The relationship between saturated and *trans* unsaturated fatty acids and LDL-cholesterol and coronary heart disease

A review undertaken for Food Standards Australia New Zealand

Chris Booker, BSc(Hons)

Jim Mann, CNZM, FRSNZ

Edgar National Centre for Diabetes Research,
Departments of Medicine and Human Nutrition,
University of Otago, New Zealand

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Introduction

The association between total cholesterol and coronary heart disease was firmly established in the 1950's and the potential of diet to influence cholesterol levels quantified in the 1960's. The landmark Seven Countries Study provided strongly suggestive evidence of a causal link between nature of dietary fat and coronary heart disease (CHD) which was mediated, at least in part, via cholesterol. In the 1970's and 1980's causality of the association was questioned by those with vested interests, but also by some distinguished researchers. However by the 1990's the role of diet, and of saturated fatty acids in particular, in the aetiology of coronary heart disease was universally acknowledged and LDL cholesterol recognised as the principal atherogenic component of LDL. The 2000 Canadian review updated a 1993 US FDA review justifying a health claim relating to dietary fatty acids and cholesterol. The specific issues relating to saturated and *trans* unsaturated fatty acids and LDL cholesterol and CHD in the Canadian review are considered in more detail in part 1 of this report; some comments in relation to the more general aspects of the 2000 review are briefly considered here.

The process followed in the preparation of the Canadian report appears to be entirely appropriate and most of the material relevant to the issues under consideration has been included and correctly interpreted. However some of the related aspects are less appropriately presented. For example a rather outdated approach is taken to the description of cardiovascular risk factors. There is no acknowledgement that they should be considered in aggregate in an attempt to determine absolute risk. The document still considers them in isolation with emphasis, for example, on cut-offs for lipid levels (table 1). The description of lipid and lipoprotein disorders was unclear, with some of the suggested cut-offs inappropriate. However of greater importance and relevance to the issues under consideration is the fact that the review does not clearly distinguish the effects of different types of dietary carbohydrate when recommending a replacement energy for dietary fat. There are repeated statements that replacing carbohydrate for fat will result in an increase in triglyceride and reduction in the protective high density lipoprotein (HDL). This in indeed the case when substantial quantities of fat are replaced by monosaccharides, disaccharides, and digestible starch. However when fat is replaced by vegetables, fruit and wholegrain cereals, rich in non-starch polysaccharides, this deleterious effect on triglyceride and HDL appears to be negligible. This issue may not have direct bearing on health claims suggesting the cardiovascular benefits of reducing saturated and *trans* fatty acids, but is of considerable relevance when considering the implementation of such advice.

Part 1: Critical appraisal of the Canadian Review

Part 1a): Selection and assessment of evidence

(i) Saturated fatty acids and LDL cholesterol

- The Canadian review has considered all the important studies relevant to this question. There are a few studies relevant to the effects of the individual saturated fatty acids which have not been included (see bullet point 6), but these would not have influenced the overall conclusions.
- The original US review has been correctly interpreted.
- The association between saturated fatty acids and LDL cholesterol is one of the most clearly established relationships between a nutrient intake and a universally accepted disease indicator.
- The evidence for a causal relationship is 'convincing', with several meta-analyses of RCT's carried out under metabolic and free-living conditions yielding broadly comparable results. Regression equations suggest an approximately 0.05 mmol/l increase in serum cholesterol for each 1% increase in saturated fatty acids. Animal experiments confirm experiments in humans and provide clear evidence of a mechanism. The effect of saturated fatty acids (SFA) on total cholesterol is almost entirely explained by changes in LDL cholesterol.
- The interpretation of the evidence cited was generally appropriate.
- Three major limitations of the available evidence were in our opinion not adequately discussed. The first relates to the effect of the individual saturated fatty acids. The review acknowledges the different effects of the major dietary SFA's, but does not adequately address the differential effect on LDL cholesterol of lauric, myristic and palmitic acids, nor the fact that lauric acid has a more marked effect on raising HDL cholesterol than myristic and palmitic acids, and is thus associated with a more favourable ratio of LDL/HDL. The negligible effect of stearic acid on LDL cholesterol is considered. This does not effect the overall conclusion since stearic acid intake is, at least in most dietary patterns, highly correlated with intake of other SFA's, and in cohort studies has been shown to increase cardiovascular risk, possibly via a thrombogenic mechanism. Second, while arguably not important with regard to health claims aimed at improving population health, it would nevertheless have been relevant to draw attention to the wide individual variation which occurs in response to change in nature of dietary fat, despite the consistent and predictable changes when considering groups of individuals. There have been many attempts made to identify genetic polymorphisms which might explain this heterogeneity in response, but none appear to be important determinants, probably because polygenic factors are responsible, and the studies have been inadequately powered to have simultaneously examined the effect of multiple genes. Finally, and perhaps of greatest relevance is the absence of a detailed discussion regarding the effects of nutrients with which SFA's are replaced. Reduction of SFA's is likely to have a greater LDL-lowering effect when replaced with n-6 polyunsaturated fats than with carbohydrate because the former have, in their own right, the potential to lower LDL, whereas the reduction seen with carbohydrate

replacement may simply be a consequence of a reduction in the SFA's. An exception to this might be if the carbohydrate is rich in soluble forms of non-starch polysaccharide which also has inherent LDL-lowering potential. However none of these limitations appreciably dilutes the claim that reduction in saturated fatty acids will reduce total and LDL cholesterol in the population at large.

- As indicated above regression equations can calculate the expected benefit in terms of total and LDL cholesterol reduction which might be expected to be achieved for each 1% reduction in SFA's. This may in turn be translated into expected clinical benefit given that each 1% reduction in cholesterol is likely to translate into at least a 2% reduction in cardiovascular events.

(ii) Saturated fatty acids and coronary heart disease

- The first papers describing the major study relating SFA's to CHD (the Seven Countries study) antedated the Canadian review. However it is surprising that the 25 year follow-up of this study (Kromhout et al, 1995) was not included amongst the cohort studies listed on pages 31-32. This study along with the study by Hu et al. (1999) provide the best prospective evidence of a direct link between SFA's and CHD. There are clinical trials which show the potential of reducing CHD risk by reducing saturated fatty acids. Admittedly several of these have considered dietary change other than a reduction in SFA's, but the Oslo Diet Heart Study (Leren, 1989) involved principally a reduction in total and saturated fatty acids. Given that there is little evidence to suggest benefit of reducing total fat it seems likely that the observed benefit derived primarily from a reduction in SFA's. The conclusions would not have been altered by the addition of these studies.
- The US review has been appropriately interpreted.
- While the evidence linking SFA's with CHD morbidity and mortality is not as consistent and impressive as the link between SFA's and LDL cholesterol, it was considered by the Canadians to be 'convincing'. The association is evident in cohort studies, and RCT's have shown clinical benefit as a result of reducing SFA intake. Not all cohort studies have confirmed the association but plausible explanations (especially with difficulties of dietary assessment) have been offered for the inconsistency. The association is biologically plausible with a clear dose-response between degree of cholesterol lowering (itself related to extent of dietary change) and clinical benefit. The issue surrounding whether this is indeed a convincingly causal association as distinct from a 'probable' one hinges around the extent to which the heterogeneity may be regarded as sufficiently explained. We were seeking confirmation of this in the new data.
- The interpretation of the cited evidence was generally appropriate. However the findings from the Oxford Vegetarian Study were not quite accurately interpreted. The appreciable reduction in CHD risk amongst the vegetarians was attributable to the lower intake of SFA rather than abstinence of meat (Mann et al., 1997). The overall conclusions are not influenced.
- The limitations of the available evidence are adequately considered.
- The review relies principally on two studies to quantify the benefit likely to accrue from reduction in SFA's. Hu et al. (1997) estimate that ~ 5% reduction

in SFA's would lead to a 17% reduction in CHD risk if SFA's were replaced by carbohydrate and 42% if replaced by unsaturated oils. Kris-Etherton and Yu (1997) estimate that an AHA Step 2 diet (saturated fat replaced by carbohydrate) would lower CHD risk by 12% whereas isoenergetic replacement of saturated fat with monounsaturated fatty acids would reduce risk by 42%. Unfortunately these estimates do not take into account nature of carbohydrate but this does not detract from the benefits which might be expected from a reduction in intake of SFA's.

(iii) *Trans* fatty acids and LDL cholesterol

- An appreciable number of studies demonstrating the effect of *trans* fatty acids on LDL cholesterol had been published at the time of the 2000 Canadian review. All major studies appear to have been included.
- Most of the dietary intervention studies examining the effects of *trans* fatty acids on LDL were of high quality and published in major journals by leading research groups. Total and LDL cholesterol appear to show a linear increase with increasing intakes of *trans* fatty acids. Lipoprotein (a) is also higher on diets high in *trans* fatty acids compared with other sources of fat. The relationship between *trans* fatty acids and LDL cholesterol can unquestionably be described as 'convincing'.
- All the evidence appears to be cited appropriately.
- There are three limitations in the available evidence.
  First is the limitation which applies to all dietary intervention studies, namely that it is impossible to guarantee 100% dietary compliance in studies including free living individuals. However the remarkable consistency of the results of the various studies and the clear dose response shown in several suggest that this cannot explain the association between total *trans* fatty acids and LDL cholesterol.
  Second, some of the studies do not fully explain how *trans* fatty acids were measured, nor do all distinguish clearly between vegetable and animal sources of *trans* fatty acids. This is a limitation which was not adequately discussed in the Canadian review. However, once again, consistency of the findings suggests that the association is a genuine one.
  Third, it is not possible to assess whether the association occurs over the whole range of intakes of *trans* fatty acids.
- Data from the experimental studies have not been extrapolated to estimate potential influence on cardiovascular risk reduction by reducing intake of *trans* fatty acids. However such estimates have been made on the basis of epidemiological data. See below.

(iv) *Trans* fatty acids and coronary heart disease

- The major epidemiological studies which have examined the association between *trans* fatty acids and coronary heart disease were considered in the Canadian review.
- When examining the various epidemiological studies, there appears to be a degree of heterogeneity with regard to the relationship between *trans* fatty acids and coronary heart disease. Since the first publication by Willett et al. (1993) based on the Nurses' Health Study which showed a clear association, there have been several case control studies, one major cross-sectional study, a Finnish cohort study (Pietinen et al., 1997) and a further longer term report based on the Nurses' Health Study from the U.S. (Hu et al., 1997). The cross-sectional study based on the Seven Countries Study described earlier and the two reports from the Finnish and US cohorts both confirmed the initial observation. Two of the four case control studies did not confirm the association. However, the Scottish study (Bolton-Smith et al., 1996) included individuals who had had cardiovascular disease for various intervals before being investigated and diet may well have changed after an acute event. Indeed a study of this nature should not be regarded as admissible evidence. The multinational EURAMIC study also found no differences between cases and controls (Aro et al., 1995). There is no clear explanation, but it is generally agreed that even 'good' case control studies may be inconclusive when examining diet-disease relationships. Of the two case control studies showing a positive association, one should probably also be discounted since it was underpowered and there was no attempt to examine for confounding variables (Siguel and Lerman, 1993). Given the impressive association observed in cross-sectional and cohort studies which show a dose-response relationship which is biologically plausible, it seems reasonable to support the Canadian conclusion that there is convincing evidence that the association between *trans* fatty acids and coronary heart disease is causal. The heterogeneity is present only amongst the case-control studies which are often discounted in the hierarchy of evidence examining diet-disease associations, and in only a single case control study (the EURAMIC study) is the lack of an association not readily explicable. However this is an association we were also anxious to pursue in the more recent data.
- The cited evidence is appropriately interpreted.
- The review does indeed consider the limitations in the available evidence. The increased risk of *trans* fatty acids as demonstrated in both cross-sectional and cohort studies stems principally from TFA derived from vegetable fat (*trans* 18:1, elaidic acid).
- It has been estimated that replacement of 2% of energy from *trans* fatty acids with evidence from *cis* unsaturated fatty acids would reduce coronary heart disease risk by over 50%. This estimate is based principally upon the data from the Nurses' Health Study.

Part 1b): Reanalysis of pivotal studies

Hu et al., 1997:

Hu FB, Stampfer MJ, Manson JE, Rimm E, Colditz GA, Rosner BA, Hennekens CH, Willett WC. Dietary fat intake and the risk of coronary heart disease in women. *N Engl J Med*, 1997, **337**(21):1491-9.

Reason for selection

This study was selected for review since it is arguably the most definitive study demonstrating the effects of dietary fat on risk of coronary heart disease.

Summary

The Nurses' Health Study involved over 80,000 women aged 34 to 59 years of age who in 1980 had no known CHD, stroke, cancer, hyperlipidaemia or diabetes. Particularly noteworthy was the fact that dietary data (obtained by means of food frequency questionnaire) was collected not only at baseline, but was also updated during 14 years of follow up during which time 939 cases of nonfatal or fatal myocardial infarction were documented. Multivariate analysis included age, smoking status, total energy intake, dietary cholesterol, percentages of energy from protein and types of dietary fat, and other risk factors. Total fat intake was not related to risk of coronary heart disease. Each increase of 5% of energy intake from saturated fat, as compared with equivalent energy intake from carbohydrates, was associated with a 17% increase in the risk of CHD (relative risk 1.17). As compared with equivalent energy from carbohydrates, the relative risk for a 2% increase in energy intake from *trans* unsaturated fat was 1.93, that for a 5% increase in energy from polyunsaturated fat was 0.62. From the data it is possible to estimate that replacement of 5% of energy from saturated fat with energy from unsaturated fats would reduce risk of CHD by 42% and that the replacement of 2% of energy from *trans* fat with energy from unhydrogenated unsaturated fats would reduce risk by 53%.

This study does indeed have some limitations. The findings are limited to women. Nutrient intakes were based on food frequency questionnaires and inevitably the *trans* fatty acid data in the food composition tables must have been incomplete. Nevertheless the repetition of the food frequency questionnaire on 4 occasions provided for superior data compared with most other prospective studies in which diet is typically assessed on only a single occasion. Sample size is impressive and over 90% were followed to the conclusion of the study.

The associations between dietary intakes of fatty acids and clinical events are compatible with the effects on lipoproteins. However the somewhat greater effects than might have been predicted on the basis of lipoprotein mediation suggest that enhanced atherogenic risk might also be mediated via other mechanisms, e.g. influencing platelet aggregability, insulin sensitivity and cardiac conductivity. The findings are also important because they place in perspective the lesser importance of dietary cholesterol and, at least in terms of CHD, the relative unimportance of total fat intake other than as a possible indicator of intake of saturated fatty acids.

Clarke et al., 1997:

Clarke R, Frost C, Collins R, Appleby P, Peto R. Dietary lipids and blood cholesterol: quantitative meta-analysis of metabolic ward studies. *BMJ*, 1997, **314**(7074):112-7.

Reason for selection

This paper was chosen since it was the first meta-analysis based on a large number of dietary experiments using appropriate statistical methods and carried out by some of the world's leading epidemiologists and statisticians with appropriate nutritional input.

Summary

The analyses were based on 395 dietary experiments carried out under strictly controlled conditions generally in metabolic wards in order to ensure compliance. Details are provided regarding the search strategies as well as inclusion and exclusion criteria. Isocaloric replacement of saturated fats by complex carbohydrates for 10% of dietary calories resulted in a reduction in total and LDL cholesterol by 0.52 and 0.36 mmol/l respectively. Isocaloric replacement of complex carbohydrates by polyunsaturated fats for 5% of dietary calories produces further decreases of 0.13 and 0.11 mmol/l. Replacement of carbohydrate by monounsaturated fats produced no significant effect on total and LDL cholesterol. Avoiding 200mg/day dietary cholesterol has the potential to further decrease total and LDL cholesterol by 0.13 and 0.10 mmol/l. The figure below (Table 2 from the original paper) shows the effect of realistic dietary change in terms of modifying dietary fatty acids and cholesterol without altering total energy in a fairly typical western diet. Much of the 0.76 mmol/l reduction in total cholesterol is achieved by a reduction in LDL cholesterol (-0.62 mmol/l, i.e. a reduction of 10-15%). The effects of *trans* fatty acids could only be examined in 40 of the experiments, but in this meta-analysis the effects of *trans* monounsaturated fats (mainly *trans* C18:1, elaidic acid) on total and LDL cholesterol did not differ appreciably from that of saturated fatty acids. As in the earlier studies stearic acid appeared not to influence total and LDL cholesterol.

There are few limitations of this study. It could not distinguish between natural *trans* unsaturates and *trans* unsaturated fatty acids from hydrogenated vegetable oils, but then relatively few studies have attempted to disentangle separate effects. The authors chose not to examine the effects of these dietary manipulations on lipoprotein fractions other than LDL cholesterol. However this is probably the most accurate attempt to quantify the effects of dietary modification on total and LDL cholesterol. It is important to emphasise that the meta-analysis relates to isocaloric exchange. Since weight loss can reduce total and LDL cholesterol appreciably greater reductions would be apparent if the changes described here were made in conjunction with energy restriction.

Table 2 Mean daily intake of dietary fats in 395 solid food experiments compared with average diet for British men and estimated changes in blood total cholesterol (from all 395 solid food experiments) and in low density lipoprotein and high density lipoprotein cholesterol (from 227 solid food experiments) associated with particular changes in intake of fats

	Mean daily intake				Mean (SE) change in blood cholesterol concentration (mmol/l)	
Dietary fat	**Experimental diets**	**British diet**	**Dietary change**	**Total**	**Low density lipoprotein**	**High density lipoprotein**
Saturated fats (% of total calories)	14.3	16.5	Replacement of saturated fat by complex carbohydrate (10% of calories)	-0.52 (0.03)	-0.36 (0.05)	-0.13 (0.02)
Poly unsaturated fat (% of total calories)	7.1	6.2	Replacement of complex carbohydrate by polyunsaturated fat (5% of calories)	-0.13 (0.02)	-0.11 (0.03)	0.03 (0.01)
Monounsaturated fat (% of total calories)	12.8	12.4	Replacement of complex carbohydrate by monounsaturated fat (5% of calories)	0.02 (0.03)	-0.04 (0.02)	0.03 (0.01)
Dietary cholesterol (mg/day)	361	390	Reduction in dietary cholesterol by 200 mg/day	-0.13 (0.02)	-0.10 (0.02)	-0.02 (0.01)
All of above			Sum of the above changes	-0.76 (0.03)	-0.62 (0.04)	-0.10 (0.02)
Total fat (% of total calories)	35.0	40.4*	Replacement of total fat by complex carbohydrate (10% of calories)	-0.20 (0.05)	-0.12 (0.06)	-0.10 (0.02)

*Total fat intake in British diet includes *trans* fatty acids and glycerol derivatives as well as sum of the fats listed in table.[20]

Source: *BMJ*, 1997, **314**(7074):112-7.

Tang et al., 1998:

Tang JL, Armitage JM, Lancaster T, Silagy CA, Fowler GH, Neil HA. Systematic review of dietary intervention trials to lower blood total cholesterol in free-living subjects.
BMJ, 1998, **316**(7139):1213-20.

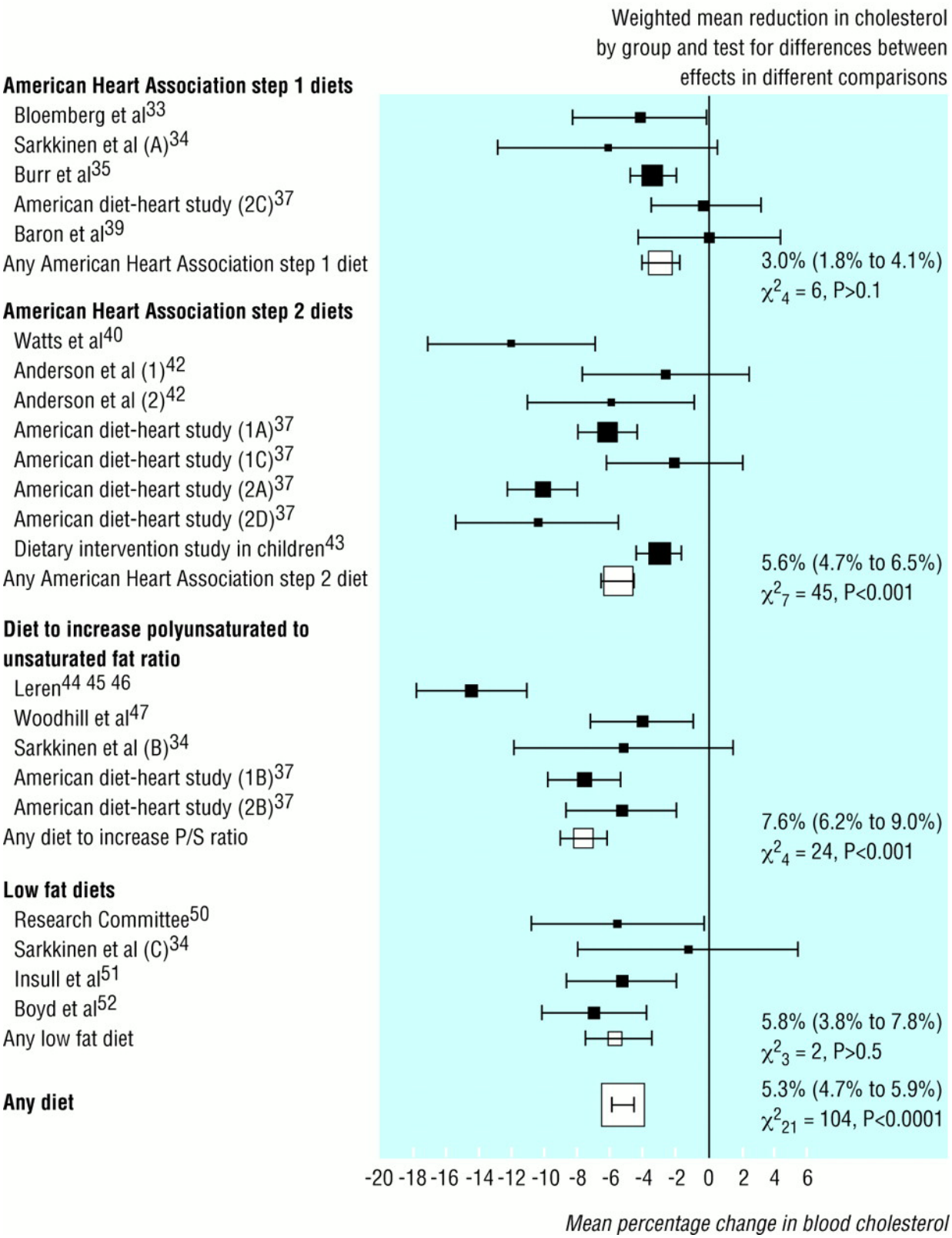
Reason for selection

This was the first systematic review of dietary intervention studies which aimed to lower cholesterol in free living subjects. Although the review considered blood total cholesterol, there is strong evidence to suggest that much of the changes in total cholesterol derive from change in LDL cholesterol.

