carbohydrate or *cis* 18:1. No significant differences in fasting fibrinogen, D-dimer (marker of fibrin degradation), factor VII coagulant concentrations or PAI-1 and tissue plasminogen activator (tPA) activity levels were reported between diets.

112. Tholstrup *et al* (2003) compared the postprandial effects of 6 meals with 50.6% energy as fat (comprising 41-47% from stearic, palmitic, oleic, linoleic, *trans* 18:1 or a mix of palmitic and myristic acids) on haemostatic factors in 16 young men. Levels of activated factor VII were lower after the stearic meal compared to the *trans* 18:1 meal ($p = 0.017$). No significant differences in postprandial factor VII coagulation activity, PAI-1 levels or activity or tPA activity were recorded between the *trans* 18:1 meal and the other meals investigated.

113. Baer *et al* (2004) carried out a randomised cross-over trial which investigated the effects of 5 diets. Diets provided 39% energy from fat, 8% of this fat energy was composed of *cis* 18:1, *trans* 18:1, stearic acid, a 50:50 mix of *trans* 18:1 and stearic acid or 12:0-16:0 SFA. A sixth diet was investigated for which 8.5% energy from fat was replaced by carbohydrate. Fibrinogen levels were significantly greater following the stearic acid diet compared with the *trans* FA diet. The high levels of *trans* FA investigated in this study (8%) compared with the average dietary *trans* FA intake in the UK (1.0-1.2% energy) should be noted.

114. Pedersen *et al* (2005) compared the effects of a PHSO diet (7% energy as *trans* FA) with a palm oil diet (11.2% energy from SFA) and another diet rich in PUFA (10.2% energy as PUFA). Effects on haemostatic function were assessed in a subset of subjects (n =9 females) that took part in this randomised, cross-over study. No significant differences in plasma fibrinogen levels, factor VII activity, or PAI-1 and tPA levels or activity were reported between diet groups.

115. In a double blind, randomised, parallel trial carried out by Tholstrup *et al* (2006) which compared the effects of two butters, one rich (~2.2% energy) and one low (~0.4% energy) in *trans* 18:1, n-7 and n-9, no differences between diets for factor VII coagulant activity or PAI-1 concentration were reported.

116. These interventions studies are largely consistent and do not provide strong evidence that *trans* FA from partially hydrogenated oils have an impact on haemostatic function at intake levels within the range, or above those, of the average UK intake. These findings are in line with the conclusions of EFSA. There is insufficient evidence to evaluate the relative effects of *trans* FA from vegetable oil origin with those of animal origin on haemostatic function.

Blood pressure and endothelial function

117. Since the report of EFSA (2004), 5 studies have reported effects of *trans* FA on blood pressure and/ or endothelial function (Table 7A, Annex 2).

118. In a randomised cross-over trial carried out in 29 healthy subjects, de Roos *et al* (2001) showed a *trans* FA diet (9.2% energy *trans* 18:1) significantly reduced flow mediated dilation of the brachial artery (-1.8%, p =0.015) compared to a diet for which *trans* 18:1 was replaced by SFA. In a subsequent acute meal study (n =21 men), de Roos *et al* (2002) reported no significant difference in acute effects of a *trans* FA rich meal (33.8% of experimental fat was *trans* 18:1) compared to a SFA rich meal on postprandial flow mediated dilation.

119. Although the above are the first studies to evaluate the effects of *trans* 18:1 on fasting and postprandial endothelial function, the levels of *trans* 18:1 provided are in excess of that found in UK diets. Concurrent differences in SFA levels between the diets prevents clear conclusions being drawn regarding effects of *trans* FA *per se*.

120. Lichtenstein *et al* (2003) carried out a randomised controlled cross-over trial which investigated the effects of 6 different experimental fats, with increasing levels of *trans* FA. The experimental fats used in this study made up two thirds of the fat intake (20% total energy) and provided a range of 0.26-26.1 g/100g *trans* 18:1 and *trans* 18:2. This study reported no significant differences in systolic or diastolic blood pressure between diets.

121. Baer *et al* (2004) carried out a randomised cross-over trial which investigated the effects of 5 diets composed of 8% energy as *cis* 18:1, *trans* 18:1, stearic acid, a 50:50 mix of *trans* 18:1 and stearic acid or 12:0-16:0 SFA. A sixth diet was investigated for which 8.5% energy from fat was replaced by carbohydrate. E-selectin levels were significantly greater following the *trans* 18:1 diet compared with the other diets ($p < 0.05$). The high levels of *trans* FA investigated in this study (8%) compared with the average dietary *trans* FA intake in the UK (<2% energy dietary energy) should be noted.

122. Dyerberg *et al* (2004) completed an 8 week RCT designed to investigate the impact of *trans* FA and n-3 PUFA intake on risk markers for CHD. Three diets were investigated; a control diet (0.9% energy as *trans* FA, 15.7% energy as SFA), a *trans* FA diet (6.8% energy from *trans* FA, 10.3% energy SFA) and a n-3 PUFA diet (0.9% energy as *trans* FA, 12.3% energy as SFA). There were no significant differences in flow mediated dilation, blood pressure, heart variability rate, arterial dilatory capacity or arterial compliance and distensibility between diet groups.

123. Few intervention studies have investigated the impact of *trans* FA on blood pressure and endothelial function. In agreement with the three studies reviewed by EFSA (2004), these additional studies show no effects of *trans* FA on blood pressure. Evidence is not available to compare the effects of animal and vegetable oil *trans* FA. Evidence for effects of *trans* FA based on relevant intake levels (0.5-3% dietary energy) is also lacking.

Inflammation

124. The effects of *trans* FA on markers of inflammation were not considered in the EFSA report, but given increasing evidence of a role for inflammation in the pathogenesis of CHD, intervention trials with these endpoints have been considered in the present report (Table 8A, Annex 2).

125. Han *et al* (2002) compared the effects of 3 diets; a soybean oil diet (0.6% energy as *trans* FA), a soybean oil stick margarine diet (6.7% energy as *trans* FA) and a butter diet (1.3% energy as *trans* FA) in a double-blind, randomised cross-over study. Production of tumour necrosis factor-α (TNFα) and interleukin-6 (IL-6) by extracted, stimulated, mononuclear cells was significantly greater following the stick margarine diet compared with the soybean oil diet ($p < 0.05$ for both outcomes). Changes in markers of immune response (delayed-type

hypersensitivity, lymphocyte proliferation and IL-2 production) were not consistent with levels of *trans* FA in these diets.

126. Lichtenstein *et al* (2003) carried out a randomised controlled cross-over trial which investigated the effects of 6 diets, with increasing levels of *trans* FA. The experimental fats used in this study made up two thirds of the fat intake and provided a range of 0.26-26.1 g/100g *trans* 18:1 and *trans* 18:2. This study reported no significant differences in CRP levels.

127. Baer *et al* (2004) carried out a randomised cross-over trial which investigated the effects of 5 diets. Diets provided 15% energy from protein, 46% energy from carbohydrate (CHO) and 39% energy from fat, with 8% of this fat energy was composed of *cis* 18:1, *trans* 18:1, stearic acid, a 50:50 mix of *trans* 18:1 and stearic acid or 12:0-16:0 SFA. A sixth diet had 8.5% energy from fat replaced by carbohydrate. CRP levels following the *trans* FA were significantly greater than the carbohydrate diet, *cis* 18:1 and the *trans* FA:stearic acid diets. IL-6 levels were also significantly increased on the *trans* 18:1 diet compared to the *cis* 18:1 diet. The high levels of *trans* FA investigated in this study (8%) compared with the average dietary *trans* FA intake in the UK (‹2% energy) should be noted.

128. A double blind, randomised, parallel trial carried out by Tholstrup *et al* (2006) which compared the effects of two butters, one rich and one low in *trans* 18:1, n-7 found no differences between the test diets in CRP concentrations.

129. Kuhnt *et al* (2007) reported findings of an RCT which compared the effects of 6 weeks supplementation of a *trans* FA rich oil (6g/day *trans* 18:1, n-11: n-12) with a control, *trans* FA free oil. No significant differences in circulating immune cells (lymphocytes, monocytes, granulocytes), subgroups of lymphocytes, TNFα, IL-1β, IL-6, IL-8, IL-10, IL-12-p70, leptin, adiponectin, secretory phospholipase A2, 6-keto-peostaglandin F1α, a marker of endothelial prostaglandin, or CRP were reported between diet groups.

130. A randomised, double-blind cross-over study by Mensink (2007) compared the effects of two diets, comparable in physical characteristics, but with greater levels of stearic acid (+1.3% energy), *cis* 18:1 (+2.9% energy), α-linolenic acid (+0.1% energy) and *trans* FA (+0.5% energy) and lower levels of palmitic acid (-4.2% energy) and alcohol (-1.2% energy) in one diet compared with another (all differences $p < 0.05$). No difference in CRP levels was recorded between the diets.

131. The above study provides the first data on the new generation of hydrogenated oils developed to have reduced *trans* FA levels and provides data based on the relevant range of *trans* FA intake (0.5-3% energy). However, *trans* FA were not the only dietary variable between groups and therefore clear conclusions cannot be drawn.

132. Only one of the 6 intervention studies described above has shown adverse effects of higher levels of *trans* FA intakes on markers of inflammation. The levels of *trans* FA used in this study were significantly higher (8% dietary energy) than current UK intakes (1.0-1.2% food energy). Current findings do not allow a comparison of effects between vegetable oil and animal sources.

Summary – *trans* FA and novel biomarkers of risk of CHD

133. Data obtained from a number of RCTs of diets of varying *trans* FA content, and of meals of varying *trans* FA composition, have not consistently demonstrated adverse effects of these FA on a range of biomarkers for CHD. Biomarkers that have been evaluated include classical biomarkers such as blood pressure and CRP, as well as emerging risk markers such as postprandial lipemia, lipid oxidation, markers of haemostasis and of circulating and *in vivo* measures of endothelial function and vascular inflammation. The studies are relatively few in number in most cases. However, their largely neutral findings to date support the conclusion that the adverse effects of *trans* FA on CHD risk is largely mediated via their actions in increasing circulating concentrations of pro-atherogenic LDL-C, whilst also decreasing concentrations of protective HDL-C.

Overall summary – relationship between *trans* FA and CHD risk

134. There is consistent evidence from prospective epidemiology to support a moderate impact of dietary *trans* FA on risk of CHD (Willet *et al*, 1993; Hu *et al*, 1997; Pietinen *et al*, 1997; Oomen *et al*, 2001; Oh *et al*, 2005). This risk operates over the range, or slightly higher than the range, of *trans* FA levels observed in the UK diet (Figure 2). There is some evidence from these dietary studies to suggest a stronger association between CHD risk and *trans* FA from vegetable oil rather than animal origin. The difference in CHD risk estimates between *trans* FA of animal and vegetable oil origin was first reported in the Nurses' Health Study by Willet *et al* (1993). However, re-estimates of risk associated with *trans* FA of animal and vegetable oil sources have not been reported in follow-up analysis (Hu *et al*, 1997; Tanasescu *et al*, 2004; Oh *et al*, 2005). Biomarker measurements of *trans* 18:2 and *trans* 16:1 in tissues and blood have been proposed to provide surrogate markers for habitual intakes of *trans* FA of vegetable oil and animal origin, respectively. However, the evidence for this does not appear to have been subjected to systematic scrutiny. A small number of studies have shown stronger association between *trans* 18:2 and CHD risk than for other *trans* isomers, although this is not a universal finding. It should be noted that 6 out of the 10 prospective studies considered in this report are based on data from the Nurses' Healthy Study which provides data on a large cohort of women from the US.

135. Consistent adverse effects of *trans* FA on LDL-C, HDL-C and total:HDL-C ratio have been demonstrated in a number of well controlled randomised trials. The

data provide a plausible biochemical mechanism to explain the pathophysiology underlying the prospective epidemiological findings. However, these trials, including a recent comparison of the effects of *trans* 18:1, n-9 and *trans* 18:1, n-7 (Chardigny *et al*, 2007), do not enable distinction between the effects on lipoproteins of *trans* FA of vegetable oil and animal origin. The latter study has shown different but adverse effects of *trans* FA from both sources.

136. The ability to quantify the increased risk of CHD attributable to that percentage of the general population currently consuming > 1% food energy as *trans* FA (61%, Annex 3), is limited by: i) the fact that estimates of risk for quintiles of intake in the region 1-2% dietary energy do not differ significantly from 1.0 (Figure 2); ii) a lack of evidence for a linear relationship between *trans* FA intake and CHD risk over the range of 1-2% dietary energy (Figure 2); and iii) the RCTs which have evaluated the impact of varying doses of *trans* FA on lipoproteins have not compared levels of intake between 1-2% dietary energy. Not withstanding these limitations in the data, estimates for risk reduction have been calculated as part of this review and are shown in section 5. The estimated risk reductions are based on data for CHD outcomes as well for impact of *trans* FA on serum lipoproteins.

Trans FA and cancer

Epidemiological studies and RCTs on *trans* FA and breast cancer

137. Prospective cohort, case-control and ecological studies reported between 1994 and 2006 were reviewed. Data from these studies are summarized in Table 9A, Annex 2. Four of the 9 prospective studies reported were outputs from the Nurses' Health Study. Most of the prospective studies assessed *trans* FA exposure by dietary questionnaire; one nested case-control study was based on analysis of erythrocyte FA while another analysed serum phospholipid FA. The 4 case-control studies used either serum or adipose tissue FA as the measure of exposure.

Ecological studies – breast cancer outcomes

138. Bakker *et al* (1997) investigated the association of breast cancer incidence and *trans* FA status across eleven populations. A statistically significant correlation was found between *trans* FA and the incidence of breast cancer in individual populations, with a Pearson correlation coefficient (r) of 0.89 (95% CI, 0.62–0.97). An increase of 1g *trans* FA per 100g FA in adipose tissue corresponded to a rise in incidence of 19.3 cases of breast cancer per 100 000 person-years.

Case-control studies – breast cancer outcomes

139. London *et al* (1993) analysed the gluteal adipose tissue of 380 US women with newly diagnosed stage I or II breast cancer and 176 with proliferative benign breast disease. Although *trans* FA levels in adipose tissue showed no statistically significant association with risk of breast cancer, this study used controls subjects who may have had breast abnormalities, which may have biased the findings. Petrek *et al* (1994) also compared the breast and abdomen tissue FA profiles of women with invasive breast cancer with those of women with a negative diagnosis for breast cancer. No differences in *trans* FA concentration were found between the case and control groups for either tissue type. However, the use of hospital control subjects reduces the robustness of the study.

140. The EURAMIC study (Kohlmeier *et al*, 1997), compared gluteal adipose tissue *trans* FA levels in 698 cases of postmenopausal primary breast cancer and matched controls. There was a strong positive correlation between the adipose tissue level of *trans* FA and breast cancer (OR 1.40; 95% CI, 1.02-1.93; p for trend 0.03). The ORs for *trans* intake after stratification by PUFA tertiles were 3.65 (95% CI, 2.17-6.14; p for trend 0.001) and 0.97 (95% CI, 0.67-1.40; p for trend 0.85) for the lowest and highest PUFA tertile, respectively.

141. Aro *et al* (2000) reported serum FA levels in 195 cases of breast cancer and 208 population-based controls identified from pre- and postmenopausal Finnish women between 1992 and 1995. The authors reported an inverse association between *trans* 18:1, n-7 and risk of breast cancer with an OR for lowest vs highest quintiles of 0.2 (95% CI, 0.1-0.6), but the authors failed to report the statistical significance of this trend. Other *trans* isomers showed no differences between the case and control populations.

Prospective studies – breast cancer outcomes

142. Holmes *et al* (1999) assessed the link between diet and breast cancer in 88 795 pre- and postmenopausal women over 14 years (1980-1994) in the Nurses' Health Study. There was a small inverse association between *trans* FA intake and risk of breast cancer, although this was not statistically significant. Additional analysis of the data that included only postmenopausal women who had not reported a diagnosis of benign breast disease also found no association between *trans* FA and breast cancer (Byrne *et al*, 2002). Similar analysis using 90 655 premenopausal women enrolled in the second phase of the Nurses' Health study (NHS II) again showed no relationship between the disease and dietary *trans* FA intake (Cho *et al*, 2003). The most recent report on the original cohort brings total follow up to 20 years, and again reports no association between *trans* FA and breast cancer (Kim *et al*, 2006).

143. Pala *et al* (2001) conducted a prospective study of erythrocyte FA and prediagnostic breast cancer (the ORDET study) in northern Italy. The 4052

postmenopausal participants were followed for an average of 5.5 years. Each woman who was diagnosed with breast cancer was matched with two randomly-selected controls, and the composition of the FA in erythrocyte membranes determined. Oleic acid and MUFA were positively associated with risk of breast cancer, but *trans* 18:1, n-9 (the only *trans* FA reported) was not associated with breast cancer risk.

144. Serum FA concentrations from 197 pre- and postmenopausal cases of breast cancer and matched population-based controls in the New York Women's Health Study were assessed by Saadatian-Elahi *et al* (2002). The authors found no association between risk of developing breast cancer and consumption of *trans* 18:1, n-9 in either pre- or postmenopausal women. No other *trans* FA were evaluated in the study.

145. Voorrips *et al* (2002) analysed the data from dietary questionnaires of 941 cases of breast cancer and 1598 subcohort controls in the Netherlands Cohort Study. There was a significant positive association between increasing *trans* FA intake and risk of breast cancer (p for trend =0.01), although the 95% CI for the lowest vs highest quintile of dietary intake of *trans* FA encompassed 1.0 (RR 1.30; 95% CI, 0.93-1.80). Further analysis to consider the effect of *trans* 18:1, n-7 showed similar results, with a highly significant trend across the quintiles of intake (p for trend =0.006), however, the 95% CI for individual quintiles were consistently non-significant (RR 1.34; 95% CI, 0.98-1.82 for lowest vs highest quintile).

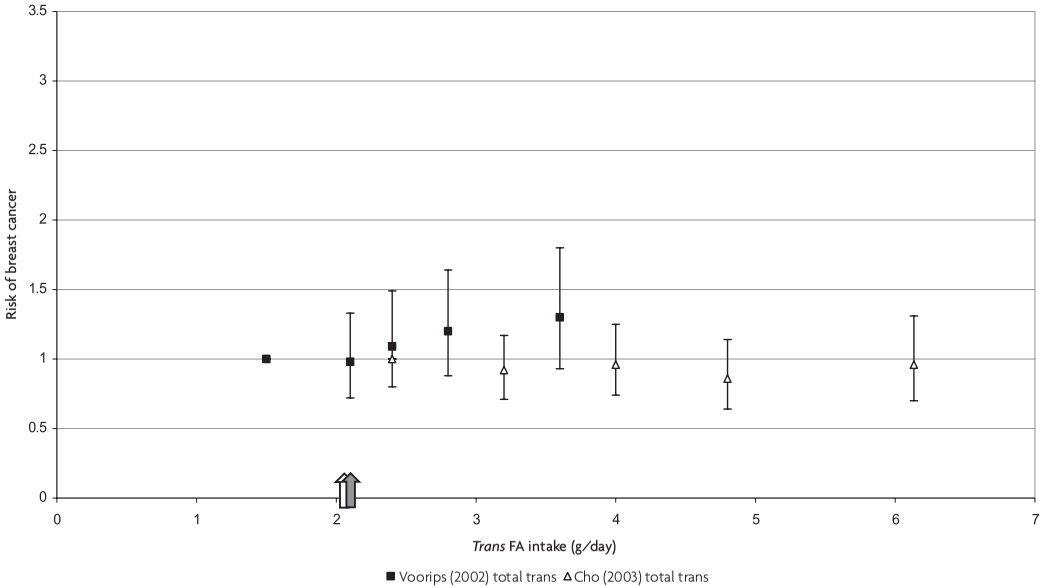
146. Rissanen *et al* (2003) studied the relationship between serum *trans* FA and risk of breast cancer in 127 incident breast cancer cases and 242 matched population-based controls from the 8196 women recruited between 1973-1976 for the Mobile Clinic Health Examination Survey in Finland. Higher serum *trans* 18:1, n-7 levels were associated with an increased risk of breast cancer (OR 3.69; 95% CI, 1.35-10.06), increasing after further adjustment for BMI, serum cholesterol, alcohol intake, education, exercise and parity (OR 4.23, 95% CI, 1.36-13.20). The relationship appeared to be slightly stronger in postmenopausal than in premenopausal women, but the trend failed to reach statistical significance in either group. There was no significant correlation with total MUFA *trans* FA.

147. A subset of women were randomly selected from a large trial (266 064 women) of breast self-examination in Shanghai by Shannon *et al* (2007). The women were followed between 1995 and 2000. Erythrocyte FA were analysed in 322 cases of breast cancer and 367 controls, matched for age and menstrual status. A strong positive association was found between the concentration of *trans* 18:1, n-7 in the erythrocytes and breast cancer (OR 2.21, 95% CI, 1.25-3.88; p for trend 0.002). No other *trans* FA were included in the study.

148. Data from these prospective studies that evaluated dietary *trans* FA intake are summarised in Figure 5.

Figure 5. Risk of breast cancer from prospective epidemiological studies that evaluated dietary intake of trans FA

Trans FA intake for women in the UK was 1.2% of food energy or 2.04 g/day (white arrow) in NDNS 2000/01 (Henderson *et al*, 2003) and in 2007 estimated to be, for all adults 19-64 years, 1% of food energy or 2.07 g/day (grey arrow). Risk of breast cancer is plotted as the RR (fully adjusted for confounding factors), with bars showing ± 95% confidence interval for intake ranges above reference (RR = 1) in each study. Data from Cho *et al* (2003) were converted from % of total energy to g/day based on an energy intake of 1700 kcal/day.



Animal models evaluating the relationship between trans FA and breast cancer

149. Selenskas *et al* (1984) investigated the effect of a high *trans* FA diet on a dimethylbenz[a]anthracene (DMBA)-induced rat mammary tumour model. The diets contained 20% fat by weight, which was either a partially hydrogenated mixture of 50% soybean oil and 50% cottonseed oil (57.5% 18:1; 22.5% *cis* monoene and 35% *trans* monoene) or a mixture of 58% olive oil, 40% cocoa butter, and 2% coconut oil (54.7% 18:1; all in *cis* configuration). Apart from differences in *trans* FA, the diets contained similar levels of other FA. Tumour incidences for the *trans* and *cis* FA diets were 32% and 40%, respectively, with no statistical differences between groups.

150. Using the same blend of *trans* and *cis* FA, Erikson *et al* (1984) examined effects of the diets on the growth and metastasis of implanted mammary tumour cells. The study also considered varying amounts of fat, with diets containing either 5% or 20% fat by weight. Cells were injected into female BALB/c mice either subcutaneously or intravenously. Mice with subcutaneous implants showed no differences in latency period, tumour growth rate or final tumour size, regardless of fat type or amount. However, in mice receiving the intravenous implants, the liver and spleen from those fed the *cis* FA diets contained significantly more viable tumour cells than did those from mice fed the *trans* FA diets.

151. Lock *et al* (2004) observed that when *trans* 18:1, n-7 is converted to CLA it exerts an anticarcinogenic effect against rat mammary tumour initiation and growth. However, the presence of *trans* 18:1, n-7 does not affect cancer development when this conversion is blocked.

Epidemiological studies and RCTs on *trans* FA and colorectal cancer

152. One prospective cohort, 1 ecological and 4 case-control studies published between 1997 and 2007 were reviewed (Table 10A, Annex 2). Apart from the ecological study, all studies used dietary assessment as the measure of exposure to *trans* FA.

Ecological studies – colorectal cancer

153. An ecological investigation of the association between colon cancer and *trans* FA status in 8 European countries and Israel was undertaken by Bakker *et al* (1997). A statistically significant correlation was found between colon cancer and the level of *trans* FA in adipose tissue (r =0. 93, 95% CI =0.74–0.98).

Case-control studies – colorectal cancer

154. The association between colorectal adenomatous polyps and the consumption of foods containing partially hydrogenated oils was examined by McKelvey *et al*

(1999). Dietary intake was obtained from a self-administered FFQ. While there was evidence of a positive association between total dietary *trans* FA and adenomas, this did not reach statistical significance (OR 1.6, 95% CI, 0.82–3.2).

155. Slatterly *et al* (2001) studied 1993 cases with colon cancer and 2410 population-based controls matched for age and sex. Dietary information was collected via a detailed diet history questionnaire. A significant positive association was found between *trans* FA consumption and colon cancer risk in women (OR 1.5; 95% CI, 1.0–2.4 for highest vs. lowest quintile), with a positive but not statistically significant association in men (OR 1.2; 95% CI, 0.9-1.7). Postmenopausal women who were not taking hormone replace therapy (HRT) had a twofold increase in risk from high levels of *trans* FA in the diet (OR 1.6; 95% CI, 1.1-2.5), while the risk of developing colon cancer was unaffected by dietary *trans* fat in women on HRT (OR 0.9; 95% CI, 0.6-1.5; p for interaction =0.06).

156. Nkondjock *et al* (2003) compared the data from the dietary questionnaires of 402 cases of colorectal cancer with 668 population-based controls in Montreal between 1989 and 1993. No relationship was found between consumption of *trans* FA and the development of colorectal cancer in either men or women.

157. Effects of FA on risk of colorectal cancer were examined in a case-control study involving 1455 cases and matched population-based controls from the Study of Colorectal Cancer in Scotland (Theodoratou *et al*, 2007). There was no association between total *trans* FA consumption and colorectal cancer after adjustment for intake of energy and total FA (OR 1.30; 95% CI, 0.97–1.75; p for trend =0.251).

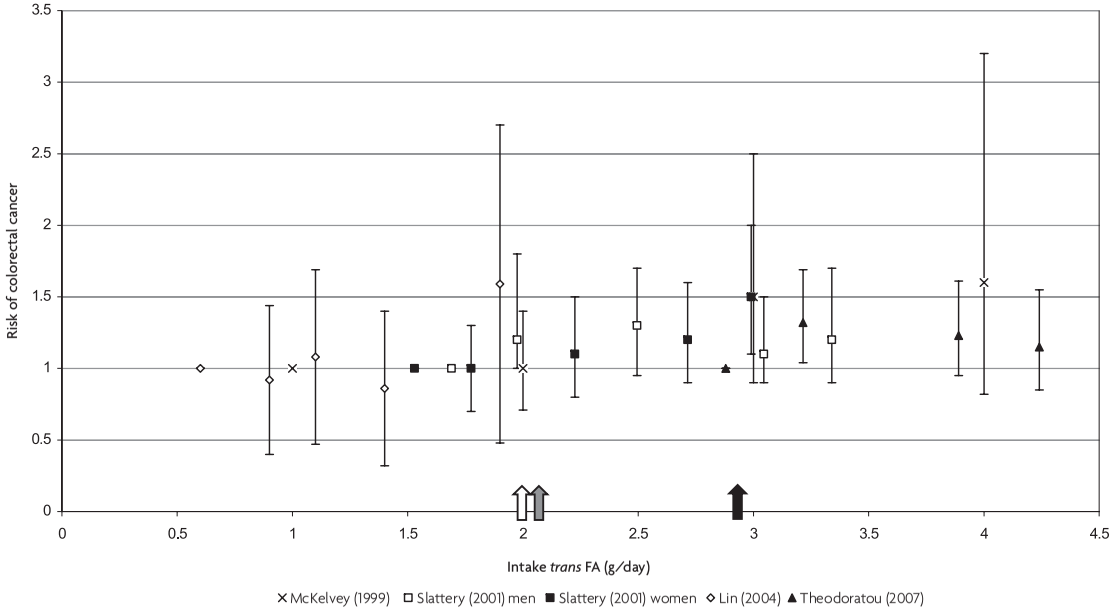
Prospective studies- colorectal cancer

158. Lin *et al* (2004) used dietary and health questionnaires from 37,547 women in the Women's Health Survey (1993 and 2003) to examine associations between diet and colorectal cancer. The authors found no statistically significant link between consumption of *trans* FA and colorectal cancer through their standard multivariate risk analysis, although the relative risks for *trans* FA intake became stronger when adjusted for consumption of other types of fat and cholesterol (highest vs lowest quintile RR 1.59; 95% CI, 0.94-2.70; p for trend =0.06). There was a strong positive association between intake of fried foods away from home and colorectal cancer risk (RR 1.86; 95% CI, 1.09-3.16; p for trend =0.01).

159. Data from these case-control and prospective cohort studies that evaluated dietary *trans* FA intake are summarised in Figure 6.

Figure 6. Risk of colorectal cancer from case-control and prospective epidemiological studies that evaluated dietary intake of *trans* FA

Trans FA intake in the UK was 1.2% of food energy or 2.04 g/day for women (white arrow) and 1.2% of food energy or 2.91 g/day for men (black arrow) in NDNS 2000/01 and in 2007 is estimated to be, for all adults 19-64 years, 1% of food energy or 2.07 g/day (grey arrow). Risk of colorectal cancer is plotted as the RR (fully adjusted for confounding factors), with bars showing ± 95% confidence interval for intake ranges above reference (RR = 1) in each study. Data from Lin *et al* (2004) were converted from % of total energy to g/day based on an energy intake of 1700 kcal/day (study only included women).



Animal models – colorectal cancer

160. Watanabe *et al* (1985) used the dimethylhydrazine model to examine the effect of *trans* fats on colon cancer in Fischer rats. A partially hydrogenated corn oil and olive oil were used at 10% by weight in the diet, and fed for 15 months. The partially hydrogenated corn oil contained 42% *trans* 18:1 and 27.2% *cis* 18:1 FA, whereas the olive oil contained 74.1% *cis* 18:1. At the end of the study, there was no statistically significant difference in colon tumour incidence, with rates of 35.3% and 31.3% in animals receiving partially hydrogenated corn oil and olive oil, respectively.

161. A similar study with a strain of animals especially susceptible to colon cancer (Wistar-Furth-Osaka) was carried out by Sugano *et al* (1989). High *trans* FA partially hydrogenated corn oil was compared with high-18:1 safflower oil at 5% of energy. The incidence of DMBA-induced tumours in small and large intestines were 63% and 75%, respectively in the animals fed partially hydrogenated corn oil and 65% and 71%, respectively, in the group fed high-18:1 safflower oil.

162. Hogan and Shamsuddin (1984) fed inbred female F344 rats (n =30 per group) a diet containing 25% *trans* 18:1 fat or 25% *cis* 18:1 fat, and injected the animals weekly with azoxymethane to induce large intestinal carcinomas. Although 4 more animals receiving the diet containing *trans* fat developed tumours than those receiving the *cis* fat diet, this difference was not statistically significant. Identical numbers of animals from each group developed extracolonic neoplasms.

163. Reddy *et al* (1985) studied the effect of increasing dietary levels of *trans* fat on azoxymethane-induced colon carcinogenesis in rats. Three diets were prepared, each containing 23.5% fat by weight, but with varying amounts of the *trans* fat mix and Oleinate, the latter used to balance the amount of 18:1 across the diets. The three diets were referred to as low-*trans*-fat (5.9% *trans* fat + 11.7% Oleinate + 5.9% corn oil), intermediate-*trans*-fat (11.7% *trans* fat + 5.9% Oleinate + 5.9% corn oil), and high-*trans*-fat (17.6% *trans* fat + 5.9% corn oil). For the low-, intermediate- and high-*trans*-fat diets, the incidences of colon tumours were 63%, 67%, and 57%, respectively, while incidences of small intestinal tumours were 40%, 43%, and 37%, respectively

Epidemiological studies and RCTs on *trans* FA and prostate cancer

164. One case-control, 2 prospective and 1 ecological studies were reviewed; 1 prospective study used serum phospholipids as the measure of exposure to *trans* FA, the others used dietary assessment (Table 11A, Annex 2).

Ecological studies – prostate cancer

165. The ecological study by Bakker *et al* (1997) also examined the association between prostate cancer and *trans* FA status measured in adipose tissue. There was no statistically significant correlation between prostate cancer and the level of *trans* FA.

Case-control studies – prostate cancer

166. Liu *et al* (2007) assessed the potential modification of risk by a functional polymorphism in the RNASEL gene (R462Q) in a study involving 1012 cases of prostate cancer and matched controls. Among Caucasians (n =834), they reported a statistically significant positive association between prostate cancer and the intake of individual and combined *trans* FA (OR 2.77; 95% CI, 1.60-4.79 for lowest vs highest quartile, p for trend =0.0003). The association remained strongly significant for all groups of *trans* isomers (16:1, 18:1 and 18:2, all p <0.005). There was no association for African American individuals. When the data for Caucasians was stratified by genotype, the association between total *trans* FA intake and prostate cancer was very strong among men with the QQ/RQ genotype (ORs of higher quartiles of 2.93 (95% CI, 1.62- 5.30), 3.13 (95% CI, 1.64-5.98) and 4.80 (95% CI, 2.29-10.08)). All groups of *trans* isomers had p values <0.005. For men with the RR genotype, neither total *trans* FA intake nor intake of any group of *trans* isomers was associated with prostate cancer.

Prospective studies – prostate cancer

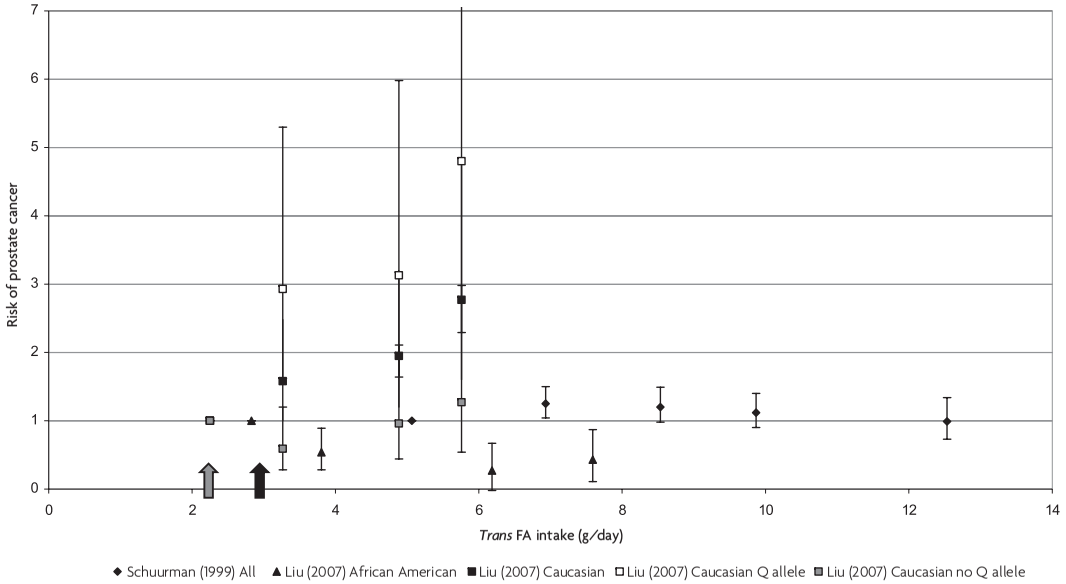
167. The Netherlands Cohort Study recruited 58,279 men between 55-69 years of age in 1986. The men completed a questionnaire on their usual diet and general risk factors for cancer, and were followed for 6.3 years. No association was found between total *trans* FA consumption and development of prostate cancer (Schuurman *et al*, 1999).

168. Serum phospholipid *trans* FA levels were compared in male subjects that had been recruited for the ß-Carotene and Retinol Efficacy Trial (CARET), a randomized trial of supplemental ß-carotene and retinol for the prevention of lung cancer among 18,314 heavy smokers and asbestos-exposed workers (King *et al*, 2005). A sample of 272 men that developed prostate cancer and 426 matched controls showed increasing prostate cancer risk with higher levels of *trans* 18:1, n-7 (OR 1.69; 95% CI, 1.03-2.77). Other *trans* C18 FA had positive trends with p-values of between 0.07 and 0.12, but none of the *trans* C16 FA were close to statistical significance.

169. Data from these case-control and prospective cohort studies that evaluated dietary *trans* FA intake are summarised in Figure 7.

Figure 7. Risk of prostate cancer from a case-control and prospective epidemiological study that evaluated dietary intake of *trans* FA

Trans FA intake in the UK for men was 1.2% of food energy or 2.91 g/day (black arrow) in NDNS 2000/01 (Henderson *et al*, 2003) and in 2007 estimated to be, for all adults 19-64 years, 1% of food energy or 2.07 g/day (grey arrow). Risk of prostate cancer is plotted as the RR (fully adjusted for confounding factors), with bars showing ± 95% confidence interval for intake ranges above reference (RR = 1) in each study. Data from Schuurman *et al* (1999) were converted from % of total energy to g/day based on an energy intake of 2400 kcal/day. Reference points for Caucasians and genotypes from Liu *et al* (2007) are all at 2.25g/day.



Epidemiological studies and RCTs on *trans* FA and other cancers (Table 12A, Annex 2)

Non-Hodgkin's Lymphoma

170. It has been suggested that a higher intake of dietary fats could decrease immune response, leading to increased risk of developing non-Hodgkin's lymphoma (NHL). Zhang *et al* (1999) used dietary and health data from the Nurses' Health Study to examine the possible links between NHL and amount and type of fat. A strong positive relationship was found between higher intakes of *trans* FA and an increased risk of the disease, with a RR for highest versus lowest quintiles of 2.4 (95% CI, 1.3–4.6; p for trend 0.01). The statistically significant association between *trans* FA consumption and development of NHL remained after further adjustments for other types of fat, protein, alcohol, and fruit and vegetable intake. The authors also considered the importance of the source of the *trans* FA, and found that the link between NHL and *trans* FA consumption was stronger for vegetable fat sources (RR 1.9; 95% CI, 1.2–3.1; p for trend =0.03) than animal fat sources (RR 1.4; 95% CI, 0.8–2.2; p for trend =0 .15). However, it must be noted that this study based *trans* FA intake on the dietary questionnaire completed in 1980, which is likely to introduce significant error due to the changes in food manufacturing and personal food choices over the course of the study.

Ovarian cancer

171. Data from the Nurses' Health Study was also used to assess the possible link between diet and risk of ovarian cancer (Bertone *et al*, 2002). In the 80 258 pre- and postmenopausal women included in the analysis, 301 cases of ovarian cancer were diagnosed. There was no association between consumption of *trans* FA and development of the disease (RR 1.03; 95% CI, 0.72-1.47; p for trend =0.87).

Pancreatic cancer

172. Michaud *et al* (2003) used the dietary and health data from the Nurses' Health Study to determine whether there was an association between diet and risk of developing pancreatic cancer. There was no correlation between the intake of *trans* FA and diagnosis of pancreatic cancer (RR 0.91, 95% CI =0.58-1.43, p for trend =0.44).

Summary – evidence for association between dietary *trans* FA and development of cancer

173. Overall, there are few studies that have assessed the relationship between *trans* FA intakes and cancer at specific sites. The cancer site for which most evidence is available on which to base a risk assessment is breast cancer, for which there are

4 case-control and 9 prospective studies reported in the literature. Of the prospective studies, 3 are outputs from the original Nurses' Health Study, which has now reported follow up of breast cancer over a period of 20 years with no evidence of an association with *trans* FA intakes (Holmes *et al*, 1999; Byrne *et al*, 2002; Kim *et al*, 2006). Three other prospective studies have reported a positive association (Voorrips *et al*, 2002; Rissanen *et al*, 2003; Shannon *et al*, 2007). Animal studies provide no evidence for an effect of *trans* FA on mammary tumourigenesis. A plausible biological mechanism to explain any adverse effect of *trans* FA on breast cancer is lacking.

174. Evidence for association between *trans* FA and cancers at other sites is sparse or limited and do not enable any meaningful risk assessment to be undertaken. This is in line with the EFSA (2004) assessment. However, recent evidence for an association between *trans* FA intakes and prostate cancer from a nested case-control study (King *et al*, 2005), and for a strong positive interaction between *trans* FA intakes and the RNASEL QQ/RQ genotype (~35% of the population) in a large case-control study (Liu *et al*, 2007), warrant further investigation. The RNASEL gene is involved in protein coding and is a mediator of interferon action. Mutations in this gene have been associated with predisposition to prostate cancer, and the gene has been identified as a candidate for the hereditary prostate cancer 1 (HPC1) allele.

175. The strong association between NHL and *trans* FA intakes reported from the Nurses' Health Study (Zhang *et al*, 1999) will require further verification by means of intake data based on more recent estimates of *trans* FA in this study population.

176. A statistically significant association was reported for vegetable oil *trans* FA but not animal *trans* FA and non-Hodgkin's Lymphoma (Zhang *et al*, 1999). Three prospective studies on breast cancer (Voorrips *et al*, 2002; Rissanen *et al*, 2003; Shannon *et al*, 2007) and a prospective study on prostate cancer (King *et al*, 2005) reported a positive association that was strongest for *trans* 18:1, n-7. However, the presence of *trans* 18:1, n-7 in both vegetable oil and animal products prevents this data from providing clear conclusions on the effects of the different sources.

Trans FA and obesity

Epidemiological studies and RCTs on *trans* FA and obesity and weight gain

177. This review evaluates case-control and prospective epidemiological studies (Table 13A, Annex 2) and RCTs and meal studies reported between 1990 and 2007 (Table 14A, Annex 2). Relevant cell and animal studies have also been included (1977-2007). The single case-control study assessed *trans* FA exposure using adipose tissue, while all prospective cohort studies quantified exposure to *trans*

FA by dietary assessment. Three of the 5 prospective cohort studies are based on data from the Nurses' Health Study. Three meal studies evaluated the acute response to *trans* FA during a single breakfast meal, while a RCT considered the longer-term effects during dietary replacement of FA for 4 weeks. The RCT and meal studies reported changes in characteristics that may influence body weight, such as fat oxidation, energy expenditure and appetite.

Case-control studies – obesity and weight gain

178. The FA content of adipose of Brazilian individuals with different BMIs was compared by Bortolotto *et al* (2005). Morbidly obese participants (BMI › 40) who were undergoing bariatric or abdominal surgery provided 32 samples of visceral fat and 31 samples of retroperitoneal fat, while 18 samples of subcutaneous fat and 9 samples of visceral fat were obtained from non-obese control subjects (BMI ‹30) who had various non-obesity-related surgical procedures. Overall, the *trans* FA content was higher in the visceral adipose tissue (8.74% ± 0.29 for obese group, 9.29% ± 0.59 for non-obese group) than either the retroperitoneal (6.40% ± 0.50) or subcutaneous tissue (6.94% ± 0.72). There was no difference in average *trans* FA levels between the groups.

Prospective studies – obesity and weight gain

179. Colditz *et al* (1990) monitored the change in body weight over 8 years (1976-1984) of 31, 940 nonsmoking women from the Nurses' Health Study. Age, relative weight, and prior weight change were more strongly associated with recent weight change than were the intake patterns of specific nutrients. All lipids other than vegetable fat were positively related to BMI, with the strongest association for *trans* FA intake (ß =0.191, *t* =9.3). This would correspond to an increase in weight of 0.52 or 0.62 kg over 8 years for an individual 1.65 or 1.8 m tall, respectively. However, when the effects of all nutrients studied were combined, they only explained 0.8% of weight change over this 8-year period. No information on the levels of *trans* FA consumption or additional statistical analysis was reported. Data from the Nurses' Health Study from the period 1986 to 1994 was also used by Field *et al* (2007) to study the association between dietary fat and weight gain among 41,518 women. Weight and diet were assessed using questionnaires at baseline and again after 8 years. Increases in dietary MUFA and PUFA over that period were not associated with weight gain, but increases in animal fat, SFA, and *trans* FA had a positive association with weight change. Among overweight women, for every 1% increase in percentage of calories from *trans* FA, there was an additional 2.3 lb (1.04 kg) weight gain over the 8 years (95% CI, 1.80-2.86; p ‹0.0001). The weight gain for normal weight women during this period was 1.2 lb (0.54 kg) for every 1% increase in *trans* FA (p ‹0.0001). Wannamethee *et al* (2004) investigated the link between alcohol intake and weight gain in the Nurses' Health Study between 1991 and 1999, and reported that weight gain associated with heavy drinking compared with light to moderate drinking appeared to be more likely in women who consumed higher

levels of *trans* FA, although this interaction was not statistically significant (p =0.10).

180. Koh-Banerjee *et al* (2003) examined dietary questionnaires and self-reported waist circumference of 16,587 men involved in the Health Professionals' Follow-up Study (HPFS). The reproducibility and validity of the self-reported measures of waist circumference was evaluated by comparing them with technician-assessed measurements taken 6 months apart in a subset of cohort participants (Rimm *et al*, 1990). The self-reported waist circumference and the average of 2 technician measurements were highly correlated (*r* =0.95). HPFS participants who developed cardiovascular disease, cancer, or diabetes were excluded, as development of those diseases may alter weight and waist measures, dietary intake, and physical activity level. The analysis found that if 2% of total energy intake from carbohydrates or PUFA was substituted for *trans* FA isoenergetically, there was a 9-year increase in waist circumference of 0.53cm or 0.52cm, respectively (p =0.007 for both substitutions). The authors performed further adjustment of the data to compensate for measurement error in the significant predictors (such as the relationship between reported dietary fat intake and actual fat intake, as determined by a validation study), after which the substitution of PUFA with *trans* FA at 2% of energy was associated with a 2.7 cm increase in waist circumference over 9 years (p <0.001).

181. The association between postpartum weight retention and diet and lifestyle factors was examined in 902 women at 6-12 months postpartum by Oken *et al* (2007). For each 0.5% increase in energy from *trans* FA consumed, the OR for retaining 5kg at 12 months was 1.33 (95% CI, 1.09 –1.62). The OR for weight retention by women who had *trans* FA consumptions below the median was 0.23 (95% CI, 0.08–0.66).

Randomised controlled trials and meal studies –obesity and weight gain

182. The oxidation rates of 7 different FA given in test meals, in 4 healthy men were examined by DeLany *et al* (2000). The participants consumed an isoenergetic weight-maintenance diet containing 40% of energy as fat for 7 days prior to the first test meal, and continued on the diet throughout the study. Each test meal included a specific 13C-FA (lauric, palmitic, stearic, *cis* 18:1, n-9, *trans* 18:1, n-9, linoleic or linolenic) at levels of 10 mg/kg body weight, provided in the form of a hot liquid meal. The participants received the test meals in random order, one every 2-4 days until all FA had been consumed. After consumption of the test meal, breath samples were collected for 9 hours, and the oxidation of each FA assessed by measuring the amount of liberated 13CO2 in the breath. The oxidation rates were in the order of lauric ›› linolenic › *trans* 18:1, n-9 › *cis* 18:1, n-9 › linoleic › palmitic › stearic. Differences in oxidation rates between the *cis* and *trans* FA were not statistically significant.

183. Lovejoy *et al* (2002) performed a randomised, double-blind, cross-over feeding study comparing three 4-week diets in 25 healthy subjects. Each diet contained 57% of energy from carbohydrate, 15% protein, and 28% fat (~ 9% of energy from *trans* 18:1, *cis* 18:1 or 16:0 FA). The 25 subjects included males and females of normal (BMI ‹25 kg/m^2) and heavier (BMI 25-30 kg/m^2) body weight. Body weight was maintained at a constant level throughout the study. Rates of oxidation for carbohydrate and fat were measured at the end of each 4-week diet, and results analysed using sex and diet order as covariates. Subjects oxidised significantly less fat on the MUFA diet (26.0 ffl 1.5 g/day) than on the *trans* FA diet (31.4 ffl 1.5 g/day) (p =0.02). Fat oxidation on the SFA diet was not significantly different from either of the other diets (29.0 ffl 1.5 g/day). There was no significant effect of diet on carbohydrate oxidation, although as expected the trend was in the opposite direction to that of fat oxidation.

184. The effect of different C18 FA on appetite and energy expenditure was assessed in 19 overweight young men (Flint *et al*, 2003) given three isoenergetic test meals containing 60% energy from fat and enriched with either PUFA, MUFA or *trans* FA (32% of energy from *trans* FA). The energy content of the meal was adjusted so that each participant consumed 0.8 g of fat/kg body weight. Energy efficiency (respiratory gas exchange) was measured continuously in a respiration chamber, and appetite rated by visual analog scales at regular intervals. After 5 hours, an ad libitum meal was served, and energy intake was registered. There were no differences in acute postprandial appetite, ad libitum energy intake or energy efficiency between the test meals.

185. Lefevre *et al* (2005) investigated the acute effects of a single isoenergetic meal containing either *cis* or *trans* 18:1 in moderately overweight but generally healthy individuals, with and without the Thr54 FABP2 allele (12 Ala/Ala; 8 Thr/Ala, 2 Thr/Thr at codon 54 in FABP2). The participants were fed a basal diet containing 24% fat for 16 days. On days 10 and 16, they were fed a large (40% of daily energy requirements) high-fat (50% of energy) breakfast meal that contained 10% energy from either *cis* or *trans* 18:1 FA. No difference was observed in fat or carbohydrate oxidation between the two meals. Sex and age were the only covariates used in the analysis.

Cell studies – obesity and weight gain (Table 15A, Annex 2)

186. Panigrahi and Sampugna (1993) investigated the effect of *trans* FA on Swiss mouse fibroblast 3T3-L1 cells, a widely-used adipocyte model. Cells were cultured in the growth media supplemented with FA complexed to bovine serum albumin. The control and test FA mixtures contained a range of FA, but the only significant difference between them was the level of C18 FA. Cell-conditioned media and cellular lipids at the preadipocyte and differentiating adipocyte stages were analyzed. At both stages of development, less fat had accumulated in cells cultured in the presence of *trans* FA (p ‹0.05), with a reduction in the total

nonpolar lipid content of the cells. The cells exposed to the *trans* FA also had higher linoleate to arachidonate (ARA) ratios ($p < 0.05$). Comparisons of the total amounts of other FA in the cells suggested that *trans* FA might have replaced MUFA in the nonpolar lipid fraction and SFA in the polar lipid fraction.

187. Cromer *et al* (1995) compared the lipolysis and glucose utilization of rat adipocytes incubated for 2 hours in media containing *cis* 18:1, n-9, *trans* 18:1, n-7 or *trans* 18:1, n-9 FA. Both *trans* isomers caused a significant reduction in the amount of glucose converted to cell lipid ($p < 0.01$) and in the oxidation of glucose to carbon dioxide ($p < 0.05$), while increasing the rate of lipolysis. The authors concluded that *trans* 18:1 FA isomers had catabolic effects on adipocyte metabolism that occurred regardless of the position of the double bond, the FA concentration in media or the FA to albumin ratio.

Animal studies – obesity and weight gain (Table 15A, Annex 2)

Rodent models

188. Privett (1977) found that the addition of 5% *trans* 18:1, n-9 or *trans* 18:2, n-9, 12 FA to the diets of EFA deficient rats lowered their growth response to linoleic acid. After 24 weeks, both *trans* FA were found to have accumulated to relatively high levels in the serum and liver (up to 17.0% and 17.9% of FA, respectively). The *trans* FA also impaired the conversion of oleic acid to eicosatrienoic acid and linoleic acid to ARA, and reduced the incorporation of eicosatrienoic acid into cholesteryl esters (CE). Serum lecithin:cholesterol acyl transferase (LCAT) activity was unaffected by *trans* 18:1, n-9 but was significantly decreased by *trans* 18:2, n-9, n-12. These results suggest that the *trans* FA affect the interconversion of unsaturated FA and the activity of LCAT and lipoprotein lipase (LPL).

189. The effect of a diet containing *cis* and *trans* FA on the FA composition and fat accumulation in mouse adipose tissue was examined by Atal *et al* (1994). Male C57Bl/6J mice were fed diets that contained 10 wt% fat (51.1% *cis* 18:1 or 25.4% *cis* 18:1 and 25.5% *trans* 18:1) Over 2-24 months, body weight, epididymal fat pad weight, perirenal fat yield, adipose tissue cellularity and FA composition were examined. Adipose tissue lipids from animals on the *trans* FA diet had a higher percentage of 14:0 and 18:2, n-6 and lower percentage of *cis*-18:1 and 20:4, n-6. The animals receiving the *trans* FA had lower body weights (16 and 24 months of age), epididymal fat pad weights (8–24 months of age), perirenal fat weights, TAG to polar lipid ratios and adipose cell size than the animals fed the *cis* FA (all $p < 0.05$).

190. To examine the energy utilisation of different FA, Colandre *et al* (2003) fed Wistar rats diets that were rich in *trans* FA, *cis* FA or SFA for 30 days (0-5.1% of energy from *trans* FA). The fats were obtained through isomerisation or hydrogenation of the *cis* FA source (maize oil), ensuring the FA chain lengths were similar in all three diets. Weight gain at the end of the study was similar between the SFA and *cis* FA groups, but was slightly (but not significantly) lower in the animals fed the *trans*

FA. Epididymal fat pads were significantly smaller in animals on the *cis* diet than either of the other diets (p =0.0007). The apparent fat absorption was 85.7% ± 3·4, 93.1% ± 0·4 and 96.7% ± 1·1 for the SFA, *trans* FA and *cis* FA diets, respectively (p <0.0001). The efficiency of energy utilization was lower in the *trans* FA (16·5 %) and SFA (15·2 %) diets than the *cis* FA diet (18.7%), but this difference was only statistically significant between SFA and *cis* FA.

Primate models

191. Kavanagh *et al* (2007) conducted a long term intervention study in which male African green monkeys (n =42) were fed maintenance diets containing 35% of energy as fat. This fat was composed of either *cis* MUFA (<1% of energy from *trans* FA) or a mixture of *cis* and *trans* isomers (~8% of energy from *trans* FA) for 6 y. The authors estimate that this period is equivalent to ~ 15 years in a human. The animals receiving the *trans* FA diet gained an additional 7.20 % ± 2.70 body weight compared to 1.78% ± 1.95 for those fed the *cis* FA diet (p =0.049). Assuming a linear relationship between weight gain and consumption of *trans* FA, this would correspond to an increase of 0.42 or 0.55 kg over 6 years for a 1% increase in *trans* FA in an individual with an initial weight of 60 or 80kg, respectively. The *trans* group also deposited more fat intra-abdominally for every cubic centimetre of fat gained, with an intra-abdominal:subcutaneous fat volume ratio of 1.67 ± 0.14 and 1.36 ± 0.09 for the *trans* and *cis* diets, respectively (p =0.018). The *trans* FA diet *also* induced significant postprandial hyperinsulineamia, with insulin concentrations more than 3-times those of the animals fed the *cis* FA (p =0.015), and a non-significant increase in fasting glucose levels (p =0.15). The monkeys fed *trans* FA also had a reduction in the phosphorylation of Akt in muscle tissue (p =0.02). The authors commented that there was no impairment in insulin receptor activation, suggesting a post-receptor defect. There was no difference in adipose tissue levels of TNF-α between the diets.

Summary – *trans* FA and obesity and weight gain

192. There are limited data available from epidemiological studies, RCTs and meal studies on which to base an assessment of risk of obesity or increased weight gain associated with variation in intakes of dietary *trans* FA. The 3 reports based on data from the Nurses' Health Study showed a small positive association (Colditz *et al*, 1990; Wannamethee *et al*, 2004; Field *et al*, 2007), with a weight increase over 8 years of approximately 0.5-1.0 kg for a 1% increase in dietary *trans* FA. Data from the HPFS showed a 2% increase in energy intake from *trans* FA was associated with a 2.7cm increase in waist circumference over 9 years (Koh-Banerjee *et al*, 2003), and a smaller study reported increased weight retention postpartum in women consuming higher levels of *trans* FA (Oken *et al*, 2007). However, the very small changes in weight or waist diameter observed over 8-9 y periods suggest that if there is a relationship between weight gain and *trans* FA intake, the effect is very small. It should be noted that in the study of Colditz *et al* (1990) the combination of all nutrients studied, including *trans* FA, only

contributed 0.8% to the variation in weight gain over the eight year period, with age, relative weight and previous weight gain making the largest contributions. It must also be noted that associations between dietary variables and body weight or weight gain are likely to be confounded by a very large number of other variables some of which have not been measured and therefore cannot be adjusted for. There are no long term RCTs that have evaluated the impact of *trans* FA intakes on weight change, but a small number of studies have investigated potential metabolic effects of *trans* FA compared with saturated and unsaturated FA using acute meal challenges. Two studies comparing FA composition reported that *trans* FA were oxidised more rapidly than *cis* isomers (DeLany *et al*, 2000; Lovejoy *et al*, 2002), but a third found no significant difference (Lefevre *et al*, 2005). Another meal study that evaluated effect of *trans* FA on appetite, energy intake and energy efficiency failed to observe any difference between diets (Flint *et al*, 2003).