Summary

19 randomised controlled trials which lasted for four weeks or more were included. The percentage reduction in blood total cholesterol attributable to various dietary manipulations is shown in the figure below for those studies which continued for at least 6 months, with an average reduction of 5.3%. When considering only the first 3 months, reduction was appreciably higher at 8.3%, but the reduction at 12 months (5.5%) was remarkably similar to that achieved after 6 months. On the basis of repeated food intake assessment, the targets for dietary change were rarely achieved, but reported changes produced cholesterol changes that were consistent with those predicted from the Keys formula.

Thus on average free-living individuals achieve roughly half the reduction in cholesterol which might be achieved by comparable dietary manipulation in a metabolic ward. The study provides evidence to suggest that the difference is principally a consequence of reduced compliance. However it is important to appreciate that even given a somewhat disappointing level of compliance reductions in total cholesterol, which may be extrapolated to reductions in LDL cholesterol, are such that they would be expected to produce considerable clinical benefit if the rule of "1% reduction in cholesterol produces a 2-3% reduction in incidence of CHD" is applied. It is noteworthy that we have been able to show changes in total and LDL cholesterol amongst free-living individuals which are more akin to that achieved in metabolic wards. However this required considerable ongoing advice and support for participants in the studies (Aitken, W, in press).



Source: BMJ, 1998, ***316:****1213-1220.*

Lichtenstein et al., 1999:

Lichtenstein AH, Ausman LM, Jalbert SM, Schaefer EJ. Effects of different forms of dietary hydrogenated fats on serum lipoprotein cholesterol levels. *N Engl J Med*, 1999, **340**(25):1933-40.
Erratum in: *N Engl J Med*, 1999, **341**(11):856.

Reason for selection

This paper was selected since it is one of the clearest demonstrations of the effect of *trans* fatty acids on lipoprotein levels, but at the same time raises the critical issue regarding amount of TFA required to exert adverse effects on lipoproteins.

Summary

This remarkable study involved 18 women and 18 men who consumed each of 6 experimental diets in random order for 35 day periods. The foods were identical in each diet. 30% of calories was derived from fat, two thirds of total fat provided by the test fats: soybean oil (<0.5g TFA/100g fat), semi-liquid margarine (0.6g /100g), soft margarine (7.4g/100g), shortening (13.6g/100g) or stick margarine (20.1g/100g). The effects were compared with those of a diet enriched with butter with a high intake of saturated fat (61.7g saturated fat/100g, 2.6g TFA/100g). Saturated fatty acid intake of the experimental diets ranged from 13g/100g in soybean oil to 16-17g/100g for the other experimental diets. Soybean oil and semi-liquid margarine resulted in 12% and 11% reductions in LDL cholesterol compared with that seen in the high butter diet. Indeed compared with butter all experimental diets were associated with lower LDL cholesterol. Of particular interest are levels on the remaining three experimental diets with comparable amounts of saturated fatty acids and increasing quantities of TFA's. Compared with soybean oil LDL cholesterol was 3% higher on the soft margarine, 5% higher on shortening and 7% higher on the stick margarine. Ratios of total and LDL cholesterol to HDL cholesterol were lowest in the soybean oil and semi-liquid margarine and highest in the butter and stick margarine.

There are two major limitations to this study. First, relatively high levels of a single fat were used particularly in the case of a stick margarine, achieving a total TFA intake which is probably greater than that typically consumed in Australia and NZ. Nevertheless the clear dose response indicating an increase in LDL cholesterol with increasing intakes of TFA provides convincing evidence of the causal association. However the clinical relevance of the findings in the context of relatively low consumption is an issue which remains to be resolved.

Second, each experimental dietary fat included a mixture of fatty acid isomers containing at least one *trans* double bond. While this does not permit evaluation of the effects of individual isomers, it does allow the assessment of commercially available hydrogenated vegetable fats. Hydrogenated fats derived from vegetable oil other than soybean oil will have different fatty acid profiles. However other data suggest that hydrogenated fats from a wider range of oils have similar effects.

These results are also important since they indicate that while shortening and margarines, which are relatively high in *trans* fatty acids but low in saturated fatty

acids, are preferable to butter in terms of LDL cholesterol, butter itself is associated with relatively favourable levels of Lp(a). The most favourable lipoprotein profiles overall are associated with diets low in saturated and *trans* unsaturated fatty acids and with relatively high proportions of *cis*-unsaturated fatty acids.

Part 1c): Consideration of the validity of the review's conclusions

- In our opinion the Canadian review provides a comprehensive and reasonably accurate summary of the evidence available at the time it was conducted. The limitations described above do not appreciably influence the overall conclusions. However we considered that it was necessary to review in the more recent data the issue as to whether the associations between saturated fatty acids and *trans* fatty acids and coronary heart disease were appropriately described as "convincing" rather than "probable".
- The review does describe the circumstances under which the studies were carried out.
- There is no doubt that the influence of total fat and cholesterol intakes on serum LDL-cholesterol and coronary heart disease are less important than the effects of saturated and *trans* fatty acids. In terms of both LDL cholesterol and coronary heart disease, there is no evidence that reducing total fat intake confers benefit beyond that resulting from the reduction in saturated fatty acids. That dietary cholesterol has a less marked effect than saturated fatty acids or total (and LDL) cholesterol has been repeatedly demonstrated since the 1960's. More recent studies have shown that even with intakes of dietary cholesterol which are appreciably greater than those typically consumed, the effect on LDL cholesterol is relatively small when intakes of saturated fatty acids is low. There is some evidence to suggest that the effect of dietary cholesterol is greater in individuals with apoE4E4 or apoE3E4 genotypes than those with other apoE genotypes and amongst those with relatively high levels of total and LDL cholesterol. However these observations do not influence the overall conclusion. There is little evidence in epidemiological studies of a meaningful association between intake of dietary cholesterol within the usual range of intakes and clinical coronary heart disease.
- The report does not consider potential undesirable effects associated with the expected dietary change. However there are no specific nutritional requirements for vegetable sources of *trans* unsaturated fat principally produced in the manufacturing process and only benefits, not undesirable effects, would be expected to accrue from their elimination from the diet were this feasible. Many saturated fats on the other hand contribute to total energy intake and are also sources of other important nutrients, notably fat soluble vitamins. However other than in the first few years of life, when substantial fat restriction may lead to inadequate energy intakes for growth and development, even substantial reductions of saturated fatty acids will not lead to energy deficits (if required *cis* unsaturated fatty acids may compensate) or deficiencies in fat soluble vitamins.
- We believe that there is convincing evidence for a causal link between saturated and *trans* unsaturated fatty acids and LDL cholesterol. Benefit in terms of reduced risk of coronary heart disease is likely to accrue from reduced intakes. However it is still somewhat open to question as to whether the evidence directly linking saturated fatty acid intake with coronary heart disease can be described as "convincing" using current criteria or whether it should more appropriately be described as "probable".
- The Canadian review is comprehensive and builds appropriately on earlier literature. Thus it is a reasonable starting point for the substantiation of a relationship between saturated and *trans* unsaturated fatty acid intake and LDL

cholesterol and coronary heart disease. Although the effects of the different saturated fatty acids are not equal we acknowledge that in terms of recommendations or claims it is inappropriate and indeed probably impossible to distinguish between the different saturated fatty acids.

- The information that will be especially sought from the more recent data relate to *trans* fatty acids and whether the adverse effects are indeed restricted to vegetable sources and also whether the associations with LDL cholesterol and CHD occur across the full range of intakes.
- Additionally the evidence directly linking saturated fatty acids and *trans* fatty acids and coronary heart disease will be evaluated in an attempt to establish whether this should be described as convincing or probable.

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Part 2: Review of evidence released since the publication of the Health Canada report

i. Literature search for current evidence

Search strategy:

A literature search was carried out on the OVID Medline 1999 to June Week 4 2005 database. (OVID search history included as Appendix 2.1).

Keyword and subject heading searches were made for the terms: *trans* fatty acid/*trans* fatty acids; dietary cholesterol; fatty acids; dietary fats; saturated fat; hydrogenated fat; meat; meat products; eggs; animal fat. Any articles identified dealing with poultry or seafood were later ignored. Results of these searches were pooled to give 27,327 articles. This pooling grouping was then crossed with a pooled search from keyword and subject heading searches for the terms: cardiovascular disease; coronary heart disease; CVD; CHD; LDL; LDL cholesterol lipoproteins; LDL lipoproteins; low density lipoprotein.

Results were limited to articles in the English language, giving a total of 1,937 articles. For ease of analysis, articles were grouped according to the categories they were indexed under on Medline, such that this set contained 13 articles indexed on Medline as Cochrane reviews; 304 indexed as review articles; 6 meta-analyses; 39 case-control studies; 65 cohort studies; 30 randomised controlled trials, and 1,480 not indexed in any of these categories.

The authors individually reviewed the titles and abstracts of all Cochrane reviews, meta-analyses, case-control, cohort and randomised controlled trial papers, and the titles of review articles individually, selecting papers deemed of interest. Lists of papers selected by each author were compared, and any discrepancies or differences in paper selection resolved after consideration of the abstract. The titles of the remainder of the 1,480 identified articles were scanned by C.B. and full text articles subsequently obtained when deemed relevant or of importance. Any additional articles not identified in the literature search, but referenced in papers or review articles which were deemed of sufficient interest or impact, were also considered in the writing of the review.

A hand search of a selection of relevant journals (see below) over the time period Jan 2000 – June 2005 was also conducted by C.B. for additional articles:
American Journal of Epidemiology; Annual Review of Nutrition; International Journal of Epidemiology; Public Health Nutrition; European Journal of Clinical Nutrition; American Journal of Clinical Nutrition.

ii. Analysis of current evidence, 1999 – 2005:

Saturated fatty acids (SFA's):

Summary:

The results of studies published since publication of the Health Canada review are generally supportive of the conclusion arrived at in that report – that increasing intake of saturated fat is convincingly associated with raised LDL cholesterol. The main development since the Health Canada report appears to be an increasing sophistication of analysis based on larger pools of data with additional examination of the effects of individual saturated fatty acids.

In addition to the further dietary intervention trials in adults, several recent randomised controlled trials have examined saturated fat intake in childhood following randomisation to low-fat or control diets. These studies suggest that reduction of saturated fat intake even very early in childhood has the potential to reduce LDL cholesterol levels and improve cardiovascular risk factors, with none reporting adverse effects on child growth and development as a result of fat restriction. Long term compliance may be difficult to sustain. It appears that saturated fat reduction in young children may only produce these beneficial effects on lipid levels in boys and not girls, with no clear explanation regarding gender differences.

Two important meta-analyses have aggregated data for the dietary intervention trials (Mensink et al., 2003; Müller et al., 2001). When considering the effect on LDL or the ratio of LDL:HDL there is no doubt that the most marked adverse effects are associated with myristic and palmitic acids. While lauric acid is certainly associated with an elevation of LDL cholesterol there is less consistency with regard to the relative effects of this fatty acid compared with myristic and palmitic acids. However while all three of these fatty acids are associated with increase in HDL cholesterol when compared with carbohydrate, the effect of lauric acid is consistently the greatest. This may to some extent mitigate the effects of LDL elevation. Although stearic acid has no effect on LDL cholesterol, there is no doubt that in aggregate saturated fatty acids are "convincingly" associated with LDL elevation. In terms of substantiating a health claim, the relative effects of the individual fatty acids are not of public health importance.

No new prospective studies of saturated fat intake and health outcomes were identified. However further analyses of the Nurses' Health Study, Seven Countries Study, the British Health and Lifestyle Survey, and a cohort of Danish men and women have been undertaken. They are generally confirmatory of an association between saturated fat intake and coronary heart disease. However discrepancies do exist and these are partially explicable. An analysis of the Nurses' Health Study cohort from 1980 to 1998 showed no significant association between saturated fat intake and coronary heart disease. The authors ascribe this weakening of the previously observed associations in this cohort to reductions in intakes of saturated fat and incidence of coronary heart disease which occurred during the 18 years subsequent to the establishment of the cohort and recording of baseline data. Data from the British Health and Lifestyle Survey showed a significant association between intake of saturated fat and coronary heart disease amongst women, with a stronger

association observed amongst older women. A 16-year follow up of Danish men and women reported an association of borderline significance between saturated fat intake and coronary heart disease in women, which was observed to be stronger amongst younger women. Both the British Health and Lifestyle Survey and the Danish cohort study showed no association of saturated fat intake and coronary heart disease in men. The prolonged period between the baseline dietary data and morbidity and mortality from coronary heart disease to which intake of saturated fatty acid was related is a likely explanation for the failure to confirm the associations convincingly demonstrated during the earlier follow up period. However inconsistencies with regard to gender and age are not readily explained.

Thus while there is a considerable body of evidence to suggest that saturated fatty acids are indeed associated with coronary heart disease and there is indeed a biologically plausible explanation, the heterogeneity observed in the recent analyses which cannot be fully explained lead to the conclusion that the association is "probably" causal rather than "convincingly" causal.

Saturated fatty acids and LDL-cholesterol:

Although the purpose of this report is to examine the link between dietary saturated and *trans* fatty acids and LDL cholesterol, the effect on HDL cholesterol has been commented on where available since this enables an examination of the effect of dietary manipulation on the overall lipoprotein pattern.

Cross sectional epidemiological studies:

Very few cross-sectional studies of saturated fatty acid intake and LDL cholesterol appear to have been undertaken in the period under review. A comparison of omnivores and vegetarians was undertaken in Hong Kong by Lee et al. (2000). Dietary assessment was by food frequency questionnaire recorded and analysed without knowledge of LDL levels. Not surprisingly, total fat intake was significantly higher in omnivores, and this was reflected in significantly higher SFA, PUFA and MUFA intakes, as well as dietary cholesterol. Other differences were a higher protein and lower carbohydrate intake, and lower P/S ratio, in omnivores than vegetarians. Total serum cholesterol and LDL cholesterol were significantly higher in omnivores, though so too was HDL cholesterol. VLDL and LDL:HDL ratio were similar in the two groups. This study was of reasonable size and appropriate design, taking into account the limitations of case control studies. Thus differences in lipids and lipoproteins between omnivores and vegetarians are confirmed but no definitive conclusions can be drawn regarding the relationship between saturated fatty acids and LDL given the many dietary differences between the two groups.

Another larger cross-sectional study was that of the EURODIAB IDDM Complications Study (Toeller et al., 1999), principally designed with diabetes complications in mind, but which also collected dietary information from diet history records and blood lipid measurements in some individuals. Regression equations for total cholesterol were based on 2762 individuals, and for LDL cholesterol on 1816 individuals, all of whom had type 1 diabetes. Energy-adjusted intake of total fat, saturated fat, and dietary cholesterol were significantly positively associated with total and LDL cholesterol. However, in a model fully adjusted for other dietary and clinical measures the significance of these associations was lost. The authors ascribed this to the effect of dietary fibre intake, which tended to decrease as saturated fat intake increased. Thus in the final regression analysis intakes of saturated fat or dietary cholesterol were not significantly associated with serum total or LDL cholesterol levels. However, it was noted that for those individuals who consumed less than 8% of energy as saturated fat the mean LDL cholesterol level was appreciably lower than individuals with higher intakes. Additionally, intakes of dietary cholesterol in excess of 500mg/day were associated with increased LDL cholesterol levels.

Childhood studies:

A number of randomised controlled trials and prospective studies examining the association of total and saturated fat intake with cholesterol levels in childhood have emerged in the last decade. However not all of these specifically measured fat, and more importantly saturated fat, intakes and hence only two randomised controlled trials are considered below: the STRIP and DISC studies, and the prospective ALSPAC study. No randomised controlled trials or prospective studies specifically examining the effect of reduction in saturated fat intake on LDL cholesterol in adults were found in the time period under review.

Childhood studies - randomised controlled trials:

The Special Turku coronary Risk factor Intervention Project (STRIP) which began 1990 has given rise to a number of publications (Niinikoski et al., 1996; Rask-Nissila et al., 2000; Viikari et al., 2004; Salo et al., 2000; Rasanen et al., 2004; Kaitosaari et al., 2003; Rask-Nissila et al., 2002; Lapinleimu et al., 1995). Families of 7-month old infants were randomised to either a control group (n = 522) or intervention (n = 540). Intervention consisted of advice aimed at reducing the child's exposure to atherosclerosis or coronary heart disease risk factors, predominantly focusing on dietary advice. The ideal diet at this age for a child was described to the parents as one comprising 30-35% fat, with equal proportions of saturated, monounsaturated and polyunsaturated acids making up this amount. No differences were observed between control and intervention groups at baseline in terms of total cholesterol, HDL cholesterol, or non-HDL cholesterol. At age 13 months a comparison of dietary intakes was made, although given the obvious difficulties involved with children being breast-fed this was restricted to children who were formula fed. Formula fed intervention children (n=60 per group) were receiving significantly less dietary saturated fat than control children (n=60). Intervention children (n=450) and control children (n=436) at 13 months had significantly different changes from baseline in total serum cholesterol, HDL cholesterol, and non-HDL cholesterol, with intervention children showing lower levels for each of these measures. These comparisons were based on non-fasting samples.

Follow-up results were published for assessments at 3 years of age (Niinikoski et al., 1996), 5 years of age (Rask-Nissila et al., 2000) and 7 years of age (Kaitosaari et al., 2003). Non-fasting blood samples were drawn yearly up to the age of 5 yrs, at which age samples were drawn after an overnight fast for the first time, allowing calculation of LDL cholesterol. The results at 5 and 7 years of age are considered below.

At 5 years of age, analysis of dietary intakes at 13 months, 24 months, 36 months, 48 months and 60 months revealed that intervention boys were consistently consuming less total fat, saturated fat and cholesterol than control boys. Polyunsaturated fat intakes were consistently significantly higher in intervention boys, with monounsaturated fat and total energy both significantly lower at one of the above time points each. Results were similar for girls, with total fat, saturated fat and cholesterol intakes significantly lower for intervention children than controls and polyunsaturated fat consistently higher. Monounsaturated fat intake was significantly lower amongst intervention girls on two occasions, and total energy intakes were significantly lower in intervention girls vs. control girls at 13 months, 48 months and 60 months.

Blood test results for boys showed that across the trial period, serum cholesterol values of intervention boys were 6% - 10% lower than control boys, and similarly non-HDL cholesterol 6% - 11% lower. HDL:total cholesterol ratios were similar between groups at all time points. Analysis of LDL cholesterol at 5 years of age showed mean levels in intervention boys were 9% lower than control boys (2.62 ± 0.55 mmol/l vs. 2.87 ± 0.61 mmol/l, $p = 0.0002$).

For the girls, mean serum cholesterol levels across the trial period were slightly lower in intervention girls and bordered on statistical significance ($p = 0.052$). Serum non-HDL cholesterol did not differ between groups such that HDL:total cholesterol levels were similar across groups as well. LDL cholesterol at 5 years showed no difference between intervention and control girls (2.94 ± 0.63 mmol/l vs. 2.99 ± 0.66 mmol/l respectively, $p = 0.53$).

The dietary and blood test results reported above were not based on the entire cohort; dietary analysis from 13 months of age was based on continually smaller numbers for each subsequent follow-up, and at 60 months was based on roughly 135 participants per gender per treatment arm (range 126 for intervention girls – 141 for control boys). Numbers for longitudinal blood test results ranged from 158 for lipid measures in intervention girls to 180/181 for lipid measures in intervention boys.