193. Animal and cell studies have reported conflicting results regarding the effect of *trans* FA on glucose and lipid oxidation and on body weight and composition. *in vitro* and ex vivo studies of adipose tissue have generally found *trans* FA to inhibit lipid synthesis and reduce lipid deposition. Two studies involving rats also found that animals fed diets high in *trans* FA had lower body weights and reduced fat deposits (Atal *et al*, 1994; Colandre *et al*, 2003). However, a recent long-term well-designed study in monkeys reported that animals consuming high levels of *trans* FA had increased weight gain and intra-abdominal fat deposition, as well as marked postprandial hyperinsulinemia (Kavanagh *et al*, 2007).

194. Overall, from epidemiological studies there is limited but consistent evidence to support a weak association between *trans* FA intake and greater body fat gain at or above the average UK intake (1.0-1.2% food energy). It should be noted that 3 of the 5 prospective studies were reports from the Nurses' Health Study cohort. There is conflicting data from animal studies, but a recent long term study in a primate model has produced data which supports a greater adipogenic effect of *trans* than *cis* MUFA, although the levels fed were some 4-fold higher than those currently consumed in the UK diet. The strength of this evidence needs to be considered against the background of lack of a plausible biological mechanism, which can explain differences in energy utilisation or fat deposition between different dietary FA. There is insufficient evidence to evaluate the relative effects of *trans* FA from vegetable oil origin with those of animal origin on weight gain. The association between *trans* FA and obesity was not examined by EFSA.

Trans FA and diabetes

Epidemiological studies and RCTs on *trans* FA and diabetes

195. This review has found no reports in the literature of case-control studies that have studied the relationship between *trans* FA intake and diabetes. Prospective

epidemiological and population studies (Table 16A, Annex 2) and RCTs (Table 17A, Annex 2) reported between 1997 and 2007 were reviewed, along with relevant cell and animal studies published between 1995 and 2007 (Table 18A, Annex 2). The 3 prospective cohort and 2 population studies quantified exposure to *trans* FA by dietary assessment. Five RCTs undertook dietary replacement of FA for between 4-6 weeks, while a meal study assessed the acute impact of exposure to *trans* FA following a single meal; these studies employed fasting and postprandial markers of glucose tolerance/insulin sensitivity as their outcomes.

Population studies – diabetes

196. A group of 38 adults with a range of ages, BMIs, and levels of glucose tolerance (5 with type 2 diabetes) completed a 3-day diet record, had fasting glucose measured and underwent a 2-hour oral glucose tolerance test (Lovejoy *et al*, 2001). All participants also provided a fasting serum sample for determination of serum CEs and phospholipid (PL) FA content, with FA concentrations in these samples thought to reflect fat intake over the past 4 to 6 weeks. Multiple linear regression models were used to examine the independent associations between energy-adjusted FA intakes and insulin resistance parameters. There was no association between either self-reported *trans* FA intake or *trans* FA enrichment in serum lipids and the participants' glucose or insulin concentrations during the oral glucose tolerance test.

197. Xu *et al* (2007) analysed data from American Indians involved in the Strong Heart Study (SHS) who had been diagnosed with diabetes for more than 1 year. While there initially appeared to be a trend towards increased *trans* FA consumption and poor glycemic control in this diabetic population, this did not reach statistical significance, and was attenuated after adjustment for additional diabetic risk factors.

Prospective studies – diabetes

198. In the Nurses' Health Study, Salmerón *et al* (2001) used the data from 84,204 women to examine risk of type 2 diabetes in a follow up of 14 years. Median *trans* FA intake ranged from 1.3 to 2.9% of energy for the lowest to highest quintiles. Energy-adjusted *trans* FA intake was positively associated with age- and BMI-adjusted risk of developing type 2 diabetes (RR of highest quintile compared with lowest was 1.26; 95% CI =1.11-1.43; p for trend =0.002). The RR was attenuated after adjustment for other diabetes risk factors (RR 1.15; 95% CI =1.01-1.32; p for trend =0.09) but regained significance after other types of fat were controlled for (RR 1.31; 95% CI, 1.10-1.56; p for trend =0.02). Further modelling of the data showed that a 2% increase in energy from *trans* FA was associated with a RR of 1.39 (95% CI, 1.15-1.67). Replacing 2% of energy from *trans* FA with carbohydrate was associated with a 28% lower risk of developing diabetes (RR 0.72; 95% CI, 0.60-0.87; p <0.001), but replacing the same amount of energy with polyunsaturated fats was associated with a 40% decrease in risk (RR =0.60, 95% CI =0.48-0.75; p <0.001).

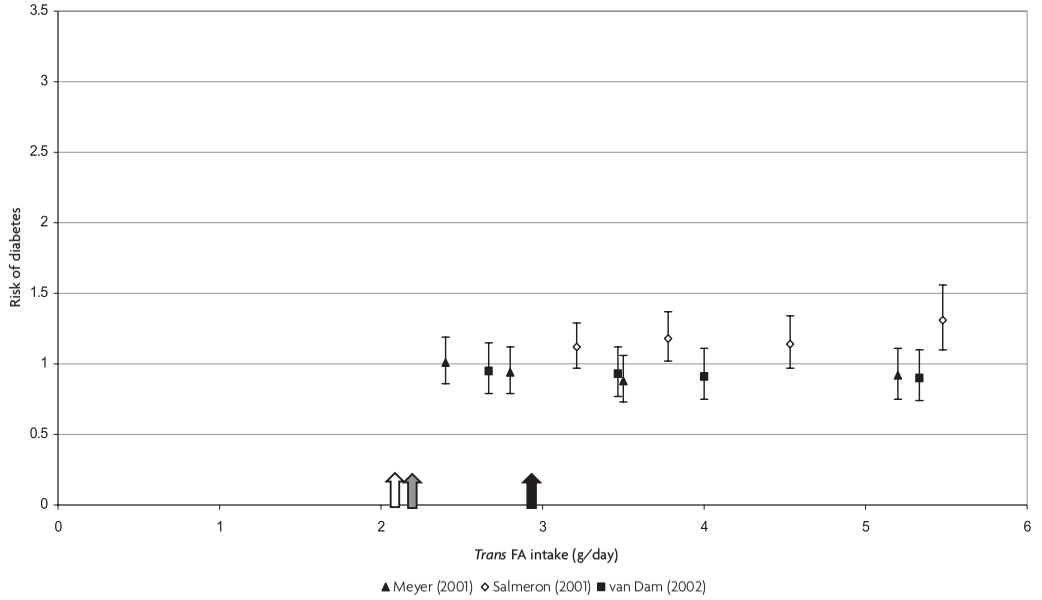
199. Meyer *et al* (2001) investigated the association between dietary *trans* FA intake and type 2 diabetes in 35, 988 women in Iowa between 1986 and 1997. Median *trans* FA intake per quintile ranged from 2.2-5.2% of energy. This study found a small inverse association between *trans* FA intake and risk of diabetes, with RRs among quintiles of intake of 1.0, 0.93, 0.90, 0.84 and 0.87 (p for trend 0.03). This inverse association was strengthened through adjustment for magnesium and cereal fibre intake (p for trend 0.004) but was no longer statistically significant when adjusted for types of FA in the diet (p for trend 0.20). The FFQ was only administered at baseline, with no subsequent dietary follow up, leading to risk of large misclassification of *trans* FA intake because of changes in fats formulations and individual food habits over the period of the study.

200. A further large study involving 4-yearly dietary and 2-yearly health questionnaires from 42,504 men (van Dam *et al*, 2002) reported a positive correlation between *trans* FA intake and risk of developing type 2 diabetes when adjusted for age and energy intake. Median *trans* FA intake was 0.7% and 2.0% of energy for the lowest and highest quintiles, respectively, with a RR value for the highest quintile of 1.39 (CI =1.16-1.67, p for trend =0.0004). The trend remained significant after further adjustment for physical activity, smoking, alcohol consumption, hypercholesterolemia, hypertension and family history. The effects of *trans* FA were no longer statistically significant after adjustment for cereal fibre, magnesium and BMI. No adjustments for other types of dietary fats were undertaken.

201. Data from prospective epidemiological studies that evaluated dietary *trans* FA intake are summarised in Figure 8.

Figure 8. Risk of diabetes from prospective epidemiological studies that evaluated dietary intake of *trans* FA

The current mean *trans* FA intake in the UK is 1.2% of food energy or 2.04 g/day for women (white arrow) and 1.2% of food energy or 2.91 g/day for men (black arrow). Risk of diabetes is plotted as the RR , with bars showing ± 95% confidence interval for intake ranges above reference (RR = 1) in each study. Data from Salmerón *et al* (2001) and van Dam *et al* (2002) were converted from % of total energy to g/day based on an energy intake of 1700 and 2400 kcal/day, respectively.



Randomised controlled trials and meal studies – diabetes

202. Christiansen *et al* (1997) conducted a randomised cross-over trial in obese subjects with type 2 diabetes, using three 6-week diets each containing 20% of total energy from SFA, MUFA or *trans* FA. Insulin and glucose levels were determined in fasting and postprandial blood samples. Postprandial insulin levels were 59% higher after the *trans* FA diet than after the MUFA diet (17.4 nmol/L/240 min, p <0.05), and 26% higher than after the baseline diet (p <0.05). C-peptide concentrations (a marker of insulin secretion) showed a similar pattern during the *trans* FA diet, and were 42% and 32% higher than the MUFA and baseline diets, respectively (both p <0.05). There were no significant differences between the SFA and the *trans* FA diets. The extreme level of *trans* FA fed in this study (20% dietary energy) compared with the average dietary *trans* FA intake of the UK (1.0-1.2% dietary energy), should be noted.

203. A randomised cross-over trial conducted by Louheranta *et al* (1999) compared a high-*trans* FA diet (5.1% of energy from *trans* FA, 80:20 *trans* 18:l and 18:2) with a high-oleic diet (5.2% *cis* 18:1 were substituted for the *trans* FA) in 14 young, healthy women. Each diet lasted 4 weeks and was preceded by a 2-week basal diet. Both experimental diets and the baseline diet supplied 36% of energy as fat, 50% as carbohydrate and 15% as protein. The authors found no significant difference between the diets for glucose effectiveness, insulin sensitivity index or acute insulin response; there was a small non-statistically significant increase in fasting insulin levels on the *trans* FA diet compared with the MUFA diet (8.1 ± 0.6 mU/L and 7.4 ± 0.5 mU/L, respectively; p =0.089).

204. Lovejoy *et al* (2002) performed a randomised, double-blind, cross-over feeding study comparing three 4-week diets in 25 healthy subjects. Each diet contained 57% of energy from carbohydrate, 15% protein, and 28% fat (~ 9% of energy from *trans* 18:1, *cis* 18:1 or 16:0 FA). The 25 subjects included males and females of normal (BMI <25 kg/m^2) and increased (BMI 25-30 kg/m^2) body weight. Body weight was maintained at a constant level throughout the study. After each 4-week diet period, pulsatile insulin secretion, insulin sensitivity index, serum lipids and fat oxidation were measured by indirect calorimetry. The study found no significant effects of diet on any of the indicators of insulin resistance, but subjects oxidized the least fat on the MUFA diet (26.0 ff11.5 g/day) and the most fat on the *trans* FA diet (31.4 ± 1.5 g/day) (p =0.02). Compared with the MUFA diet, the overweight subjects demonstrated 11% and 24% decreases in insulin sensitivity on the *trans* and SFA diets, respectively, but this difference between the groups did not reach statistical significance.

205. The effect of 6 different types of fats on glucose homeostasis was investigated by Lichtenstein *et al* (2003), with each diet followed for 35 days. The diets provided 30% of energy from fat, with 20% substituted for the 6 different fat sources. The sources were soybean oil, semi-liquid margarine, soft margarine, PHSO, traditional stick margarine and butter. There were small differences between the diets for

some measurements, but these were not consistent with the levels of *trans* FA present. The authors concluded that there was no association between *trans* FA and glucose metabolism.

206. Lefevre *et al* (2005) investigated the acute effects of a single meal containing either *cis* or *trans* 18:1 FA in individuals with and without the Thr54 FABP2 allele (12 Ala/Ala; 8 Thr/Ala, 2 Thr/Thr at codon 54 in FABP2). Thr/Ala and Thr/Thr genotypes were combined into one group for data analysis. The participants were moderately overweight but generally healthy, and were fed a basal diet containing 24% fat for 16 days. On days 10 and 16, they were fed a high-fat (50% of energy) breakfast meal that contained 10% energy from either 18:1 *cis* or *trans* FA. Irrespective of genotype, the *trans* meal increased insulin levels to a greater extent than the *cis* meal ($p < 0.05$). An index of relative insulin sensitivity (product of postprandial changes in insulin and glucose concentrations) was also greater after the consumption of the *trans* meal than the *cis* meal ($p < 0.05$). Individuals with either Thr/Ala or Thr/Thr genotypes demonstrated a 2-fold greater postprandial glucose response ($p < 0.05$) and a 70% greater postprandial insulin response ($p = 0.15$) than Ala/Ala genotypes. The authors observed that these results were consistent with impaired insulin-mediated glucose uptake, due perhaps to fatty acid–induced insulin resistance in the muscle. A significant genotype by meal interaction was observed for TAG fractional synthetic rate ($p < 0.05$). This rate was not affected by the FA composition of the test meal for individuals in the Ala54 group, while individuals in the Thr54 group had a 20% higher TAG fractional synthetic rate after consumption of the *trans* FA meal than after the *cis* FA meal ($p < 0.05$). The study concluded that acute exposure to *trans* FA can significantly increase insulin resistance, and that consumption of *trans* FA by individuals who have the FABP2 Thr54 allele may contribute to increased partitioning of glucose to TAGs and insulin resistance.

207. The effects of hydrogenated and interesterified soybean oil on glucose and insulin metabolism were compared by Sundram *et al* (2007). Thirty participants were fed diets containing ~ 35% fat, primarily from palm olein (POL), PHSO or interesterified soybean oil (IE). The diet containing the PHSO provided 3.2% of energy from *trans* FA. Each diet was consumed in a random order, and lasted for 4 weeks. Fasting glucose increased modestly after the PHSO diet relative to POL ($p < 0.05$), but was highest in the IE diet ($p < 0.001$). Using the POL diet as a reference, fasting insulin decreased by 10% and 22% on the PHSO and IE diets, respectively ($p > 0.05$ and $p < 0.001$). Postprandial glucose was higher in the IE diet, but similar in the PHSO and POL diets. Postprandial 2hr insulin was lower in both IE and PHSO compared with the POL diet, but C-peptide levels were only significantly lower for the IE fat. Overall, the authors concluded that adverse effects on glucose metabolism were most significant in the diet containing the interesterified soybean oil relative to the dietary treatments that were higher in SFA or *trans* FA.

Cell studies – diabetes

208. Rat adipocytes incubated for 2 hours in media containing purified 18:1 FA isomers showed that both *trans* 18:1, n-9 and *trans* 18:1, n-7 FA reduced the amount of glucose converted to cell lipid ($p < 0.01$) and inhibited the oxidation of glucose to carbon dioxide ($p < 0.05$) compared with *cis* 18:1, n-9 (Cromer *et al*, 1995).

209. Alstrup *et al* (1999) assessed the acute effect of different *cis* and *trans* FA on insulin release and glucose oxidation in isolated mouse islets. While all FA caused an increase in GSIS, the *trans* isomers elicited a higher level of insulin secretion than their *cis* counterparts ($p < 0.05$). The rate of glucose oxidation at high glucose concentrations was suppressed by *cis* FA ($p < 0.05$) but not affected by the *trans* isomers. The authors also considered the effect of longer-term exposure of the islet cells to a range of 18:1 isomers (Alstrup *et al*, 2004). Basal insulin release was higher in cells exposed to the *cis* 18:1, n-7 ($p < 0.05$), but there was no difference between *cis* and *trans* 18:1, n-9. GSIS was not altered by either *cis* or *trans* 18:1, n-7 or by *cis* 18:1, n-9, but was stimulated by 0.3 to 0.4 mmol/L *trans* 18:1, n-9. In contrast to the observations at the shorter exposure times, the cells incubated with the FA isomers for 3 days showed no differences in glucose oxidation, but FA oxidation was higher in the presence of the *trans* isomers ($p < 0.05$).

Animal studies – diabetes

210. Stein *et al* (1997) found that although *trans* 16:1 resulted in 7.6 x higher levels of GSIS than the corresponding *cis* isomer in the perfused pancreas of Sprague-Dawley rats, the difference did not quite reach statistical significance ($p = 0.07$). There was no difference between the effects of *cis* or *trans* 18:1 FA.

211. Insulin action and adipocyte plasma membrane fatty acid composition and fluidity in rats fed different levels of *trans* FA were compared by Ibrahim *et al* (2005). Higher levels of insulin secretion, as well as reduced membrane fluidity, were observed in the groups receiving dietary *trans* FA. In further studies (Natrajan *et al*, 2005), the authors showed the mRNA expression of resistin was upregulated, and peroxisome proliferative activated receptor-gamma (PPARg) and LPL were down regulated in the animals receiving high *trans* FA diets. Due to variations in dietary levels of other FA (SFA, MUFA, PUFA), it is not possible to conclude that these differences were due to the higher levels of *trans* FA in some of the diets.

212. Bernal *et al* (2006) evaluated the glucose metabolites and gluco-regulatory enzyme activities in skeletal muscle and liver of Wister rats fed diets that contained 17% (by weight) *cis* or *trans* FA. No significant differences in glucose metabolism or enzyme activity were observed.

Summary – *trans* FA and diabetes

213. There are limited data available to assess evidence for an association between *trans* FA intakes and incidence of diabetes. Of the 3 large prospective cohort studies, 2 showed a positive association. In these studies, the impact of *trans* FA on risk of diabetes were similar, with ORs of 1.31 (Salmerón *et al*, 2001) for a range of *trans* FA intakes from 1.3-2.9% dietary energy, and 1.39 for *trans* FA intakes from 0.7- 2.0% dietary energy (van Dam *et al*, 2002). The latter range of intakes is slightly higher than that of current UK intakes, but it provides a reasonable basis for comparison. However, in this study the association was no longer significant after adjustment for diet and BMI. In the third study, an inverse association between *trans* fatty acid intakes and diabetes was reported, but these findings were limited by high likelihood of misclassification due to the collection of dietary data only at baseline (Meyer *et al*, 2001). Neither of the 2 population-based studies found any relationship between *trans* FA intake and parameters associated with diabetes.

214. No effect on insulin sensitivity or glucose tolerance was found in 4 RCTs or meal studies of healthy individuals fed moderately high *trans* diets. However, postprandial hyperinsulinemia was observed in obese subjects with type 2 diabetes fed diets containing 20% energy *trans* FA (Christiansen *et al*, 1997). In an acute meal study (Lefevre *et al*, 2005), a significant increase in insulin resistance was observed following meals high in *trans* FA (10% dietary energy). In the same study, other adverse effects of *trans* FA (on triglyceride synthesis) were shown to be genotype dependent, with greater adverse response for individuals carrying the FABP2 Thr54 allele (~28% of the population).

215. The data from isolated pancreatic islets studies suggest that there is a differential effect of *trans* compared with *cis* FA on the regulation of insulin secretion, with *trans* FA potentiating glucose-stimulated insulin secretion more than *cis*-isomers of identical chain length. There are also concordant data from cells and human studies, which suggest increased rates of oxidation of *trans*- compared with *cis*-unsaturated FA. There are limited data from animal studies; existing studies are of insufficient quantity or quality to be able to draw definite conclusions.

216. Overall there is limited, weak evidence to suggest that *trans* FA have adverse effects on insulin sensitivity and type 2 diabetes. It is clear that further epidemiological, RCTs and mechanistic studies are required before firmer conclusions can be drawn, particularly at *trans* FA intakes relevant to the UK. Evidence is not available to compare the effects of animal and vegetable oil sources of *trans* FA.

Trans FA and early development

Epidemiological studies and RCTs on *trans* FA and early development (Table 19A, Annex 2)

Trans FA exposure during pregnancy

217. Significant positive correlations have been reported between maternal and fetal plasma *trans* FA (Elias & Innis, 2001; Innis, 2003). Early studies suggested that the placenta acted as a barrier for *trans* FA (Johnston *et al*, 1958) but later work contradicted these findings (Koletzko & Muller, 1990; van Houwelingen & Hornstra, 1994) and led the International Life Sciences Institute Expert Panel on *Trans* Fatty Acids and Early Development (Carlson *et al*, 1997) to conclude that "*trans* fatty acids are transferred by the placenta to the fetus and incorporated into fetal tissues". As humans do not synthesise *trans* FA, fetal *trans* FA must have originated in the diet of the mother. This assertion has been supported by Hornstra *et al* (2006), who reported a significant and positive association between maternal intake of *trans* FA (measured using duplicate portion analysis) and *trans* FA concentration in fetal cord plasma phospholipids. Elias and Innis (2001) reported that maternal plasma TAGs and PLs were higher in *trans* FA than in the infant, but that the infants had higher concentrations of *trans* FA in plasma CE ($p < 0.05$).

218. Levels of about 0.5–3% *trans* FA have been reported in blood lipid fractions of preterm and full-term babies (Koletzko, 1992; Decsi *et al*, 2001; Elias & Innis, 2001), as well as 0.1–0.9% *trans* 18:1 in adipose tissue of preterm babies (Ohlrogge *et al*, 1982). *Trans* FA have been shown to inhibit the synthesis of long-chain PUFA (such as ARA and DHA) from their EFA precursors (Sugano & Ikeda, 1996) and it has been reported that *trans* FA isomers displace EFA in neonatal blood and cord tissue, despite apparent adequate maternal intake of EFA (Koletzko, 1992; Al *et al*, 1996; Hornstra, 2000). This is of concern as long-chain PUFA are necessary for fetal growth and development. The association between PUFA and early development was reviewed in the SACN report "Advice on fish consumption: benefits and risks"(SACN 2004).

219. Koletzko (1992) reported a negative relationship between *trans* FA in plasma lipids and birth weight. However, there was no adjustment for confounding factors. Another study of full-term infants, which also did not undertake adjustments, failed to show any association (Decsi *et al*, 2001). Elias and Innis (2001) observed a negative relationship between *trans* FA levels in the CEs from infant cord arterial plasma samples and the length of gestation (unadjusted data). No statistically significant relationships were observed with birth weight or birth length. Van Houwelingen and Hornstra (1994) reported preliminary findings of a negative relationship between *trans* 18:1 in UA vessel walls and infant birth weight and head circumference, but these associations disappeared after correction for gestational

age (Hornstra, 2000). Hornstra *et al* (2006) observed a significant negative relationship between *trans* 18:1, n-9 levels in neonatal cord plasma and arterial and venous walls and the head circumference and body length at birth in the Maastricht Essential Fatty Acid Birth (MEFAB) cohort, in which adjustment was undertaken for a range of confounding factors. Preliminary data from the Amsterdam Born Children and their Development (ABCD) cohort appeared to show a negative association between maternal plasma *trans* 18:1, n-9 levels and the birth weight of full-term children (Hornstra *et al*, 2006), but the association lost significance after adjustment for maternal sociodemographic factors.

220. Very few studies have directly investigated the relationship between *trans* FA and neonatal neurological health. In full-term infants, *trans* FA in umbilical artery (UA) and vein (UV) FA were reported to negatively correlate with neurological function (Dijck-Brouwer *et al*, 2005), with a Spearman correlation coefficient of -0.3 between neurological status and C18 *trans* FA levels in UA FA ($p < 0.05$). However, there was not a statistically significant difference between the infants with normal and abnormal neurological status when analysed by a Pearson χ^2 test, and there was no adjustment for confounding factors. There was no association between general movements (an indicator of neurological status) and umbilical cord *trans* FA level at 3 months of age (Bouwstra *et al*, 2006b). However, more comprehensive neurological testing at 18 months showed a strong negative correlation between levels of umbilical *trans* FA and a neurologic optimality score (Bouwstra *et al*, 2006a). Both the latter studies adjusted for a number of socioeconomic factors.

221. It appears that the possible effect of neonatal *trans* FA status on neurological health in infants and toddlers attenuates with age, with well-adjusted studies in older children finding no relationship between neonatal *trans* FA and cognitive function at either 4 (Ghys *et al*, 2002) or 7 (Bakker *et al*, 2003) years of age.

Trans FA exposure postpartum

222. After birth, the sole source of nutrition for many infants is maternal breast milk. A range of *trans* FA isomers from vegetable oil and animal sources have been found in human breast milk (Chen *et al*, 1995), and dietary intervention studies have shown that changes in maternal dietary *trans* FA intake are rapidly reflected in the *trans* FA levels of the breast milk (Aitchison *et al*, 1977; Craig-Schmidt *et al*, 1984). A strong positive association between *trans* FA from breast milk and infant plasma triglycerides ($r = 0.82$, $p < 0.001$) was observed by Innis & King (1999). Chen *et al* (1995) estimated that maternal dietary intakes of 1.1 and 3.9% of energy from *trans* FA corresponded to 3.1 and 6.9g *trans* FA/100 g breast milk fat, respectively. As breast milk typically contains 3-5% fat, a typical infant consuming 780ml of the milk a day would be exposed to 0.7-1.2 g *trans* FA/day for a maternal *trans* FA intake of 1.1% of energy.

223. During breast-feeding, *trans* FA present in maternal milk are absorbed by the feeding infant and stored in various tissues and organs, with preferential storage in the adipose tissue (Pettersen & Opstvedt, 1992), and the lowest or negligible incorporation in the brain (Cook, 1981; Pettersen & Opstvedt, 1992). By weaning, the *trans* FA levels in the infant tissues are similar to the *trans* FA levels found in the milk, with the exception of the brain (Pettersen & Opstvedt, 1989), suggesting the presence of a protective mechanism that limits the incorporation of *trans* isomers in the central nervous system.

224. Similar to the observations in newborns, *trans* FA incorporation in infant tissues have been reported to result in a reduction in the proportion of long-chain PUFA, particularly ARA and DHA (Pettersen & Opstvedt, 1992). This effect has been reported in the plasma PLs of healthy children between 1 and 15 years of age (Decsi & Koletzko, 1995), and suggests that the *trans* FA are negatively affecting the elongation and the desaturation of EFA.

Summary – *trans* FA on early development

225. While there is evidence that *trans* FA from the maternal diet accumulate in the fetal and infant tissue via placental transport or consumption of breast milk, there is limited and contradictory information as to effects on the health of the child. A negative relationship between blood *trans* FA and birth weight was found in one study in premature infants (Koletzko, 1992) and one in full-term infants (Hornstra *et al*, 2006, MEFAB study), but no relationship in similar studies in full-term infants (Decsi *et al*, 2001; Elias & Innis, 2001). Only the data from the MEFAB study was adjusted for socioeconomic status and other confounding factors. A negative effect was also reported between neurologic optimum status and umbilical *trans* FA in studies on newborns (Dijck-Brouwer *et al*, 2005) and at 18 months (Bouwstra, 2006a), but no significant effect was seen on general movement at 3 months of age (Bouwstra, 2006b). Although the study in newborns reported unadjusted data, the studies at 3 and 18 months included a range of confounding factors such as socioeconomic and educational status.

226. The lack of well-adjusted studies providing consistent results prevents any conclusions regarding the effect of *trans* FA on the physical or neurological health of the fetus or young children. There are consistent reports that the level of *trans* FA in plasma and tissue lipids is inversely proportional to the levels of long-chain ω-6 PUFA, and that the *trans* FA may interfere with the metabolism of EFA. However, because individual tissue FA levels are expressed as % of total FA, changes in the content of one FA will result in reciprocal changes in at least one (if not more) other FA. Such data require cautious interpretation as a basis for proposing effects of *trans* FA on long-chain ω-6 PUFA metabolism. This area requires further investigation by well-designed studies.

Trans FA and other health issues

Epidemiological studies and RCTs on *trans* FA and other health issues (Table 20A, Annex 2)

227. The potential association between *trans* FA intake and a number of health issues have been investigated in prospective epidemiological and population studies. A strong positive association was reported for gallstone formation (OR for lowest vs highest quintile was 1.23; 95% CI, 1.04-1.44; p for trend 0.03), with additional analysis by isomer showing that the relationship was restricted to *trans* 18:1 FA (Tsai *et al*, 2005). Positive trends were also seen in studies on Alzheimer's disease (Morris *et al*, 2003), cognitive decline (Morris *et al*, 2004) and ovulatory infertility (Chavarro *et al*, 2007), but they failed to reach statistical significance. However, the latter study reported that obtaining 2% of energy from *trans* FA instead of carbohydrates was associated with a 73% greater risk of ovulatory infertility after adjustment for risk factors (p for trend 0.02). One multi-centre population study found a significant association between allergic diseases and *trans* FA intake in 13-14 year old children (Weiland *et al*, 1999), but another population study in Germany showed no relationship (Kompauer *et al*, 2005). No association was found for cataract formation (Lu *et al*, 2005), dementia (Engelhrt *et al*, 2002), multiple sclerosis (Zhang *et al*, 2000) or Parkinson's disease (Chen *et al*, 2003; de Lau *et al*, 2005).

228. Although some studies do provide preliminary evidence of possible associations between *trans* FA intake and the above mentioned disease states, the small number of studies precludes the development of any firm conclusions regarding the potential relationships.

4. CLA and health

Introduction

229. Conjugated linoleic acids are a group of isomers of linoleic acid, with the conjugated structure referring to the fact that these FA have the double bonds on adjacent carbon atoms, with no interceding methylene group (CH_2) group. The double bonds present can be in the *trans/trans, trans/cis*, *cis/trans* or *cis/cis* configuration (Christie *et al*, 2003). Intakes in the UK diet are estimated to be in the range of 100-200mg per day (Lawson *et al*, 2001). Animal fats are the almost exclusive source of CLA in the diet, being produced as a result of the animal biohydrogenation of linoleic acid, where they represent up to 2% of total FA present. Approximately 90% of CLAs present are in the *cis*-9, *trans*-11 orientation, which is referred to as rumenic acid with the *trans*-10, *cis*-12 CLA being the second most abundant.

230. Despite the fact that CLAs contain double bonds in the *trans* orientation, numerous studies in animal and cell culture models conducted over the last 15 years, have demonstrated anticarcinogenic, antiadipogenic, antiatherogenic and antidiabotogenic benefits of CLAs. These studies have been largely conducted using commercially available mixed isomer preparations containing equal amounts 40-45% of the *cis*-9 *trans*-11 and *trans*-10 *cis*-12 isomers. More recent evidence using a purified form of the isomers indicates that the two isomers may have divergent effects. However, despite the biopotency demonstrated in animal models, the studies in humans have shown modest, neutral or often deleterious effects reported. This section will not be in the form of on all-encompassing review of all the available evidence, but will rather provide an overview of:

- the main conclusions of a number of recent expert reviews in the area of CLA and health (Wahle *et al*, 2004; Bhattacharya *et al*, 2006; Salas-Salvado *et al*, 2006; Tricon & Yaqoob, 2006; Gebauer *et al*, 2007)
- the only meta-analysis in the area of CLA, a 2007 publication which considers its role in reducing fat-mass in humans (Whigham *et al*, 2007)
- the available limited data for a link between CLA intake and cancer risk in humans (Aro *et al*, 2000; Chajes *et al*, 2002; Voorrips *et al*, 2002; Larsson *et al*, 2005), as these are only covered to a limited extend in the above mentioned reviews.

CLA and body composition

231. Since the original demonstration of the ability of 0.5% dietary CLA to decrease fat mass by 50-60% in mice (Park *et al*, 1997), numerous further studies in rodent and

other animal and cell models have demonstrated the ability of CLA to reduce adipose fat accumulation. This effect has been attributed to a range of mechanisms including decreased adipocyte differentiation, size and fat uptake, increased fat oxidation and reduced lipogenesis, with the CLA influencing the gene expression of key regulators of these metabolic pathways. Feeding of isolated isomers has demonstrated that the effect is largely attributable to the *trans*-10, *cis*-12 isoform.

232. A number of studies in humans feeding doses of 0.7 to 6.8g CLA have produced highly inconsistent findings, with less than half showing any significant effects. This loss of efficacy in humans versus the large effects seen in rodent models is likely to be attributable to a number of reasons: (1) inherent differences in adipose tissue metabolism in the two models; (2) lower dosage used per kg body weight; (3) the animal studies have been generally conducted in growing animals (the impact of CLA in adult animals has not been as dramatic as in young animals) whereas the human trials have been conducted in adults. However, the meta-analysis of Whigham *et al* (2007) does indicate a weak significant benefit. This analysis considered 18 intervention studies, which have examined the efficacy of CLA in reducing fat mass in humans. An overall significant treatment effect was evident with fat loss compared with placebo of -0.024kg/gCLA/week (p =0.03). The effect was linear up to 6 months, with the effect decreasing thereafter, indicating an adaptation to treatment.

CLA and insulin sensitivity

233. The literature suggests a general lack of consistency between different animal models, and when comparing data from human clinical trials, regarding the impact of individual CLA isomers on insulin sensitivity. There are studies which have demonstrated deleterious effects of the *trans*-10, *cis*-12 isoform on a number of biomarkers of insulin sensitivity, which may be associated with its effect on adipose tissue fat metabolism (Risérus *et al*, 2002; Risérus *et al*, 2004). There is a need for longer term studies in this area feeding the purified *trans* isomers.

CLA, blood lipid levels and atherogenesis

234. Animal evidence demonstrates consistent benefits of CLA with respect to regression of atherosclerosis and the effects on blood lipid profile. Human studies that have examined the impact on blood lipids have produced neutral or negative findings, with a moderately consistent body of evidence suggesting a negative effect of the *trans*-10, *cis*-12 on HDL-C levels.

CLA and carcinogenesis

235. Despite a large body of evidence demonstrating efficacy, and plausible mechanisms for the anticarcinogenic actions of CLA (Field & Schley, 2004), there is a distinct lack of data linking CLA intake with cancer risk in humans, with only 4 studies currently available in the literature, 2 case-control (Aro *et al*, 2000; Chajes *et al*, 2002) and 2 prospective studies (Voorrips *et al*, 2002; Larsson *et al*, 2005). Three of these studies focus exclusively on breast cancer. Aro and co-workers observed a significant reduction in breast cancer risk (OR 0.4, 95% CI, 0.2-0.9; p for trend <0.01) with CLA status determined by analysing plasma CLA levels (Aro *et al*, 2000). In contrast, no significant association between adipose tissue CLA and breast cancer risk was observed in a French case-control trial, with evidence of a trend towards an increase in risk from tertile 1 to tertile 3 (Chajes *et al*, 2002). This is consistent with the finding in the Netherlands Cohort Prospective Study, which reports a modest significant positive association between CLA intake, as assessed by FFQ, and breast cancer risk (RR 1.24, 95% CI, 0.91-1.69; p for trend =0.02) (Voorrips *et al*, 2002). In the Swedish Mammography Cohort, CLA intake as assessed by FFQ was associated with a reduced risk of colorectal cancer following 14.8 years of follow-up (RR 0.71, 95% CI, 0.55-0.91, p for trend =0.004) (Larson *et al*, 2005).

236. Although there is currently weak evidence to suggest that CLA intake may be associated with modest loss of fat mass, there is insufficient evidence to evaluate the impact of CLA in humans.

5. Overall summary and conclusions

237. Following a request from the Secretary of State for Health, this report considered: UK intake of *trans* FA; the evidence regarding effects of *trans* FA on CHD since the EFSA (2004) and WHO/FAO Expert Consultation (2003) reports on this issue; the evidence relating to other health effects of *trans* FA, particularly cancer, obesity and diabetes; whether, on the basis of present evidence, it is possible to distinguish the health effects of *trans* FA from vegetable oil versus those of animal origin; and whether present advice that *trans* FA intakes should not exceed on average 2% of food energy (COMA, 1994) should be revised.

238. *Trans* FA naturally occur at low levels in dairy products and meats from ruminant animals. They are also produced by the industrial hydrogenation of vegetable oils, a process that has been used to produce the semi-solid and solid fats that are now widely used in food manufacture (e.g. margarines, biscuits) and catering outlets. *Trans* FA are also formed during high temperature treatment of oils and during deodorisation of unsaturated oils to remove unstable by-products of oxidation.

239. The SACN *Framework for the Evaluation of Evidence* (SACN 2002) was used as the basis to identify and assess evidence published on CHD since the EFSA (2004) and WHO/FAO Expert Consultation (2003) reports, and to review published evidence for the other main diseases considered here (cancer, obesity, diabetes). The evidence base for this report was mainly restricted to retrospective and prospective epidemiology and RCTs in humans. In the epidemiology, measures of exposure included both direct measures of dietary *trans* FA intakes, as well as levels of *trans* FA in blood and tissues, which are taken to provide surrogate biomarkers of *trans* FA intakes.

240. The average adult (19-64 years) intake of *trans* FA in the UK was reported as being 2.2% food energy in 1986/87 (NDNS 1986/87), but had declined to 1.2% food energy by 2000/2001 (NDNS, 2000/1). Recent estimation of intake based on the reported consumption data from 2000/01, but using new composition data provided by industry, has given an estimated value of 1.00% food energy for the mean intake of *trans* FA in the UK adult population (FSA 2007). This figure is likely to be an overestimate of actual current intake as it was not possible in the time available to take account of all the reductions in *trans* FA levels in the model.

241. There is consistent evidence from prospective epidemiology to support a moderate impact of dietary *trans* FA on risk of CHD for ranges of intakes similar to, or slightly higher than, *trans* FA levels observed in the UK diet. Adverse effects of *trans* FA on LDL-C, HDL-C and total:HDL-C ratio have been consistently demonstrated in a number of well-controlled randomised trials. There is therefore a plausible biochemical mechanism to explain the pathophysiology underlying the prospective epidemiological findings.

242. Data obtained from a number of RCTs and meal studies using varying *trans* FA contents have not consistently demonstrated adverse effects of these FA on CHD biomarkers other than serum lipoproteins. These include classical biomarkers such as blood pressure and CRP, as well as emerging risk markers such as postprandial lipemia, lipid oxidation, markers of haemostasis, endothelial function and vascular inflammation. While the number of studies is limited for many biomarkers, their findings largely support the conclusion that the adverse effects of *trans* FA on CHD risk is primarily mediated via their actions in increasing circulating concentrations of pro-atherogenic LDL-C, whilst also decreasing concentrations of protective HDL-C.

243. The ability to quantify the increased risk of CHD attributable to that percentage of the general population currently consuming > 1% dietary energy as *trans* FA is limited by: i) estimates of risk from epidemiology for quintiles of intake in the region 1-2% dietary energy do not differ significantly from 1.0; ii) a lack of evidence for a linear relationship between *trans* FA intake and CHD risk over the range of 1-2% dietary energy (Figure 2); and iii) RCTs that have evaluated the impact of varying doses of *trans* FA on lipoproteins have not compared levels of intake between 1-2% dietary energy.

244. It should be noted that of the 10 published outputs from prospective studies which have reported on the association between *trans* FA intakes or biomarker levels and CHD risk, 6 have been obtained from the same study population (the Nurses' Health Study). There are potential limitations in estimating risks based on a preponderance of evidence from the Nurses' Health Study, since the study cohort includes only women and the dietary data are derived from an FFQ which shows relatively poor correlation with fat intakes estimated using more reliable measures. Extrapolation of risk estimates from a 20 year follow up of this study cohort (Oh *et al*, 2005), suggests that a decrease of 1% energy from *trans* FA would decrease risk of CHD by up to 15-16%. Similar extrapolation using the pooled variance-weighted risk from a meta-analysis of studies that included both men and women (6-14 y follow up) suggests that a 1% decrease in energy from *trans* FA would be associated with a 12.5% decrease in risk of CHD (Oomen *et al*, 2001).

245. Based on an average UK intake of 1.2% food energy (NDNS 2000/2001), it is estimated that to reduce *trans* FA intakes of the population so that every individual had a *trans* FA intake of < 1% food energy would require the average intake to be reduced by 0.6% of food energy as *trans* FA. Extrapolation from the meta-analysis of prospective studies (Oomen *et al*, 2001) would indicate that this decrease in *trans* FA intake would result in a 7.5% reduction in risk of CHD. Prospective studies assess total mortality or morbidity, and therefore consider the total effect of all contributors to disease pathology. Estimates for CHD risk based solely on the impact of *trans* FA on LDL-C and HDL-C and on the total: HDL-C ratio, rather than total disease incidence, suggest that the reduction in CHD risk arising from an average reduction in *trans* FA of 0.6% energy would be in the

region of 4.0%. This figure is lower than the risk reduction calculated from the epidemiological data, suggesting that either the CHD risk that can be specifically attributed to changes in lipoprotein profiles does not include the contribution of other pathophysiological pathways, or that the epidemiological risk estimate has been inflated by some unmeasured confounding.

246. Applying this same approach, but using the more recent estimates of UK *trans* FA intakes based on updates food composition data (FSA 2007), the estimated reductions in CHD risk based on an average UK intake of 1.00% food energy would be 5% and 2.8% for extrapolations from epidemiological and lipoprotein data, respectively.

247. Care must be taken when considering all of these estimates of risk reduction as they are based, in the case of some studies, on intake ranges higher than the current UK levels which assume a linear dose-response between *trans* FA intake and CHD risk. The latter assumption may not be valid given the distribution of the risk estimates at intake levels relevant to the UK population (Figure 2). The lack of linearity at the lower end of the range of *trans* FA intakes, suggest the actual reduction in risk is likely to be lower than the values estimated above.

248. There is some evidence from dietary data to suggest a more significant association between risk of CHD and *trans* FA of vegetable oil compared with that of animal origin. It should be noted that these conclusions were drawn from early prospective studies, including that of Willett *et al* (1993). Later outputs from the Nurses' Health Study have not reported separate associations for the dietary *trans* FA from vegetable or animal origin (Oh *et al*, 2005), although they have reported RRs for specific *trans* FA isomers in erythrocytes (Sun *et al*, 2007a; Sun *et al*, 2007b). It has been proposed that biomarker measurements of *trans* 18:2 and *trans* 16:1 in tissues and blood may provide surrogate markers for habitual intakes of *trans* FA of vegetable oil and animal origin, respectively. However, the evidence for this does not appear to have been subjected to systematic scrutiny. Until the validity of the use of these tissue biomarkers has been sufficiently well established, it may be misleading to use them to distinguish between *trans* FA of vegetable oil and animal origin.

249. There is weak and inconsistent evidence for a relationship between *trans* FA and breast or colorectal cancer. Evidence for an association between *trans* FA and prostate cancer is limited, but a recent large case-control study has shown a strong interaction between risk and *trans* FA intake for a particular genotype that make up ~35% of the population. This potential association requires further investigation. The strong association between non-Hodgkin's lymphoma and *trans* FA intakes reported in a single study require further verification by means of intake data based on more recent estimates of *trans* FA intake.

250. There are limited data available upon which to assess the risk of obesity or increased weight gain associated with increased intakes of dietary *trans* FA. The reports from 3 prospective cohorts show a small positive relationship between *trans* FA intake and increased weight or waist circumference. However, in the studies that reported positive associations, the effect size was small (a weight increase over 8 years of approximately 0.5-1.0 kg for a 1% increase in dietary *trans* FA) when considered in the context of the extended time periods investigated. Although relatively large effects on weight gain were reported by a long-term study involving primates, the level of *trans* FA used in this study (8% dietary energy) was much higher than the average UK intake (1.0-1.2% food energy).

251. The evidence for an association between *trans* FA intakes and incidence of diabetes is limited. Prospective cohort studies have reported inconsistent results, with two showing a positive association of moderate effect size, but the association lost significance after adjustment in one study. One of the studies, which did show a positive association, did not adjust for the effects of other fatty acids. No effect on insulin sensitivity or glucose tolerance was found in 4 RCTs or meal studies of healthy individuals. However, postprandial hyperinsulinaemia was observed in obese subjects with type 2 diabetes fed very high *trans* FA diets (20% dietary energy). An acute meal study reported a significantly higher insulin response following meals high in *trans* FA (10% dietary energy), with other adverse effects of *trans* FA appearing to be genotype dependent. Data from isolated islet studies suggest that there is a differential effect of *trans* FA compared with *cis* FA on the regulation of insulin secretion, with *trans* FA potentiating glucose-stimulated insulin secretion more than *cis*-isomers of identical chain length.

252. The assessment of the possible relationship between *trans* FA and early development is hindered by a lack of studies that are of suitable size and rigorous design which include adequate adjustment for potential confounding factors. Amongst the limited data available, the level of *trans* FA in plasma and tissue lipids is reported to be inversely proportional to the levels of long-chain ω-6 PUFA, with the conclusion that, indirectly, *trans* FA may interfere with the metabolism of essential fatty acids. This may be important as long-chain PUFA have been shown to be important in fetal growth and development, and may have longer-term effects on physical health and behaviour. The data require cautious interpretation because the use of FA compositional data from observational studies to indicate the effects of *trans* FA on long-chain ω-6 PUFA metabolism lacks rigour. This area requires further investigation by well-designed studies.

253. The potential association between *trans* FA intake and a number of health issues has been investigated in prospective epidemiological and population studies. Although a significant positive association was reported for gallstone formation, this has only been examined in one study. Trends towards positive associations were reported for Alzheimer's disease, cognitive decline and ovulatory infertility, but these failed to reach statistical significance.

254. Although this report has not been able to distinguish differences in risks attributable to *trans* FA of vegetable and animal origin, foods of animal origin that contain *trans* FA (dairy products, beef, lamb) are valuable sources of other nutrients such as protein, calcium and iron. These products currently account for roughly 40-50% of total *trans* FA intakes, although that proportionate figure is increasing as the *trans* FA levels in manufactured foods fall due to action taken by food manufacturers to reduce *trans* FA levels to the minimum required to maintain the quality of the product. Any recommendation for further reductions in *trans* FA intake levels should take account of the overall contribution these animal products make to the intake of key nutrients by the UK population.

255. At a horizon scanning meeting in 2003, SACN considered the need for an updated risk assessment on the health effects of *trans* FA. The Committee agreed that the original risk assessments made by COMA in 1994 remained appropriate and that the recommendation that *trans* FA intakes should not, on average exceed 2% food energy, should continue. The Committee considered that, although reductions in SFA intakes in the UK diet had been achieved since 1994, they remained above the target set by COMA (1994) and were considered to pose a greater risk to health than *trans* FA.

256. In considering the impact of changes in *trans* FA on targets for SFA, this review has also briefly considered the current intake levels of SFA (13.3% food energy) compared with the target intake of 11% food energy, and the dietary changes that would be required to achieve these intake levels by the UK population as a whole. There are already some indications that efforts by industry to reduce *trans* FA levels may have compromised efforts to achieve the dietary target for SFA, with reports that the reformulation of fats to remove *trans* FA may have resulted in increased SFA levels. This is of concern given the priority for reducing SFA as a population measure for reducing CHD risk. It is therefore important to monitor and assess changes in the overall lipid profile of the diet (*trans* FA, SFA, MUFA and PUFA) and their impacts on lipoprotein profiles of the population (LDL-C and HDL-C), so that adverse consequences can be identified.

Conclusions

257. The previous recommendation made by COMA that, on average, *trans* FA should contribute no more than 2% food energy, was based on epidemiological evidence of adverse effects of these fatty acids on risk of CHD. Since that report, epidemiological evidence based on up to 20 years of follow-up in prospective studies has remained consistent for an adverse effect of *trans* FA on CHD risk, although estimates of the size of the effect are smaller now than in 1994. Much of this evidence is based on a single large cohort of women in the USA. Evidence from RCTs that has emerged since 1994 has provided strong support for adverse effects of *trans* FA on LDL-C (increases) and HDL-C (decreases). In addition, the evidence for cardioprotective effects of HDL-C has strengthened over the same

time period, resulting in greater recognition of the potential hazards of *trans* FA due to their unique properties in reducing HDL-C compared with other FA classes.

258. In most cases, the data that are available are for ranges of intakes slightly higher than those of current UK intakes. It is concluded that there is sufficient evidence upon which to base a risk estimate for CHD, but not for other diseases. The reduction in risk of CHD that would be obtained if all the population were to reduce *trans* FA intake to ‹ 1% energy is estimated to be in the region of 7.5%. However, the overall impact may be less than this since this estimate is based on mean intake levels of 1.2% food energy (Henderson *et al*), whereas current intake levels (FSA 2007) may be closer to 1.0% food energy due to continuing efforts by the food industry to reduce levels of *trans* FA in manufactured foods. In the latter case, the reduction in risk is estimated to be in the region of 5%.

259. There is insufficient evidence to make reliable risk assessments for adverse effects of *trans* FA on risk of diseases other than CHD. Recent epidemiological data on the potential impact of *trans* FA on some types of cancers (colon, prostate, non-Hodgkin's lymphoma) and diabetes is inconsistent, and further research is required. There is some, but very limited, data to suggest adverse effects of *trans* FA on body weight and body fat accumulation. However, the data are sparse and the impact cannot presently be quantified with any accuracy. There is currently no putative mechanism that could explain differential effects of *trans* FA versus *cis* FA on energy balance and adipose tissue deposition. On the basis of this evidence, this review has concluded that a specific recommendation for further reduction in *trans* FA based on potential adverse effects on body weight and obesity cannot be made.

260. Taking into account the totality of the evidence reviewed in this report the Committee endorse the recommendation made by COMA in 1994 that average *trans* FA intakes should be no more than 2% of food energy intake. The Committee agreed that there is currently no firm scientific basis for revising the recommendations.

261. Steps taken since the COMA report (1994) to reduce levels of *trans* FA in manufactured foods are likely to have contributed to a reduction in risk of CHD for the UK population as a whole. A recommendation that no individual in the UK population should have a *trans* FA intake › 1% food energy may have adverse consequences for the overall lipid profile of the diet, including increasing SFA intake, and may also impact adversely on the consumption of animal products.

6. Recommendations

262. There is consistent evidence to support a moderate effect of *trans* FA on risk of CHD. The primary mechanism for this effect appears to be via changes in the serum lipoprotein profile, although inflammatory responses and endothelial function may also be negatively affected by dietary *trans* FA.

263. The evidence relating *trans* FA intakes to risk of diseases other than CHD is limited, and no reliable risk assessments can be made. However, future reports on these associations should be monitored, particularly the effect of *trans* FA on insulin sensitivity and diabetes, and the *trans* FA-genotype interaction with risk of prostate cancer.

264. This review endorses the current recommendation set by COMA (1994), that the average *trans* FA intake should not exceed 2% of food energy, as there is currently no firm scientific basis for its revision.

265. The current data provides insufficient evidence to justify the differentiation of *trans* FA from vegetable oil and animal sources based on the isomeric forms of the *trans* FA. There are also inadequate data to demonstrate that *trans* FA from different dietary sources have differential effects on CHD risk or lipoprotein profiles.

266. The impact of reformulations of fats within the diet should be monitored to ensure there are no unintended adverse consequences for dietary lipid profiles and related CHD risk factors.

Recommendations for further research

267. Outputs from the Nurses' Health Study provide 6 of the 10 prospective epidemiological reports on the association between *trans* FA and risk of CHD, as well as much of the data for associations between *trans* FA and diabetes (the largest of 3 studies) and weight gain (the largest of 2 studies). Reliance on outputs from a single large US study cohort as the basis for formulating public health recommendations within the UK is unsatisfactory. Further research is required using large cohorts which include both genders and which consider other biological variables such as high-risk genotypes. There may be a particular advantage in studying UK/European cohorts, in whom there may also be greater possibility of estimating risk of CHD at the lower end of the *trans* FA intake range.

268. There is a gap in the literature that would be filled by well-designed RCTs assessing the impact of *trans* FA on CHD and other disease risk factors at intake levels relevant to the current UK population (0.5-3% food energy).

269. Further research is required to distinguish the metabolic and health effects of different *trans* FA isomers. There is also a need for evidence to support the use of tissue and blood levels of specific *trans* FA isomers as markers of dietary origin, i.e. animal verses vegetable oil origin.

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Annex 1: List of abbreviations

△	changes in
95% CI	95% confidence interval
Akt	Protein Kinase B
ARA	arachidonic acid
BMI	body mass index, kg/m2
body wt	body weight
CARET	ß-Carotene and Retinol Efficacy Trial
CE	cholesteryl esters
CHAP	The Chicago Health and Aging Project
CHD	coronary heart disease
CHO	Carbohydrate
CHS	Cardiovascular Health Study
CLA	conjugated linoleic acid, 18:1
COMA	Committee on Medical Aspects of Food and Nutrition Policy
CRP	C-reactive protein
CVD	cardiovascular disease
DHA	docosahexaenoic acid
DMBA	dimethylbenz[a]anthracene
DRV	Dietary reference value
EFA	essential fatty acid
EFSA	European Food Safety Authority
EFS	Expenditure Food Survey
EURAMIC	European Community Multicenter Study on Antioxidants, Myocardial Infarction, and Breast Cancer

FA	fatty acid
FFQ	food frequency questionnaire
FSA	Food Standards Agency
GSIS	glucose-stimulated insulin secretion
HDL-C	High density lipoprotein cholesterol
HOMA index	Homeostasis Model Assessment index, a measure of insulin sensitivity
HPFS	Health Professionals' Follow-up Study
HRT	hormone replacement therapy
IE	interesterified soybean oil
IHD	ischemic heart disease
IL-12-p70	Interleukin-12-p70
IL-1β	Interleukin-1β
IL-2, IL-6, IL-8, IL-10	Interleukin-2, 6, 8 or 10
IWHS	Iowa Women's Health Study
Lp (a)	Lipoprotein (a)
LCAT	lecithin:cholesterol acyl transferase
LDL-C	Low density lipoprotein cholesterol
LIDNS	Low Income Diet Nutrition Survey
linoleic acid	*cis* 18:2 n-6
linolenic acid	*cis* 18:3 n-2
LPL	lipoprotein lipase
MEFAB	Maastricht Essential Fatty Acid Birth Cohort
MI	myocardial infarction
mRNA	messenger Ribonucleic Acid
MUFA	monounsaturated fatty acid

MV	Multivariate
NDNS	National Diet and Nutrition Survey
NEFA	non-esterified fatty acids
NHL	non-Hodgkin's lymphoma
NHS	Nurses' Health Study
NHS II	Nurses' Health Study, phase II
NLCS	The Netherlands Cohort Study
NR	not reported
NS	not significant
NSAIDs	nonsteroidal anti-inflammatory drugs
cis 18:1 n-9	oleic acid
OR	odds ratio
ORDET	Italian prospective Study on Hormones, Diet and Breast Cancer
PAI-1	plasminogen activator inhibitor-1
PHSO	partially hydrogenated soybean oil
PL	phospholipid
POL	palm olein
PPARg	peroxisome proliferative activated receptor gamma
PUFA	polyunsaturated fatty acid
Q1, Q2, Q3, Q4, Q5	quartiles (25%) or quintiles (20%) of the distribution of intake or tissue level of a nutrient in a population
r	Pearson correlation coefficient
RCT	randomised controlled trial
RR	relative risk
SACN	Scientific Advisory Committee on Nutrition

SD	standard deviation
SEM	standard error of the mean
SFA	saturated fatty acid
SHS	Strong Heart Study
sICAM-1	soluble intercellular adhesion molecule–1
sVCAM-1	soluble vascular adhesion molecule11
T1, T2, T3	tertiles (33.3%) of the distribution of intake or tissue level of a nutrient in a population
TAG	triacylglyerol
TC	total cholesterol
TNF-α	tumour necrosis factor-alpha
TDS	Total Diet Survey
tPA	tissue plasminogen activator
trans 18:1 n-7	vaccenic acid
trans 18:1 n-9	elaidic acid
trans FA	*trans* fatty acid (s)
TRANSFAIR	Trans fatty acid intake and risk factors for cardiovascular disease in Europe
UA	umbilical artery
UV	umbilical vein
VLDL-C	Very low density lipoprotein cholesterol
WHO	World Health Organisation
β	Standardised coefficient or β coefficient
ω-3	omega-3
ω-6	omega-6

Annex 2:
Studies considered relating to trans FA and health

Table 1A. Case control studies investigating the association of *trans* FA with risk of CHD

Reference	Subject population	Measure of exposure	Quartile Range and Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Aro *et al* (1995)	Eight European countries MI cases n =671 Controls n =717	Adipose tissue *trans* FA	Total adipose tissue *trans* FA 18:1 (median, % fatty acid) Q1 = 0.45 OR = 1 Q2 = 1.29 OR = 0.68 (0.41-1.13) Q3 = 1.80 OR = 1.05 (0.63-1.75) Q4 = 2.51 OR = 0.97 (0.56-1.67)	ns	Age, centre, smoking, BMI and number of cases vs. controls
Baylin *et al* (2003)	Costa Rican 482 case-control pairs	Adipose tissue *trans* FA	**Total** adipose tissue *trans* FA (median, g/100g) Q1= 1.84 OR = 1 Q2 = 2.46 OR = 1.34 (0.73-2.47) Q3 = 2.98 OR = 2.05 (1.06-3.98) Q4 = 3.57 OR = 2.22 (1.14-4.33) Q5 = 4.40 OR = 2.94 (1.36-6.37)	0.004	Sex, age, residence, income, history of diabetes, history of hypertension, physical activity, smoking, years living in house, adipose tissue α-linolenic acid^, alcohol^, vitamin E^, SFA^,total energy intake^, BMI, W:H, multivitamin use, folate, fibre and cholesterol intake.
			C16:1 adipose tissue *trans* FA (median, g/100g) Q1 = 0.044 OR = 1 Q2 = 0.070 OR = 1.57 (0.83-2.98) Q3 = 0.080 OR = 1.39 (0.73-2.66) Q4 = 0.096 OR = 1.34 (0.65-2.79) Q5 = 0.115 OR = 2.58 (1.22-5.43)	0.025	As above

Reference	Subject population	Measure of exposure	Quartile Range and Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
			C18:1 adipose tissue *trans* FA (median, g/100g) Q1= 0.98 OR = 1 Q2 = 1.31 OR = 1.14 (0.57-2.26) Q3 = 1.61 OR = 1.12 (0.54-2.32) Q4 = 1.97 OR = 1.26 (0.58-2.71) Q5 = 2.54 OR = 0.75 (0.34-1.65)	ns	As above
			C18:2 adipose tissue *trans* FA (median, g/100g) Q1= 0.75 OR = 1 Q2 = 0.98 OR = 0.96 (0.49-1.89) Q3 = 1.20 OR = 2.09 (0.98-4.48) Q4 = 1.50 OR = 3.51 (1.49-8.29) Q5 = 2.04 OR = 5.05 (1.86-13.72)	0.0005	As above
Clifton *et al* (2004)	Australian population Case n =209 (adipose sample n =79) Control n =174 (adipose sample n =167)	*Trans* FA assessed by FFQ and adipose tissue	Dietary *trans* FA intake (median, g/day) Q1 = 1.55 RR[1] = 1 Q2 = 2.41 RR = 1.0 (0.53-1.88) Q3 = 3.03 RR = 1.3 (0.69-2.44) Q4 = 3.7 RR = 1.23 (0.65 – 2.23) Q5 = 5.46 RR = 2.25 (1.16-4.32) *trans*FA 18:1 (n-7) was an independent predictor of first MI	ns (w/o SFA adjustment p for trend =0.01) p =0.03	Energy, % energy as fat, protein, CHO, SFA, PUFA, BMI, age, sex, blood pressure, lipids, smoking, job classification Blood lipids, dietary intake and other adipose tissue FA

[1] The RR stated are those which have been fully adjusted for confounding factors. When a significant difference in an associated risk was reported, the specific factors that were adjusted for in the model have been shown within the table. The p trend values refer to the level of difference between the highest and lowest tertile/quartile/quintile of intake.