Extending these findings to 7 years of follow-up (Kaitosaari et al., 2003) total fat intake was the same amongst intervention vs. control boys, although saturated fat intake was lower in intervention boys (11.5% total energy vs. 13.6%, $p < 0.001$). Both total fat and saturated fat intakes were lower in intervention girls compared to control girls. Serum lipid measures revealed no significant differences between control and intervention girls, and in fact lipid measures did not even tend towards any differences, with the lowest p-value being 0.48 for comparison of triglycerides. On the other hand, intervention boys at 7 years had significantly lower serum cholesterol and LDL cholesterol, and a significantly higher HDL:total cholesterol ratio than control boys.

It appears from the results of the STRIP project that reduction of saturated fat intake across a number of years from early infancy through childhood has the ability to consistently reduce non-HDL and LDL cholesterol in boys, but not girls. No adverse effects on behavioural or cognitive development of children was observed (Rask-Nissila et al., 2002).

Considering older age groups, the Dietary Intervention Study in Children (DISC) (Obarzanek et al., 2001a) enrolled 663 children aged 8-10 yrs in 1987 to randomised dietary intervention or control groups. The study was conducted in the U.S. with six participating centres. Children were followed either until age 18 yrs (15.3%) or until a screening visit prior to age 18 (84.7%). Mean length of follow-up was 7.4 years. Dietary aims in the intervention group were for 28% of total energy from fat, less than 8% from saturated fat, and less than 75mg of cholesterol per 1000 kcal energy, thus similar to the National Cholesterol Education Programme Step 2 diet. Children were required to be mildly hypercholesterolaemic, with the average LDL after two screening visits above or equal to the 80th percentile but below the 98th percentile for age and sex. The intervention programme was delivered by nutritionists or

behaviourists in either group or individual settings, and additionally by phone contact at varying intervals over the study period –initially more intense with follow-up tapering off as the study progressed. Lipid measurements were made at baseline, 1 year, 3 years, 5 years and final visit, with two different fasting samples collected 1 month apart at baseline and 3 years to reduce variance.

Mean age (SD) at baseline was 9.5 (0.72) yrs and 17.0 (0.91) yrs at final visit, and did not differ between groups. Intakes of total and saturated fat were significantly lower at years 1, 3, 5 and final visit in the intervention group, with dietary cholesterol also significantly lower except at the visit.

An interesting finding in this trial was that serum LDL cholesterol fell significantly in the usual care as well as intervention group, at least up to 5 years of follow-up, and then climbed in both at final visit. LDL cholesterol levels were however significantly lower in the intervention group at 1 and 3 years, but not 5 years and at final visit. In a sub-analysis of those who attended all scheduled clinic visits (n=461) these differences at 1 and 3 years were larger, and LDL cholesterol levels were significantly different at 5 years but remained similar at final visit.

Regarding concerns of fat restriction influencing growth and development, again other measures of height, weight, sexual maturation by Tanner score, age of menarche in girls, serum ferritin, and triglycerides were not different at any points between groups.

The lack of statistical significance at the later time points in this study, which occurred in parallel with decreasing interventional contact, are highly suggestive that a lack of dietary adherence and compliance to dietary aims may have contributed to the lack of significant difference between groups at these points.

These results suggest that some dietary changes occurred in the usual care group, no doubt as a result of the knowledge that the children had marginally raised cholesterol levels, and would have received a degree of intervention to combat this in their usual clinical care.

Childhood studies - prospective studies:

The Avon Longitudinal Study of Parents and Children (ALSPAC) (Cowin et al., 2001; Rogers et al., 2001) enrolled approximately 15,000 mothers; a proportion of these enrolled in the last six months of study initiation were included in a sub-study called Children In Focus, which forms the basis of the dietary data reported here. Amongst these, diet records were assessed at 18, 31 and 43 months. Non-fasting venous blood samples were obtained at 31 and 43 months and analysed for total and HDL cholesterol. At the 18-month dietary assessment, complete records were obtained for 517 boys and 434 girls, and at 43 months 488 boys and 375 girls.

No association was found between dietary fat intake at 43 months and blood lipid measures at the same time. However a relationship was found with dietary intake reported at 18 months and blood lipid measures made at 31 months, with total cholesterol levels at 31 months significantly associated with quartile of fat intake at 18 months. There was an interaction of gender of marginal significance (p=0.052) where

the effect of dietary fat on total cholesterol seemed to be greater for boys. A trend towards a positive association between intake of dietary fat at 18 months and non-HDL cholesterol at 31 months was also observed, of borderline significance at p=0.054 (Rogers et al., 2001). Taking dietary intakes as continuous variables rather than quartiles, Pearson correlation coefficients were calculated for dietary intake variables and lipid measures (Cowin et al., 2001). Saturated fat intake at 18 months was significantly correlated with total cholesterol level at 31 months in boys (Pearson correlation coefficient (p-value) = 0.211 (0.002)) but not girls (coefficient (p-value) = 0.015 (0.840)). The same relationship was found for calculated LDL cholesterol and saturated fat intake in boys (correlation coefficient (p-value) = 0.174 (0.042)) but again not in girls (correlation coefficient (p-value) = -0.009 (0.927)). These gender differences are in agreement with those found in the STRIP study detailed above.

As dietary fats play an important role in early development there were concerns that fat restriction could have an adverse effect on growth. However in the ALSPAC study, no association was found between dietary fat intake and the growth measures of height and BMI.

These three studies offer interesting insights into the effect of reduction of saturated fat intake in children. Of particular interest is the striking sexual divergence of the effect of dietary modification in the STRIP and ALSPAC projects. Despite significant reductions in saturated fat intake across all time points measured in the STRIP study, girls in the intervention group showed no reduction in LDL cholesterol measured at 5 and 7 years, or total or non-HDL cholesterol measured prior to 5 years of age. Boys exposed to the dietary intervention on the other hand showed significant and persistent reductions in non-HDL cholesterol as measured before 5 years of age, and LDL cholesterol at ages 5 and 7 yrs. The reasons for this difference between boys and girls are not known, although the authors speculate on possible hormonal influences or the effect of differences in physical activity levels reported between boys and girls.

There are a number of potential confounding factors in the link between reduction in dietary saturated fat and blood lipids in the studies mentioned above. As shown in the DISC study, significant changes can occur in the population without intervention, as occurred in the control group in this study. One possible reason for this is that subjects were mildly hypercholesterolaemic and thus were likely to have received intervention to reduce cholesterol levels in the course of their usual care. Dietary saturated fat intake did fall in the usual care group, but not nearly as much as the intervention group. A comparison of means and 95% confidence intervals of lipid measures suggests that dietary saturated fat intakes at 5-year follow-up and the final visit, while being significantly different, were not sufficiently different to result in statistically significant differences in LDL between groups, as it can clearly be seen that mean LDL cholesterol levels tended to be higher in the usual care group than intervention at 5-years and final follow-up. This absence of statistical significance could be due to a lack of intensity in the intervention being offered during the later years of the trial, especially given the trial was terminated prematurely due to lack of funding. The results pertaining to 1-year and 3-year follow-up points in this study certainly suggest that a reduction in saturated fat intake in children from ages 8 - 10 yrs can produce a reduction in LDL cholesterol, however the 5-year and final visit results, and significant drop in LDL cholesterol in the usual care group mean that these differences in LDL cholesterol cannot be easily attributed to changes in saturated fat

intake alone. Unfortunately, results for this study are also presented as pooled results for girls and boys with statistical adjustment for sex, and thus cannot be compared with the results of the STRIP and ALSPAC projects with regard to gender differences.

Thus these three intervention trials in children confirm earlier observational studies and show the effect of reducing saturated fatty acids on total and LDL cholesterol. However they do show the difficulty in achieving long term compliance to the type of dietary advice necessary to bring about the required changes. The reason for the different responses between boys and girls remains elusive.

Metabolic/Intervention studies:

One of the more important studies published in the 1999-2005 time period was the KANWU study (Rivellese et al., 2003; Vessby et al., 2001). The study took its name from the five centres involved – Kuopio, Aarhus, Naples, Wollongong, Uppsala – and thus was a multinational multicentre study consisting of controlled dietary intervention for 90 days in healthy men and women. One hundred and sixty-two subjects were included provided they were otherwise healthy with the exception of some being overweight. The study design consisted of randomisation to either a saturated fat or monounsaturated fat diet (plus a further division within each of these into placebo or n-3 fatty acid supplementation) which was followed for 3 months. The diets were well controlled, and analysis of nutrient intakes at the end of the dietary period showed that total energy, total fat, polyunsaturated fat, protein and carbohydrate intake did not differ across groups. Thus the only difference in source of dietary energy arose from substitution of saturated or monounsaturated fat. Saturated fat intake was 17.6 ± 2.5 % total energy and 9.6 ± 1.8 % total energy in the saturated and monounsaturated diets respectively, with monounsaturated fat intakes being 13.1 ± 2.5 % total energy and 21.2 ± 4.2 % total energy respectively. Given the difference in saturated fat intakes, dietary cholesterol also differed at 322 ± 91 mg/day vs. 254 ± 80 mg/day in saturated fat vs. monounsaturated fat diets respectively. Thus, the diets represented an approximately 8% variation in total energy from either saturated or monounsaturated fat.

Mean difference (95% CI, p-value) in total serum cholesterol was 0.28 (0.12 – 0.45, p=0.0007) mmol/l and for LDL cholesterol was 0.34 (0.19 – 0.49, p=0.0001) with the saturated fat diet being the higher for both of these measures. No effect was seen on triglyceride or HDL levels, with respective differences (95% CI, p-value) for saturated fat diet minus monounsaturated fat diet values of 0.01 (-0.15 to 0.17, p=0.4879) mmol/l and -0.01 (-0.08 to 0.07, p=0.6708) mmol/l.

The Dietary Approaches to Stop Hypertension (DASH) trial had as one of its secondary outcome measures the effects of the DASH diet on lipid levels (Obarzanek et al., 2001b). The study was a randomised intervention conducted in four sites, comparing a control diet with the DASH diet and a diet high in fruit and vegetables. The DASH diet comprised a higher proportion of total energy from protein than control, 10% less energy from total fat, and more energy from carbohydrate. Saturated fat comprised 14% of total energy in the control diet vs. 7% in the DASH diet. Of particular interest is that the diet high in fruit and vegetables had an almost identical macronutrient composition and contribution of energy from MUFA, PUFA and saturated fat as the control diet.

A strength of the study is the large sample size of 459 participants. Dietary intervention lasted for 8 weeks and consisted of participants consuming one prepared meal onsite per day, at which time they were provided with additional meals and snacks to cover them until their next visit. Energy intakes were adjusted as needed so that body weight was kept constant, and after randomisation baseline characteristics and lipid profiles of dietary groups were not significantly different.

The DASH diet resulted in significantly lower total cholesterol relative to the control diet, with a mean difference (95% CI) of 0.35 (0.22, 0.49) mmol/l. Likewise LDL

cholesterol was 0.28 (0.16, 0.40) mmol/l lower in the DASH diet group. HDL was also significantly lower at 0.09 (0.06, 0.13) mmol/l mean difference. These equate to reductions of 7.3% in total cholesterol, 9.0% in LDL cholesterol and 7.5% in HDL cholesterol. Triglyceride, total:HDL cholesterol ratio and LDL:HDL ratio showed no significant differences between DASH and control diet groups. Separate analyses by race, baseline cholesterol concentration and sex revealed the same trends, with the interaction of greater reductions in total and LDL cholesterol seen in men than women. No significant differential reduction was seen based on differing baseline levels of total cholesterol, LDL cholesterol or triglyceride. However, greater reduction in HDL was observed for those with higher baseline HDL levels, a finding of merit given that for those with already low HDL levels at baseline – placing them at a higher cardiovascular risk – any further reduction would only amplify the effect of their already low HDL levels on cardiovascular risk.

Of interest are the findings relating to the fruit and vegetable diet, in which no significant differences were seen in any lipid measures compared with the control diet. Given their identical compositions in terms of macronutrients and types of dietary fat this is perhaps not surprising, and contributes to the evidence which suggests that the higher fibre and lower dietary cholesterol intake do not have an appreciable effect on lipid measures.

With regard to smaller, shorter studies published between 2000 and 2005, Judd et al., (2002) conducted a randomised dietary crossover trial in 50 men – arguably still a reasonable sample size for a study of this design. The results of the study are more pertinent to the role of dietary *trans* fatty acids, hence the study is described in detail below under '*Trans* fatty acids and LDL cholesterol: Metabolic/Intervention studies'. One of the six diets under investigation provided 18% of total energy from lauric, myristic and palmitic acids combined, with another 2.7% from stearic acid. This diet produced one of the highest total serum cholesterol levels of the diets under investigation, significantly more than diets with high amounts of carbohydrate, oleic acid, or stearic acid. Similarly, LDL cholesterol was significantly higher on this diet than seen on diets high in carbohydrate or oleic acid.

Poppitt et al. (2002) conducted a smaller trial in 20 healthy young men. The main aim of the study was to compare the effects of two diets differing only in the type of butter, one a standard butter, the other produced by modifying bovine diets to produce a butter lower in saturated fat and higher in poly- and monounsaturated fats. The two diets did not differ in macronutrient composition, however the modified butter diet had significantly lower saturated fat (15% total energy compared to 20% total energy). The modified butter fat diet had lower amounts of C10:0, C14:0 and C16:0 than the control but C12:0 and C18:0 content was greater. The modified butter diet also provided significantly less dietary cholesterol. Given the same composition of the diets in terms of macronutrients, the modified butter diet thus represented a reduction in saturated fat with increases in PUFA and MUFA. The study was a randomised, cross-over trial in which not only was all food and drink provided, but subjects also lived in the metabolic unit of the study site for the diet periods hence guaranteeing a very high level of dietary compliance. Dietary intervention periods were 3 weeks, separated by a minimum washout period of 4 weeks.

At randomisation there were no significant differences in baseline variables for either of the dietary groups, and importantly there was no change in body weight in either group across dietary periods which would otherwise influence lipid profiles.

In terms of lipid profiles, the only significant treatment effects between normal and modified butter diets were that the modified butter diet resulted in lower total cholesterol and LDL cholesterol levels than the standard butter diet. Blood samples were collected throughout the treatment period as well as at baseline and the end of dietary periods. Significant differences in total and LDL cholesterol between diets achieved statistical significance only at the end of the 3 week dietary intervention period.

The amounts of saturated fat used in this study (15% and 20% total energy) are relatively high compared to current usual recommendations. However the replacement of 5% of total energy from saturated fat for polyunsaturated and monounsaturated fat represents a realistic change for free-living individuals, especially in the case of having a particularly high intake of saturated fat.

In a number of studies the effect of replacing dietary saturated fat has not surprisingly varied depending on which dietary components replace energy lost by reduction in saturated fat. In two trials, Hodson et al. (2001) examined the effect of replacing dietary saturated fat with either n-6 polyunsaturated fatty acids or monounsaturated fat. The two separate trials were both very similar in design: a randomised cross-over dietary intervention, with subjects switching between two diets consumed for 2 and a half weeks each without an intervening washout period.

In trial I (n = 29) a diet high in saturated fat was compared with an n-6 polyunsaturated fatty acid diet. Trial II (n = 42) compared the same saturated fat diet with a diet high in monounsaturated fat. All diets had total fat intakes of approximately 30% of total energy. Subjects were Human Nutrition students, and were asked to self-select a background diet which would be modified to achieve the dietary goals for each phase of the trials. Subjects were provided with a recipe book for ideas and provided with specific spreads and oils depending on diet allocation. For the high saturated fat diet, subjects were asked to use butter as a spread and in baking and cooking and to consume high fat dairy foods. In the n-6 polyunsaturated fat diet participants were provided with safflower and sunflower oils to use as a spread and in baking and cooking, and similarly subjects on the monounsaturated fat diet were given canola oil and spreads. Dietary intake was assessed by 3 day weighed diet record.

Participants in trial I (saturated vs. n-6 polyunsaturated fats) were not significantly different in age, weight and BMI from those in trial II (saturated vs. monounsaturated fats). Mean saturated fat intake (SD) assessed by diet records in saturated fat diets were 17.5 (4.2) % in trial I and 17.7 (4.2) % in trial II, compared with 8.5 (2.8) % on the polyunsaturated fat diet in trial I and 8.4 (2.8) % on the monounsaturated fat diet in trial II. Proportion of energy from total fat was comparable between the diets, although total energy intake was reduced on both the polyunsaturated and monounsaturated diets relative to the saturated fat diet, with 1.6MJ (358 kcal) less on the polyunsaturated and 1.7MJ (406 kcal) less on the monounsaturated fat diets,

apparently largely due to changes in alcohol intake. Mean body weight however did not change on the mono- or polyunsaturated diets relative to the saturated fat diets.

Total cholesterol levels were significantly reduced on the poly- and monounsaturated fat diets compared with the saturated fat diet, by 19% and 12% respectively ($p<0.001$ for both comparisons). LDL cholesterol was also significantly lower, with a reduction of 22% ($p<0.001$) on the polyunsaturated fat diet, and 15% ($p<0.001$) on the monounsaturated fat diet.

Metabolic/Intervention studies – individual saturated fatty acids

A number of studies conducted prior to 2000 suggested that individual fatty acids exert differing effects on lipid profiles. In the time period under review in this report, only a handful of studies which specifically examined individual fatty acids were identified. Unfortunately the majority of these were underpowered, or there were methodological issues such as weight loss, and thus it was not possible to draw definitive conclusions from their results.

In a study conducted in Colombia, Bautista et al. (2001) examined the effects of palm oil, high in palmitic acid, in the context of high and moderate cholesterol diets. Twenty-eight healthy male university students were randomised to a 4 x 4 double-blind dietary cross-over trial. The four diets were: high palm oil-high cholesterol; high palm oil-low cholesterol; moderate palm oil-moderate cholesterol; and low palm oil-moderate cholesterol. All diets contained 57% of energy from carbohydrates, 12% from protein and 31% from fats. Being university students, diets were prepared in the cafeteria of a university residence and subjects were served breakfast, lunch and dinner on-site, with staff keeping records of dietary compliance. In all other respects subjects were free-living individuals and were also allowed to consume food outside of the study, intake of which was recorded and nutrient intake estimated from Colombian food comparison tables. Diets were consumed for 4 week periods. All subjects completed the study, and 90% of all diets provided were consumed by participants; compliance did not differ between the four diets or with diet order. Foods consumed outside of the study were mostly bottled drinks, sweets and bread. Across the study period an average but non-significant reduction in body weight of 1.1 kg was observed ($p=0.09$).

Considering the high vs. low dietary cholesterol comparison, high cholesterol diets led to a significant increase in total and LDL cholesterol, but no change in HDL cholesterol. Similarly high palm oil vs. low palm oil produced an increase in total and LDL cholesterol but no effect on HDL cholesterol. The effects and level of significance of dietary palm oil was greater than dietary cholesterol, with the high vs. low cholesterol diets producing a 0.21 mmol/l increase in total cholesterol ($p=0.009$) and 0.16 mmol/l increase in LDL cholesterol ($p=0.047$), whereas the high vs. low palm oil diets produced an increase in total cholesterol of 0.39 mmol/l ($p<0.001$) and LDL cholesterol of 0.38 mmol/l ($p<0.001$).

One aspect considered in depth by the authors was the variability in responses between individuals. Large variation in individual response to a high cholesterol diet has been reported in a number of other studies, and was also seen here. Lipid response

to the high palm oil diet varied amongst participants but was less striking than the variability in responses to dietary cholesterol. Furthermore, responses to dietary cholesterol correlated with responses to dietary palm oil, with Pearson's correlation coefficients of 0.46 ($p=0.02$) for total cholesterol and 0.41 ($p=0.03$) for LDL cholesterol. Extent of total cholesterol response to dietary palm oil was also correlated with baseline total cholesterol, a relationship not observed when considering total cholesterol response to dietary cholesterol.

Other data published in recent years regarding the influence of individual saturated fatty acids include those of Tholstrup et al. (2003), who observed an increase in HDL cholesterol within 24 hours of a myristic acid enriched diet, but not after a stearic acid enriched diet. Kelly et al. (2002) observed a significant decrease in LDL cholesterol after a diet enriched with stearic acid, but not a diet enriched with palmitic acid. These studies were both cross-over design in healthy young males, although were also both small with 9 and 10 subjects.

Predictive modelling equations:

Mensink et al. (2003) conducted a meta-analysis of 60 controlled trials. Articles published between January 1970 and December 1998 were included, and had to meet the following criteria: food intake clearly described with dietary fatty acids as the sole variable; dietary cholesterol constant between comparison diets; parallel, cross-over or Latin-square design; diet adherence for at least 13 days to allow time for changes in lipid measures to take place; adults with no disturbances of lipid metabolism or diabetes included. A total of 60 studies were selected for the meta-analysis.

The estimated regression coefficient (95% CI) for the effect of replacement of 1% of total energy from carbohydrates with 1% of energy from SFA's on LDL cholesterol was 0.032 (0.025, 0.039) mmol/l. Corresponding regression coefficients for total cholesterol and total:HDL cholesterol ratio were 0.036 (0.029, 0.043) mmol/l, and 0.003 (-0.008, 0.013) respectively. Thus, replacement of carbohydrates with SFA's was predicted to significantly increase LDL cholesterol and total cholesterol, although produce no significant change in total:HDL cholesterol ratio.

Intakes of individual saturated fatty acids were reported for 35 studies, with mean intakes being: 1.1% of total energy for lauric acid, 1.3% for myristic, 6.2% for palmitic, and 3.0% for stearic.

Lauric acid appeared to be the most potent individual saturated fatty acid when considering the increase in LDL and total cholesterol, although this was also true for HDL cholesterol, such that replacement of carbohydrates with lauric acid actually decreased total:HDL cholesterol ratio. No effect of myristic, palmitic or stearic acids was predicted on total:HDL cholesterol ratio when these fatty acids replaced carbohydrate constituting 1% of total energy.

Regarding the effect of substitution of 1% of total energy from carbohydrates with individual saturated fatty acids on total cholesterol and LDL cholesterol, there was a trend toward greater increases with decreasing chain length. Regression coefficients are shown in the table below.

Regression coefficients (95% CI) for substitution of 1% of total energy from carbohydrates with individual saturated fatty acids from Mensink et al. (2003)

Saturated fatty acid	LDL cholesterol	Total cholesterol
Lauric (C12:0)	0.052 (0.026, 0.078)	0.069 (0.040, 0.097)
Myristic (C14:0)	0.048 (0.027, 0.069)	0.059 (0.036, 0.082)
Palmitic (C16:0)	0.039 (0.027, 0.051)	0.041 (0.028, 0.054)
Stearic (C18:0)	-0.004 (-0.019, 0.011)	-0.010 (-0.026, 0.006)

Adapted from Am J Clin Nutr, 2003, **77**(5):1146-55.

It should be noted that stearic acid was not associated with a change in total or LDL cholesterol when replacing carbohydrate. Also, as mentioned above, while lauric acid resulted in the greatest increase in LDL cholesterol, it had a greater relative effect on HDL cholesterol so that it was predicted to decrease total:HDL cholesterol ratio relative to carbohydrates. None of the other individual saturated fatty acids influenced total:HDL cholesterol ratio.

Thus in summary the results of this meta-analysis suggest that myristic acid has the greatest detrimental effect on the overall lipid profile, followed by palmitic acid. Stearic acid was not predicted to significantly alter lipid levels, and while lauric acid showed the greatest ability to raise LDL cholesterol, total:HDL cholesterol ratio is lower.

Müller et al. (2001) also published predictive equations relating to the effect of individual dietary saturated fatty acids on LDL cholesterol. The key focus of the paper was on *trans* fatty acids, but analyses relating to the effects of lauric, myristic and palmitic acids were included.

Predictive models were derived from the results of four studies carried out from 1993 – 1998 by the authors at the University College of Akershus in Norway. These studies involved Latin-square or crossover designs with participants receiving the experimental different diets in random order. Blood samples were taken after 3 weeks of dietary intervention in three studies and after 2 weeks in the fourth. One study involved only male participants and the other three only female participants, with the number of participants ranging from 16 to 31, mean ages 22 to 31 years, mean BMI 23 to 26 kg/m^2, and mean baseline serum cholesterol 4.44 to 5.35 mmol/l. Thus the studies were relatively small and conducted in healthy young normal weight participants. In the final regression analysis, the results for four overweight participants in one of the trials were excluded due to significant weight loss of 1.7 – 7 kg during the study.

Predictive equations were generated which included lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids, *trans* fatty acids, and also included terms for oleic, linoleic and alpha-linolenic polyunsaturated fatty acids (using coefficients derived by Yu et al. (1995)). Stearic acid (C18:0) was not included in the analysis as the authors considered that there was sufficient previously published evidence that stearic acid had no effect on total or LDL cholesterol levels.

Regression coefficients (SD) for the effect of each 1% change of total energy from individual saturated fatty acids on LDL cholesterol were: 0.01 (0.17) for lauric acid; 0.071 (0.011) for myristic acid; and 0.047 (0.032) for palmitic acid. Similar results were obtained for total cholesterol.

The developed models were tested against other datasets by applying them to the results of seven dietary studies which included the fatty acid composition of the experimental diets (including *trans* fatty acid intakes) and were deemed to be well-designed. The Pearson correlation coefficient between predicted and observed total cholesterol in these studies was $r = 0.981$. A correlation coefficient specifically for LDL cholesterol in these studies is not given.

It should be noted that there was a large uncertainty for the coefficient for lauric (C12:0) acid in this study, and the results for this fatty acid should thus be interpreted with caution. The models developed seem to behave as robust predictors of change in lipid profile when compared with results from seven other dietary interventions.

The authors concluded that C12:0 did not raise serum total cholesterol as much as myristic (C14:0) or palmitic (C16:0) saturated fatty acids. When considering

isoenergetic comparison of the individual saturated fatty acids, myristic acid was predicted to cause the greatest increase in LDL cholesterol followed by palmitic acid.

These results are generally consistent with those of Mensink et al. (2003) in as much as myristic and palmitic are clearly the most important fatty acids in determining elevation of total and LDL cholesterol. The intake of lauric acid in most populations is lower and the effect on LDL appears to be less consistent. However it is of interest that this fatty acid increases HDL to a greater extent than the other saturated fatty acids and is this associated with a relatively favourable ratio of total:HDL cholesterol.

SFA's and coronary heart disease:

Cross sectional epidemiological studies:

The EURODIAB-IDDM study (Toeller et al., 1999), outlined above under 'Saturated fatty acids and LDL cholesterol: cross sectional epidemiological studies', also gathered data on diagnosed cardiovascular disease. Out of a sample of 2868 individuals with type 1 diabetes, 286 cases of CVD were identified. As with the link between dietary saturated fat and LDL cholesterol, odds ratios for cardiovascular disease adjusted for total energy intake were significantly higher with increasing intakes of total fat, saturated fat and cholesterol. However in multivariate analysis taking into account other dietary and clinical variables these associations were no longer statistically significant.

A cross-sectional study of saturated fat intake and risk of nonfatal MI was conducted in Costa Rica by Kabagambe et al. (2003). Four hundred and eighty-five male and female survivors of first MI were enlisted as cases and 508 controls matched by age, sex and area of residence were identified. Dietary information was collected with the use of a previously developed and validated food frequency questionnaire, administered during home visits by trained personnel.

Cases reported significantly higher intakes of total fat, animal fat, saturated fat, individual saturated fatty acids, cholesterol and energy than controls. Mean saturated fat intake (SD) as percentage of total energy was 11.7 (2.8) % for controls and 12.4 (2.8) % for cases. Multivariate models for risk of MI were generated using the highest quintile of intake vs. the lowest quintile with adjustment for established CVD risk factors and dietary confounders. Attempts were also made to identify the effects of individual saturated fatty acids, pooling lauric and myristic acid due to their low intake levels.

Relative risk (95% CI) of MI for the highest quintile of total saturated fat intake vs. lowest was 3.00 (1.54 – 5.84). Odds ratios (95% CI) for MI for a 1% change in total energy from lauric plus myristic acid were 1.08 (0.65 – 1.81), 1.16 (1.01 – 1.32) for palmitic acid, and 1.98 (1.08 – 3.65) for stearic acid. The corresponding OR for a 1% increase in energy from total saturated fat (reflecting mainly palmitic, this being the most abundant individual fatty acid) was 1.12 (1.03 – 1.21).

This study was of sufficient size to show potential links between dietary saturated fat and nonfatal MI, and additionally was well-conducted. Increasing saturated fat intake was clearly shown to raise risk of MI, and this also held true for individual saturated fatty acids. It is interesting to note, as previously mentioned in Part 1 of this report, that although the above metabolic studies generally do not support a link between stearic acid and LDL cholesterol, it emerged as conferring the greatest risk per percentage of total energy of all the individual saturated fatty acids (with the effects of lauric or myristic acid analysed as a combined group). This suggests that stearic acid may exert effects on cardiovascular risk other than via effects on lipid profile.

Prospective studies:

Oh et al. (2005) reported on the effects of dietary fat intake in the Nurses' Health Study. A previous paper from the same study (Hu et al., 1997), described above under the heading 'Part 1b: Reanalysis of pivotal studies', reported on dietary fat intake and risk of CHD over 14 years of follow-up. This paper extended the time period to 20 years of follow-up. Dietary intake was assessed via posted food frequency questionnaires, the first being a 61-item questionnaire sent out in 1980, which was extended to 116 items in 1984, followed by similar questionnaires in 1986, 1990, 1994 and 1998. The endpoint was nonfatal myocardial infarction or fatal CHD between 1980 and June 1, 2000. After exclusion of those with incomplete FFQ's or previous diagnosis of CHD, cancer, diabetes or hypercholesterolaemia before 1980, 78,778 women were included in the analysis. Over the 20-year follow-up period 1,241 cases of nonfatal MI and 525 CHD deaths were recorded (total 1,766 incident cases).

From 1980 to 1998 fat intake fell from 39% of total energy to 29%. Saturated fat intake ranged from 10.1% of total energy in the lowest quintile to 17.6% of total energy in the highest quintile of intake.

A comprehensive multivariate relative risk calculation was made between saturated fatty acid intake quintiles, calculated based on a cumulative average intake from all available food frequency questionnaires, and incident CHD including numerous possible confounding factors.

Multivariate relative risk of incident coronary heart disease was 0.97 (0.73-1.27) for the highest vs. the lowest quintile of saturated fat intake.

This is in contrast with a previous 14-year follow of the Nurses' Health Study (Hu et al., 1997) which did indeed show an association. In this previous analysis each increase of energy intake of 5% from saturated fat, replacing carbohydrate, was associated with a relative risk (95% CI) of coronary heart disease of 1.17 (0.97 – 1.41, p-value = 0.10). The authors suggest that the lack of association in this latest analysis (Oh et al., 2005) could be due to declining intakes of saturated fat and incidence of coronary heart disease weakening the associations found previously.

Boniface et al. (2002) reported on 16-year follow-up of the 1984-85 Health and Lifestyle Survey in Great Britain. Initial response rate to a face-to-face interview, physical measurements, and self-reported food frequency questionnaire was 73.5%. Ninety-seven percent of these were flagged for on-going monitoring of death certificate information. This study took into account coronary heart disease deaths amongst those aged 40-75yrs at the initial interview up until December 2000. Cox survival analyses for survival without CHD death relative to fat intake were made for men and women separately as well as combined, after adjustment for confounding factors of age, alcohol intake, smoking, body shape (BMI or waist-hip ratio), exercise, blood pressure, social class and deprivation index.

An association was found between total dietary fat and saturated fat and CHD death amongst women but not for men. Multivariate relative risk (95% CI) of CHD death for an increase of 100 g/week of saturated fat was 1.40 (1.09 – 1.79) for women. The

corresponding risk estimate for men was 1.00 (0.86 – 1.18). An analysis according to age suggested that this relationship was stronger in older women.

A similar 16-year follow up study was reported by Jakobsen et al. (2004). 3,686 Danish men and women were identified through their participation in four other cohort studies: the 1914 and 1936 cohorts, and the MONICA-I and MONICA-III cohorts. Median length of follow up was 16 years (range 7-22 years). Participants filled in 7 day weighed food records (or a small proportion interviewed by dietitian) and hospitalisation and death records obtained from the National Patient Registry and Cause of Death Registry.

Cox's proportional hazard regression models were developed and a comprehensive fully-adjusted model included confounding factors of fat intake, total energy intake, protein, other types of dietary fatty acids, family history of MI, smoking, physical activity, education, alcohol intake, dietary fibre, and dietary cholesterol, as well as further adjustments for blood pressure and BMI.

Three hundred and twenty-six cases of fatal or nonfatal CHD were identified (98 women and 228 men). Hazard ratio for development of CHD in women with an increase of 5% energy from saturated fat (replacing carbohydrate) was of borderline significance at 1.36 (0.98, 1.88). A further examination of the data according to age group revealed an appreciable and significant increase in risk in the younger women (hazard ratio (95% CI) for a 5% increase in saturated fat intake = 2.68 (1.40, 5.12)) but not in the age group over 60 years (HR (95% CI) = 1.22 (0.86, 1.71)). As in the Boniface et al. study, no significant association between saturated or total fat intake and risk of coronary heart disease was seen in men.

Despite their similar designs and length of follow-up it is interesting to note that Boniface et al. reported a stronger association in women 60 yrs of age and older, whereas Jakobsen et al. found a stronger effect amongst women under 60 yrs, with no significant effects seen in women over 60 yrs. There is no obvious explanation for this discrepancy nor why the relationship should be present in women but not in men.

A follow-up of the Seven Countries study was published in 2000 which considered the effect of dietary and lifestyle variables on all-cause mortality after 25-years of follow-up (Kromhout et al., 2000). It is interesting to note that despite all the limitations of this study the model that best predicted observed all cause mortality rates was one which included saturated fat intake, vitamin C and smoking. Based on this analysis, a 5% reduction in saturated fat intake at a population level would lead to a 4.7% reduction in age-adjusted all cause mortality.

Thus although there is support in the more recently published data for the association between saturated fatty acids and cardiovascular disease, there is heterogeneity in the results of the studies, particularly with regard to the different effect in men and women and in different age groups. There is no clear explanation for this heterogeneity.

***Trans* fatty acids:**

Summary:

There has been much debate regarding the link between dietary TFA and adverse health outcomes since the time of the Health Canada report. Numerous editorials, comments and reviews were retrieved while searching the evidence published since 1999; a selection are summarised in table 2.2 at the end of this document. In some countries government action and legislation regarding food labelling and *trans* fatty acid content of industrially produced foods has taken place on the basis of reports from agencies such as the Danish Nutrition Council, European Food Standards Agency, and the US Food and Drug Administration.

Numerous dietary intervention and metabolic studies published between 2000 and 2005 on the effects of dietary *trans* fatty acids support their role in increasing LDL cholesterol. While there is still some uncertainty as to whether this increase is more, less or equal to that of saturated fat, the association between intakes of *trans* fatty acids and LDL cholesterol is unquestionably "convincing". In addition, while some studies covered in the time period under review showed no significant effect of dietary *trans* fatty acids on HDL cholesterol levels, the majority document a decrease in HDL cholesterol when *trans* fatty acids replace dietary carbohydrate, and meta-analyses confirm a more adverse effect on overall lipoprotein profile (LDL:HDL ratio) than that conferred by saturated fatty acids. For the purposes of substantiation of a health claim the much debated issue regarding the relative adverse effects of *trans* and saturated fatty acids on LDL cholesterol is of little relevance.

Two cross-sectional studies examining dietary *trans* fatty acids and LDL cholesterol have been published during the period 2000 – 2005; one carried out in Costa Rica, the other a multinational European collaboration, the TRANSFAIR study. The Costa Rican study found no evidence for an association between *trans* fatty acid intake and LDL cholesterol. The TRANSFAIR study however reported an inverse relationship between total *trans* fatty acid intake and LDL cholesterol levels, with differing effects for different *trans* isomers: t18:1 and t18:2 were inversely associated with LDL cholesterol levels, while t14:1 (n-9) and t22:1 showed a positive association. It is noteworthy that the mean intakes of *trans* fatty acids reported in both of these studies were very low, with *trans* fatty acids contributing less than 1% of total energy. It is conceivable that there may be a threshold effect whereby low levels of *trans* fatty acid intake do not effect lipid levels as do higher intakes. However regardless of the explanation of these findings, such cross-sectional studies do not negate the findings of the more powerful intervention studies.

Recent evidence from the major prospective cohorts the Nurses' Health Study and Zutphen Cohort study show a clear link between dietary *trans* fatty acid intake and incidence of fatal and nonfatal cardiovascular disease. The report from the Zutphen Cohort Study also examined the pooled effect of dietary *trans* fatty acids from these two studies and the previously published Health Professional's Study and Alpha-Tocopherol Beta-Carotene Cancer Prevention Study. There is also a clear positive association between dietary *trans* fatty acids and cardiovascular disease across these four large cohort studies, with evidence for a dose response relationship.

Two case control studies considered in the report from Health Canada found no association between adipose *trans* fatty acids and incidence of myocardial infarction or sudden cardiac death (Roberts et al., 1995; Aro et al., 1995). In contrast three similar studies reported since 2000 have reported a clear association of increased risk of nonfatal or fatal myocardial infarction with increasing dietary intakes or adipose tissue content of *trans* fatty acids. No explanation for this disparity is offered by the authors of three recent studies, nor indeed could we find a definitive one. It should be noted that in the EURAMIC study (Aro et al., 1995) a trend towards a positive association between adipose *trans* fatty acids and acute myocardial infarction was present after exclusion of two outlying centres with low adipose *trans* fatty acid levels. The study by Roberts et al. is the only one considered here, and in the Health Canada report, to examine the link between adipose tissue *trans* fatty acids and sudden cardiac death. Thus samples from cases were taken during post-mortem examinations (median time of 24 hours after death) rather than from survivors of first acute MI. Furthermore samples were collected from the anterior abdominal wall and not from abdomen or buttock tissue aspirates as in other similar studies. Whether these differences in methodology could account for the differences in results is unknown.

Despite the strength of the evidence from the cohort studies the heterogeneity in the data suggest that at the present time it may be more appropriate to describe the association as “probable” rather than “convincing”.

Trans fatty acids and LDL-cholesterol:

Cross sectional epidemiologic studies

Kim et al. (2003) examined *trans* fatty intake and LDL particle size in a cross-sectional study of adults in Costa Rica. Participation rates were high at 85% for men and 93% for women, resulting in a final sample size of 202 men and 212 women. Dietary information was assessed using a semi-quantitative food frequency questionnaire. *Trans* fatty acid intake was higher in urban areas than rural, and also in women than men, although intakes in this population were particularly low in general, with intakes barely reaching 1% of total energy. Although specifically examining the effect of *trans* fatty acid intake on LDL particle size, the authors did not find a significant correlation between *trans* fatty acid intake measured by food frequency questionnaire and plasma LDL or HDL cholesterol levels[*]. Thus, it is conceivable that low levels of *trans* fatty acid intake may not appreciably influence LDL cholesterol levels.

Another important cross-sectional epidemiologic study was the multi-national European TRANSFAIR study which included an investigation of the relationship between *trans* fatty acids and cardiovascular risk factors (van de Vijver et al., 2000). Subjects were 327 men and 299 women aged 50 – 65 yrs in Finland, Iceland, Netherlands, France, Sweden, Spain, Greece, and Portugal. Response rates were variable between countries (20 - 66% for men and 25 - 72% for women). Those who had recently altered their diet or were on certain medications were excluded. Blood lipids were determined from a fasting venous sample. Total TFA amounted to 2.40 g/d or 0.87% total energy in men and 1.98 g/d or 0.95% total energy in women. Lowest intake of TFA was reported in Portugal at 0.57% total energy and highest in Iceland at 1.65% total energy.

In half of the men and women participating, adipose tissue samples were collected from the buttock for the purposes of validating food frequency questionnaire data. Significant correlations between dietary intake and adipose tissue measurement were 0.67 for total *trans* fatty acid intake, 0.36 for t16:1 intake, and 0.63 for t18:1 intake ($p < 0.01$ for each).

Linear regression models were developed separately for men and women, using TFA intake as a continuous variable and serum lipid levels as the outcome measure. Mean total cholesterol levels were 5.7 ± 1.0 mmol/l for men and 6.2 ± 1.1 mmol/l for women. Mean daily intake of total *trans* fatty acids was low in this study, with women having a slightly higher intake than men at 0.95 ± 0.55 % total energy in women vs. 0.87 ± 0.48 % of total energy in men. An analysis of lipid levels across quintiles of *trans* fatty acid intake revealed no association between total *trans* fatty acid intake with LDL or HDL cholesterol, or LDL:HDL ratio. After adjustment for other fatty acids, total cholesterol was found to have an inverse relationship with total *trans* fatty acid intake.

[*] The authors also point out that in a previous study by Lichtenstein et al. (1999) (covered in the Health Canada review) no association was found between *trans* fatty acid intake and LDL or HDL cholesterol levels when comparing groups with a low intake of *trans* fatty acids

(0.55% of total energy vs. 0.91% of total energy; see analysis of Lichtenstein et al., 1999 paper in Part 1 above).
Linear regression models for individual *trans* fatty acids were developed. Positive associations between fatty acid intake and LDL cholesterol were found for t14:1 (n-9) and t22:1. An inverse association was found for t18:1 and t18:2 after adjustment for intake of other fatty acids. Regarding effects on HDL cholesterol, the only isomer with a significant association was t16:1 (n-9) which showed an inverse relationship with HDL cholesterol levels. The combined effects of these isomers on LDL and HDL cholesterol levels were seen in regression models for LDL:HDL ratio, with the three above mentioned individual *trans* fatty acids, t14:1 (n-9), t16:1 (n-9) and t22:1, showing positive associations with LDL:HDL cholesterol ratio.

The TRANSFAIR study is the only published study reporting an inverse association of either total or LDL cholesterol with *trans* fatty acid intake. Positive associations with LDL cholesterol levels were reported for t14:1 (n-9) and t22:1, however these particular *trans* fatty acids contribute a very small proportion of dietary *trans* fatty acids. Potential limitations of the TRANSFAIR study include the relatively low response rate in some countries, with the lowest rates for any country being 20% for men and 25% for women, thus those surveyed may not be a representative sample of the population, especially relevant as while a being multinational study conducted across Europe, a sample size of 329 men and 299 women would not even be a particularly large study in any of the individual countries alone.