Reference	Subject population	Measure of exposure	Quartile Range and Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Colón-Ramos *et al* (2006)	Costa Rican 1797 case control matched pairs 1994-1999 (n =954) 2000-2003 (n =2638)	Adipose tissue *trans* FA	**1994-1999** **Total** adipose tissue *trans* FA (median, g/100g) Q1= 1.85 OR = 1 Q2 = 2.47 OR = 1.37 (0.80-2.35) Q3 = 2.99 OR = 1.91 (1.08-3.37) Q4 = 3.58 OR = 1.86 (1.04-3.32) Q5 = 4.40 OR = 3.28 (1.68-6.42) **2000-2003** **Total** adipose tissue *trans* FA (median, g/100g) Q1 = 1.84 OR = 1 Q2 = 2.26 OR = 0.78 (0.58-1.04) Q3 = 2.57 OR = 1.03 (0.80-1.43) Q4 = 2.88 OR = 0.88 (0.65-1.18) Q5 = 3.42 OR = 1.03 (0.75-1.42)	<0.001 ns	Sex, age, residence, income, history of diabetes, history of hypertension, physical activity, smoking, alcohol, adipose tissue α-linolenic acid^, vitamin E^, SFA^, total energy intake^, education, weight, height, W:H, multivitamin use, folate, fibre and cholesterol intake.

Reference	Subject population	Measure of exposure	Quartile Range and Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
			1994-1999 **C18:1** adipose tissue *trans* FA (median, g/100g) Q1 = 0.93 OR = 1 Q2 = 1.30 OR = 1.84 (1.09-3.11) Q3 = 1.61 OR = 1.54 (0.87-2.71) Q4 = 1.96 OR = 2.13 (1.21-3.74) Q5 = 2.54 OR = 1.75 (0.97-3.15) **2000-2003** **C18:1** adipose tissue *trans* FA (median, g/100g) Q1 = 0.85 OR = 1 Q2 = 1.10 OR = 0.94 (0.71-1.24) Q3 = 1.29 OR = 0.92 (0.69-1.23) Q4 = 1.52 OR = 0.97 (0.72-1.30) Q5 = 1.94 OR = 1.02 (0.75-1.37)	ns ns	As above

Reference	Subject population	Measure of exposure	Quartile Range and Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
			1994-1999 **C18:2** adipose tissue *trans* FA (median, g/100g) Q1 = 0.75 OR = 1 Q2 = 0.98 OR = 1.04 (0.60-1.80) Q3 = 1.20 OR = 2.16 (1.18-3.96) Q4 = 1.50 OR = 2.86 (1.50-5.45) Q5 = 2.02 OR = 4.76 (2.24-10.11) **2000-2003** **C18:2** adipose tissue *trans* FA (median, g/100g) Q1 = 0.74 OR = 1 Q2 = 0.91 OR = 1.09 (0.81-1.46) Q3 = 1.05 OR = 1.10 (0.81-1.50) Q4 = 1.19 OR = 1.05 (0.75-1.46) Q5 = 1.40 OR = 1.15 (0.80-1.64)	<0.001 ns	As above
Lopes *et al* (2007)	Portuguese case-control study Cases n =297, controls n =310; adipose tissue biopsy provided by n =49 cases and n =49 controls	*Trans* FA assessed by FFQ and adipose tissue * levels from both methods did not correlate (r =-0.2)	Dietary *trans* FA intake (median, g/day) Q1 = 0.34 OR = 1 Q2 = 0.58 OR = 0.79 (0.47-1.33) Q3 = 1.20 OR = 0.66 (0.38-1.14) Q4 = 1.38 OR = 0.81 (0.48-1.37) Adipose tissue *trans* FA (median % total fatty acid content) Q1 = 0.62 OR = 1 Q2 = 0.77 OR = 0.25 (0.06-0.96) Q3 = 0.93 OR = 0.04 (0.006-0.32)	ns 0.001	Age, family history of acute MI, smoking, years of education, physical activity, BMI, energy intake Age, family history of acute MI, smoking, years of education, physical activity, BMI, energy intake

BMI, body mass index; MI, myocardial infarction; W:H, waist to hip ratio; SFA, saturated fatty acids; CHO, carbohydrate; PUFA, polyunsaturated fatty acids; w/o, without; ; ^ When these factors included in multivariate analysis trend became significant, or level of significance was increased

Table 2A. Prospective cohort studies investigating the association of *trans* FA with risk of CHD

Reference	Subject population	Measure of exposure	Quartile Range and Relative Risk[1] (95% CI)	Trend	Factors adjusted for in analysis
Willett *et al* (1993) NHS	n =85,095 431 incident CHD cases^^ 8 y follow up	Dietary *trans* FA assessed by FFQ	Dietary *trans* FA (median, g/day) Q1 = 2.4 RR = 1 Q2 = 3.2 RR = 1.23 (0.50-1.79) Q3 = 3.9 RR = 1.11 (0.79-1.68) Q4 = 4.5 RR = 1.36 (0.89-2.09) Q5 = 5.7 RR = 1.67 (1.05-2.66) *Trans* from vegetable fats / *Trans* from animal fats Q1= RR = 1 / RR =1 Q2 = RR = 1.43 (1.00-2.04) / RR = 0.76 (0.51-1.12) Q3 = RR = 1.11 (0.74-1.66) / RR = 0.69 (0.43-1.10) Q4 = RR = 1.39 (0.91-2.13) / RR = 0.55 (0.31-0.96) Q5 = RR = 1.78 (1.12-2.83) / RR = 0.59 (0.30-1.17)	p =0.002 Veg *trans* p =0.009 Animal *trans* p =0.230	Age, smoking, BMI, hypertension, alcohol intake, menopausal status, postmenopausal oestrogen use, energy intake, family history of myocardial infarction, intake of SFA, MUFA and linoleic acid and multivitamin use
Hu *et al* (1997) NHS	n =80,082 939 incident CHD cases^^ 14 y follow up	Dietary *trans* FA assessed by FFQ	Dietary *trans* FA (median, % of energy) Q1 = 1.3 RR = 1 Q2 = 1.7 RR = 1.09 (0.87-1.37) Q3 = 2.0 RR = 1.16 (0.91-1.47) Q4 = 2.4 RR = 1.24 (0.96-1.60) Q5 = 2.9 RR = 1.53 (1.16-2.02)	p =0.002	Age, time period, BMI, smoking status, menopausal status, parental history of MI incidence, multivitamin, vitamin E, alcohol, history of hypertension, aspirin use, physical activity, % energy from protein, total energy intake, dietary cholesterol, SFA, PUFA and MUFA intake.

[1] The RR stated are those which have been fully adjusted for confounding factors. When a significant difference in an associated risk was reported, the specific factors that were adjusted for in the model have been shown within the table. The p trend values refer to the level of difference between the highest and lowest tertile/quartile/quintile of intake.

Reference	Subject population	Measure of exposure	Quartile Range and Relative Risk[1] (95% CI)	Trend	Factors adjusted for in analysis
Tanasescu *et al* (2004) NHS	n =5672 (diagnosed with diabetes since baseline) 619 incident CHD cases^^ 18 y follow up	Dietary *trans* FA assessed by FFQ	Dietary *trans* FA (median, % of energy) Q1 = 1.3 RR = 1 Q2 = 1.7 RR = 0.90 (0.68-1.19) Q3 = 2.1 RR = 1.15 (0.87-1.52) Q4 = 2.4 RR = 0.95 (0.69-1.30) Q5 = 3.0 RR = 1.03 (0.73-1.44)	p =0.74	Age, smoking status, menopausal hormone use, parental history of MI incidence, alcohol intake, physical activity, BMI, energy intake, protein, fibre, multivitamin use, vitamin E supplementation, diabetic medication, SFA, MUFA, PUFA and cholesterol intake
Oh *et al* (2005) NHS	n =78,778 1766 incident CHD cases^^ 20 y follow up	Dietary *trans* FA assessed by FFQ	Dietary *trans* FA (median, % of energy) Q1 = 1.3 RR = 1 Q2 = 1.6 RR = 1.08 (0.92-1.26) Q3 = 1.9 RR = 1.29 (1.09-1.53) Q4 = 2.2 RR = 1.19 (0.99-1.44) Q5 = 2.8 RR = 1.33 (1.07-1.6)	p =0.01	Age, BMI, smoking status, menopausal status, hormone use, parental history of MI incidence, multivitamin, vitamin E, alcohol, history of hypertension, aspirin use, physical activity, % energy from protein, total energy intake, dietary cholesterol, SFA, PUFA,MUFA intake, α-linolenic acid, marine n-3 FA, cereal fibre and fruit and vegetable intake
Lemaitre *et al* (2006) CHS	Nested case-control study in CHS (cases n =214, controls n =214)	Plasma phospholipids *trans* FA	Plasma phospholipid *trans* FA *Trans*FA 18:2 *Trans*FA 18:1 Q1 = OR = 1 Q1 = OR = 1 Q2 = OR = 0.87 (0.41-1.84) Q2 = OR = 0.29 (0.14-0.61) Q3 = OR = 1.08 (0.52-2.28) Q2 = OR = 0.29 (0.15-0.70) Q4 = OR = 3.20 (1.42- 7.20) Q4 = OR = 0.45 (0.21- 0.97) Q5 = OR = 4.52 (1.83-11.20) Q5 = OR = 0.38 (0.17-0.86) Total *trans* FA and *trans* 16:1 not associated with IHD	p for trend not stated	Age, gender, presence of cardiovascular disease, clinic site, time of blood draw, diabetes mellitus, education, smoking status, congestive heart failure, history of stroke, plasma phospholipid levels of docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), *trans* FA 18:2 and *trans* FA 18:1

Reference	Subject population	Measure of exposure	Quartile Range and RRisk Ratio (95% CI)	Trend	Factors adjusted for in analysis
Sun *et al* (2007a)	Nested case-control study in NHS (cases^^ n =166, controls n =327)	Erythrocyte *trans* FA	Erythrocyte total *trans* FA (mean % fatty acid content) Q1 = 1.17 RR = 1 Q2 = 1.50 RR = 1.6 (0.7-3.6) Q3 = 1.72 RR = 1.6 (0.7-3.4) Q4 = 2.23 RR = 3.3 (1.5-7.2) Erythrocyte total *trans* FA 18:1 (mean % fatty acid content) Q1 = 0.77 RR = 1 Q2 = 1.03 RR = 1.1 (0.5-2.4) Q3 = 1.21 RR = 1.3 (0.6-2.7) Q4 = 1.62 RR = 3.1 (1.5-6.7) Erythrocyte total *trans* FA 18:2 (mean % fatty acid content) Q1 = 0.25 RR = 1 Q2 = 0.31 RR = 1.5 (0.7-3.4) Q3 = 0.38 RR = 2.5 (1.1-5.7) Q4 = 0.50 RR = 2.8 (1.2-6.3)	p<0.01 p<0.01 p<0.01	Age, smoking status, fasting status at blood draw, BMI, menopausal status and hormone use, physical activity, alcohol intake, aspirin use, parental history of MI, history of hypertension, hypercholesterolemia and diabetes, long chain n-3 and total n-6 FA in erythrocytes
Sun *et al* (2007b)	As above	Plasma and erythrocyte *trans* FA	Plasma *trans* FA 16:1 n-7 (mean % fatty acid content) Matched MV T1 = 0.11 RR = 1 RR = 1 T2 = 0.15 RR = 0.77 (0.49-1.20) RR = 0.79 (0.46-1.36) T3 = 0.20 RR = 0.61 (0.39-0.97) RR = 0.79 (0.44-1.42) Erythrocyte *trans* FA 16:1 n-7 (mean % fatty acid content) MV T1 = 0.10 RR = 1 T2 = 0.14 RR = 0.79 (0.44-1.43) T3 = 0.17 RR = 0.98 (0.53-1.83)	Matched p =0.04 MV p = ns p =ns	Matched: Age, smoking status, fasting status at blood draw As above plus: BMI, menopausal status and hormone use, physical activity, alcohol intake, aspirin use, parental history of MI, history of hypertension, hypercholesterolemia and diabetes, linoleic acid, total *trans* FA in erythrocytes/plasma

NHS, Nurses' Health Study; CHS, cardiovascular health study; (European Food Safety Authority, 2004) veg, *trans* from vegetable sources; BMI, body mass index; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; MV, multivariate adjustment; IHD, ischemic heart disease; ^^incident CHD cases include non fatal MI and fatal CHD

Table 3A. Randomised controlled trials investigating the association of trans FA with fasting lipoproteins

Reference	Subject population (men/women)	Design	Time period for study	Diet name	Fatty acid composition (% of daily energy intake)				Outcomes				Other
					S	M	P	T	TC	HDL-C	LDL-C	TAG	
Han *et al* (2002)	8/11 HC	X	32 days/ diet	SO Stick marg Butter	7.3 8.5 16.7	8.1 8.5 8.1	12.5 6.3 2.4	0.6 6.7 1.3	mg/dl 227 [c] 245 [b] 257 [a]	mg/dl 45 [b] 42 [c] 48 [a]	mg/dl 150 [c] 167 [b] 177 [a]	mg/dl 158 166 153	TC:HDL significantly ↑ on stick marg diet
Lichtenstein *et al* (2003)	18/18 HC	X	5 weeks/ diet	SO S-L marg Soft marg PH-short. Stick marg Butter	0.26 g/100g trans 18:1 & 18:2 0.6 g/100g trans 18:1 & 18:2 9.4 g/100g trans 18:1 & 18:2 13.6 g/100g trans 18:1 & 18:2 26.1 g/100g trans 18:1 & 18:2 2.6 g/100g trans 18:1 & 18:2 (experimental fats provided 20% energy)				Results graphically displayed therefore individual result not available for tabulation				TAG significantly ↑ after stick marg vs. S-L marg TC significantly ↑ after butter and stick marg. vs. SO, S-L marg, soft marg. LDL-C significantly ↑ after PH-short, stick marg and butter vs. SO and S-L marg VLDL-C significantly ↑ after stick marg vs S-L marg
Dyerberg *et al* (2004)	89/0 79 completed H	P	8 weeks	*Trans* FA n-3PUFA Control	10.3 12.3 15.7	10.2 10.4 10.2	5.7 6.5 6.2	6.8 0.9 0.9	mmol/l 4.60 5.02 5.27	mmol/l 1.26 [a] 1.29 [a,b] 1.31 [b]	mmol/l 2.81 3.28 3.46	mmol/l 1.17 [a,b] 0.99 [a] 1.14 [b]	
Tholstrup *et al*, (2006)	42/0 H	P	5 weeks	Hi *Trans* FA Lo *Trans* FA	22.5 24.4	14.9 9.6	3.8 3.6	~2.2 ~0.4	mmol/ 4.57 a 4.87 [b]	mmol/l 1.39 a 1.54 [b]	mmol/l 3.17 3.44	mmol/l 1.01 0.89	

Reference	Subject population (men/women)	Design	Time period for study	Diet name	Fatty acid composition (% of daily energy intake)				Outcomes				Other
					S	M	P	T	TC	HDL-C	LDL-C	TAG	
Lichtenstein *et al*, (2006)	14/16 HC	X	5 weeks/ diet	SO LoSFA-SO HiOA-SO LoALA-SO PH-SO	6.5 4.9 5.8 6.8 7.3	6.5 6.2 18.9 7.0 10.0	12.7 14.6 2.8 13.5 8.7	0.6 0.6 0.3 0.5 2.5	mmol/l 5.72 [b] 5.59 [b] 5.71 [b] 5.74 [b] 6.01 [a]	mmol/l 1.32 [a,b] 1.32 [b] 1.36 [a] 1.32 [b] 1.32 [a,b]	mmol/l 3.66 [b] 3.53 [b] 3.70 [b] 3.71 [a,b] 3.92 [a]	mmol/l 1.67 1.65 1.61 1.73 1.73	PH-SO ↔ difference in VLDL-C, HDL2, LDL-C:ApoB, HDL-C:ApoA1
Vega-López *et al* (2006)	5/10 HC	X	5 weeks/ diet	PH-SO SO Palm oil Canola oil	8.56 7.30 14.83 6.38	7.51 7.20 10.24 13.72	8.13 12.5 3.51 8.74	4.15 0.55 0.60 0.98	mg/dl 235 [a] 220 [b] 240 [a] 210 [b]	mg/dl 48 49 50 48	mg/dl 162 [a] 145 [b] 165 [a] 140 [b]	mg/dl 129 123 120 120	
Sundram *et al* (2007)	M/F Gender breakdown not stated for 30 completers Initially 11/21 H	X	4 weeks/ diet	Palm olein PH SO IE	13.7 9.1 18.2	13.6 12.4 5.9	3.6 5.8 7.0	0 3.2 0	mmol/l 4.93 5.03 4.89	mmol/l 1.43 [a] 1.32 [b] 1.30 [a,b]	mmol/l 3.08 [a] 3.30 [b] 3.20 [a,b]	mmol/l 0.91 0.88 0.86	↔ difference in VLDL cholesterol between diets
Mensink *et al* (2007)	11/33 H	X	3 weeks/ diet	HI OA *trans* HI palmitic	12.3 15.1	9.4 6.5	6.2 6.2	0.7 0.2	mmol/l 5.60 [a] 6.03 [b]	mmol/l 1.55 [a] 1.62 [b]	mmol/l 3.49 [a] 3.84 [b]	mmol/l 1.20 1.25	
Chardigny *et al* (2007)	19/21 H	X	3 weeks/ diet	PH *trans* FA Nat *trans* FA	Total trans FA ~4% each diet 2.43% energy as *trans* 18:1 , n-9 3.43% energy as trans 18:1, n-7				mg/dl 170.8 [a] 182.7 [b]	mg/dl 66.6 [a] 68.5 [b]	mg/dl 88.3 a 96.3 [b]	mg/dl 79.4 a 89.5 [b]	Gender specific analysis showed differences in outcome found in women only

[a,b] different subscripts denote significant difference between outcomes in same column. S, saturated fatty acids; M, monounsaturated fatty acids; P, polyunsaturated fatty acids; T, *trans* fatty acids; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; TAG, triacylglycerol; ↔ no difference; ↑ increased; X, Cross-over or Latin square design; P, parallel; H, healthy; HC, hypercholesterolemic (LDL>130mg/dl); SO, soybean oil; S-L semi liquid; marg, margarine; PH-short, partially hydrogenated shortening; Lo, low; Hi, high; LoSFA-SO, low saturated fat soybean oil; HiOA-SO, high oleic soybean oil; LoALA-SO low α-linolenic soybean oil; PH-SO, partially hydrogenated soybean oil; OA oleic acid; Nat, animal trans FA; PH, partially hydrogenated; ns, not significant; ~ calculated from data presented in original paper

Table 4A. Randomised controlled studies investigating the association of *trans* FA and postprandial triacylglycerol (lipemia)

Reference	Subject population (men/women)	Design	Time period for study	Diet name	Composition of test meals	Outcome
Sanders *et al* (2000)	11/5 H	X	Acute meal	*Cis* 18:1 *Trans* 18:1 Stearic Palmitic MCT Low fat	65% energy as fat (low fat 7% energy as fat) g/meal of specific fa 68.6g 34.1g 35.6g 36.9 37.7g 10g fat	3 h: TAG significantly ↑ after OA, *trans* 18:1, palmitic vs stearic, MCT and low fat meals 7 h: TAG significantly ↑ after OA, *trans* 18:1, palmitic vs low fat meal
Thostrup *et al* (2001)	16/0 H	X	Acute meal	Stearic P&M *Cis* 18:1 *Trans* 18:1 Linoleic	50.6% energy as fat % total by wgt of specific fa in test TAG 47 21.5 myristic, 23.9% palmitic 82.5 44.7 42.9	↔ Difference in postprandial lipemia between *trans* and *cis* 18:1 meals
Gatto *et al* (2003)	19/0 H	X	Acute meal	*Trans* 18:1 *Cis* 18:1	75% energy as fat 10% energy as *trans* 18:1 10% energy as *cis* 18:1	Cholesteryl ester transport ↑ significantly after *trans* meal vs *cis* meal TRL formed after trans meal significantly ↑ lipoprotein (a) content vs cis meal ↔ difference in TAG, TRL TAG, TC, HDL-C
Lefevre *et al* (2005)	10/12 H, OW	X	Acute meal	*Trans* 18:1 *Cis* 18:1	50% energy as fat 20% energy *cis* 18:1 10% energy as *cis*, 10% energy *trans* 18:1	↔ difference in postprandial TAG

Reference	Subject population (men/women)	Design	Time period for study	Diet name	Composition of test meals	Outcome
Cantwell *et al* (2006)	8/0 H	X	Acute meal	PH-fish oil Palm oil Lard	~45% energy as fat 23% energy as *trans* FA 0% trans FA, 29.6% energy as SFA 0.79% trans FA, 27.1% SFA	↔ difference in postprandial TAG, TRL-TAG, TC, NEFA

Fa, fatty acid; H, healthy; OW, overweight; X, Cross-over or Latin square design; PH, partially hydrogenated; wgt, weight; TAG, triacylglycerol; TRL, triacylglycerol rich lipoproteins; NEFA, non esterified fatty acids; TC, total cholesterol; ↔ no difference, ↑ increased

Table 5A. Randomised controlled trials investigating the association of *trans* FA with oxidative stress

Reference	Subject population (men/women)	Design	Time period for study	Diet name	Fatty acid composition (% of daily energy intake)				8-iso-$PGF_{2\alpha}$ nmol/mmol Cr or ng/ml	15-kd-$PGF_{2\alpha}$ nmol/mmol Cr	8-oxoG nmol/mmol Cr
					S	M	P	T			
Kuhnt *et al* (2006)	12/12 H	P	6 weeks	*Trans* FA low *trans* FA	~7.4 ~7.8	~9.1 ~9.9	~6.9 ~8.6	~2.5 ~0.1	0.54[a] 0.38[b]	0.22 0.20	0.95 1.02
Tholstrup *et al* (2006)	42/0 H	P	5 weeks	Hi *trans* FA Lo *trans* FA	22.5 24.4	14.9 9.6	3.8 3.6	~2.2 ~0.4	0.99 1.05		

[a,b] different subscripts denote significant difference between outcomes in same column; S, saturated fatty acids; M, monounsaturated fatty acids; P, polyunsaturated fatty acids; T, *trans* fatty acids; H, healthy; P, parallel design; ~ calculated from data presented in original paper

Table 6A. Randomised controlled trials investigating the association of *trans* FA with haemostatic function

Reference	Subject population (men/women)	Design	Time period for study	Diet name	Fatty acid composition (% of daily energy intake)				Haemostatic function				Other
					S	M	P	T					
Turpeinen *et al* (1998) H	31/49	P	5 weeks	*Trans* 18:1 Stearic acid	7.1 15.0	11.7 11.3	2.9 3.5	8.7 0.4	ADP P-A (2µmol/l) 75.3 78.8	Collagen P-A (2µg/ml) 97.6 [a] 100.9 [b]		P-TBX B_2 (ng/ml) 54.3 67.2	↔ differences in *in vivo* platelet aggregation, (β-thromboglobulin and 2,3-dinor-6-keto-PGF1α levels)
Armstrong *et al* (2000) H	88/0	X	6 weeks/ diet	LO *trans* 18:3 HI *trans* 18:3	60 mg trans 18:3 /day 1410 mg trans 18:3 /day				PAI-1 (AU/ml) 11.1 10.3	FVII (%) 98.5 95.6	FVIIc (%) 102.9 97.6	Fibrinogen (g/l) 2.9 2.9	↔ collagen induced platelet aggregation ↔ *in vitro* thromboxane B_2 production by platelets
Sanders *et al* (2003) H	29/0	X	2 weeks/ diet	*Trans* 18:1 *cis* 18:1 CHO	9.5 10.2 10.9	12.3 20.7 12.8	6.1 6.5 6.4	9.6 0.1 0.1	PAI-1 (U/10^3/l) 12.67 12.84 13.92	t-PA (U/10^3/l) 1.86 1.86 1.68	FVIIc (%) 101 101 101	Fibrinogen (g/l) 2.41 2.37 2.43	↔ D-dimer concentration
Tholstrup *et al* (2003) H	16/0	X	Acute meal	Stearic Palmitic *Cis* 18:1 Linoleic *Trans* 18:1 P&M	50.6% energy as fat 41-47% provided by experimental fats				Individual data points not provided				activated factor VII ↓ stearic meal compared to the *trans* FA meal (p =0.017). ↔ in postprandial factor VII coagulation activity, PAI-1 levels or activity or tPA activity

Reference	Subject population (men/women)	Design	Time period for study	Diet name	Fatty acid composition (% of daily energy intake)				Haemostatic function		Other
					S	M	P	T			
Baer *et al* (2004)	50/0	X	5 weeks/ diet	CHO *cis* 18:1 *trans* 18:1 *trans* & SA SA 12:0-16:0	39% energy as fat, 8% made up from experimental fat 421 g/day CHO 61.2 g/day cis 18:1 28.8 g/day trans 18:1 23.8 (SA)/14.5 g/day *trans* 37.8 g/day SA 62.6 g/day 12:0-16:0				Fibrinogen (g/l) 2.61[a] 2.58[a] 2.58[a] 2.58[a,b] 2.73[b] 2.53[a]		
Pedersen *et al* (2005)	0/27 Haemostatic measures (n =9)	X	17 days/diet	PH-SO Palm oil PUFA	3.8 11.2 6.1	10.6 11.8 11.7	5.5 5.7 10.2	7.0 0.1 0.1	Individual data points not provided		↔ between diets for plasma fibrinogen levels, factor VII activity, or PAI-1 and tPA levels or activity.
Tholstrup *et al* (2006)	42/0 H	P	5 weeks/ diet	HI *Trans* 18:1 n-7 LO *Trans* 18:1 n-7	22.5 24.4	14.9 9.6	3.8 3.6	~2.2 ~0.4	PAI-1 (ng/ml) 7.79 8.69	FVIIc (%) 95 90	