While the low response rate may to some extent account for the findings, the most plausible explanation for the absence of an association between *trans* fatty acids and total and LDL cholesterol may be the very low intakes of *trans* fatty acids in both the Costa Rican and the TRANSFAIR studies. There is no clear explanation as to why t14:1 (n-9) and t22:1 differed from other *trans* fatty acids.

Prospective studies

A Finnish prospective study of a low-saturated fat, low-cholesterol diet in children also provides some evidence on the effect of dietary *trans* fatty acids in children. Salo et al. (2000) published a paper examining the effect of dietary *trans* fatty acids in the STRIP project (described above on page 25 under 'Saturated fat and LDL cholesterol: Childhood randomised controlled trials'). Given that in the intervention group margarine intake was encouraged, it was assumed that *trans* fatty acid intake would higher amongst those participants than in the control group.

In the entire cohort at 3 years of age (402 intervention children, 411 control) intake of *trans* fatty acids were indeed significantly different between arms, although low in both groups, at 0.8% total energy in the intervention group vs. 0.6% total energy in the control group ($p<0.001$). Although HDL levels were on average lower in intervention children, so too were non-HDL cholesterol levels, no doubt due to the lower saturated fat intake in this group, and HDL:total cholesterol ratios were almost identical. Importantly, while differences were found between the two groups it was not possible to distinguish between the effects of saturated fatty acids and *trans* fatty acids. Thus, while no specific detrimental effects could be ascribed to *trans* fatty acids in this study the results should be interpreted with caution. One possible positive interpretation of these findings it that low intakes of *trans* fatty acids do not appear to be detrimental to lipid profiles of young children (nor for that matter on other cognitive or behavioural parameters measured in the study (cited in accompanying editorial by Deckelbaum and Williams, 2000)).

Metabolic/Intervention studies

Vermunt et al. (2001) examined the effects of dietary alpha-linolenic *trans* fatty acid on plasma lipids and lipoproteins in 91 healthy men, in a multi-centre dietary intervention carried out in Scotland, France, and the Netherlands. After a 6-week run-in period on a '*trans*-free diet' (less than 1g *trans* fatty acids per 100g total fatty acids), subjects were randomized to either a low *trans* or high *trans* fatty acid diet for the following 6 weeks. Subjects were advised to avoid other *trans* fatty acid containing foods during the study period and dietary intake was assessed by 4 day diet records at the end of both run-in and experimental periods (weeks 5 and 11). Subjects on the high *trans* diet consumed an average of 1.41 g/d of *trans* alpha-linolenic acid. Mean (SD) body weight increased in subjects on the high *trans* diet by 0.2 (1.1) kg, and fell in those on the low *trans* diet by 0.6 (2.4) kg, and these changes were significantly different between groups ($p = 0.03$). After correction for changes in body weight and intakes of saturated and monounsaturated fats, the high *trans* group showed a non-significant increase in plasma LDL, and a non-significant decrease in HDL. Although these changes failed to reach significance, at $p = 0.10$ and $p = 0.22$ respectively, LDL to HDL cholesterol ratio increased by a mean change of 8.1% (1.4 – 15.3%, $p = 0.02$) on the high *trans* diet vs. low *trans* diet. Likewise, the treatment effect of high vs. low *trans* diets on total cholesterol to HDL ratio was an increase of 5.1% (0.4 – 9.9%, $p = 0.03$). These differences were seen in each of the three study centres, and were evident after three weeks consumption of experimental diets, providing evidence that the specific *trans* fatty acid alpha-linolenic acid in itself has an adverse effect on blood lipids.

Judd et al. (2002) conducted a large well-controlled study examining the effect of *trans* fatty acid intake on plasma lipids and lipoproteins in men, concentrating on the relative effects of dietary replacement of saturated fats, carbohydrate, or polyunsaturated fatty acids with *trans* fatty acids. Fifty generally healthy men were enrolled and randomly assigned to six experimental diets in a Latin-square design. Average age was 42 yrs and mean BMI 26.2 kg/m^2. Diets were followed for 5 weeks each. All meals were provided, with breakfast and dinner meals being consumed on-site, and carry-out lunch meals and all weekend and snack foods provided. Six diets were used which consisted of a carbohydrate enriched diet (CHO), deriving 30.5% of total energy from fats, and five other diets varying in fat content. The other five diets aimed to provide 38.9% total energy from fat (actual range 38.2% - 39.9%) and thus derived approximately an extra 8% of total energy from fat and 8% less from carbohydrate than the CHO diet, and varied principally in the composition of dietary fats: oleic acid diet (OL) which was enriched to provide 8% of total energy from oleic acid; LMP diet which comprised 8% enrichment with lauric, myristic and palmitic acids; a diet with 8% enrichment from stearic acid (STE); a diet of 8% enrichment with *trans* fatty acids (TFA) of a range of t18:1 isomers; and a diet enriched with 4% from stearic acid and 4% from *trans* fatty acids (TFA/STE). There was no significant effect of diet order on lipid measures.

TFA, TFA/STE, and LMP diets were all associated with significantly higher total cholesterol levels than the other three diets with respective means (SD): 4.991 ± 0.088 mmol/l; 4.981 ± 0.088 mmol/l; 4.957 ± 0.088 mmol/l. LDL cholesterol was again higher on TFA and TFA/STE diets than on the other diets, with respective means (SD) of 3.36 ± 0.08 mmol/l and 3.32 ± 0.083 mmol/l. The TFA, TFA/STE, and STE

diets were all associated with low HDL levels, respectively: 1.159 ± 0.041 mmol/l; 1.174 ± 0.041 mmol/l; 1.156 ± 0.041 mmol/l. Total cholesterol to HDL ratios were thus significantly highest on the TFA and TFA/STE diets at 4.5 ± 0.1 and 4.4 ± 0.1 respectively.

Those dietary effects which were of significance at the p = 0.01 level were summarised as percentage changes with respect to substitution of other dietary components in Table 6 in the original paper. Those of interest to this report are summarised in the table below.

Of particular relevance to this discussion is the confirmation that large quantities of *trans* fatty acids increase LDL to a greater extent than that of saturated fatty acids. However once the intake of *trans* fatty acids is as great as 4% total energy, little further increase in LDL cholesterol is seen when intake of *trans* fatty acids is greater.

Selected results from Table 6 of Judd et al., 2002.

	TFA diet	**TFA/STE diet**	**STE diet**
% total energy from:			
Trans fatty acids	8.3	4.2	0.3
Stearic acid	2.8	6.9	10.9
Effect on plasma lipid by replacement of 8% of energy from:			
Total cholesterol			
Lauric, Myristic + Palmitic acids	NS	NS	- 3.7 %
Carbohydrate	+ 5.8 %	+ 5.6 %	NS
Stearic acid	+ 4.5 %	+ 4.3 %	-
LDL cholesterol			
Lauric, Myristic + Palmitic acids	+ 4.8 %	NS	NS
Carbohydrate	+ 10.1 %	+ 8.7 %	NS
Stearic acid	+ 8.4 %	+ 7.1 %	-
HDL cholesterol			
Lauric, Myristic + Palmitic acids	- 11.1 %	- 9.9 %	- 11.3 %
Carbohydrate	NS	NS	- 3.3 %
Stearic acid	NS	NS	-

NS: not significant at p = 0.01 level

*Adapted from Lipids, 2002, **37**:123-131.*

One study which directly compared the relative effects on serum lipids of saturated vs. *trans* fatty acids was de Roos et al. (2001). Twenty-nine volunteers (men and women) were given 2 different diets – a high *trans* and high saturated fat diet – for four weeks each in a randomised crossover design. The high saturated fat diet had slightly higher total fat content (41.0% of total energy vs. 37.4%), and derived 22.9% of total energy from saturated fat vs. 12.9% of total energy in the *trans* fatty acid diet. The *trans* diet had a fairly substantial level of *trans* fatty acid intake at 9.4% of total energy, with the saturated fat diet providing only 0.4% of total energy from *trans* fatty acids. Serum LDL cholesterol and triglycerides were the same on both diets. Mean (SD) serum HDL cholesterol was 1.87 (0.46) mmol/l on the saturated fat diet and 1.49 (0.33) mmol/l on the *trans* fatty acid diet, reflecting a mean decrease (95% CI) of 0.39

(0.28, 0.50) mmol/l or 21%. Total cholesterol concentrations differed solely as a result of the difference in HDL concentrations.

Dyerberg et al. (2004) conducted an 8 week dietary intervention study in healthy males. Study design was a randomised, double-blind parallel dietary intervention for 8 weeks with 12 weeks of follow-up. Diets under analysis were a *trans* fatty acid enriched diet, an n-3 polyunsaturated fat enriched diet, and a control fat diet. Seventy-nine participants completed intervention with mean age across diet groups ranging from 35.3 yrs to 39.2 yrs. Intakes at the end of intervention revealed the *trans* diet group were consuming 6.8% of total energy from *trans* fatty acids vs. 0.9% in the control group. Controls consumed higher amounts of saturated, monounsaturated and polyunsaturated fatty acids, but total fat intake was comparable, at 33.9% in the *trans* diet group vs. 34.8% in the control group. Experimental outcomes included platelet membrane fatty acid composition as measured by gas chromatography, and heart rate variability measured by 24 hour Holter monitor.

Not surprisingly, platelet membrane fatty acid composition was significantly higher in t18:1, t18:2, and total *trans* fatty acid content after consumption of a diet high in *trans* fatty acids, and interestingly t18:1 levels were still significantly higher than baseline at follow-up 12 weeks after finishing the diet (mean (SD) platelet membrane t18:1 as percentage of total fatty acid content 0.91(0.03) at baseline vs. 1.1 (0.04) at 12 weeks follow-up).

With regard to effect on plasma lipids, the high *trans* diet group showed a decrease at end of dietary intervention in HDL levels compared with baseline, and the change was significantly different from the changes in HDL seen pre- vs. post-diet in the control group.

One potential weakness of this study is that due to the randomised parallel design, random allocation resulted in the *trans* diet and control diet groups differing in some respects. While BMI, proportion of non-smokers, physical activity and fish intake measures were similar, mean age in controls was slightly older (37.6 ± 10.6 yrs vs. 35.3 ± 10.3 yrs) and baseline lipid measures differed substantially, with controls having higher total and LDL cholesterol, lower HDL cholesterol, and hence significantly different LDL:HDL ratios.

Two small studies identified between 2000 and 2005 which made use of deuterium to measure metabolism and catabolism of blood lipids are described below. Whilst small studies, they are included in this report as they are of a very few studies which examined potential biological mechanisms which could lead to differences in lipid levels between diets, by examining HDL, LDL and cholesterol synthesis and catabolism rates.

A small study conducted by Matthan et al. (2004) used a randomized dietary cross-over design with three different diets followed for a period of 5 weeks each. Only eight postmenopausal women were included in the study, which limits the extent to which the results can be generalized to the greater population. The diets differed in that two-thirds of the fat content was derived from either soybean oil (unsaturated fat diet), stick margarine (hydrogenated fat diet) or butter (saturated fat diet). The dietary composition of macronutrients was kept relatively, although not exactly, consistent

across the three diets, as shown in the table below, with dietary fat providing approximately 29% of total daily energy intake.

Adapted from Matthan et al. (2004). Values are % of total daily energy intake.

	Unsaturated fat diet	Hydrogenated fat diet	Saturated fat diet
Carbohydrate	55.8	53.5	54.0
Protein	15.7	16.7	16.9
Fat	28.5	29.7	29.1
of which was *trans*	0.6	6.7	1.3

*Adapted from Arterioscler Thromb Vasc Biol, 2004, **24**:1092-97.*

Measurement of HDL and LDL catabolism was achieved by an experimental protocol, carried out after the 5 week consumption of each diet, whereby a gradual intravenous infusion over 15 hours of deuterated leucine (5,5,5-$^{2}H_3$-L-leucine) was combined with hourly 'meals' – these were identical small servings which in total provided the daily caloric intake for each dietary phase. Blood samples were collected via a second intravenous line for measurement of lipid and lipoprotein characteristics of interest.

The hydrogenated fat diet produced levels of total and LDL cholesterol intermediary between the other two diets, such that compared to the unsaturated fat diet, total cholesterol was 7% higher on the hydrogenated fat diet, and 11% higher on the saturated fat diet; likewise LDL cholesterol was 12% higher on the hydrogenated fat diet, and 15% higher on the saturated fat diet. However a significant increase in HDL was observed on the saturated fat diet relative to the unsaturated, which did not occur with the hydrogenated fat diet, such that the hydrogenated fat diet showed the poorest ratio of total cholesterol to HDL cholesterol.

Mean (SD) of blood lipids adapted from Table 2 in Matthan et al. (2004)

	Unsaturated fat diet	Hydrogenated fat diet	Saturated fat diet
Total cholesterol	4.85 (0.73)	5.16 (0.57)	5.39 (0.76)
LDL-C	2.99 (0.57)	3.27 (0.39)	3.43 (0.72)
HDL-C	1.32 (0.39)	1.30 (0.35)	1.39 (0.33)
Total chol:HDL ratio*	3.67	3.97	3.88

*calculated by the authors of this report based on mean values only.

*Adapted from Arterioscler Thromb Vasc Biol, 2004, **24**:1092-97.*

The results of lipid production and degradation comparisons, as measured by the infused deuterated leucine experiment at the end of each diet phase, showed that the fractional catabolic rate of HDL was significantly higher on the hydrogenated fat diet compared to the other two diets. HDL production rates did not differ between diets, suggesting that the reduced HDL levels seen on the high *trans* fatty acid diet arose from increased HDL catabolism rather than lower HDL production rates.

Regarding LDL measures, LDL production rates based on apoB-100 were similar on all three diets, with both the saturated and hydrogenated fat diets showing a significantly lower LDL fractional catabolic rate, thus giving rise to the higher LDL levels seen on these two diets relative to the unsaturated fat diet. Importantly, the saturated fat and hydrogenated fat diets were not significantly different in terms of either LDL production or fractional catabolic rates. This suggests that saturated and

trans fatty acids produce a similar effect of raising LDL cholesterol, however the poorer lipid profile observed on the hydrogenated fat diet in this study appears to have arisen via *trans* fatty acids increasing HDL catabolism.

Another small study making use of deuterium was conducted by French, Sundram and Clandinin (2002) in 10 Malaysian women. A diet high in *trans* fatty acids gave significantly greater rises in LDL cholesterol than a diet high in saturated fat (11.5% increase over high-saturated fat diet, $p < 0.05$). In this study deuterium was used to measure cholesterol fractional synthetic rate. A high *trans* diet produced a significantly greater fractional synthetic rate of free cholesterol than a diet high in saturated fat. The fractional synthetic rate of total cholesterol was also greater on a high *trans* diet in 8 out of the 10 subjects than on a high saturated fat diet, but did not reach statistical significance.

Idris and Sundram (2002) examined the effects of dietary *trans* fatty acids in *Cynomolgus* monkeys, allowing tight control of dietary intake. Nine monkeys were randomly rotated through four dietary regimes for 6 weeks each in two different phases. In the first phase, different blends of oils were the main difference between these four diets, with only one diet having significant amounts of *trans* fatty acids, derived from partially hydrogenated soybean oil, which is frequently used for human consumption. In the diet high in *trans* fatty acids, total *trans* fatty acids contributed 9.3% of total energy intake, with 7.4% from t18:1(n-9), 1.1% from t18:1(n-11), and 0.5% from t18:1(n-13). For the second phase, the same four dietary regimes were used, with the addition of dietary cholesterol at 0.1% of total energy to each diet.

The high *trans* diet (without added cholesterol) produced significantly higher total cholesterol, LDL cholesterol and significantly lower HDL cholesterol, resulting in the poorest LDL:HDL ratio of all four diets. The addition of dietary cholesterol in the second phase of the experiment resulted in the high *trans* diet producing significantly higher total cholesterol and LDL cholesterol than without dietary cholesterol (also seen in the other three diets) although no decrease in HDL cholesterol was seen, such that the LDL:HDL ratio was no longer the poorest of the four diets. The authors give no possible explanation as to why the addition of dietary cholesterol appears to have ameliorated the HDL-lowering effects of dietary *trans* fatty acids. These results also seem to suggest that, at least in non-human primates, dietary *trans* fatty acids are much more atherogenic when consumed as part of a diet which is also high in cholesterol.

Predictive modelling equations

An authoritative meta-analysis of the effects of dietary *trans* fatty acids was published in 2003 in the American Journal of Clinical Nutrition (Mensink et al., 2003). As described above under 'Saturated fatty acids and LDL cholesterol: Predictive modelling equations', articles published between January 1970 and December 1998 were included, and had to meet the following criteria: food intake clearly described with dietary fatty acids as the sole variable; dietary cholesterol constant between comparison diets; parallel, cross-over or Latin-square design; diets adherence for at least 13 days to allow time for changes in lipid measures to take place; adults with no disturbances of lipid metabolism or diabetes included. A total of 60 studies were selected for the meta-analysis, of which only 8 were included that specifically measures *trans* fatty acids. Thus in essence the modelled effect of dietary *trans* fatty acids was only based on these 8 studies, with models that estimated the influence of *trans* fatty acids by predicting the changes in lipid measures that would occur with replacement with polyunsaturated or monounsaturated fatty acids or carbohydrates. The relative effects of each of these other replacement dietary components was based on the modelling results of the full meta-analysis of 60 trials. In the 8 studies that measured *trans* fatty acids intakes ranged from 0% to 10.9% of total energy.

Trans fatty acids emerged as the most detrimental of the fatty acids examined in the study (which included an examination of the effects of the individual saturated fatty acids). Regression coefficients (95% CI) for the effect of *trans* fatty acids vs. carbohydrate of 1% of energy were 0.022 (0.0005, 0.038) for total:HDL cholesterol ratio, 0.031 (0.020, 0.042) for total cholesterol, and 0.040 (0.020, 0.060) for LDL cholesterol. No effect on HDL cholesterol ratio was predicted (regression coefficient (95% CI): 0.000 (-0.007, 0.006).

It was predicted that thus replacement of *trans* fatty acids accounting for 1% of total energy would decrease the total:HDL cholesterol ratio by 0.019 when replaced with saturated fatty acids, 0.048 for *cis* monounsaturated fatty acids, and 0.054 for *cis* polyunsaturated fatty acids.

Given these results, replacement of *trans* fatty acids constituting 1% of total energy with carbohydrate would have the same effect on total:HDL cholesterol ratio as replacement of 7.3% of saturated fatty acids with carbohydrate. The authors state that given an 'average US diet' which derives 2.6% of total energy from *trans* fatty acids and 13% from saturated fatty acids, replacement of the 2.6% of *trans* fatty acids with carbohydrates would have a greater beneficial effect on total:HDL cholesterol ratio than replacing the total amount of saturated fatty acids with carbohydrate. The authors conclude that 'the replacement of *trans* fatty acids with unsaturated fatty acids from unhydrogenated oils is the single most effective measure for improving blood lipid profiles.'

Another study which produced predictive equations on the effects of dietary *trans* fatty acids on LDL cholesterol was that of Müller et al. (2001). Their impetus was that most published studies of predictive equations for LDL cholesterol were based only on the effects of either total saturated fatty acids, or individual saturated fatty acids, ignoring the influence of *trans* fatty acids. The design of the study is included above under 'Saturated fatty acids and LDL cholesterol: predictive modelling equations'.

One weakness of the study was that the dataset from which the models were derived consisted only of four studies which had been carried out from 1993 – 1998 by the authors at the University College of Akershus in Norway.

Trans fatty acids were incorporated into predictive equations which included lauric (C12:0), myristic (C14:0) and palmitic (C16:0) acids. Stearic acid (C18:0) was not included in the analysis. *Trans* fatty acids from partially hydrogenated soybean oil (labelled '*TRANS* V') were distinguished from *trans* fatty acids from fish oil (labelled '*TRANS* F'), and regarded as consisting of the individual *trans* fatty acids: *TRANS* V: t16:1, t18:1; *TRANS* F: t16:1, t18:1, t18:2, t20:1, t20:2, t22:2, t24:1.

Regression coefficients (SD) for the effect of each 1% change of total energy per *trans* fatty acid on LDL cholesterol in mmol/l were 0.025 (0.042) for *TRANS* V, and 0.043 (0.028) for *TRANS* F. In the final predictive equation, comparing isoenergetic amounts of dietary fatty acids, the saturated fatty acid myristic acid produced the greatest rise in LDL cholesterol followed by palmitic acid. *Trans* fatty acids derived from fish oil (*TRANS* F) closely followed palmitic, in turn closely followed by *trans* fatty acids from vegetable oil (*TRANS* V). Similar results were obtained for the prediction of total cholesterol. The final equation is given below.

$$\Delta\text{LDL cholesterol} = 0.01\,(\Delta\text{C12:0}) + 0.071\,(\Delta\text{C14:0}) + 0.047\,(\Delta\text{C16:0}) + 0.043\,(\Delta\textit{TRANS}\text{ F}) + 0.025\,(\Delta\textit{TRANS}\text{ V}) - 0.0044\,(\Delta\textit{cis}\text{ 18:1}) - 0.017\,(\Delta\textit{cis}\text{ 18:2, }\textit{cis}\text{ 18:3})^{*}$$

* Note that predictive values for oleic (*cis* 18:1), linoleic (*cis* 18:2) and alpha-linolenic (*cis* 18:3) were taken from Yu et al. (1995). LDL cholesterol expressed as change in mmol/l per change in dietary fatty acid constituting 1% total energy. Stearic acid (C18:0) was not included in the analysis.

Trans fatty acids and coronary heart disease:

i. Cross sectional studies of trans fatty acid intake and nonfatal MI

Three cross sectional studies examining the link between dietary and/or adipose tissue *trans* fatty acids and nonfatal MI were identified since publication of the Health Canada report. They will be described below in order of publication. Each of these had similar experimental designs including subjects who had a first nonfatal MI and data relating to *trans* fatty acid intake derived from food frequency or dietary questionnaires or adipose tissue samples.

Pedersen et al. (2000) conducted a case control study of men and postmenopausal women admitted for a first acute MI in Oslo, Norway in 1996. Patients with previous MI or serious disease such as diabetes, which may alter their dietary patterns, or significant recent weight loss, were excluded. Participation rates were high with only 5% of those asked to participate refusing. Controls were matched for age, sex and geographical location. Adipose tissue samples were collected from the buttock, and validated food frequency questionnaires completed during interviews with participants. Power calculations were based on the EURAMIC study (Aro et al., 1995; reviewed in the Health Canada report) and 112 cases and 107 controls were recruited. Two patients had previously diagnosed coronary heart disease but were included as they reported not changing their diet. Odds ratios were calculated for the highest versus the lowest quintiles of dietary intake, with consideration of possible confounders including age, sex, waist-to-hip ratio, current smoking, and family history of CHD.

The correlation between adipose tissue *trans* fatty acid and dietary *trans* fatty acid intake was found to be 0.295. Adjusted odds ratio (95% CI) for a first MI for the highest vs. the lowest adipose tissue *trans* fatty acid levels were 2.25 (0.78 – 6.48), with a significant trend across groups at p=0.03.

Levels of *trans* fatty acids in this study were highly correlated with levels of linoleic and linolenic fatty acids, probably due to a common dietary source such as margarine. Adjustment of the above odds ratios for content of either linoleic or linolenic acids in the adipose tissue abolished the significance of the increased risk associated with higher *trans* fatty acids.

A 2003 paper in the Journal of Nutrition (Baylin et al., 2003) examined the association between *trans* fatty acid intake and non-fatal myocardial infarction in a Costa Rican population. Subjects included men and women who attended hospitals in the metropolitan area of San Jose, Costa Rica, and were diagnosed as having had an acute MI by two independent cardiologists. Cases were excluded if they had had any previous admission related to cardiovascular disease, or if they died during the hospitalization arising from the MI. Five hundred and thirty cases were matched by age, sex, and area of residence to 531 controls. Cases and controls were both visited in their homes for collection of dietary and health information, clinical measures, and adipose tissue biopsy. Participation rates were exceptionally high in both groups with 97% of cases and 90% of controls agreeing to participate. The association of *trans* fatty acids with nonfatal MI was principally based on adipose tissue *trans* fatty acid, with a biopsy from the upper buttock. Food frequency questionnaires were

administered to examine possible confounding of results by other dietary factors for which good biomarkers of intake were not available. The major source of 18:1 and 18:2 *trans* fatty acids in the Costa Rican diet is considered by the authors to be derived from partially hydrogenated soybean oil, used by over 40% of subjects.

Multivariate odds ratios for nonfatal MI were determined using conditional logistic regression including the possible confounding factors: age, sex, residence, income, history of diabetes, history of hypertension, physical activity, smoking, years living in the same house, adipose alpha-linolenic acid, intake of alcohol, vitamin E, saturated fat and total energy. Further adjustments for BMI, waist-to-hip ratio, multivitamin use, folate of fibre intake were also examined and found not to influence OR's of any of the associations with *trans* fatty acids reported.

Total adipose tissue *trans* fatty acid was associated with an increased risk of MI, with the highest quintile of adipose tissue TFA exhibiting a multivariate OR (95% CI) for MI of 2.94 (1.36 – 6.37) when compared to the lowest quintile of adipose tissue TFA. The trend across quintiles was highly significant at $p = 0.004$.

When considering individual *trans* fatty acids the most striking effects were seen for t18:2. An increased risk associated with t16:1 was also apparent in the highest quartile of intake. No effect was seen with t18:1.

The same trend of increased risk of nonfatal MI with adipose tissue TFA has been reported by Clifton et al. (2004), also published in the Journal of Nutrition. This study had a very similar design, but with the added interesting feature of coinciding with the withdrawal of *trans* fatty acids from commercially available margarine.

Cases were men and women admitted for heart disease between 1995 and 1997 at any of the four major hospitals in Adelaide. Subjects with a previous diagnosis of heart disease, angina or diabetes were excluded. All blood samples were taken within 4hr of development of chest pain. Adipose tissue samples were taken from abdominal subcutaneous fat at the umbilicus level within 2 weeks of discharge. Food frequency and other lifestyle questionnaires were administered by a dietitian before discharge. Statistical analysis was performed using logistic regression, including the potential confounders: total energy intake, % energy as fat, protein, carbohydrate, saturated fat, polyunsaturated fat, BMI, age, sex, blood pressure, lipids (however HDL was not measured), smoking, job classification, and all other measured fatty acids.

209 cases and 174 controls were recruited. Total adipose TFA was higher in cases than controls, a similar effect was seen for each of the *trans* fatty acids t18:1 (n-9), t18:1 (n-8) and t18:1 (n-7). In logistic regression adipose tissue t18:1 (n-7) was an independent predictor of first heart attack.[*]

[*] This interpretation and discussion of the results of Clifton et al. (2004) are based on the published erratum in *J Nutr* **134:**1848 (2004) which states: 'throughout the article "*trans* 18:1(n-11)" should read "*trans* 18:1(n-7)" and "*trans* 18:1(n-10)" should read "*trans* 18:1(n-8)"'.

Using dietary data from food frequency questionnaires, the highest quintile of TFA intake had a risk of MI twice that of the lowest quintile, however this was abolished after correction for saturated fat intake. Thus, adipose TFA appeared to be a more powerful predictor than TFA from dietary intake.

In June 1996, a year after commencement of recruiting, food manufacturers in Australia withdrew TFA from the production of popular margarine brands. The authors state that prior to this, margarine provided approximately 50% of the dietary intake of TFA. Thus an analysis pre- and post-withdrawal has interesting bearing on the influence of TFA in the food supply.

Dietary margarine intake assessed by food frequency questionnaire did not differ before or after this withdrawal in either cases or controls. When an analysis was done of biopsies taken before June 1996, cases had a higher level of *trans* 18:1 (n-9) and 18:1 (n-7) than controls. For those samples taken after June 1996, cases and controls did not differ in any of the adipose tissue TFA's.

Of interest is the observation that: t16:1 fatty acid levels were found to be higher in cases than controls, indicating a possible role of animal derived TFA in the increased risk of MI. However, although the highest quintile of TFA intake from dietary data had a risk of MI twice that of the lowest quintile, this group also had a higher saturated fat intake, and after correction for saturated fat, this association disappeared.

The studies described above examining the link between adipose tissue *trans* fatty acids and myocardial infarction all suggest a powerful connection between the two. It is noteworthy that the European EURAMIC study (covered in the Health Canada report) found no association between adipose tissue t18:1 fatty acids and MI in cases and controls across Europe. However, two Spanish centres displayed adipose fatty acid profiles quite different from other centres with low mean adipose t18:1 levels and high mean adipose oleic acid levels. When these two centres were excluded, a positive association was observed suggesting an increased risk of MI with higher adipose t18:1 levels. The authors concluded that they 'cannot exclude the possibility that the contribution of *trans* fatty acids to risk of MI is significant in countries with high intakes of *trans* fatty acids'. Thus the only truly negative case control study is that by Roberts et al. considered in the Health Canada report.

Lemaitre et al. (2002) reported a study examining the association of *trans* fatty acids with myocardial infarction, though unlike the above studies, using red blood cell membrane fatty acid content as a marker of dietary *trans* fatty acid intake rather than adipose tissue content. The study was conducted in King County, Washington, US, with a specific interest in whether *trans* 18:1 isomers or *trans* 18:2 isomers were more strongly associated with MI. Cases were identified between October 1998 and June 1999. Response rate amongst cases was 77% and 59% for controls, giving final samples sizes of 179 cases and 285 controls. Mean age (SD) of cases was 59.5 (10.3) yrs and controls 57.8 (10.3) yrs, with over 80% of both groups being male.

Red blood cell (RBC) membrane fatty acid content was measured by gas chromatography, which detected nine different fatty acids in samples: the t18:1 isomers (n-9), (n-10), (n-11), (n-12) and a mix of (n-6) to (n-8); the t18:2 isomers (*cis* 9, *trans* 12) and (*trans* 9, *cis* 12); and the t16:1 isomers (n-7) and (n-9). Of note is that no conjugated linoleic acid (CLA) isomers were detected in membrane samples.

Dietary intakes were not measured amongst all cases or controls, but instead in a sub-sample a 17-item food frequency questionnaire was given to spouses of cases and controls. The red blood cell membrane total *trans* fatty acid level was related to intake

assessed by questionnaire amongst 111 controls with $r = 0.5$, $p < 0.001$. The different *trans* 18:1 and 18:2 isomers were additionally correlated to intake of different foods. An analysis of 4-day food records completed by 55 controls showed no correlation of RBC membrane *trans* fatty acids and total energy intake or percentage of energy from saturated fat.

Odds ratios were calculated based on increases in RBC membrane *trans* fatty acid content equal to the interquartile range of controls. After adjustment for traditional risk factors, an increase of one interquartile range of membrane t18:1 was significantly associated with increased risk of MI, although membrane *trans* fatty acid content was found to be inversely related to membrane content of the long-chain n-3 polyunsaturated fatty acids docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), which was also different between cases and controls. After further adjustment for membrane DHA and EPA levels, the association of membrane t18:1 with MI was no longer significant. However the corresponding OR (95% CI) after the same adjustments for an interquartile range increase in membrane t18:2 was highly significant at 2.66 (1.58 – 4.46). In a model assessing risk of MI which included both membrane t18:1 and t18:2, only t18:2 independently emerged as significantly associated with MI.

Analysis across quartiles of membrane t18:2 was also suggestive of a linear dose-response relationship, with OR (95% CI) of MI for increasing quartiles compared with the lower quartile of 1.41 (0.66 – 3.00), 2.39 (1.07 – 5.35) and 4.22 (1.65 – 10.8).

i.b. Methodological considerations:

Adipose tissue TFA and dietary intake:

In order for a health claim regarding dietary *trans* fatty acids and CHD to be made on the basis of the studies reported above, which have derived associations using adipose tissue *trans* fatty acids, it is important to demonstrate that adipose tissue *trans* fatty acids are a reliable marker of dietary *trans* fatty acids, and that dietary *trans* fatty acids are incorporated into adipose tissue.

A pioneering study by Schrock and Connor in 1975 demonstrated a high level of incorporation of dietary 18:1 *trans* fatty acids into adipose tissue in New Zealand white rabbits, albeit at a particularly high *trans* fatty acid intake (Schrock and Connor, 1975).

A similar study into the effect of controlled dietary fatty acid variation and adipose tissue composition in humans wasn't conducted until 2002, when Baylin et al. provided a comprehensive validation of adipose tissue fatty acids as biomarkers of dietary *trans* fatty acid intake (Baylin et al., 2002). These researchers analyzed the respective adipose and dietary correlations of 35 detectable fatty acids using adipose samples from the upper buttock. *Trans* fatty acids in general were the most highly correlated fatty acid group. Aside from t14:1(n-5) and t16:1(n-7) which had relatively low correlations of 0.09 and 0.12 respectively, t18:1 isomers had correlations between 0.30 and 0.43, with total t18:1 having a pooled correlation coefficient of 0.43. *Trans* 18:2 isomers had higher correlations between 0.53 and 0.61. The only other fatty acids with comparable correlation coefficients were linoleic (18:2(n-6)) at 0.58 and alpha-linolenic (18:3(n-3)) at 0.34.

In this study sample, total *trans* fatty acids accounted for 3.1% of total adipose fatty acids, and *trans* fatty acid contributed 4.8% of total dietary fat and 4.0 g/d of energy-adjusted dietary intake; these levels are comparable with dietary intake reported by a number of studies worldwide, suggesting these findings could be widely generalized to other populations.

In epidemiological studies such as those above which have measured both dietary intake and adipose tissue *trans* fatty acid levels, correlations between the two have varied across studies. In the Nurses' Health Survey, two such comparisons between adipose *trans* fatty acid content and *trans* fatty acid intake estimated from a FFQ were undertaken, producing correlations of 0.50 and 0.41 (Garland et al., 1998). Cantwell (2000) provides a review of studies which have correlated adipose tissue *trans* fatty acids with dietary assessment, with correlation coefficients ranging from 0.29 (Hunter et al., 1992) to 0.51 (London et al., 1991). Lemaitre et al. (1998) examined correlations between adipose and dietary *trans* fatty acids, finding that after adjustment for energy intake, age and BMI, correlation coefficients for *trans* fatty acid isomers were generally higher amongst men. For total *trans* fatty acids, the correlation coefficient for women (n=27) was 0.52, and for men (n=24) 0.76. *Trans* 16:1 fatty acids gave the lowest correlation of the *trans* fatty acids for both sexes at 0.30 for women and 0.42 for men.

Site of adipose tissue collection: abdominal vs. buttock adipose tissue

In the three studies summarised above linking adipose tissue *trans* fatty acids and nonfatal MI published since 1999, Clifton et al. (2004) used abdominal adipose samples whilst both Pedersen et al. (2000) and Baylin et al. (2003) used buttock adipose samples. As shown by Baylin et al. (2002), buttock adipose tissue *trans* fatty acids are highly correlated with dietary intake. While one can assume abdominal adipose composition would not differ appreciably, it would useful be to have data to quantify this. The only study identified by the authors of this report to compare abdominal vs. buttock adipose tissue content is that of Mamalakis et al. (2002). Unfortunately this study did not include dietary intake data to examine whether dietary *trans* fatty acids are preferentially deposited into abdominal or buttock adipose tissue. Moreover, this study was conducted in school aged children (mean age 13 yrs) and thus the findings are of limited relevance to the link between adipose tissue *trans* fatty acids and MI in adulthood, as the effect of changes in circulating hormones post-puberty on adipose tissue composition is unknown. Briefly, the study found that abdominal *trans* fatty acids were significantly higher than buttock *trans* fatty acids in girls, but not boys, and also in the pooled gender analysis. Although girls and boys had the same abdominal *trans* fatty acid content, girls had significantly lower buttock *trans* fatty acid content than boys.

Red blood cell (erythrocyte) membrane fatty acid composition as a biomarker of TFA intake

In the study by Lemaitre et al. (2002) the intake of dietary *trans* fatty acids measured by FFQ in 111 controls gave a good correlation with red blood cell membrane *trans* fatty acid content ($r = 0.5$, $p < 0.001$), suggesting red blood cell membrane content is a valid biomarker of *trans* fatty acid intake. The results of other studies are supportive of this. Barnard et al. (1990) showed dietary *trans* fatty acids were incorporated into red blood cell membranes in monkeys. Brosche et al. (1986) showed changes in red blood cell membrane *trans* fatty acid content mirroring changes in dietary intake in elderly subjects. Emken et al. (1979) reported uptake of over 24hrs of deuterium labelled *trans* fatty acids into red blood cell membranes in young men, and additionally noted that uptake into red blood cell membranes was less, and slower, than uptake into plasma phospholipids.

ii. Prospective studies of trans fatty acid intake and coronary heart disease

An important prospective study released since the Health Canada report is that of the Zutphen Elderly Study (Oomen et al., Lancet 2001). In this Dutch study, a significant source of dietary *trans* fatty acid was partly hydrogenated fish oil, in contrast to the partially hydrogenated vegetable oils more frequently used in the US (Hulshof et al., 1999).

The Zutphen cohort was established in 1960 with 878 men born between 1900 and 1919 and formed the Dutch component of the Seven Countries Study. Dietary surveys were conducted in 1985, 1990 and 1995. Clearly an appreciable number of the original cohort had died by this time, and further exclusion of those with diagnosed MI or angina resulted in dietary information being available for 667 men in 1985, 435 in 1990, and 225 in 1995. *Trans* fatty acid intake was determined with the use of time-specific tables of Dutch foods. National data on composition of edible fats was available for 1985 and 1990 from the Wageningen University in the Netherlands, and for 1995 from the large European TRANSFAIR study. Vital status was determined from municipal registries and cause of death from either Statistics Netherlands, hospital discharge data, or general practitioners. During the 10 year follow up period, 98 cases of coronary heart disease were identified, with 49 of these being cardiac deaths (46 primary cause of death; 3 secondary cause of death).

Trans fatty acid intake in this cohort was higher than reported for other studies conducted internationally, with total *trans* fatty acid intake in 1985 accounting for 4.3% of total energy, and falling to 1.9% by 1995.

Cox's proportional hazard analysis was used to calculate relative risks, with a fully-adjusted analysis taking into account age, energy intake, BMI, smoking, alcohol intake as a categorical variable, vitamin supplements, intake of saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, cholesterol and fibre.

Fully-adjusted relative risk (95% CI) for coronary heart disease amongst the highest tertile of *trans* fatty acid intake was 2.00 (2.07 – 3.75) when compared to the lowest tertile, with a significant trend across tertiles (p=0.03).

An analysis was also conducted examining the effect of a difference at baseline of 2% of energy from total *trans* fatty acids. For a diagnosis of coronary heart disease a fully adjusted RR (95% CI) of 1.28 (1.01 – 1.61) was found, and for fatal coronary heart disease 1.33 (0.96 – 1.86).

When considering *trans* fatty acids from different sources, the relative risks (95% CI) of coronary heart disease for a difference at baseline of 0.5% of total energy from either ruminant *trans* fatty acids, manufactured t18:1 acids, and other manufactured *trans* fatty acids were similar at: 1.17 (0.69 – 1.98), 1.05 (0.94 – 1.17), and 1.07 (0.99 – 1.15) respectively.

The authors conducted a pooled analysis of their results combined with three previous prospective studies – the Nurses' Health Study 14 year follow-up (Hu et al., 1997), the Health Professionals follow-up study (Ascherio et al., 1996), and the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (Pietinen et al., 1997) all of

which measured dietary *trans* fatty acid intake and incidence of CHD, and which are each covered in the Health Canada report. The pooled fully adjusted relative risk (95% CI) of coronary heart disease based on an increase of 2% of energy in *trans* fatty acids at baseline was 1.25 (1.11-1.40).

Oh et al. (2005) reported on the effects of dietary fat intake after 20 years of follow-up in the Nurses' Health Study (methods detailed above in 'Saturated fatty acids and coronary heart disease: prospective studies'). *Trans* fatty acid intake decreased slightly over the follow-up period from 2.2% of total energy in 1980 to 1.6% in 1998. A comprehensive multivariate relative risk calculation (also detailed above) was made between *trans* fatty acid intake quintiles and incident CHD. Relative risk (95% CI) of incident CHD for the highest quintile of *trans* fatty acid intake (median 2.8% of total energy) vs. the lowest quintile (median 1.3% of total energy) was 1.33 (1.07 – 1.66). The fourth highest quintile (2.2% of total energy) was of borderline significance at a RR (95% CI) of 1.19 (0.99 – 1.44), and a highly significant trend across quintiles existed at p=0.01. Risk of CHD differed by age and BMI group, with women younger than 65 yrs and women of BMI < 25 kg/m^2 at greater risk of CHD, although in both cases the interaction of CHD risk and age/BMI was not significant.

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Part 3. Relevance to Australia and New Zealand

Information on population intakes of saturated fat, and food sources of saturated fat such as meat and dairy products, is readily available in both countries from National Nutrition Surveys conducted at fairly regular intervals. Information on *trans* fatty acid intakes is sparse. Whilst the respective National Nutrition Surveys of Australia and New Zealand include data on the intakes of industrially produced foodstuffs, such as margarines, and meat from ruminant animals, which are common sources of dietary *trans* fatty acids, there have been few attempts to derive estimated intakes of *trans* fatty acids from these. Given this paucity of population-level data, other published measures of *trans* fatty acid intake in the Australasian population measured in specific groups as part of scientific studies are also considered below.

Data on saturated fat intake from the most recent Australian Nutrition Survey (McLennan and Podger, 1995a + b), which is now a decade old, indicated a mean saturated fat intake for males aged 19 yrs and over of 39.0 g/day or 12.7 % total energy, and 26.7 g/day or 12.7% total energy for females aged 19 yrs and over. Total fat intake contributed approximately a third of total energy across all ages. Regarding food sources of dietary saturated fat, milk and milk products were the largest contributors to dietary saturated fat intake accounting for 26.7% and 27.2% of saturated fat intake for males and females respectively. Meat (including poultry) was the second highest contributor at 22.5% and 18.7% of saturated fat intake for males and females respectively. Fats and oils, potentially also a large source of dietary *trans* fatty acids, accounted for 8.9% of saturated fat intake for both genders. The values quoted here are for persons aged 19 yrs and over.