[a,b] different subscripts denote significant difference between outcomes in same column; S, saturated fatty acids; M, monounsaturated fatty acids; P, polyunsaturated fatty acids; T, trans fatty acids; H, healthy; X, Cross-over or Latin square design; LO, low; HI, high; CHO, carbohydrate; P&M, palmitic and myristic acid mix; PH-SO, partially hydrogenated soybean oil; PUFA, polyunsaturated fatty acids; PAI-1, plasminogen activator inhibitor-1; tPA, tissue plasminogen activator; FVIIc, factor VII coagulant activity; ADP-PA, ADP induced platelet aggregation; P-TBX B_2, platelet thromboxane B_2 production; Collagen P-A, collagen induced platelet activation; ↔ no difference; ↓ decreased

Table 7A. Randomised controlled trials investigating the association of trans FA with blood pressure and endothelial function

Reference	Subject population (men/ women)	Design	Time period for study	Diet name	Fatty acid composition (% of daily energy intake)				Blood pressure/ Endothelial function		Other
					S	M	P	T			
de Roos *et al* (2001)	10/19 H	X	4 weeks/ diet	*Trans* 18:1 SFA	12.9 22.9	8.4 7.9	4.7 6.9	9.2 0.3	FMD (%) 4.4 [a] 6.2 [b]		
de Roos *et al* (2002)	21/0 H	X	Acute meal	*Trans* 18:1 Palm oil	33.8 g/100g *trans* 18:1 0.4 g/100g *trans* 18:1				FMD (%) 2.87 3.00		
Lichtenstein *et al* (2003)	18/18 HC	X	5 weeks/ diet	SO S-L marg Soft marg PH-short. Stick marg Butter	0.26 g/100g *trans* 18:1 & 18:2 0.6 g/100g *trans* 18:1 & 18:2 9.4 g/100g *trans* 18:1 & 18:2 13.6 g/100g *trans* 18:1 & 18:2 26.1 g/100g *trans* 18:1 & 18:2 2.6 g/100g *trans* 18:1 & 18:2 (experimental fats provided 20% energy)				SBP mmHg 125 125 127 123 121 124	DBP mmHg 77 78 79 77 76 77	
Baer *et al* (2004)	50/0 H	X	5 weeks/ diet	CHO *cis* 18:1 *trans* 18:1 *trans* & SA SA 12:0-16:0	39% energy as fat, 8% made up from experimental fat 421 g/day CHO 61.2 g/day cis 18:1 28.8 g/day trans 18:1 23.8 (SA)/14.5 g/day *trans* 37.8 g/day SA 62.6 g/day 12:0-16:0				E-selectin (ng/ml) 38.8 [a,b] 37.9 [a] 42.4 [c] 40.4 [b] 38.2 [b] 41.1 [b]		

Reference	Subject population (men/ women)	Design	Time period for study	Diet name	Fatty acid composition (% of daily energy intake)				Blood pressure/ Endothelial function				Other
					S	M	P	T					
									SBP mmHg	DBP mmHg	MAP mmHg	FMD (%)	↔ heart variability rate between diets
Dyerberg *et al* (2004)	89/0 79 completed H	P	8 weeks/ diet	*Trans* FA	10.3	10.2	5.7	6.8	124.8	72.5	89.9[a,b]		
				n-3PUFA	12.3	10.4	6.5	0.9	127.6	76.8	93.7[b]	103.8	↔ arterial dilatory capacity, arterial compliance and distensibility
				Control	15.7	10.2	6.2	0.9	123.8	74.3	90.9[a]	103.6	
												103.3	

[a,b] different subscripts denote significant difference between outcomes in same column; S, saturated fatty acids; M, monounsaturated fatty acids; P, polyunsaturated fatty acids; T, trans fatty acids; H, healthy; HC, hypercholesterolemic (LDL>130mg/dl); X, Cross-over or Latin square design; SFA; Saturated fatty acids; SO, soybean oil; S-L semi liquid; marg, margarine; PH-short, partially hydrogenated shortening; CHO, carbohydrate; SA, stearic acid; SBP, systolic blood pressure; DBP, diastolic blood pressure; FMD, flow mediated dilation; MAP, mean arterial pressure; ↔ no difference

Table 8A. Randomised controlled trials investigating the association of trans FA with inflammation

Reference	Subject population (men/women)	Design	Time period for study	Diet name	Fatty acid composition (% of daily energy intake)				Inflammatory outcome	
					S	M	P	T		
Han *et al* (2002)	8/11 HC	X	32 days/diet	SO Stick marg Butter	7.3 8.5 16.7	8.1 8.5 8.1	12.5 6.3 2.4	0.6 6.7 1.3	IL-6 and TNFα produced by extracted, stimulated mononuclear cells significantly ↑ in stick marg diet compared to SO diet ($p<0.05$) ↔ delayed-hypersensitivity skin response, lymphocyte proliferation or IL-2 production by extracted monocytes.	
Lichtenstein *et al* (2003)	18/18 HC	X	5 weeks/ diet	SO S-L marg Soft marg PH-short. Stick marg Butter	0.26 g/100g trans 18:1 & 18:2 0.6 g/100g trans 18:1 & 18:2 9.4 g/100g trans 18:1 & 18:2 13.6 g/100g trans 18:1 & 18:2 26.1 g/100g trans 18:1 & 18:2 2.6 g/100g trans 18:1 & 18:2 (experimental fats provided 20% energy, two thirds of fat intake)				CRP (mg/dl) 2.45 2.70 2.30 2.70 2.18 2.24	
Baer *et al* (2004)	50/0 H	X	5 weeks/ diet	CHO *cis* 18:1 *trans* 18:1 *trans* & SA SA 12:0-16:0	39% energy as fat, 8% made up from experimental fat 421 g/day CHO 61.2 g/day *cis* 18:1 28.8 g/day *trans* 18:1 23.8 (SA)/14.5 g/day *trans* 37.8 g/day SA 62.6 g/day 12:0-16:0				CRP (mg/l) 1.06[a] 0.97[a] 1.04[b] 0.95[a] 0.98[a,b] 1.02[a,b]	IL-6 (pg/ml) 1.43[a,b] 1.36[a] 1.55[b] 1.57[a,b] 1.56[b] 1.56[b]

Reference	Subject population (men/women)	Design	Time period for study	Diet name	Fatty acid composition (% of daily energy intake)				Inflammatory outcome
					S	M	P	T	
Tholstrup *et al* (2006)	42/0 H	P	5 weeks	HI *Trans* 18:1 n-7 LO *Trans* 18:1 n-7	22.5 24.4	14.9 9.6	3.8 3.6	~2.2 ~0.4	CRP (mg/l) 0.77 0.80
Kuhnt *et al* (2007)	12/12 H	P	6 weeks	*Trans* 18:1 n-11, n-12 low *trans* FA	~7.4 ~7.8	~9.1 ~9.9	~6.9 ~8.6	~2.5 ~0.1	↔ circulating immune cells (lymphocytes, monocytes, granulocytes), subgroups of lymphocytes, TNFα, IL-1β, IL-6, IL-8, IL-10, IL-12-p70, leptin, adiponectin, secretory phospholipase A2, 6-keto-prostaglandin F1α, a marker of endothelial prostaglandin or CRP between diet groups.
Mensink (2007)	11/33 H	X	3 weeks	HI oleic, low *trans* FA HI palmitic, *trans FA* free	12.3 15.1	9.4 6.5	6.2 6.2	0.7 0.2	CRP (mg/l) 0.87 0.80

[a,b] different subscripts denote significant difference between outcomes in same column; S, saturated fatty acids; M, monounsaturated fatty acids; P, polyunsaturated fatty acids; T, trans fatty acids; H, healthy; HC, hypercholesterolemic (LDL>130mg/dl); X, Cross-over or Latin square design; P, parallel; SO, soybean oil; S-L semi liquid; marg, margarine; PH-short, partially hydrogenated shortening; CHO, carbohydrate; SA, stearic acid HI, high; Lo, low; IL-6, interleukin 6; TNFα, tumour necrosis factor α; CRP, C-reactive protein; ↑, increased; ↔, no difference

Table 9A. Human studies investigating the association of trans FA with breast cancer

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Population studies						
Bakker *et al* (1997) Ecological Europe and Israel	10 countries M/F Gender breakdown not stated Cancer data 1982-1987 FA sample 1991-1992	Adipose tissue *trans* FA	% *trans* FA of total FA in adipose tissue Lowest centre (Granada) 0.13 g/100g total FA Highest centre (Zeist) 1.98 g/100g total FA	Pearson correlation coefficient r = 0.89 (0.62-0.97)	N/A	Age, sex, study centre and laboratory methods
Case-control studies						
London *et al* (1993) Case-control USA	556 case/397 hospital-based controls	Gluteal adipose *trans* FA	% *trans* FA of total FA in adipose tissue Total *trans* FA Q 1 = 2.74 Q 2 = 3.43 Q 3 = 3.98 Q 4 = 4.58 Q 5 = 5.42	 1.0 1.7 (1.1-2.8) 1.0 (0.6-1.6) 1.2 (0.8-2.0) 1.2 (0.7-1.9)	p =0.94	Risk factors for breast cancer[2], menopause, weight 5 years before study, alcohol intake, and prior history of benign breast disease
Petrek *et al* (1994) Case-control USA	154 case/125 hospital-based controls	Breast and abdomen tissue *trans* FA	% *trans* FA for quartiles not given. Whole population (mean ± SD): Cases 3.80% ± 1.13 Controls 4.07% ± 1.14	 1.00 0.686 (0.330-1.43) 0.411 (0.202-0.838) 0.528 (0.257-1.08)	p =0.13	Menopausal status and body mass index

[1] The RR stated are those which have been fully adjusted for confounding factors. When a significant difference in an associated risk was reported, the specific factors that were adjusted for in the model have been shown within the table. The p trend values refer to the level of difference between the highest and lowest tertile/quartile/quintile of intake.

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Kohlmeier *et al* (1997) Case-control (EURAMIC) Europe (5 countries)	698 cases/698 postmenopausal population-based controls	Gluteal adipose *trans* FA	Percentile of % total *trans* FA in adipose tissue: 25th 0.68% 75th 1.60% % PUFA in tissue: T1 < 12.14 T2 12.14-15.09 T3 >15.09	Whole population, 25th vs 75th percentile: 1.40 (1.02-1.93) OR for *trans* FA levels, stratified by % PUFA: 3.65 (2.17-6.14) 1.88 (1.14-3.09) 0.97 (0.67-1.40)	p =0.035 p =0.001 p =0.013 p =0.85	Standard confounding factors[1], smoking, age, socioeconomic status study centre, hormone replacement therapy and adipose PUFA levels
Aro *et al* (2000) Case-control Finland	195 cases/208 population-based controls	Serum *trans* FA	% trans 18:1, n-7 acid of total FA in serum Q1 = 0.17 Q2 = 0.23 Q3 = 0.27 Q4 = 0.31 Q5 = 0.40	1.00 0.5 (0.2-1.3) 0.2 (0.1-0.6) 0.4 (0.1-0.9) 0.2 (0.1-0.6)	Not reported	Age (at time of study, menarche, first full-term pregnancy), area (rural/urban), oral contraceptive use, HRT, family history of breast cancer, history of benign breast disease, education, alcohol intake, smoking, physical activity, waist-to-hip ratio, and BMI
Prospective epidemiological studies						
Holmes *et al* (1999) Prospective cohort (NHS) USA	88 795 pre- and postmenopausal women 2956 events 1980-1994	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA. Intake of *trans* FA for cohort not given RR for increase of 1% of energy from total *trans* FA: All women 0.92 (0.86-0.98) Premenopausal 1.00 (0.88-1.11) Postmenopausal 0.91 (0.84-0.99)		Not reported	Standard confounding factors[1], risk factors for breast cancer[2], age at menopause, vitamin A intake, time period, weight change since age 18 years, BMI at age 18 years, menopausal status, HRT, history of benign breast disease

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Pala *et al* (2001) Prospective nested case-control (ORDET) Italy	71 cases/142 postmenopausal population-based controls 1987-1995	Erythrocyte membrane *trans* FA	% 18:1, n-9 of total FA in membrane T1 <0.25% T2 ≥ 0.25-0.36% T3 ≥0.36%	1.00 2.35 (1.00-5.49) 0.7 (0.30–1.64)	p =0.42	Age (at time of study, at menarche, menopause, first birth), BMI, waist-hip ratio, months of lactation, parity, and educational level were considered but none exerted a major confounding effect for *trans* FA level. Therefore, the authors chose to only present only unadjusted OR.
Bryne *et al* (2002) Prospective cohort (NHS) USA	31 673 women, postmenopausal 1071 events 1980-1994	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Total *trans* FA level of quintiles not given. Total *trans* FA (mean ± SD) for cohort: = 1.4% ± 0.5	1.0 0.95 (0.78-1.15) 1.01 (0.83-1.23) 0.89 (0.72-1.10) 0.91 (0.73-1.13)	p =0.33	Standard confounding factors[1], risk factors for breast cancer[2], use of postmenopausal hormones, body mass index at age 18, weight change since age 18, vitamin A intake, and other fat subtypes

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Saadatian-Elahi *et al* (2002) Prospective nested case-control (New York Women's Health Study) USA	197 cases/197 population-based controls 1985-1991	Serum *trans* FA	% 18:1, n-9 of total FA in serum Levels of total *trans* FA quartiles not given. Premenopausal cohort (mean ± SD) Cases 0.4% ± 0.2 Controls 0.5% ± 0.8 Postmenopausal cohort (mean ± SD) Cases 0.4% ± 0.8 Controls 0.3% ± 0.1	All women 1.00 1.01 (0.52-1.98) 1.08 (0.55-2.11) 0.66 (0.33-1.31) Premenopausal 1.00 1.40 (0.47-4.18) 0.94 (0.31-2.88) 1.02 (0.36-2.88) Postmenopausal 1.00 0.67 (0.27-1.68) 1.07 (0.42-2.73) 0.36 (0.13-1.03)	p =0.25 p =0.80 p =0.33	Age at first full-term birth, family history of breast cancer, history of benign breast disease, and total cholesterol.

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Voorrips *et al* (2002) Prospective nested case-control (Netherlands Cohort Study) The Netherlands	941 cases/1598 population-based controls 1986-1992	Dietary *trans* FA assessed by FFQ and diet record	g/day intake of *trans* FA **Total** *trans* FA Q1 = 1.5 Q2 = 2.1 Q3 = 2.4 Q4 = 2.8 Q5 = 3.6 **Trans 18:1, n-7** Q1 = 0.3 Q2 = 0.5 Q3 = 0.7 Q4 = 0.9 Q5 = 1.2 **Other 18:1 trans isomers** Q1= 0.4 Q2 = 0.8 Q3 = 1.1 Q4 = 1.5 Q5 = 2.3	 1.00 0.98 (0.72-1.33) 1.09 (0.80-1.49) 1.20 (0.88-1.64) 1.30 (0.93-1.80) 1.00 1.16 (0.84-1.59) 1.19 (0.87-1.62) 1.36 (1.00-1.85) 1.34 (0.98-1.82) 1.00 0.80 (0.59-1.09) 1.10 (0.75-1.38) 1.01 (0.74-1.37) 0.89 (0.65-1.21)	 p =0.01 p =0.006 p =0.91	Standard confounding factors[1], risk factors for breast cancer[2], age at menopause, history of benign breast disease, oral contraceptive use, BMI, education, smoking and energy-adjusted fat intake.
Cho *et al* (2003) Prospective cohort (NHS II) USA	90 655 women, premenopausal 714 events 1991-1999	Dietary *trans* FA assessed by FFQ	% of energy from *trans* FA Total *trans* FA Q 1 =0.9 Q 2 =1.2 Q 3 =1.5 Q 4 =1.8 Q 5 =2.3	 1.00 0.92 (0.71-1.17) 0.96 (0.74-1.25) 0.86 (0.64-1.14) 0.96 (0.70-1.31)	 p =0.38	Standard confounding factors[1], risk factors for breast cancer[2], history of benign breast disease, smoking, oral contraceptives, menopausal status, energy, protein intake, other types of fat and cholesterol.

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Rissanen *et al* (2003) Prospective nested case-control (Mobile Clinic Health Examination Survey) Finland	127 cases/242 population-based controls 1973-1991	Serum *trans* FA	% *trans* FA of total FA in serum Whole population **Trans 18:1** T1 < 0.85 T2 0.85-1.15 T3 <1.15 Whole population **Trans 18:1, n-7** T1 <0.32 T2 0.32-0.41 T3 > 0.41 Postmenopausal **Trans 18:1** T1 <0.32 T2 0.32-0.41 T3 > 0.41 Postmenopausal **Trans 18:1, n-7** T1 <0.32 T2 0.32-0.41 T3 > 0.41	 1.00 0.91 (0.36-2.26) 1.47 (0.65-3.32) 1.00 1.65 (0.66-4.10) 3.69 (1.35-10.06) 1.00 1.82 (0.51-6.48) 7.90 (1.46-42.69) 1.00 1.52 (0.40-5.83) 2.05 (0.54-7.77)	 p =0.18 p =0.17 p =0.49 p =0.22	Adjusted for standard confounding factors[1], smoking, serum cholesterol, number of pregnancies, parity, leisure-time exercise and education; if no significant difference between adjusted and unadjusted results the latter were reported. The results presented here were unadjusted.
Kim *et al* (2006) Prospective cohort (NHS) USA	80,375 women, postmenopausal 3,537 events 1980-2000	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Intake of *trans* FA for cohort not given RR for increase of 1% of energy from total *trans* FA: All postmenopausal women 0.99 (0.91-1.08) Stratified by retrospective premenopausal intake 1.08 (1.01-1.15)		Not reported	Standard confounding factors[2], risk factors for breast cancer[3], time period,weight change since age 18 years, body mass index at age 18 years, age at menopause, HRT and benign breast disease.

[1] The RR stated are those which have been fully adjusted for confounding factors. When a significant difference in an associated risk was reported, the specific factors that were adjusted for in the model have been shown within the table. The p trend values refer to the level of difference between the highest and lowest tertile/quartile/quintile of intake.

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Shannon *et al* (2007) Prospective nested case-control China	322 cases/367 population-based controls 1995-2000	Erythrocyte *trans* FA	% *trans* 18:1, n-7 in membrane Q 1 ≤ 0.85 Q 2 < 0.85 to ≥ 0.93 Q 3 <0.93 to ≥ 1.01 Q 4 ≥ 1.01	1.00 1.09 (0.68-1.76) 1.52 (0.94-2.44) 1.82 (1.12-2.96)	p =0.002	Year of interview, age (at time of study and at first birth), duration of breastfeeding, time since last induced abortion and duration of intrauterine device use.

[2] Standard confounding factors: energy intake and alcohol consumption;

[3] Risk factors for breast cancer: age at time of study, age at menarche, age at first birth, height, family history of breast cancer, parity. HRT, hormone replacement therapy; NHS, Nurses' Health Study; NHS II, Nurse's Health Study second phase

Table 10A. Human studies investigating the association of *trans* FA with colorectal cancer

Reference	Subject population	Measure of exposure	Trans FA intake/level	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Population studies						
Bakker *et al* (1997) Ecological Europe and Israel	10 countries Male & female Cancer data 1982-1987 FA sampling 1991-1992	Adipose tissue *trans* FA	% *trans* FA of total FA in adipose tissue Lowest centre (Granada) 0.13 g/100g total FA Highest centre (Zeist) 1.98 g/100g total FA	Pearson correlation coefficient r = 0.93 (0.74-0.98)	N/A	Age, sex, study centre and laboratory methods
Case-control studies						
McKelvey *et al* (1999) Case-control USA	516 cases/551 controls Male & female	Dietary *trans* FA assessed by FFQ	g/day *trans* FA intake Total *trans* FA Group 1 < 2 Group 2 2-<4 Group 3 4-<6 Group 4 >6	 1.00 1.0 (0.71-1.4) 1.5 (0.9-2.5) 1.6 (0.82-3.2)	Not reported	Additional confounding factors[2], age, sex, BMI, red meat consumption, vegetable consumption and use of NSAIDs.

[1] The RR stated are those which have been fully adjusted for confounding factors. When a significant difference in an associated risk was reported, the specific factors that were adjusted for in the model have been shown within the table. The p trend values refer to the level of difference between the highest and lowest tertile/quartile/quintile of intake.

Reference	Subject population	Measure of exposure	Trans FA intake/level	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Slattery *et al* (2001) Case-control USA	1993 cases/2410 controls Male & female	Dietary *trans* FA assessed by FFQ	*trans* FA g /1000 kcal energy intake Total *trans* FA - Men Q1 =≤ 1.69 Q2 =1.70-2.25 Q3 =2.26-2.73 Q4 =2.74-3.34 Q5 =›3.34 Total *trans* FA - Women Q1 = ≤ 1.53 Q2 =1.54-2.01 Q3 =2.02-2.43 Q4 =2.44-2.99 Q5 =› 2.99 Women and HRT Q1 + HRT Q5 + HRT Q1 no HRT Q5 no HRT	 1.00 1.2 (1.0-1.8) 1.3 (0.95-1.7) 1.1 (0.9-1.5) 1.2 (0.9-1.7) 1.00 1.00 (0.7-1.3) 1.1 (0.8-1.5) 1.2 (0.9-1.6) 1.5 (1.1-2.0) 1:00 0.9 (0.6-1.5) 0.8 (0.6-1.3) 1.6 (1.1-2.5)	 p =0.34 p =0.04 p for interaction =0.06	Age at diagnosis, body size, physical activity, aspirin and/or NSAID use, energy intake, and dietary calcium
Nkondjock *et al* (2003) Case-control Canada	402 cases/668 population-based controls Male & female	Dietary *trans* FA assessed by FFQ	% of energy from *trans* FA Total *trans* FA Q1 ‹0.32 Q2 = 0.32-0.79 Q3 = 0.80-1.60 Q4 ›1.60	 1.00 0.92 (0.64-1.31) 0.64 (0.44-0.93) 0.83 (0.58–1.19)	 p =0.309	Standard confounding factors[1], marital status, and physical activity. Total energy, HRT, fibre, vitamin C and E intakes were not significantly different between cases and controls, and were not included.

Reference	Subject population	Measure of exposure	Trans FA intake/level	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Theodoratou *et al* (2007) Case-control Scotland	1455 cases/1455 population-based controls Male &female	Dietary *trans* FA assessed by FFQ	g/day *trans* FA intake Total *trans* FA Q1 < 2.88 g/day Q2 = 2.89-3.54 Q3 = 3.55-4.23 Q4 > 4.24 g/day	 1.00 1.32 (1.04-1.69 1.23 (0.95-1.61) 1.15 (0.85–1.55)	 p =0.548	Standard confounding factors[1], additional confounding factors[2], use of NSAID, fibre intake and total FA.
			trans MUFA Q1 < 2.21 Q2 = 2.22-2.72 Q3 = 2.73-3.23 Q4 >3.24	 1.00 1.39 (1.08-1.78) 1.39 (1.07-1.81) 1.30 (0.97–1.75)	 p =0.251	Fully adjusted for all factors.
			Q1 < 2.21 Q2 = 2.22-2.72 Q3 = 2.73-3.23 Q4 >3.24	1.00 1.42 (1.12-1.80) 1.45 (1.15-1.82) 1.38 (1.09-1.74)	p =0.012	Without adjustment for energy and FA

Reference	Subject population	Measure of exposure	Trans FA intake/level	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Prospective studies						
Lin *et al* (2004) Prospective cohort (Women's Health Study) USA	37 547 women 202 events 1993-2003	Dietary *trans* FA assessed by FFQ	% of energy from *trans* FA Total *trans* FA Q1 = 0.6 Q2 = 0.9 Q3 = 1.1 Q4 = 1.4 Q5 = 1.9 *Trans* 18:1, n-9 Q1 = 0.5, Q5 = 1.7 *Trans* 18:2 Q1 = 0.03, Q5 = 0.09	 1.00 0.92 (0.59-1.44) 1.08 (0.72-1.69) 0.86 (0.55-1.40) 1.59 (0.94-2.70) 1.94 (0.92- 2.58) 1.58 (0.94- 2.67)	 p =0.18 p =0.08 p =0.09	 Standard confounding factors[2], additional confounding factors[3], random treatment assignment, history of colorectal polyps and HRT. Additional adjustment for other types of fat and cholesterol were made for isomer analysis.

[2] Standard confounding factors: age, BMI, family history of colorectal cancer
[3] Additional confounding factors: smoking, physical activity, energy intake, alcohol intake
HRT, hormone replacement therapy; NSAID, non steroidal anti inflammatory drugs; NHS, Nurses' Health Study

Table 11A. Human studies investigating the association of *trans* FA with prostate cancer

Reference	Subject population	Measure of exposure	Trans FA intake/level	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Population studies						
Bakker *et al* (1997) Ecological Europe and Israel	10 countries Male & female Cancer data 1982-1987 FA sampling 1991-1992	Adipose tissue *trans* FA	% *trans* FA of total FA in adipose tissue Lowest centre (Granada) 0.13 g/100g total FA Highest centre (Zeist) 1.98 g/100g total FA	Pearson correlation coefficient (r) = 0.50 (-0.15-0.85)	N/A	Age, sex, study centre and laboratory methods

[1] The RR stated are those which have been fully adjusted for confounding factors. When a significant difference in an associated risk was reported, the specific factors that were adjusted for in the model have been shown within the table. The p trend values refer to the level of difference between the highest and lowest tertile/quartile/quintile of intake.

Reference	Subject population	Measure of exposure	Trans FA intake/level	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Case-control studies						
Liu *et al* (2007) Case-control USA	1012 case/1012 population-based controls	Dietary *trans* FA assessed by FFQ	g/day *trans* FA intake Total *trans* FA **African American** <2.83 2.83-4.78 4.78-7.59 ≥ 7.59 **Caucasians** <2.52 2.52-4.01 4.01-5.76 ≥ 5.76 **QQ or RQ genotype** <2.52 2.52-4.01 4.01-5.76 ≥ 5.76 **RR[5] genotype** <2.52 2.52-4.01 4.01-5.76 ≥ 5.76	 1.00 0.54 (0.19-1.49) 0.27 (0.07-0.99) 0.43 (0.10-1.78) 1.00 1.58 (1.00-2.48) 1.95 (1.20-3.19) 2.77 (1.60-4.79) 1.00 2.93 (1.62-5.30) 3.13 (1.64-5.98) 4.80 (2.29-10.08) 1.00 0.59 (0.28-1.24) 0.96 (0.44-2.11) 1.27 (0.54-2.98)	 p =0.21 p =0.0003 p =0.0001 p =0.58	Age, race, medical institution and total energy intake. Some analyses were also adjusted for genotype.

Reference	Subject population	Measure of exposure	Trans FA intake/level	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Prospective studies						
Schuurman *et al* (1999) Prospective cohort (Netherlands Cohort Study) The Netherlands	58 279 men 642 events 1986-1992	Dietary *trans* FA assessed by FFQ	% of energy from *trans* FA Total *trans* FA Q1<1.9% Q2 = 2.6 Q3 = 3.2 Q4 =3.7 Q5 >4.7	1.00 1.25 (0.90-1.72) 1.20 (0.86-1.65) 1.12 (0.80-1.55) 0.99 (0.70–1.40)	p =0.72	Age, family history of prostate carcinoma, socioeconomic status, total energy intake, and total energy-adjusted fat intake
King *et al* (2005) Prospective nested case- control (CARET) USA	272 cases/426 population-based controls	Serum phospholipid *trans* FA	% *trans* FA of total FA in serum phopholipid **Trans 18:1, n-7** Q1 › 0.31 Q2 = 0.31-0.42 Q3 = 0.43-0.55 Q4 › 0.55 **Trans 18:1, n-9** Q1 › 0.21 Q2 = 0.21-0.27 Q3 = 0.28-38 Q4 › 0.38 No significant effect for any other *trans* isomer (16:1 t9, 16:1 t7, 18:1 t8, 18:1 t10, 18:1 t12)	1.0 1.14 (0.71-1.84) 1.20 (0.73-1.97) 1.69 (1.03-2.77) 1.0 1.05 (0.67-1.67) 1.37 (0.86-2.17) 1.39 (0.87-2.28)	p =0.04 p =0.10	Asbestos exposure, period of enrollment, enrollment center, enrollment age group, year of randomization, ethnicity, baseline smoking status, age during study, BMI, alcohol use.