Data from the latest New Zealand Nutrition Survey regarding saturated fat intake are also somewhat out of date, the survey having been conducted in 1996/97 (Russell et al., 1999). Mean intake of saturated fat was 15% of total energy for both males and females and similar across ages. Absolute intake of saturated fat was higher for men given their higher total energy intake. Proportion of energy from total fat intake was higher for Maori females (36% total energy) compared with females of NZ European and other ethnicities (34% total energy). Mean saturated fat intake was also higher for Maori women (39 g/day, 16.0% total energy) than NZ European women and women from other ethnicities (31 g/day, 14.7% total energy). Likewise Maori men had a higher proportion of total energy derived from total fat (37% total energy) than men of NZ European and other ethnicities (35% total energy), however proportion from saturated fat was comparable across ethnicities, at 52 g/day or 15.7% total energy for Maori men compared with 49 g/day or 15.2% total energy for men of NZ European or other ethnicities. Median intake of total fat or saturated fat did not appear to be related to NZ deprivation index score. Saturated fat intake data for Maori should be interpreted with caution due to the smaller number of Maori studied. With regard to comparisons over time, total fat intake had decreased from the previous Life In New Zealand survey (Russell and Wilson, 1991) conducted in 1989, from 37.5% to 34.9% of total energy. However comparison between the surveys should be interpreted with caution because of slight differences in methodologies.

Thus it appears that amongst the populations of Australia and New Zealand there exists a fairly substantial potential for reduction of saturated fat intake.

The study by Clifton et al. (2004) described in Part 2 under the heading '*Trans* fatty acids and coronary heart disease: cross-sectional studies' was conducted in Adelaide between 1995 and 1997. The authors report that the *trans* fatty acid content of many commercially produced margarines dropped in June 1996. Given the importance of margarines as a source of *trans* fatty acids, intakes are likely to be lower after this time than before. However estimated dietary intakes of TFA for participants enlisted pre- and post-withdrawal are not presented separately but pooled. It appears that two-thirds of participants were enlisted after June 1996. Total mean (SD) TFA intake is given as 3.52 (1.82) g/d or 1.2 % of total energy for cases and 3.01 (1.29) g/d or 1.1 % total energy for controls. Approximately 10% of *trans* fatty acids were derived from animal sources for both cases and controls, approximately 23% from dairy sources, and the remaining majority from margarines and mixed dishes. Given that the data from some of these subjects was collected prior to June 1996, it is reasonable to believe that actual intakes post-June 1996 were slightly lower than the total intakes reported here. Mansour et al. (2001) also conducted a very small study in 10 volunteers from Geelong, Australia, in which usual mean (SD) intake of *trans* fatty acids was 4.4 (0.9) g/day or 1.9 % total energy. Of course, the data from both these studies are now outdated.

The paucity of reliable estimates of population intakes of *trans* fatty acids in Australasia has been raised before by Food Standards Australia New Zealand (Landells, 2005). Apart from the estimates of Clifton et al. and Mansour et al. the most recent estimates on intakes in Australia were noted to have been published in 1994 from simulated Australian diets or based on food frequency questionnaire data collected in 1987, and for New Zealand from the 1989/1990 Life In New Zealand (LINZ) survey. Mean *trans* fatty acid intakes from simulated Australian diets were calculated to range from 6.4 g/day or 2.5 % total energy for males and 4.4 g/day or 2.1 % total energy for females, to 13.6 g/day or 5.3 % total energy for males and 10.5 g/day or 5.1 % total energy for females, based on different assumptions about the *trans* fatty acid content of available foods. The authors conclude that intakes 'are likely to be less than 2 – 2.5 % energy' (Noakes and Nestel, 1994). Data from the LINZ survey suggest intakes in New Zealand to be slightly less at 5.4 g/day for males and 3.4 g/day for females. Based on the mean total energy intakes published from the LINZ survey (Wilson et al., 1990) of 10,400 kJ for men and 6,800 kJ for women, these figures equate to 1.9 % total energy for both sexes. Thus although current intakes may not be excessive it is necessary to ensure that levels do not increase and indeed benefit may accrue from reduction to even lower levels.

Another important factor to consider in the case of Australia and New Zealand is the multi-cultural nature of their populations. A great degree of dietary variation between ethnic groups has been documented in both countries (Ferguson, 2002; Harris et al., 2004; Metcalfe et al., 1998; Russell et al., 1999; Swinburn et al., 1998; Thomson and Shaw, 2002). Given the high coronary heart disease rates amongst the indigenous populations and some more recent migrant groups, higher intakes of saturated fatty

Where intakes of saturated or *trans* unsaturated fatty acids as percentages of total energy intake have not been given in the original papers or reports described above, they have been converted from intakes in grams per day using the conversion factors of 1g *trans* fatty acids = 9 kcal or 17 kJ, based on the conversion factors for fat reported in McCance and Widdowson's Composition of Foods (Food Standards Agency, 2002).

acids and possibly *trans* fatty acids may represent an even greater cause for concern than is the case for European populations.

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Part 4. Other relevant biomarkers of disease outcome

Nutritional factors including saturated fatty acids and *trans* unsaturated fatty acids have been related to several biomarkers for coronary heart disease other than lipoproteins.

Perhaps one of the most striking associations is the "convincing" relationship between saturated fatty acids and insulin resistance. Insulin resistance is a key abnormality underlying the metabolic syndrome and type 2 diabetes, critically important determinants of cardiovascular disease, and has been shown in prospective studies to predict coronary heart disease (Zethelius et al., 2005). Carefully controlled nutritional intervention studies have clearly shown the ability of saturated fatty acids (when compared with the effect of monounsaturated fatty acids) to increase insulin resistance, measured by the intravenous glucose tolerance test (KANWU study, Vessby et al., 2001). There is no doubt about the relationships between markers of inflammation (e.g. CRP, TNF-α, interleukin 6) and coronary heart disease (Wilson et al., 2004; Pai et al., 2004). There is a reasonable body of evidence derived from cross-sectional studies which links dietary saturated fat and CRP (King et al., 2003), though the findings are not entirely consistent (Fredrikson et al., 2004). One intervention trial carried out in Canada has shown the potential of dietary modification to reduce CRP levels (Jenkins et al., 2003). However in addition to substantial reduction of dietary saturated fatty acids, the trial involved substantial increases in dietary fibre and changes in other fatty acids. Thus evidence for the association between saturated fatty acids and inflammatory markers, while of considerable interest, is for the present most appropriately regarded as "possible".

Trans unsaturated fatty acids have also been linked with markers of inflammation in cross-sectional analyses in participants in the Nurses' Health Study (Mozaffarian et al., 2004). Intakes of *trans* 18:1 and *trans* 18:2 (but not *trans* 16:1) were positively correlated with TNF-α. The associations of TFA consumption with coronary artery disease risk are greater than would be predicted by effects of serum lipoproteins alone. In this cross-sectional study relations between TFA intake and TNF receptor concentrations were partly attenuated by adjustment for serum lipid concentration suggesting a further independent effect mediated by promotion of the inflammatory response.

In a further analysis based on the same study, Lopez-Garcia et al. (2005) additionally found associations between TFA intakes and CRP as well as E-selectin and soluble cell adhesion molecules (sICAM and sVCAM). Thus while there appears to be evidence for a link between *trans* fatty acids and markers of endothelial function it is insufficient to form conclusions.

Obesity and increased tendency to thrombosis are unquestionably associated with increased risk of cardiovascular disease. High intakes of saturated fatty acids have been linked with both of these states, but it has not been possible to disentangle the effects of saturated fat from total fat, once again leaving the evidence classified as insufficient.

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Part 5. Overall conclusions

a) Public summary

Coronary heart disease (CHD) mortality rates have fallen during the last several decades, but it remains a major cause of serious illness and death in adults in Australia and New Zealand. The underlying pathology in most cases is atherosclerosis which involves an accumulation of lipoproteins, platelets, monocytes, endothelial cells, and smooth muscle cells in the walls of arteries, following damage to the layer of cells lining the artery. Atherosclerosis results in narrowing of the arteries and consequently reduction in the blood supply to heart muscle. A clot or thrombus may be superimposed on the atherosclerotic lesions, leading to a total obstruction to the blood supply and consequently death of the section of heart muscle supplied by the artery. This process leads to coronary thrombosis or myocardial infarction, whereas reduction of the blood supply leads to angina. The pathology is believed to result from an interaction between genetic and environmental factors. However it is noteworthy that cholesterol derived principally from low density lipoproteins is an important constituent of the atherosclerotic plaque, and total and LDL cholesterol are the most clearly established of the many potentially modifiable risk factors for CHD. LDL cholesterol is the major contributor to total blood cholesterol, hence total cholesterol is often used as a surrogate for LDL cholesterol since it is more easily measured. A clear dose response effect is apparent for total cholesterol in prospective epidemiological studies examining determinants of CHD, and CHD is extremely uncommon in populations with low mean cholesterol levels. Randomised controlled clinical trials have shown a benefit in terms of CHD risk reduction that is proportional to the reduction in cholesterol levels, regardless of whether this is achieved by drug therapy or dietary modification. Thus, LDL cholesterol is the most convincing of the biomarkers for CHD and it is generally accepted that measures able to reduce LDL cholesterol will reduce CHD risk.

The starting point for the present review was a similar process undertaken in Canada in 2000 by Ratnayake and McDonald. The report updated a 1993 United States report and reaffirmed the observation, first made in the 1950's, that saturated fatty acids were important determinants of total and LDL cholesterol. The association can unquestionably be described as 'convincing', with much evidence derived from randomised controlled trials and a clear dose response effect apparent with increasing amounts of SFA. The report indicated the differential effect of the different dietary saturated fatty acids on lipoproteins, with stearic acid having a negligible effect on LDL compared with the cholesterol raising effect of lauric, myristic and palmitic acids. However given the fact that stearic acid often co-exists with the other saturated fatty acids, and that stearic acid may promote thrombogenesis and so enhance atherosclerotic risks, this does not detract from the overall association and certainly not from the benefits in terms of LDL cholesterol of lowering total intake of saturated fatty acids. The Canadian review did not consider in detail the appreciable variation in individual response to a reduction in saturated fatty acids, though the population risk reduction which would be expected to accrue from reduction in saturated fatty acid intake should not be underestimated. Finally, it should be noted that the extent of LDL cholesterol reduction achieved by lowering intake of saturated fatty acids is dependent upon the source of replacement energy. Replacing saturated fatty acids with

polyunsaturated fatty acids would result in appreciably greater reductions in LDL cholesterol than replacement with either carbohydrate or monounsaturated fatty acids. Not replacing a reduction in saturated fatty acids, partially or totally, and resultant weight loss would also result in additional reduction of LDL cholesterol.

There is rather less direct evidence for the association between saturated fatty acids and CHD. While the studies generally suggest a relationship between saturated fatty acids and coronary heart disease and while there are certainly several plausible hypotheses, there are some inconsistencies in the data which cannot all be easily explained. Thus while we believe a reduction in saturated fatty acids is highly likely to reduce not only LDL cholesterol and other coronary heart disease risk factors but also cardiovascular disease, the current evidence for the direct association between saturated fatty acids and coronary heart disease is arguably more appropriately described as "probable" rather than "convincing".

Far fewer data exist for *trans* fatty acids. However a series of well conducted studies including randomised controlled trials show an association between *trans* fatty acids and LDL cholesterol. There are however two major limitations regarding the studies covered by the Canadian review and more recent data. Many of the studies do not distinguish between animal (largely occurring naturally) sources of *trans* fatty acids and vegetable sources, largely produced by the hydrogenation of vegetable derived oils. Furthermore it is not clear whether the effect of *trans* fatty acids on LDL cholesterol is biologically meaningful at low levels of intakes, such as that likely to be found in Australia and New Zealand. These limitations do not preclude the conclusion that the association between *trans* fatty acids and LDL cholesterol is a "convincing" one.

Fewer data exist relating *trans* fatty acids directly to coronary heart disease than is the case for saturated fatty acids. However, there are also fewer inconsistencies than is the case with studies linking saturated fatty acids and coronary heart disease. While one case control study found a lack of association between *trans* fatty acids and sudden cardiac death, three recent case control studies are confirmatory of an association, and another suggestive of a trend towards a positive association. Despite the apparent strength of evidence some inconsistencies remain, and we therefore believe it may be more appropriate to describe the association between *trans* fatty acids and coronary heart disease as "probable" rather than "convincing".

Table 2.1: Key studies of the effects of saturated and *trans* fatty acids on cardiovascular risk factors, coronary heart disease and myocardial infarction cited in Part 2. Multiple references for the same study are listed under the study title or acronym.

Authors	Study type, country	Subjects, gender and age	Study design: Duration and Methods Intervention and Endpoints	Dietary saturated or *trans* fatty acid intake (g/d or % En)	Results and Comments
Bautista et al. (2001)	Dietary cross-over trial, Columbia.	28 men, mean age 24.6 yrs (range 20-34 yrs)	Double-blind 4 x 4 dietary cross-over trial, comparing diets high vs. low in palm oil in the context of high vs. low dietary cholesterol	High palm oil diet provided 8.8% energy from palmitic acid	High palm oil diet produced significantly higher total and LDL cholesterol with mean differences (95% CI) of 0.39 (0.19, 0.59) mmol/l and 0.38 (0.21, 0.55) mmol/l for high vs. low palm oil diets. Response to diets was highly varied between individuals and total cholesterol response to palm oil was related to baseline cholesterol levels.
Baylin, A, et al. (2003)	Case control, Costa Rica	482 cases of first non-fatal MI and 482 controls. Case and control groups consisted of 26% women, 74% men. Mean age of cases and controls 57 yrs.	Cases enrolled in hospital unit, all adipose sample collection and food frequency questionnaire administration for cases and controls done in participant's home. Endpoints: Adipose tissue composition, dietary intake by food frequency questionnaire, and clinical outcome of first MI.	Not stated, *trans* fatty acid determined from adipose samples only.	Multivariate OR (95% CI) for first MI increased significantly across quintiles of total adipose tissue *trans* fatty acid content, up to 2.94 (1.36 – 6.37). A significant effect for t16:1 adipose tissue content was only seen in the highest quintile, possibly suggestive of a threshold effect. No effect of adipose tissue t18:1 was seen, but adipose tissue t18:2 significantly predicted first MI.

					Significant positive association between adipose tissue *trans* fatty acid and income is an observation of potential interest.
Boniface and Tefft. (2002)	Cohort study, Britain	Men (n=1225) and women (n=1451) aged 40-75 yrs at baseline.	Subjects randomly selected for the Health and Lifestyle Survey in Great Britain in 1984/85. Subjects interviewed at baseline regarding dietary habits. Followed up for 16 years with death certificate monitoring.	Mean saturated fat intake 329 g/week for men and 241 g/week for women.	Cox survival analysis adjusted for alcohol, smoking, exercise, and socioeconomic status gave relative risks (95% CI) of death from CHD for an 100 g/week increase in saturated fat intake of 1.00 (0.86, 1.18) for men and 1.40 (1.09, 1.79) for women.
Clifton, Keogh and Noakes (2004)	Case control, Australia	209 cases of first non-fatal MI and 179 controls Both men and women Mean age of cases and controls 56.3 yrs.	Food frequency questionnaires administered before discharge from first non-fatal MI. Adipose tissue samples collected within 2 weeks of discharge. Endpoints: Adipose tissue composition, dietary intakes, and clinical outcome of first MI.	*Trans* fatty acid consumption decreased after manufacturers of popular margarines produced *trans* fat-free products	Cases had significantly higher adipose tissue *trans* fatty acids than controls. Logistic regression showed t18:1 (n-11) in adipose tissue was an independent predictor of first MI. *Trans* fatty acids from margarine according to dietary surveys significantly correlated with t18:1 (n-9) in adipose tissue. From dietary data, neither total dietary *trans* fatty acids nor % energy from *trans* fatty acids predicted first MI after correction for dietary saturated fat and total energy intake. Authors state that animal sources of *trans* fatty acid are the major dietary contributor to *trans* fatty acid intake in Australia, given the withdrawal of *trans* fatty acids from margarines in 1996.

Cowin et al., (2001)	Cohort study, England	Children enrolled in the Avon Longitudinal Study of Pregnancy And Childhood (ALSPAC). 214 boys, 175 girls.	Data collected at 18 months of age, 31 months and 43 months. Diet assessed by 3 day unweighted dietary record. Non-fasting blood sample collected at 31 months.	Saturated fat intake in lowest and highest quintiles were 21.1 g/day and 23.9 g/day respectively for boys and 21.0 g/day and 21.3 g/day respectively for girls.	In boys, total cholesterol concentrations positively related to saturated fat intake ($r=0.211$, $p = 0.002$). No significant associations between total or LDL cholesterol and fat intake were seen in girls.
Dyerberg et al., (2004)	Randomised, double-blind, parallel dietary intervention, Denmark	79 males. Mean age 35.3 years in high *trans* fatty acid diet group, 37.6 yrs in control group	Comparison of three diets: control diet, one high in *trans* fatty acids and one high in n-3 polyunsaturated fatty acids.	High *trans* fatty acid diet provided 10.3% of total energy as saturated fat and 6.8% from *trans* fatty acids. Control diet provided 15.7% total energy from saturated fat, 0.9% from *trans* fatty acids.	High *trans* diet produced increases in total and LDL cholesterol relative to baseline, as did control diet. Relative to the control diet, high *trans* diet produced significantly lower HDL concentrations, but not significantly different effects on total or LDL cholesterol.
French, Sundram and Clandinin, 2002)	Dietary intervention, Malaysia	3 men and 3 women in experiment 1, mean age 25 yrs; 10 women in experiment 2.	Experiment 1: 8 experimental diets fed for 21 days each, with 7 day washout period. 30% of energy from total fat, of which 10% was from palmitic acid. Dietary linolenic acid varied between 8 diets. Experiment 2: A high saturated and high *trans* diet was fed for 30 days, separated by 4 week	High saturated fat diet provided 13% of total energy from saturated fat, with 10.6% from palmitic acid. High *trans* diet gave 8% of total energy from saturated fat, 5.6% from *trans* fatty acids.	Total cholesterol increased by 6.6% after the *trans* diet relative to saturated fat diet, and an 11.5% increase in LDL cholesterol relative to saturated fat diet. Total cholesterol synthetic rate was significantly higher on high *trans* diet compared to high saturated fat, with a significantly greater fractional synthetic rate of free cholesterol underlying this difference.

			washout. At the end of each dietary period a deuterium experiment was carried to determine cholesterol synthesis and catabolism.		
Hodson, Skeaff and Chisholm, (2001)	Dietary intervention, New Zealand	University students studying Human Nutrition. 29 men and women in study 1, 42 in study 2.	High saturated fat diet compared with n-6 polyunsaturated fat diet in study 1, and then with monounsaturated fat diet in study 2.	Saturated fat diets provided 33.3% and 34.0% of total energy from saturated fat in experiments 1 and 2 respectively.	Total cholesterol decreased by 19% on the polyunsaturated fat diet and 12% on monounsaturated fat diet relative to high saturated fat diet. LDL cholesterol decreased by 22% on polyunsaturated and 15% on monounsaturated fat diets relative to saturated fat diet.
Idris, C. and Sundram, K. (2002)	Cross-over RCT, Malaysia	9 non-human primates Mean age unknown	6 weeks per diet, with a 4-week washout period between each. Eight diets in total, the following four prepared diets alone or with added cholesterol: *trans*; AHA (american heart association); palm olein (POL); lauric-myristic blend (LM)	9.3 % of energy from *trans* fatty acids in '*trans*' diet. Levels undetectable in others.	'*trans*' diet gave significantly higher LDL and LDL:HDL ratio than other three diets without addition of cholesterol. Addition of cholesterol resulted in *trans* diet being either not significantly different, or significantly lower in LDL and LDL:HDL ratio than other three diet preparations t18:1 n-9 followed by t18:1 n-11 were the most abundant *trans* fatty acids found in plasma of monkeys when on the '*trans*' diet without cholesterol. Addition of cholesterol raised t18:1 n-12 and t18:1 n-13 to levels comparable to t18:1 n-11