Table 12A. Human studies investigating the association of trans FA with other types of cancer

Reference	Subject population	Measure of exposure	Trans FA intake/level	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Non-Hodgkins Lymphoma						
Zhang *et al* (1999) Prospective cohort (NHS) USA	88 410 women 199 events 1980-1994	Dietary *trans* FA assessed by FFQ	% of energy from *trans* FA **Total trans FA** Q1 = 1.3 Q2 = 1.8 Q3 = 2.2 Q4 = 2.6 Q5 = 3.2 **Vegetable trans FA** Q1 = 0.5 Q2 = 0.9 Q3 = 1.2 Q4 = 1.6 Q5 = 2.3 **Animal trans FA** Q1 = 0.5 Q2 = 0.7 Q3 = 0.9 Q4 = 1.1 Q5 = 1.3	 1.0 (CI not given for Q1-Q4) 1.3 1.8 1.4 2.4 (1.3–4.6) 1.0 1.7 1.8 1.8 1.9 (1.2–3.1) 1.0 1.4 1.3 1.7 1.4 (0.8–2.2)	 p =0.01 p =0.03 p =0.15	Age, total energy, length of follow-up, geographic region, smoking, height, intake of other fat types, dietary protein, alcohol intake, fruit and vegetable intake

Reference	Subject population	Measure of exposure	Trans FA intake/level	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Ovarian cancer						
Bertone *et al* (2002) Prospective cohort (NHS) USA	80 258 women 449 events 1980-1996	Dietary *trans* FA assessed by FFQ	No values for *trans* FA intake given.	1.00 1.03 (0.72-1.46) 1.08 (0.76-1.53) 1.03 (0.72-1.47)	p =0.87	Age (at time of study and at menarche), parity, oral contraceptive use and duration, menopausal status, HRT, tubal ligation, and smoking.
Pancreatic cancer						
Michaud *et al* (2003) Prospective cohort (NHS) USA	88 802 women 178 events 1980-1998	Dietary *trans* FA assessed by FFQ	g/day *trans* FA intake Total *trans* FA Q1 = 2.5 Q2 = 3.3 Q3 = 3.9 Q4 = 4.6 Q5 = 5.7	1.00 0.97 (0.62-1.50) 0.98 (0.64-1.50) 0.72 (0.44-1.18) 0.91 (0.58-1.43)	p =0.44	Smoking, BMI, history of diabetes mellitus, caloric intake, height, physical activity, menopausal status, and glycemic load intake.

NHS, Nurses' Health Study

[1] The RR stated are those which have been fully adjusted for confounding factors. When a significant difference in an associated risk was reported, the specific factors that were adjusted for in the model have been shown within the table. The p trend values refer to the level of difference between the highest and lowest tertile/quartile/quintile of intake.

Table 13A. Epidemiological studies investigating the association of trans FA with changes in body weight

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Bortolotto *et al* (2005) Case-control Brazil	~33 cases/18 controls (paper not clear) Morbidly obese and non-obese men and women	Adipose tissue *trans* FA	% *trans* FA of total FA in adipose tissue Level of total *trans* FA for quartiles not given. Trans FA in visceral adipose tissue of whole population (mean ± SD): Cases 8.74% ± 0.29 Controls 9.29% ± 0.59	Not reported. There was no difference between the case and control groups	ns	Not reported.
Colditz *et al* (1990) Prospective cohort (NHS) USA	31 940 women 1976-1984	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Intake of *trans* FA for cohort not reported.	Intake of *trans* FA was related to changes in BMI over 8 years with a coefficient (β) of 0.191 (t =9.3)	Not reported	Age and total calorie intake

[1] The RR stated are those which have been fully adjusted for confounding factors. When a significant difference in an associated risk was reported, the specific factors that were adjusted for in the model have been shown within the table. The p trend values refer to the level of difference between the highest and lowest tertile/quartile/quintile of intake.

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Koh-Banerjee *et al* (2003) Prospective cohort (HPFS) USA	16 587 men 1987-1996	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Mean intake of *trans* FA for cohort 1.3 ±Ð0.6%	9-year Δ waist circumference as a result of changing the source of 2% of energy to *trans* FA: Replacing carbohydrates = 0.53cm ± 0.19 Replacing PUFA = 0.52cm ± 0.19 Replacing PUFA = 2.7cm	 p =0.007 p =0.007 p <0.001	Age, baseline waist circumference, baseline and Δ BMI, baseline and Δ total calories, baseline and Δ alcohol consumption, baseline and Δ total physical activity, Δ smoking, baseline and Δ intakes of total fat. Replacement of carbohydrates analysis also adjusted for baseline and Δ intakes of protein and all fat subtypes Further adjustment for measurement errors in significant predictors
Wannamethee *et al* (2004) Prospective cohort (NHS) USA	49 324 women 1991-1999	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA *Trans* FA intake of cohort or individual tertiles not reported.	OR for weight gain ≥ 5kg stratified by alcohol intake (0g/day alcohol used as reference level for OR calculations): Alcohol 0.1-4.9g/day T1 = 0.93 (0.66-0.99) T2 = 0.91 (0.84-0.98) T3 = 0.96 (0.90-1.03) Alcohol ≥ 30g/day T1 = 0.91 (0.71-1.16) T2 = 1.21 (0.86-1.70) T3 = 1.24 (0.85-1.81)	P for interaction=0.10	Age, cigarette smoking, level of physical activity, race, initial weight, previous weight change, height, spousal education at 1999, total non-alcohol calorie intake, trans fat, saturated fat, total fibre, protein and sucrose

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Oken *et al* (2007) Prospective cohort (Project Viva) USA	902 women, postpartum 1999-2003	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Median intake of *trans* FA at 6 months postpartum Those who retained ≥ 5kg at 12 months: 1.3 ± 0.6 Those who retained < 5kg at 12 months: 1.1 ± 0.5	OR for retaining ≥ 5kg at 12 months post partum: per 0.5% increase in energy from *trans* FA: 1.33 (1.09 –1.62) if consumed below the median level of *trans* FA: 0.23 (0.08–0.66)	Not reported	Maternal age, race/ethnicity, parity, education, household income, parity, pre-pregnancy BMI, gestational weight gain, breastfeeding, and smoking
Field *et al* (2007) Prospective cohort (NHS) USA	41 518 women 1984-1996	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Mean intake of *trans* FA for cohort 1.7 ±0.5%	Change in body weight (lb) for a 1% increase in percentage of calories from *trans* fat: Normal weight women +1.22 (CI not reported) Overweight women + 2.33 (1.80 to 2.86)	 p <0.0001 p <0.0001	Age, BMI in 1986, activity level in 1986, menopausal status, smoking status, time spent sitting, protein intake, Δ protein intake, intake of different fat types.

HPFS, Health Professionals' Follow-up Study; NHS, Nurses' Health Study; Δ, changes in; ns, not significant

Table 14A. Randomised controlled trials investigating the association of trans FA with changes in body weight

Reference	Subject population (men/women)	Design	Time period for study	Test diet/ meal	Fatty acid composition (% of daily/meal energy intake)				Lipid oxidation	Other parameters
					S	M	P	T		
Delany *et al* (2000)	4/0 Healthy, normal weight	X	7 days baseline diet prior to first test meal; test meal every 2-4 days with baseline diet for remaining meals throughout study	Laurate Palmitate Stearate Oleate Elaidate Linoleate Linolenate	10 mg/kg body wt of the specified ^{13}C-labeled fatty acid, ≥98% chemically pure.				40.6 ± 7.0a breath 13CO2/9hr 15.8 ± 2.8 ^{c,d} 13.0 ± 4.7 ^{d} 17.9 ± 3.8 ^{c,d} 20.5 ± 3.0 ^{b,c} 19.8 ± 5.4 ^{b,c} 27.0 ± 7.0 ^{b}	N/A
Lovejoy *et al* (2002)	13/12 Healthy but some overweight	X	4 weeks/diet	SFA MUFA *Trans* FA	11.3 5.8 7.3	9.3 15.2 8.4	6.4 6.3 4.0	0.0 0.0 7.3	29.0 ± 1.5 ^{a,b} g/day 26.0 ± 1.5 ^{a} 31.4 ± 1.5 ^{b}	No changes in insulin metabolism (see diabetes section)
Flint *et al* (2003)	19/0 Healthy but overweight	X	Free-living situation, test breakfast meal given after overnight fast, 3 day washout between meals	PUFA* MUFA *Trans* FA	6.5 4.6 18.1	10.3 49.8 8.1	42.1 NR NR	0.6 1.44 32.4	Not measured	No changes in postprandial appetite, ad libitum energy intake or energy efficiency
Lefevre *et al* (2005)	10/12 Healthy but overweight	X	16 days baseline diet, test breakfast meal on days 10 and 16	*Cis* 18:1 *Trans* 18:1	15 15	20 10	15 15	0 10	16.0 ± 1.2 kJ/8 h/kg LBM 16.9 ± 1.5	Acute insulin resistance and hyperinsulinemia on *trans* FA diet (see diabetes section)

LBM, lean body mass; X, Cross-over or Latin square design; NR = not reported

*Fatty acid composition of the diets was reported as a function of specific fatty acids (16:0, 18:0. 18:1, 18:1t, 18:2t, 18:2, 18:3) and thus included the category 'other', which made up 0.9% of PUFA diet, 7.0% of the MUFA diet and 2.4% of the trans FA diet.

Means within columns with different superscript letters are significantly different, $p < 0.05$

Table 15A. Cell and animal studies investigating the association of *trans* fatty acids with changes in body weight

Reference	Model	Study design	Types of fat used in study	Outcome
Cell studies				
Panigrahi and Sampugna (1993)	Swiss mouse fibroblast 3T3-L1 cells (adipocyte model)	Cells cultured in growth media supplemented with FA complexed to bovine serum albumin	Mixture of FA, differing only in terms of C18 FA. Control: 0 wt% *trans* 18:1, 46 wt% *cis* 18:1, 6 wt% 18:0 Test: 18.6 wt% *trans* 18:1, 27.8 wt% *cis* 18:1, 8 wt% 18:0	Cells cultured in the presence of *trans* FA had lower levels of polar and non-polar lipids accumulated in the cells ($p < 0.05$), and higher ratios of linoleate to ARA ($p < 0.05$).
Cromer *et al* (1995)	Rat adipocytes	Cells incubated for 2 hours in media containing	Purified oleic acid, *trans* 18:1, n-9 and 18:1, n-7	The *trans* FA reduced the conversion of glucose to cell lipid and the oxidation of glucose to carbon dioxide, while increasing the rate of lipolysis (all $p < 0.05$).
Alstrup *et al* (1999)	Isolated islets from Naval Medical Research Institute mice	Cells incubated for between 45 min and overnight in media containing 0.1-2.0 mmol/L specific 18:1 isomers	Purified oleic acid, *cis/trans* 18:1, n-9 and 18:1, n-7.	The *cis* isomers suppressed the oxidation of glucose by the islet cells at high glucose concentrations ($P < 0.05$), but the *trans* isomers had no effect.
Alstrup *et al*, 2004)	Isolated islets from Naval Medical Research Institute mice	Cells incubated for 3 days in media containing 0.1-2.0 mmol/L specific 18:1 isomers	Purified oleic acid, *cis/trans* 18:1, n-9 and 18:1, n-7.	No differences in glucose oxidation. FA oxidation was higher in the presence of the *trans* isomers than the *cis* isomers ($p < 0.05$).
Animal studies				
Privett (1977)	Essential fatty acid deficient rats	24 weeks fed standard diet containing 10% fat. In the control diets, the fat came solely from safflower oil or hydrogenated coconut oil, in the test diets 5% of the fat was substituted for purified *trans* FA.	Purified elaidiate (18:1 t9) or linolelaidate (18:2 t9 t12)	*Trans* FA appeared to impair the interconversion of unsaturated FA and reduce the activity of lipoprotein lipase. Linolelaidate reduced the activity of serum lecithin:cholesterol acyl transferase ($p < 0.05$).

Reference	Model	Study design	Types of fat used in study	Outcome
Atal *et al* (1994)	Male C57Bl/6J mice	The animals were fed diets containing 10 wt% control or test fat for up to 24 months	Mixtures of fats were blended to produce two diets that were approximately the same, except that 50% of the *cis* 18:1 in the control diet was substituted for *trans* 18:1 in the test diet	The *trans* diet reduced the total body weight as well as the weight of epididymal fat pads and perirenal fat (all $p < 0.05$). The animals on the *trans* diet also had lower triacylglycerol to polar lipid ratios and adipose cell size (both $p < 0.05$).
Colandre *et al* (2003)	Wistar rats	Fed diets containing 20% fat for 30 days. 17% of the fat was a maize oil-derived product that contained varying levels of *trans*, *cis*, and SFA.	Maize oil (0.66 wt% *trans* FA, 13.94 wt% SFA, 85.40 wt% *cis* FA), hydrogenated maize oil (10.47 wt% *trans* FA, 71.44 wt% SFA, 18.09 wt% *cis* FA), isomerised maize oil (30.00 wt% *trans* FA, 14.38 wt% SFA, 55.62 wt% *cis* FA)	Energy utilization and apparent fat absorption were lower in *trans* FA than the cis diet (p = ns and <0.05, respectively), but were lowest in the SFA diet. Animals on the *cis* diet had significantly smaller epididymal fat pads ($p = 0.0007$) and lower serum and hepatic TAG levels (both $p < 0.05$).
Kavanagh *et al* (2007)	Male African green monkeys	Fed maintenance diets containing 35% of energy as a fat blend enriched with either *cis* or *trans* FA for 6 years. Overall, ~8% of dietary energy was from *trans* FA	Blend of partially-hydrogenated and non-hydrogenated oils that provided similar FA profiles, except that the *cis* diet contained 51.1% *cis* 18:1 while the *trans* diet contained 26.6% *cis* and 20.4% *trans* 18:1.	Animals on *trans* FA diet gained 7.20 % ± 2.70 weight, those on the *cis* FA diet gained 1.78% ± 1.95 ($p = 0.049$). The intra-abdominal:subcutaneous fat volume ratio was 1.67 ± 0.14 and 1.36 ± 0.09 for the *trans* and *cis* diets, respectively ($p = 0.018$). The *trans* FA diet also induced postprandial hyperinsulineamia ($p = 0.015$) and reduced Akt phosphorylation in muscle tissue ($p = 0.02$).

Table 16A. Epidemiological studies investigating the association of trans FA with diabetes

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Lovejoy *et al* (2001) Cross-sectional study USA	38 (19 males)	Dietary *trans* FA assessed by 3 day food record	g *trans* FA/day Dietary *trans* FA intake (mean ± SD): Women 4.6 ± 0.6 g/day Men 6.0 ± 0.6 g/day	Pearson correlation coefficient (r) was between -0.02 and 0.28 for trans FA intake and metabolic measurements, and was not significant for any variable	No association	Age, % body fat, fat consumption
Xu *et al* (2007) Population study (SHS) USA	1284 (420 males) Diabetic American Indians	Dietary *trans* FA assessed by 24-hour recall	% energy from *trans* FA Total *trans* FA – (levels for women, quintile levels not given for total cohort) Q 1 ≤ 1.2 Q 2 = 1.3-1.8 Q 3 = 1.9-2.4 Q 4 = 2.5-3.3 Q 5 >3.3	OR for poor glycemic control: 1.00 1.00 (0.64-1.58) 1.52 (0.95-2.43) 1.27 (0.8-2.03) 1.18 (0.75-1.87)	p =0.37	Standard confounding factors1, sex, study centre, BMI, duration of diabetes, diabetes treatment and sex × smoking status
Salmeron *et al* (2001) Prospective cohort (NHS) USA	84 204 women 2507 events 1980-1994	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Total *trans* FA Q 1 = 1.3 Q 2 = 1.7 Q 3 = 2.0 Q 4 = 2.4 Q 5 = 2.9 RR[1] 1.39 (1.15-1.67) for a 2% increase in energy from *trans* FA	 1.0 1.12 (0.97-1.29) 1.18 (1.02-1.37) 1.14 (0.97-1.34) 1.31 (1.10-1.56)	p =0.02	Standard confounding factors[2], BMI, time period, parental history of diabetes, percentage of energy from protein, dietary cholesterol and intake of other fats

[1] The RR stated are those which have been fully adjusted for confounding factors. When a significant difference in an associated risk was reported, the specific factors that were adjusted for in the model have been shown within the table. The p trend values refer to the level of difference between the highest and lowest tertile/quartile/quintile of intake.

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk[1] or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Meyer *et al* (2001) Prospective cohort USA	35 988 women 1890 events 1986-1997	Dietary *trans* FA assessed by FFQ	g *trans* FA/day Total *trans* FA Q 1 = 2.2 Q 2 = 2.4 Q 3 = 2.8 Q 4 = 3.5 Q 5 = 5.2	 1.0 1.01 (0.86-1.19) 0.94 (0.79-1.12) 0.88 (0.73-1.06) 0.92 (0.75-1.11)	p =0.20	Standard confounding factors[2], waist-to-hip ratio, BMI, education, marital status, residential area, HRT, dietary magnesium and cereal fibre, types of fat, and cholesterol.
van Dam *et al* (2002) Prospective cohort USA	42 504 men 1321 events 1986-1994	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Total *trans* FA Q 1 = 0.7 Q 2 = 1.0 Q 3 = 1.3 Q 4 = 1.5 Q 5 = 2.0 Q 1 = 0.7 Q 2 = 1.0 Q 3 = 1.3 Q 4 = 1.5 Q 5 = 2.0	 1.00 1.17 (0.98-1.41) 1.22 (1.02-1.46) 1.28 (1.07-1.53) 1.28 (1.07-1.53) 1.00 0.95 (0.79-1.15) 0.93 (0.77-1.12) 0.91 (0.75-1.11) 0.90 (0.74-1.10)	p =0.009 p =0.33	Standard confounding factors[2], time period, hypercholesterolemia, hypertension, family history of type 2 diabetes Additional adjustment for cereal fibre and magnesium intake, BMI

[2] Standard confounding factors = age, total energy intake, physical activity, cigarette smoking, alcohol consumption

SHS, Strong Heart Study; NHS, Nurses' Health Study

Table 17A. Randomised controlled trials investigating the association of *trans* FA with diabetes

Reference	Subject population (men/women)	Design	Time period for study	Diet name	Fatty acid composition (% of daily energy intake)				Fasting glucose	Fasting insulin	Postprandial changes
					S	M	P	T			
Christiansen *et al* (1997)	9/7 Obese and diabetic	X	6 weeks/ diet	SFA *Cis* MUFA *Trans* MUFA	20.0 5.0 5.0	2.5 2.0 0.0	5.0 5.0 5.0	2.5 0.0 20.0	8.9 mmol/L 8.1 8.7	113 pmol/L 99 102	Levels of serum insulin and C-peptide were lower on *cis* MUFA diet than other diets ($P < 0.05$), and slightly lower on *trans* MUFA diet than SFA diet (NS)
Louheranta *et al* (1999)	0/14 Healthy	X	4 weeks/ diet	MUFA *Trans* FA	10.9 11.0	18.6 12.5	5.8 5.2	0.0 5.1	5.0 mmol/L 5.0	7.4 mU/L 8.1	No difference in acute insulin response, glucose effectiveness or insulin sensitivity index
Lovejoy *et al* (2002)	13/12 Healthy but some overweight	X	4 weeks/ diet	SFA MUFA *Trans* FA	11.3 5.8 7.3	9.3 15.2 8.4	6.4 6.3 4.0	0.0 0.0 7.3	4.8 mmol/L 4.9 4.7	24.0 pmol/L 26.2 25.2	No significant differences in insulin sensitivity or secretion. Overweight subjects had 11% reduction in insulin sensitivity on MUFA compared with *trans* FA diets (NS)
Lichtenstein *et al* (2003)	18/18 Healthy but overweight	X	35 days/ diet	Soybean oil SL marg Soft marg PHSO Stick marg Butter	0.26g/100g trans 18:1 & 18:2 0.6g/100g trans 18:1 & 18:2 9.4g/100g trans 18:1 & 18:2 13.6g/100g trans 18:1 & 18:2 26.1g/100g trans 18:1 & 18:2 2.6 g/100g trans 18:1 & 18:2 (experimental fats provided 20% energy)				92 mg/dL 92 91 94 92 94	11.2[a] U/mL 11.6[a] 11.1[a] 13.0[b] 11.2[a] 12.4[ab]	No differences in C-reactive protein. The HOMA index, a measure of insulin sensitivity, was highest on shortening diet, and lowest on the stick margarine diet

Reference	Subject population (men/women)	Design	Time period for study	Diet name	Fatty acid composition (% of daily energy intake)				Fasting glucose	Fasting insulin	Postprandial changes
					S	M	P	T			
Lefevre *et al* (2005)	10/12 Healthy but overweight	X	16 days baseline diet, test breakfast meal on days 10 and 16	*Cis* 18:1 *Trans* 18:1	15 15	20 10	15 15	0 10	N/A – single meal measuring acute response		Acute insulin resistance. Serum insulin levels higher after *trans* FA meal than *cis* FA meal ($P < 0.05$). No differences in C-peptide. Possible increased risk for individuals with FABP2 Thr54 allele
Sundram *et al* (2007)	30 participants Healthy and normal weight	X	4 weeks/ diet	POL PHSO IE	13.7 9.1 18.2	13.6 12.4 5.9	3.6 5.8 7.0	0 3.2 0	5.6a,mmol/L 5.9[b] 6.7[c]	10.1[a]ulU/mL 9.1[a,b] 7.9[b]	Postprandial glucose increased on IE diet, no difference between PHSO and POL diets. Postprandial 2hr insulin was lower in IE and PHSO diets. C-peptide levels were lower with the IE diet.

NS, not significant; X, Cross-over or Latin square design; SL, semi-liquid; marg, margarine; POL, palm olein; PHSO, partially hydrogenated soybean oil; IE, interesterified soybean oil. Means within columns with different superscript letters are significantly different, $p < 0.05$

Table 18A. Cell and animal studies investigating the association of trans FA with diabetes

Reference	Model	Study design	Types of fat used in study	Outcome
Cell studies				
Cromer *et al* (1995)	Rat adipocytes	Cells incubated for 2 hours in media containing	Purified oleic acid, *trans* 18:1, n-7, *trans* 18:1, n-9	Both the *trans* FA suppressed the conversion of glucose to cell lipid ($p < 0.01$) and carbon dioxide ($p < 0.05$)
Alstrup *et al* (1999)	Isolated islets from Naval Medical Research Institute mice	Cells incubated for between 45 min and overnight in media containing 0.1-2.0 mmol/L specific 18:1 isomers	Purified oleic acid, *cis* and *trans* 18:1, n-7 and 18:1, n-9.	Cells exposed to *trans* FA showed higher rates of GSIS than the *cis* isomers ($p < 0.05$). Glucose oxidation at high glucose concentrations was reduced in the presence of *cis* but not *trans* isomers ($p < 0.05$).
Alstrup *et al* (2004)	Isolated islets from Naval Medical Research Institute mice	Cells incubated for 3 days in media containing 0.1-2.0 mmol/L specific 18:1 isomers	Purified oleic acid, *cis* and *trans* 18:1, n-7 and 18:1, n-9	GSIS was not altered by either *cis* or *trans* 18:1, n-7 or by oleic acid, but was stimulated by 0.3 to 0.4 mmol/L and *trans* 18:1, n-9 ($p < 0.05$).
Animal studies				
Stein *et al* (1997)	Sprague-Dawley rats	Animals maintained on standard chow diet containing 4% fat prior to study. Pancreatic perfusion was performed in the presence of various FA isomers	Purified *cis* and *trans* isomers of 16:1 and 18:1 FA.	*Trans* 16:1 caused an increase GSIS compared with *cis* 16:1 ($p = 0.07$), but the 18:1 isomers showed no differential effects.
Ibrahim *et al* (2005)	Wistar/NIN rats	Animals were fed for 12 weeks on diets that contained 10% fat from blends of groundnut, palmolein, vanaspati and safflower oils	Oils were blended to provide diets containing ~3% 18:1 *trans* FA and a range of other FA	The *trans* FA diets increased insulin secretion and reduced membrane fluidity (both $p < 0.05$).

Reference	Model	Study design	Types of fat used in study	Outcome
Natrajan *et al* (2005)	Wistar/NIN rats	Animals were fed for 3 months on diets that contained 10% fat from blends of groundnut, palmolein, vanaspati and safflower oils	Oils were blended to provide diets containing ~3% 18:1 *trans* FA and a range of other FA	*Trans* FA upregulated the mRNA expression of resistin but down regulated PPARg and LPL (p values not reported).
Bernal *et al* (2006)	Wister rats	Fed diets containing 20% fat for 30 days. 17% of the fat was a maize oil-derived product that contained varying levels of *trans, cis,* and SFA.	Maize oil (0.66 wt% *trans* FA, 13.94 wt% SFA, 85.40 wt% *cis* FA), hydrogenated maize oil (10.47 wt% *trans* FA, 71.44 wt% SFA, 18.09 wt% *cis* FA), isomerised maize oil (30.00 wt% *trans* FA, 14.38 wt% SFA, 55.62 wt% *cis* FA)	No significant differences in glucose metabolism or enzyme activity were observed between the diets

GSIS, glucose-stimulated insulin secretion

Table 19A. Epidemiological studies investigating the association of trans FA with early human growth and development

Reference	Subject population	Measure of exposure	Key indicators of health	Outcome
Physical well-being				
Koletzko *et al* (1992)	29 premature infants	Plasma PL, CE, and TAG *trans* FA levels	% *trans* FA of total FA Levels of long-chain PUFA and evidence of their biosynthesis Birth weight and birth length	Total *trans* FA in plasma lipids were not proportional to concentrations of linoleic or α-linolenic acids, but showed an inverse association with ω-3 and ω-6 long-chain PUFA (correlation coefficients for ω-6 long-chain PUFA and PL = -0.40, CE = -0.41, TAG = -0.47, all p <0.01) and with the product/substrate ratios for long-chain PUFA biosynthesis. Level of *trans* FA (mean ± SEM) and correlations: PL (1.08% ± 0.07), correlated with birth weight r = -0.42, p <0.01 CE (1.95% ± 0.17), correlated with birth weight r = -0.40, p <0.05 TAG (1.62% ± 0.10), correlated with birth weight r = -0.28, p <0.05 No adjustment for confounding factors reported
Desci and Koletzko (1995)	53 healthy children 1-15 years old	Plasma PL *trans* FA levels	% *trans* FA of total FA Levels of long-chain PUFA and evidence of their biosynthesis	Total *trans* FA in PL = 1.78% ± 0.10 There was no correlation between *trans* FA and linoleic acid, but significant negative associations between C18:1 *trans* FA and total ω-6 long-chain PUFA (r =-0.30, p = 0.029), ARA (r =-0.33, p =0.015), and ARA/linoleic acid ratio (r =-0.28, p =0.045). No adjustment for confounding factors reported
Decsi *et al* (2001)	42 newborns	Umbilical cord plasma PL, CE, TAG and NEFA *trans* FA levels	% *trans* FA of total FA Birth weight and birth length	Level of *trans* FA (mean ± SEM) and correlations with health indicators: PL (0.49% ± 0.02), no correlation with birth weight or length CE (2.47% ± 0.02), no correlation with birth weight or length TAG (1.73% ± 0.09), no correlation with birth weight or length NEFA (1.59% ± 0.07), no correlation with birth weight or length No adjustment for confounding factors reported

Reference	Subject population	Measure of exposure	Key indicators of health	Outcome
Elias and Innis (2001)	70 newborns	Umbilical cord plasma PL, CE and TAG *trans* FA levels	% *trans* FA of total FA Levels of long-chain PUFA and evidence of their biosynthesis Birth weight, birth length, length of gestation	Inverse association between infant TAG *trans* FA and linoleic acid and DHA ($p < 0.001$ and $p < 0.05$, respectively) and between CE *trans* FA and linoleic acid ($p < 0.01$) and AA ($p < 0.05$). Level of *trans* FA (mean ± SEM) and correlations with health indicators: PL (0.67% ± 0.03): Birth weight r = -0.06 (NS); birth length r = -0.12 (NS); gestation length r = -0.09 (NS) CE (2.04% ± 0.01): Birth weight r = -0.19 (NS); birth length r = -0.11 (NS); gestation length r = -0.33 (p = 0.006) TAG (2.83% ± 0.19): Birth weight r = -0.06 (NS); birth length r = -0.03 (NS); gestation length r = -0.14 (NS) No adjustment for confounding factors reported.
Hornstra *et al* (2006)	Maastricht Essential Fatty Acid Birth cohort	Umbilical cord plasma, erythrocyte, arterial and venous wall PLs	Birth weight, birth length, head circumference	Negative relationship between plasma and *trans* 18:1, n-9 and head circumference (β =-2.57, p =0.010), arterial and venous wall *trans* 18:1, n-9 and birth length (β =-3.34, p =0.025; β =-5.53, p =0.027; respectively). These relationships were strengthened upon adjustment for confounding factors (neonatal sex, gestation age, maternal age, length, BMI at beginning of study, race, smoking, alcohol use, weight gain during pregnancy, socio-economic status and parity). After further adjustment for ARA and DHA, additional relationships became significant: plasma and arterial wall *trans* 18:1, n-9 and head circumference; arterial and venous wall *trans* 18:1, n-9 and birth length (all β -2.14 to -4.89, $p < 0.05$).
	Amsterdam Born Children and their Development (ABCD) cohort	Plasma PLs	Birth weight, birth length, head circumference	The maternal proportion of elaidic acid in plasma PLs was negatively associated with the birth weight of full-term children, although this lost significance upon adjustment for sociodemographic factors.

Reference	Subject population	Measure of exposure	Key indicators of health	Outcome
Neurological well-being				
Dijck-Brouwer *et al* (2005)	317 full-term infants, 10-14 days after birth	Umbilical artery (UA) and vein (UV) *trans* FA levels	Neurological status as determined by a 'neurological optimality score' (NOS)	NOS scores were positively associated with MUFA, EFA and DHA status in UV (all $p < 0.05$). There was a negative association between NOS and 18 *trans* FA, but this was not statistically significant. The median % C18 *trans* FA by NOS score (Pearson Ð2 test): Site normal abnormal p value UV 0.70% 0.72% ns UA 0.67% 0.74% ns There was a statistically significant Spearman's Ð correlation coefficient of approximately -0.3 between the NOS scores and UA C18 *trans* FA levels. No adjustment for confounding factors reported.
Bouwstra *et al* (2006b)	474 infants at 3 months of age	Umbilical vein (UV) *trans* FA levels	Quality of general movements (GMs)	Infants with mildly abnormal GMs had a lower EFA index ($p = 0.003$), lower ARA levels ($p = 0.03$), higher total n-9 fatty acid ($p = 0.001$), and higher total MUFA levels ($p = 0.003$) in the umbilical artery compared with infants with normal GMs. Contribution of *trans* FA status at birth to the occurrence of mildly abnormal GMs, corrected for potential confounders such as postnatal feeding group: $\beta = 1.8$ (95% CI, 0.37– 8.9; p =ns) No association was found between GMs and the level of *trans* FA.

Reference	Subject population	Measure of exposure	Key indicators of health	Outcome
Bouwstra *et al* (2006a)	317 breast-fed, formula-fed, and long-chain PUFA formula–fed children at 18 months of age	Umbilical vein (UV) *trans* FA levels	Neurological status as determined by 'neurologic optimality score' (NOS), the 'Bayley Psychomotor Developmental Index' (PDI) and 'Mental Developmental Index' (MDI).	NOS was significantly reduced in infants with a UV DHA content in the lowest quartile ($p = 0.02$). NOS showed a strong negative association with total *trans* FA, with children in the highest quartile of trans FA having a significantly lower NOS ($p < 0.001$). There were also significant differences between the 1st/3rd and 2nd/4th quartiles ($p < 0.05$). In a multivariate analysis assessing correlation of *trans* FA with NOS: Total *trans*-FA $\beta = -0.52$, $p = 0.03$ C18 *trans*-FA $\beta = -0.16$, $p = 0.02$ *Trans*-18:1n-9 or 7 ns *Trans,cis*-18:2n-6 $\beta = -0.20$, $p = 0.001$ *Trans*-16:1n-7 $\beta = -0.14$, $p = 0.02$ (Adjusted for a range of confounding factors, including education of the father, HOME score, weight of gravida before pregnancy, uncertain or unreliable date of last menstrual period, breast-feeding for more than 6 wk, and age of assessment) No associations were found between ARA, DHA and total *trans*-FA with PDI or MDI.

ARA, arachidonic acid; DHA, docosahexaenoic acid; EFA, essential fatty acid; PL, phospholipids; CE, cholesteryl esters; TAG, triacylglycerol; NEFA, non-esterified fatty acids; ns, non significant

Table 20A. Epidemiological studies investigating the association of trans FA with assorted health issues

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Allergic diseases						
Weiland *et al* (1999) Population study Europe and UK (10 countries)	55 centres in 10 countries Children ages 13-14 years old	Reported *trans* FA intake for country from TRANSFAIR study	% energy from *trans* FA *Trans* FA intakes for specific countries not reported. Range of *trans* FA intakes read from x-axis of graphs presented in the paper: 0.5-1.4%	Asthma β = 20.9 Allergic rhinoconjunctivitis β = 8.2 Atopic eczema β = 12.4	p <0.001 p <0.001 p <0.001	GDP for country
Kompauer *et al* (2005) Population study Germany	740 men and women 20-64 years of age	Serum phospholipids *trans* FA	% *trans* FA in total FA Quartile levels of *trans* FA not reported Cohort mean ± SD 0·36% ± 0·15	Hay fever 1.00 0·89 (0·43–1·84) 1·56 (0·81–3·01) 0·77 (0·36–1·63) Allergic sensitisation 1.00 0·98 (0·60–1·61) 1·11 (0·68–1·80) 0·67 (0·40–1·13)	Not reported	Age, sex, education, smoking, BMI, energy intake

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Alzheimer's disease						
Morris *et al* (2003) Prospective cohort (CHAP) USA	815 men and women over 65 years of age 131 events 1993-1997	Dietary *trans* FA assessed by FFQ	g/day *trans* FA Total *trans* FA: Q1 = 1.8 Q2 = 2.3 Q3 = 3.0 Q4 = 3.7 Q5 = 4.8	 1.0 3.4 (1.3-8.8) 4.2 (1.4-12.2) 3.1 (0.9-10.5) 5.2 (1.5-18.5)	p =0.09	Age, period of observation, sex, race, education, APOE genotype, interaction between race and genotype, types of fat
Cataract formation						
Lu *et al* (2005) Prospective cohort (NHS) USA	71 083 women 4196 events 1984-2000	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Total *trans* FA Q1 = 1.1 Q2 = 1.4 Q3 = 1.6 Q4 = 1.8 Q5 = 2.2	 1.0 1.01 (0.92-1.11) 0.97 (0.88-1.06) 1.02 (0.93-1.13) 1.11 (1.00-1.23)	p =0.06	Age, smoking, energy intake, alcohol intake, lutein and zeaxanthin intake, BMI, physical activity, menopausal status, HRT, hypertension, physician visits in past year and state of residency
Cognitive function						
Morris *et al* (2004) Prospective cohort (CHAP) USA	2560 men and women over 65 years of age 6 y follow-up	Dietary *trans* FA assessed by FFQ	g/day *trans* FA Total *trans* FA: Q1 = 2.1 Q2 = 2.7 Q3 = 3.2 Q4 = 3.9 Q5 = 4.9	Annual adjusted change in cognitive function 1.0 -0.007 -0.013 -0.015 -0.020	p =0.07	Age, period of observation, sex, race, education, total energy intake, vitamin E and vitamin C intakes, alcohol intake, smoking, hypertension, types of fat

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Dementia						
Engelhart *et al* (2002) Prospective cohort (Rotterdam Study) The Netherlands	5 395 men and women over 55 years of age 197 events 1993-1999	Dietary *trans* FA assessed by FFQ	g/day *trans* FA Total *trans* FA for cohort (mean ± SD): 2.7 ± 1.0	For every 1 standard deviation increase in *trans* FA intake RR^{7} = 0.9 (0.77-1.06)	Not reported	Age, sex, education, total energy intake, vitamin E intake
Gallstone formation						
Tsai *et al* (2005) Prospective cohort (HPFS) USA	45 912 men 2 365 events 1986-2000	Dietary *trans* FA assessed by FFQ	g/day *trans* FA Total *trans* FA Q1 = 1.4 Q2 = 2.2 Q3 = 2.7 Q4 = 3.3 Q5 = 4.5 *Trans* 18:1 FA (Quintile levels not reported)	 1.00 1.11 (0.96-1.28) 1.24 (1.07-1.44) 1.19 (1.02-1.39) 1.23 (1.04-1.44) 1.00 1.14 (0.99-1.31) 1.22 (1.05-1.41) 1.19 (1.02-1.39) 1.24 (1.06-1.45)	 p =0.03 p =0.02	Age, periods of follow-up, BMI, weight change during the past 2 years, physical activity, dietary fiber, diabetes, thiazide diuretics, nonsteroid anti-inflammatory drugs, smoking, alcohol intake, caffeine intake, total energy intake and types of fat.

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Multiple sclerosis						
Zhang *et al* (2000) Prospective cohort (NHS and NHS II) USA	NHS – 92 422 women 121 events 1982-1994 NHS II – 95 389 women 74 events 1993-1995	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Total *trans* FA – NHS Q1 = 1.3 Q2 = 1.8 Q3 = 2.2 Q4 = 2.6 Q5 = 3.2 Total *trans* FA – NHS II Q1 = 0.9 Q2 = 1.3 Q3 = 1.5 Q4 = 1.9 Q5 = 2.4	 1.0 1.2 (0.7-2.2) 1.4 (0.8-2.5) 1.4 (0.7-2.5) 1.4 (0.7-2.5) 1.0 1.4 (0.7-2.9) 1.2 (0.5-2.5) 1.4 (0.7-3.0) 1.2 (0.5-2.5)	 p =0.31 p =0.75	Age, geographic location at birth, smoking and total energy

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Ovulatory infertility						
Chavarro *et al* (2007) Prospective cohort (NHS II) USA	18 555 married premenopausal women attempting pregnancy or becoming pregnant 438 events 1991-1999	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Total *trans* FA Q1 = 0.9 Q2 = 1.2 Q3 = 1.4 Q4 = 1.7 Q5 = 2.3	1.00 0.87 (0.64-1.20) 1.11 (0.79-1.55) 1.21 (0.85-1.73) 1.31 (0.88-1.95) Substitution of 2% of energy from carbohydrates for 2% of energy from *trans* FA: 1.73 (1.09-2.73)	p =0.09 p =0.02	Age, energy intake, BMI, parity, smoking, physical activity, contraceptive use, use of multivitamins, alcohol intake, coffee consumption, retinol intake, iron intake, α-carotene intake, protein intake and types of fat

Reference	Subject population	Measure of exposure	Trans FA intake/level (median of group unless otherwise specified)	Adjusted Relative Risk or Odds Ratio (95% CI)	Trend	Factors adjusted for in analysis
Parkinson's disease						
Chen *et al* (2003) Prospective cohort (NHS and HPFS) USA	NHS – 88 563 women 1980-1998 HPFS – 47 331 men 1986-1988	Dietary *trans* FA assessed by FFQ	% energy from *trans* FA Total *trans* FA – NHS Q1 = 1.3 Q2 = 1.8 Q3 = 2.2 Q4 = 2.6 Q5 = 3.2 Total *trans* FA – HPFS Q1 = 0.7 Q2 = 1.0 Q3 = 1.2 Q4 = 1.5 Q5 = 1.9	 1.00 1.07 (0.69-1.65) 0.84 (0.52-1.34) 0.62 (0.37-1.05) 1.01 (0.64-1.60) 1.00 0.66 (0.41-1.07) 1.04 (0.67-1.60) 1.15 (0.75-1.77) 1.10 (0.71-1.70)	 p =0.3 p =0.5	Age, length of follow-up, smoking, energy intake, alcohol consumption and caffeine intake
de Lau *et al* (2005) Prospective cohort (Rotterdam Study) The Netherlands	5 289 men and women over 55 years of age 51 events 1993-1999	Dietary *trans* FA assessed by FFQ	g/day *trans* FA Total *trans* FA T1 = 1.6 T2 = 2.4 T3 = 3.7	1.00 OR for T2 not reported 0.84 (0.61-1.17)	Not reported	Age, sex, smoking and vitamin E intake

HPFS, Health Professionals' Follow-up Study; NHS, Nurses' Health Study; CHAP, The Chicago Health and Aging Project; HRT, hormone replacement therapy

Annex 3: *Trans* FA intake in the UK

Total *trans* FA intakes for the general population and sub-groups

1. The National Diet and Nutrition Surveys (NDNS) provide data on fat intakes in the general population. The most recent NDNS data on adults aged 19-64 years was collected in 2000/01 (Henderson *et al*, 2003) and on children aged 4-18 years in 1997 (Gregory *et al*, 2000). The Low Income Diet and Nutrition Survey (LIDNS) provides data on fat intakes in low income materially deprived adults and children. Data were collected in 2003-2005 (Nelson *et al*, 2007). Tables 1 & 2 show intakes of saturated and *trans* FA split into age/gender groups.

Table 1: Saturated FA and trans FA intakes by age & gender: general population[a]

Age, yrs	Gender	% food energy from SFA		% food energy from *trans* FA	
		Mean	Upper 2.5 percentile	Mean	Upper 2.5 percentile
4 – 10	Boys	14.2	19.0	1.3	2.0
	Girls	14.5	19.3	1.3	2.0
11 – 18	Boys	13.5	17.9	1.3	2.1
	Girls	13.6	18.5	1.3	2.1
19 – 64	Men	13.4	19.0	1.2	2.1
	Women	13.2	20.0	1.2	2.1

a Source: NDNS young people 4 – 18 yrs 1997 (Gregory *et al*, 2000); NDNS adults 19 – 64 yrs 2000/01 (Henderson *et al*, 2003)

Table 2: Saturated FA and *trans* FA intakes by age and gender: low income population[a]

Age, yrs	Gender	% food energy from SFA		% food energy from *trans* FA	
		Mean	Upper 2.5 percentile	Mean	Upper 2.5 percentile
2 – 10	Boys	14.6	21.9	1.2	2.1
	Girls	14.4	20.5	1.1	1.8
11 – 18	Boys	13.7	18.6	1.2	1.9
	Girls	13.5	19.0	1.2	2.4
19 – 64	Men	13.3	21.7	1.3	2.6
	Women	13.3	21.2	1.2	2.6

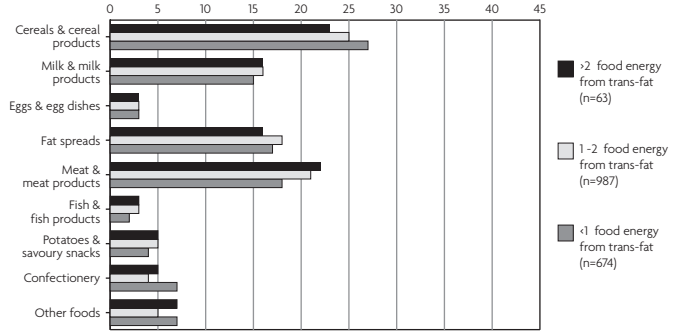
a Source: Low Income Diet and Nutrition Survey 2003/05 (Nelson *et al*, 2007)

2. In the NDNS (Henderson *et al*, 2003), all subgroups had average *trans* FA intakes less than the UK Dietary Reference Value (DRV) of 2% food energy, while mean intakes of SFA exceeded the DRV in all subgroups (Table 1) (COMA, 1994). Similar results were reported by the LIDNS (Nelson *et al*, 2007) (Table 2).

3. The similarities between the results presented in Table 1 and Table 2 indicate that there are no significant differences in the average proportion of energy from total fat, SFA or *trans* FA between the general and low income populations. However, a higher proportion of low income adults (7.5% of adults (Nelson *et al*, 2007)) exceeded 2% food energy from *trans* FA compared with the general population (3% of adults (Henderson *et al*, 2003)).

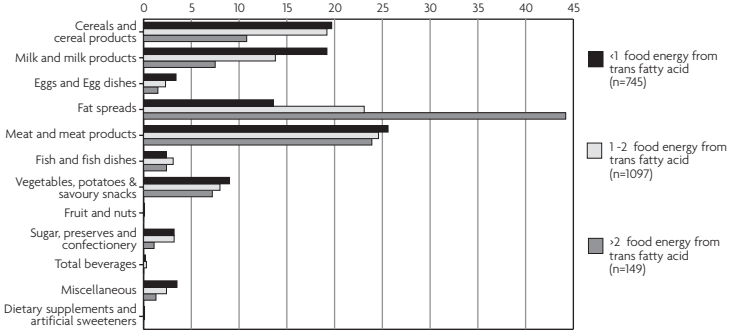
4. Figure 1 shows that there was little variation in the main sources of *trans* FA intake between the general adult population who met the WHO recommendation of 1% food energy from *trans* FA and those who exceeded the UK DRV of 2%. Figure 2 shows a similar pattern for low income adults, with the exception of fat spreads, which provided over 40% of *trans* FA intake in the 149 adults who exceeded the DRV. Consumption of a few fat spread products with relatively high *trans* FA levels was the main factor contributing to the average intake exceeding the DRV in this group. It should be noted that the levels of *trans* FA in spreads has reduced significantly since this data was collected, and the *trans* FA contribution from these products is now likely to be much lower.

Figure 1. Percentage contribution of food types to *trans* FA intakes for adults 19-64 years with low, medium and high intakes.



Source: NDNS adults 19-64 years 2000/01 (Henderson *et al*, 2003)

Figure 2. Low Income Diet and Nutrition Survey – percentage contribution of food types to *trans* FA intakes for adults 19-64 years with low, medium and high intakes.



Source: LIDNS adults 19-64 years 2003/05 (Nelson *et al*, 2007)

Time trends in intakes of total fat, saturated fat and *trans* FA

5. The NDNS provides comparable data on intakes of total fat, SFA and *trans* FA for adults in 1986/87 (Gregory *et al*, 1990) and 2000/01 (Henderson *et al*, 2003). Table 3 shows that the proportion of food energy derived from total fat was markedly lower in 2000/01 (35% for women; 36% for men) compared with 1986/87 (40% for men and women). A similar pattern was seen for SFA (13% of food energy in 2000/01 compared with 17% in 1986/87) and for *trans* FA (1.2% of food energy in 2000/01 compared with 2.2% in 1986/87). The mean proportion of food energy from *trans* FA for adults in 2000/01 was well below the UK DRV of 2%.

Table 3: Total fat, SFA and *trans* FA intakes in 1986/87 and 2000/01

Men	1986/87[a]			2000/01[b]		
	% food energy			% food energy		
	Lower 2.5 percentile	Mean	Upper 2.5 percentile	Lower 2.5 percentile	Mean	Upper 2.5 percentile
Total fat	30.6	40.4	49.5	24.0	35.8	46.6
SFA	10.6	16.5	22.4	7.8	13.4	19.0
Trans FA	1.06	2.19	4.08	0.5	1.2	2.1
Women	1986/87[a]			2000/01[b]		
	Lower 2.5 percentile	Mean	Upper 2.5 percentile	Lower 2.5 percentile	Mean	Upper 2.5 percentile
Total fat	28.7	40.3	50.1	22.0	34.9	47.9
SFA	10.7	17.0	23.4	7.2	13.2	20.0
Trans FA	0.92	2.16	3.91	0.4	1.2	2.1

a Aged 16-64 years. Dietary and Nutritional Survey of British Adults 1986/87 (Gregory *et al*, 1990)
b Aged 19-64 years National Diet and Nutrition Survey adults 19-64 years 2000/01 (Henderson *et al*, 2003)

6. The fall in *trans* FA intake between the two surveys is due to a number of factors:

- Reformulation work by manufacturers in the late 1990s onwards to remove partially hydrogenated vegetable oils[1] reduced the levels of *trans* FA in many margarines and reduced and low fat spreads.

- There was a fall in consumption of some of the main contributors to *trans* FA in the diet – mean consumption of biscuits, buns, cakes, pastries and fruit pies was 29% lower in 2000/01 (Henderson *et al*, 2003) than in 1986/87 (Gregory *et al*, 1990), mainly due to a fall in consumption of cakes and pastries, and mean consumption of fat spreads was a third lower due to a 50% drop in butter consumption.

- Composition values for *trans* FA used in the 2000/01 (Henderson *et al*, 2003) survey, based mainly on analysis in the mid 1990s, differed from and tended to be lower than the values used in the 1986/87 survey (Gregory *et al*, 1990), a higher proportion of which were estimated values. In particular the values used for meat, milk and dairy products were generally (though not universally) higher in the 1986/87 survey (Gregory *et al*, 1990). In the case of meat this is partly due to higher total fat values in the older dataset. It is not clear to what extent these differences reflect real changes in the *trans* FA content of these foods but it is likely that at least some of the apparent changes are due to the replacement of estimated values used in 1986/87 (Gregory *et al*, 1990) with analytical values for the 2000/01 (Henderson *et al*, 2003) survey.

7. It should be noted that the 2000/01 intakes (Henderson at al, 2003) do not reflect any manufacturer reformulations of biscuits, buns, cakes and pastries or crisps and savoury snacks to reduce *trans* FA levels that may have taken place since the last comprehensive analysis of these product groups in the early 1990s (Table 5).

1 Partially hydrogenated vegetable oils contain trans fats, which are produced during the process of hydrogenation used to turn liquid oil into solid fat.

Table 4: Age of *trans* FA composition data

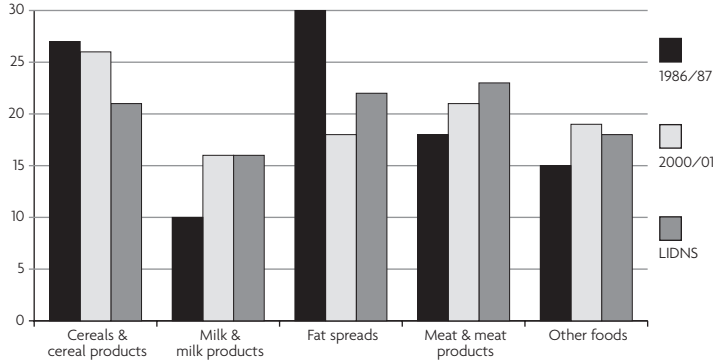
Food group	Date of most recent fatty acid analysis
Biscuits, cakes and pastries	1992
Pasteurised milk	1995
Other milks and cream	1998
Cheese	1999
Chilled and frozen desserts	1997/98
Carcase meat	1992-1995
Meat products	1991-1995
Ethnic takeaway foods	1997
Crisps and savoury snacks	1989/91
Confectionery	1992
Chips	1980s
Potato products	1990s

8. For products for which no analytical data are available, the FA profile was estimated based on manufacturers'/retailers' data for total fat and SFA (usually from the product label) and the FA profile of similar foods. Any claims on the label about *trans* FA or hydrogenated fat levels was taken into account when estimating the FA profile. FA profiles for reduced and low fat spreads were updated using manufacturer's data collected prior to the 2000/01 NDNS (Henderson *et al*, 2003). Products that claimed to be low in *trans* FA were coded separately from other products.

Primary dietary sources of *trans* FA

9. Figure 3 shows the main contributors to *trans* FA intakes in adults in 2000/01 (Henderson *et al*, 2003) compared with 1986/87 (Gregory *et al*, 1990). In 1986/87, 30% of *trans* FA came from spreads (Gregory *et al*, 1990) (including butter), but this had dropped to 18% of intake by 2000/01 (Henderson *et al*, 2003). This was due both to reformulation of many fat spreads in the late 1990s to reduce/eliminate hydrogenated fat and to an overall decrease in consumption of this food group. The contribution of cereals and cereal products was unchanged at just over a quarter of total intake in both surveys, mainly from biscuits, buns, cakes and pastries. While mean consumption of biscuits, buns, cakes, and pastries fell between 1986/87 and 2000/01, their relative contribution to *trans* FA intakes remained stable as it was offset by the decline in fat spreads. The relative contributions of milk and milk products and meat and meat products both increased slightly.

Figure 3. Percentage contribution of food types to adult *trans* FA intakes



Source: Dietary and Nutritional Survey of British adults 1986/87(Gregory *et al*, 1990); NDNS adults 19-64 years 2000/01 (Henderson *et al*, 2003); LIDNS 19 years and above 2003/05 (Nelson *et al*, 2007).
Note: 'Other foods' includes chips, potato products and savoury snacks, confectionery, fish products, eggs and egg dishes.

Estimation of current *trans* FA intake using data supplied in 2007

10. Industry was asked to provide data to the FSA on current *trans* FA levels in product categories. The majority of data provided by industry were maximum *trans* FA levels in product categories but in some cases an average level was provided for a category. The current values for *trans* FA provided by industry were compared with the range of values that had been used for that food group in the NDNS 2000/01 (Henderson *et al*, 2003). New values provided by industry were used in the re-estimates if they were either average levels for a category or if they were maximum levels that were lower than the majority of existing values. Updated levels of *trans* FA for food group that were used in the re-estimation are shown in Table 5.

Table 5. Current *trans* FA levels in food categories as used by the updated estimate (FSA 2007)

Food categories	g *trans* FA /100g product
Biscuits (maximum)	1
Buns, cakes, pastries and fruit pies (maximum)	1
Reduced and low fat spreads and soft margarine (maximum)	1
Ice cream (average)	0.2

11. Values for confectionery, savoury snacks and processed potato products were also provided by industry but were not used in the re-estimates as they were maximum levels for each category and were higher than the majority of existing values. The rationale for this approach (rather than replacing the existing values) is that the existing values were generally based on analysis and it is consistent with specific information provided by industry that *trans* FA levels have reduced in products over time. Other data provided by industry for individual products could not be applied to entire product groups.

12. Using consumption data from the NDNS adults 2000/01 (Henderson *et al*, 2002) a new value for *trans* FA intake was estimated at 1.00% of food energy for all adults aged 19-64. Because it was not possible to take account of all the reductions in *trans* FA levels as described above, this figure is likely to be an overestimate of actual intake. Details of data provided by industry and the revised calculations can be found respectively in the FSA Board paper (www.food.gov.uk) and a separate paper published by FSA (FSA, 2007).

Contribution of animal and vegetable oil sources to *trans* FA intake

13. At present there are no methods of analysis applicable to a wide range of foods that can distinguish between *trans* FA, which are naturally present in foods (e.g. in animal products), and those formed during the processing of vegetable oils. This is because of the overlap in *trans* FA profiles of animal fats and hydrogenated oils and the varying proportions of *trans* FA isomers among different hydrogenated fats.

14. An estimate of the contribution of animal and vegetable oil sources to total *trans* FA intake has been made by identifying the main source of *trans* FA in each NDNS food group. This estimate, based on the 2000/01 NDNS (Henderson *et al*, 2003), suggests that around 55-65% of *trans* FA intake is derived from vegetable oil, with the remainder from animal sources.

Trends in intakes of individual *trans* FA isomers

15. There are no data available from NDNS or LIDNS on intakes of individual *trans* FA isomers nor any differentiation between naturally occurring *trans* FA and those present as a result of hydrogenation processes. Analysis of UK Total Diet Study samples provides some information on intakes of individual *trans* FA isomers at a population level (Table 6).

Table 6: Estimated population average intakes of total *trans* FA, individual *trans* FA and conjugated linoleic acid (CLA) based on analysis of Total Diet Study samples from 1991 and 1995.

	Population mean intake, g/day	
	1991	1995
Total *trans* FA	3.57	3.36
Of which:		
18:2 *trans*	0.58	0.41
18:3 *trans*	0.06	0.31
16:1 *trans*	0.27	0.16
18:1 *trans*	2.33	2.18
20:1 *trans*	0.24	0.17
22:1 *trans*	<0.01	0.13
18:2 conjugated	0.23	0.28

Source: Fatty acids and iodine in 1995 UK Total Diet samples. MAFF FSIS 127. 1997

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