Jakobsen et al., (2004)	Cohort study, Denmark	3,686 Danish men and women aged 30 – 71 years, derived from four other cohort studies: 1914 and 1936 cohorts, MONICA I and III cohorts.	Follow up of participants recruited between 1964 and 1991, median follow up length 16 years (range 7 – 22 yrs). Diet assessed by 7 day weighed food record or interview. 98 women and 228 men died of coronary heart disease over follow up period.	Intake of saturated fat in women ranged from 14.1% total energy to 24.8% total energy in 10^{th} and 90^{th} centiles. Intakes in men ranged from 14.5% to 24.8% total energy in 10^{th} and 90^{th} centiles.	Multivariate relative risk (95% CI) for coronary heart disease mortality for a 5% increase in saturated fat contribution to total energy (replacing carbohydrates) was 1.36 (0.98, 1.88) for women and 1.03 (0.78, 1.37) for men. Analysis by age showed a greater effect amongst women aged less than 60 years.
Judd et al. (2002)	Dietary intervention, Latin-square design, USA	50 men, mean age 42 years	Six diets consumed for 5 weeks each. All food provided by study personnel. Diets were either a carbohydrate diet, or for the other five diets replacement of 8% of total energy from carbohydrate with oleic acid, stearic acid, a mixture of lauric, myristic and palmitic acids, *trans* fatty acids, or stearic and *trans* fatty acids.	*Trans* fatty acid intake was 0.2% of total energy on carbohydrate diet, 4.2% on combined *trans* fatty acid/stearic acid diet, and 8.3% on *trans* fatty acid diet. Individual saturated fatty acid intakes varied across diets.	The *trans* fatty acid, combined *trans* fatty acid and stearic acid, and combined lauric, myristic and palmitic acid diets gave the highest total cholesterol levels. LDL cholesterol was significantly higher on the high *trans* and combined *trans* and stearic acid diets. The high *trans* and combined *trans* and stearic acid diets gave the worst total:HDL cholesterol ratios.
Kabagambe et al., (2003)	Case control study, Costa Rica	Cases (n= 485) were survivors of first MI, both genders, mean age 58 years. Controls (n = 508) were matched on age, sex and area of residence.	Cases enrolled from metropolitan area of San Jose between 1995 and 1998. Dietary data collected with food frequency questionnaire.	Total saturated fat intake was 11.7% in controls and 12.4% in cases.	Multivariate risk for first MI was two to four fold higher in the highest quintile of intake vs. lowest for each of the individual saturated fatty acids. Subjects in the highest quintile of *trans* fatty acid intake (at 2.5% of total energy) had a relative risk (95% CI) of first MI of 1.83 (1.04, 3.25) compared to lowest quintile of intake. Odds ratios

					(95% CI) of first MI for 1% increase in total energy from lauric and myristic acids, palmitic acid, and stearic acid were: 1.08 (0.65, 1.81), 1.16 (1.01, 1.32), and 1.98 (1.08, 3.65) respectively.
Kaitosaari et al., (2003) - see STRIP project					
KANWU study	Randomised controlled dietary intervention, international	162 participants across five centres, slightly more men than women. Mean age 48-49 years across randomised groups. Subjects normal to moderately overweight, mean BMI 26 to 27 kg/m^2 across groups.	90 day dietary intervention of either a diet high in saturated fat, or high in monounsaturated fat with further divisions within each to n-3 supplementation or placebo supplements.	Diets were not significantly different in macronutrient composition and differed only in amounts of saturated and monounsaturated fat. Saturated fat intake was 17.6% total energy on high saturated fat diet, 9.6% total energy on monounsaturated fat diet.	High saturated fat diet significantly increased cholesterol content of LDL fraction relative to baseline, whereas it was significantly decreased on the monounsaturated fat diet. The difference between diets was highly significant. Mean treatment effect on LDL cholesterol levels was 0.34 (0.19, 0.49) mmol/l (p = 0.0001)
Kim and Campos, (2003)	Cross-sectional study, Costa Rica	Randomly selected subjects of 202 men and 212 women.	Diet assessed by semi-quantitative food frequency questionnaire. Principal measure of interest in the study was LDL particle size.	*Trans* fatty acid intakes generally very low, at 0.86% total energy in urban areas and 0.56% total energy in rural areas.	No significant association was found between LDL and HDL concentrations and *trans* fatty acid intake.
Kromhout et al., (2000)	Cohort study (Seven Countries	12,763 men aged 40-59 yrs enrolled from 1958 to 1964.	Diet assessed in random sub-samples of 14 of the 16 cohorts between 1959 and 1964, and in	Saturated fat intake ranged from 3.9% of total energy in Japan	Multivariate linear regression showed that total mortality over 25 years was most reliably predicted by saturated fat

	Study), International		1970 for the remaining two, by weighed diet record.	to 22.7% of total energy in Finland.	intake, smoking and vitamin C. A drop of 5% of energy intake from saturated fat was predicted to give a 4.7% decrease in all cause mortality. It should be noted the inclusion of saturated fat in the mortality prediction models was based on previously published analyses showing an effect of saturated fat on mortality.
Lee et al., (2000)	Cross-sectional study, Hong Kong	194 omnivores identified by random survey, matched to 60 volunteer vegetarians. Mean age approximately 40 years	Dietary assessment done by food frequency questionnaire. Blood samples collected for analysis of serum lipids and fatty acids.	Saturated fat intake 5.9 g/1000 kcal in vegetarians and 9.1 g/1000 kcal in omnivores	Total cholesterol and LDL concentrations higher in omnivores than vegetarians, although groups were not significantly different in terms of LDL:HDL ratio.
Lemaitre et al., (2002)	Case control study, USA	Cases were cardiac arrest patients enrolled in Seattle from 1988 to 1999 (n = 179). Controls were matched for age and sex (n = 285).	Fatty acid composition of red blood cell membranes determined by gas chromatography. Correlations with dietary fatty acid intakes ascertained in 111 controls who completed a food frequency questionnaire.	Not stated.	Total red blood cell membrane *trans* fatty acid content association with a higher risk of MI. *Trans* 18:1 were not associated with increased risk (Odds ratio (95% CI) for an increase of one interquartile range 0.77 (0.48, 1.24)). *Trans* 18:2 membrane fatty acids gave a greater risk of 3.05 (1.71, 5.44).
Matthan, N. et al. (2004)	Randomised cross-over dietary intervention, USA	8 postmenopausal women Mean age not stated (all over 50 yrs)	Three diets – 'unsaturated fat', 'saturated fat', 'hydrogenated fat', each followed for 5 week periods. Endpoints:	Unsaturated fat diet provided 0.6 % of total energy from *trans* fatty acids, saturated fat 1.3%, and hydrogenated fat	Hydrogenated fat diet resulted in significantly higher total and LDL cholesterol than unsaturated fat diet, but significantly lower than saturated fat diet. However hydrogenated fat diet resulted in significantly lower HDL

			Lipid and lipoprotein measures, HDL fatty acid profile and composition, apolipoproteins	6.7%.	than saturated fat diet. HDL cholesterol esters and phospholipid profile showed higher levels of t18:1 acids on the hydrogenated diet at the expense of monounsaturated acids. Data suggested that lower HDL on the hydrogenated fat diet was due to a higher rate of HDL catabolism. Agrees with earlier study (Journal of Nutrition,1997, **127**, 531S) which found the same in cebus monkeys.
Mensink, R. et al. (2003)	Meta-analysis of controlled dietary trials, international with majority of studies in USA.	All subjects in trials under consideration at least 17 years of age, total of studies included had 1672 volunteers, with 70% male, 30% female	60 controlled trails published between Jan 1970 and Dec 1998, which had: parallel, cross-over or Latin square design; only adult subjects; at least 13 days of dietary intervention; dietary fatty acids as only variable under alteration and food intake well controlled	For studies that measured total cholesterol, mean total fat intake was 34.3% (range 4.5 - 53.0%) and mean intake of saturated fat 10.2% (2.2 – 24.4%) of total energy.	Regression coefficient for a 1% change of total energy from carbohydrates for saturated fat, taken from a pool of 43 studies describing 102 different diets predicted a 1% increase in saturated fat would result in a mean (95% CI) increase in LDL of 0.032 (0.025, 0.039) mmol/l ($p = <0.001$).

Obarzanek, E. et al. (2001a)	Childhood randomised controlled trial (DISC study), USA	663 children aged 8 – 10 years with elevated LDL randomised.	Comparison of control and intervention arms: serum lipid measurements taken at 1 year, 3, years, 5 years and end of study. Mean length of follow up 7.4 years. Intervention group advice sessions started off more intense and frequent, gradually reducing with time.	Intervention group underwent behavioural diet sessions with the aim of achieving a total fat intake of 28% of total energy, with less than 8% of total energy from saturated fat (similar to NCEP step 2 diet).	Intervention group consumed less total fat and saturated fat than control group. Intervention children tended to have lower LDL cholesterol measures at all time points after baseline, but were only significantly lower at 1 year and 3 years, and not the final two time points at 5 years and end of study.
Obarzanek, E. et al. (2001b)	Randomised controlled outpatient feeding intervention (DASH study), USA	436 participants in 4 centres. Mean age 44.6 yrs, 60% African American, approximately equal numbers of men and women.	Comparison of a control diet, a diet high in fruit and vegetables, and the DASH diet (Dietary Approaches to Stop Hypertension).	Control diet provided 37% total energy from fat, with 14% from saturated fat. Fruit and vegetable diet provided 37% total energy from fat with 13% from saturated fat. DASH diet provided 27% total energy from fat with 7% from saturated fat.	DASH diet resulted in significantly lower total cholesterol, LDL and HDL cholesterol. No significant changes were seen in these measures on the fruit and vegetable diet. Men showed a greater reduction in total and LDL cholesterol on the DASH diet than women.
Oh, K. et al. (2005)	Case control (nested in Nurses Health Study), USA	78,778 women followed over 20 years: 1,766 CHD cases 30 – 55 years at entry in 1976.	Food frequency questionnaire administered in 1980, ‘86, ‘90, ’98, analysis used an updated average intake based on these. Endpoints:	Median intake of 1.3 % of total energy from *trans* fatty acids in lowest intake quintile, 2.8 % in highest quintile	Multivariate relative risk (95% CI) of CHD in highest quintile of *trans* fatty acid intake of 1.33 (1.07, 1.66). P-value for trend across quintile in multivariate model = 0.01. Intake of *trans* fat decreased from 1980

		Median age (or mean age, not clearly stated) of highest and lowest quintiles of *trans* fatty acid intake 55 and 57 years respectively.	Non-fatal MI or fatal CHD between 1980 and June 2000.		to 1998 from 2.2 % total energy to 1.6 % total energy (relative reduction of 40%). *trans* fat intake most strongly related to risk of CHD in women less than 65 years, and women with a lower BMI, although interaction effects were not significant.
Oomen, C. et al. (2001)	Prospective cohort study (Zutphen Cohort study), Netherlands	667 elderly men Mean age 71.1 years	Dietary surveys conducted in 1985, 1990 and 1995. Endpoints: Fatal or non-fatal coronary heart disease	Average fatty acid intake decreased from 4.3% total energy in 1985 to 1.9% total energy in 1995, largely due to t18:1 reduction.	Relative risk (95% CI) of CHD at 10 years follow-up after multivariate adjustment was 2.00 (2.07 – 3.75) in highest tertile of baseline *trans* fatty acid consumption compared to lowest tertile. Also gives pooled RR of CHD of four prospective cohort studies (includes this one): an increase of 2% in total energy from *trans* fatty acid gives a RR (95% CI) of CHD of 1.28 (1.01 – 1.61)
Pedersen, J. et al. (2000)	Case control, Norway	100 patients and 98 controls. Men and post-menopausal women Mean age 62.4 years (range 45 – 75 years)	Recruited in 1996 after first non-fatal MI. Endpoints: Adipose tissue fatty acid measured, anthropometric and clinical measures, dietary and CVD risk factor questionnaire	Mean *trans* fatty acid intake in highest quintile was 4.75 % of total energy	Adipose tissue t18:1, t20:1 and total *trans* fatty acids higher in cases than controls. OR (95% CI) for MI in highest intake quintile 2.25 (0.78 – 6.48), p-value for trend = 0.03 adjusting for age, sex, waist-hip ratio, smoking and family history of CVD. Further adjustment for α - linoleic and α - linolenic acid reduced the OR.

Poppitt, S. et al. (2002)	Dietary controlled trial, New Zealand	20 normal weight healthy males	Two diets compared with two dietary intervention periods of 3 weeks, separated by a minimum 4 week washout. Subjects lived in the research unit during diet periods. Diets varied only in the type of butter included – standard butter vs. a modified butter produced by a different bovine feeding regimen. Modified butter lower in total saturated fat and lauric, myristic and palmitic acids but slightly higher in stearic acid.	Control diet provided 40% of total energy from total fat with 20% from saturated fat. Modified butter diet provided 39% total energy from total fat, with 15% from saturated fat. Dietary cholesterol was also lower on the modified butter diet.	Modified butter diet resulted in significantly lower total and LDL cholesterol, this emerged as significant at 3 weeks of dietary intervention. No significant difference between treatments was seen in effects on HDL cholesterol. As a percentage change from baseline, control diet resulted in a 2.4% decrease in LDL cholesterol, and modified butter diet a 9.5% decrease.
Rasanen, M. et al. (2004) - See STRIP project					
Rask-Nissila, L. (2000; 2002) - See STRIP project					
Rivellese, A. et al. (2003) - See KANWU study.					
Rogers, I. et al. (2001)	Childhood cohort study (ALSPAC study), Britain	A proportion of the whole cohort was enrolled in a sub-study, Children In Focus. 951 children included at 18 months of age follow-up, 805 children at 43 months of age follow-up.	Diet assessed at 18 and 43 months of age using unweighed 3 day diet records. Nonfasting blood samples collected at 31 and 43 months of age.	Fat intake at 18 months of age ranged from 31.2% of total energy in the lowest quartile to 43.1% of total energy in the highest, and at 43 months from 30.4% of total energy to 41.8% across quartiles.	No association was seen between quartile of fat intake at 43 months and blood lipids at 43 months. Significant associations were seen with quartile of fat intake at 18 months and total cholesterol level at 31 months. A similar effect of quartile of fat intake and non-HDL cholesterol was seen of marginal significance at $p = 0.054$. A gender interaction of borderline significance was seen ($p = 0.052$) with the cholesterol response across quartiles

					of fat intake seeming to differ between genders.
Salo, P. et al. (2000) - see STRIP project					
STRIP project	Childhood cohort study Finland.	540 children in intervention arm, 522 in control arm.	Individualised counselling sessions given to intervention families regarding dietary and health advice, aimed at reducing saturated fat intake. Blood and clinical measurements taken at various points throughout study, as well as behavioural and cognitive assessment.	Dietary advice in intervention children aimed to meet Nordic Dietary Recommendations for children from the age of three of 30% total energy from fat, with 10% or less from saturated fat.	Intervention boys had 0.20 to 0.39 mmol/l lower total cholesterol than control boys throughout 7 years of follow-up. No differences in cholesterol levels were seen in intervention girls relative to control girls.
Toeller, M. et al. (1999)	Cohort of diabetes patients, Europe	2,868 subjects with type 1 diabetes. Mean age 32.9 yrs. Models for total cholesterol included 2,762 subjects, and 1,816 for LDL cholesterol. Approximately equal gender mix.	Diet assessed by 3-day diet records. Incidence of CVD determined by clinical records or resting ECG. Regression analyses performed to determine links between fat intake and incidence of CVD.	Mean intake of saturated fat in terms of percentage of total energy across quartiles were 10.1%, 12.7%, 15.1% and 17.9%.	Energy-adjusted OR for incidence of CVD increased across quartiles and was highly significant ($p = 0.02$), however a fully adjusted model had ORs above 1.0 but none of the quartiles had 95% CIs which excluded 1.0. The beneficial effects of consuming low intakes of saturated fat were restricted to those consuming less than 8% of total energy from saturated fat; mean LDL in this group was significantly lower than the rest of subjects.

van de Vijver, L. et al. (2000)	Cross-sectional study (TRANSFAIR study), eight European countries	327 men and 299 women aged 50-65 years.	Diet assessed by diet history questionnaire. Adipose tissue samples collected from upper buttock and fatty acid composition of adipose tissue determined by gas chromatography.	Mean *trans* fatty acid intakes 0.87% of total energy for men and 0.95% of total energy for women. Highest consumption was in Iceland, lowest in Mediterranean countries.	After adjustment for cardiovascular risk factors, no association was found between total *trans* fatty acid intake and LDL or HDL cholesterol or LDL:HDL ratio. Adjustment for other fatty acids showed a significant inverse relationship between *trans* fatty acid intake and total cholesterol, contributed to mainly by t18:1, the most abundant isomer consumed. Individual *trans* fatty acid isomers varied in their relationship with total and LDL cholesterol.
Vermunt, S. et al. (2001)	Randomised dietary intervention, France, Scotland, Netherlands, UK.	88 healthy men aged between 18 and 55 years.	Subjects consumed a *trans* fatty acid free diet for 6 weeks as a run-in and then were randomised to either a high- or low- *trans* alpha-linolenic acid diet.	High *trans* diet gave significantly lower intakes of saturated fatty acids and monounsaturated fatty acids. High *trans* diet gave a mean of 1410 mg/day of *trans* alpha-linolenic acid vs. 60 mg/day on low *trans* diet.	Mean treatment effect (95% CI) of high vs. low *trans* diets was an increase of 4.7 (-0.8, 10.5) % in LDL ($p = 0.10$) and increase in total:HDL cholesterol ratio on high *trans* diet of 5.1 (0.4, 9.9) % ($p = 0.03$). Mean body weight increased slightly in high *trans* group and decreased slightly in low *trans* group such that the change was significantly different between groups (mean difference 0.6 (SD 2.4) kg, 95% CI: 0.1, 1.1; $p = 0.03$) which may have influenced results.
Vessby, B. et al. (2001) - see KANWU study					

Table 2.2: Relevant reviews, editorial comments and other documents consulted regarding the link between dietary *trans* fatty acids and coronary heart disease/ cardiovascular risk factors, published 1999 – 2005.

Author	Agency or citation	Article type	No. refs	Title and Sections	Comments
Ascherio, A.	*Am J Med*, 2002, **113**(9b): S9-S12	Review/ Symposium	11	'Epidemiologic studies on dietary fats and coronary heart disease' - Total fat - Saturated fat - *Trans* fatty acids - Monounsaturated fatty acids - Polyunsaturated fatty acids - Conclusion	
Ascherio, A. et al.	*N Engl J Med*, 1999, **340**:1994-1998	Sounding Board	31	*'Trans* fatty acids and coronary heart disease' - Metabolic studies - Epidemiologic Studies - Conclusions	Provides figure of pooled studies of change in LDL:HDL cholesterol ratio vs. percentage of energy from saturated or *trans* fatty acids
Brousseau, M. and Schaefer, E.	*Curr Atheroscl Rep*, 2000, **2**:487-493.	Review	48	'Diet and coronary heart disease: clinical trials' - Introduction - Selected dietary intervention trials using CHD morbidity and mortality as endpoints - Selected dietary intervention trials using angiography as an endpoint - Conclusions	Notes studies of particular importance and a summary of their findings in references

Hu, F. and Willett, W,	*JAMA,* 2002, **288**:2569-2578	Clinical Cardiology Review	147	'Optimal Diets for prevention of coronary heart disease' - Methods - Dietary fat - Carbohydrates - Folate - Specific foods and dietary patterns - Combined effects of diet and lifestyle - Areas of uncertainty - Conclusions	
Hu, F. et al.	*J Am Coll Nutr*, 2001, **20**:5-19.	Review	143	'Types of dietary fat and risk of coronary heart disease: a critical review' - Introduction - Major types of dietary fat - Nut consumption and risk of CHD - Intervention trials of dietary fat - Fish and marine n-3 fatty acids - Alpha-linolenic acid (ALA) - The balance between n-3 and n-6 fatty acids - Dietary cholesterol and eggs - Conclusions	Provides a review of individual saturated fatty acids, *trans* fatty acids, and studies with clinical endpoints or angiographic endpoints
Katan, M.	*Nutr Rev,* 2000, **58**:188-191.	Review and Editorial Comment	25	*'Trans* fatty acids and plasma lipoproteins' *not divided into sections*	Adapts table from Ascherio et al. (1999)
Lichtenstein, A.	*Curr Opin Lipidol*, 2003, **14**:1-2	Editorial review	13	*'Trans* fatty acids: where are the dietary recommendations?' - Introduction - How significant a contribution do *trans* fatty acids	

				make to total energy intake? - Are dietary *trans* fatty acids worse than saturated fatty acids? - How low is practical to recommend for *trans* fatty acid intakes? - Do we need a new rubric for dietary saturated and *trans* fatty acid recommendations?	
Lichtenstein, A.	*Curr Opin Lipidol,* 2000, **11**:37-42.	Review	47	*'Trans* fatty acids and cardiovascular disease risk' - Introduction - Lipid, lipoprotein, apolipoprotein and fatty acid levels - Plasma fatty acid levels - Hemostatic factors - Current intake - Adipose tissue levels and dietary intake - Other effects of *trans* fatty acids - Conclusion	Reviews papers from 1998 to publication (2000); Notes references of particular importance and brief summary of their findings
Reddy, K. and Katan, M.	*Public Health Nutr*, 2004, **7**(1A):167-186.	Review	149	'Diet, nutrition and the prevention of hypertension and cardiovascular diseases' - Global dimensions of the CVD epidemic - Diet and CVD: methodological issues in the study of causal associations - Nutrients and CVD - Minerals: blood pressure and CVD - Food items and food groups - Dietary patterns and composite dietary interventions - Implications for policy - Diet and CVD: summary of evidence and recommendations	

Stender, S, and Dyerberg, J.	Danish Nutrition Council, 2003.	Report	78	'The influence of *trans* fatty acids on health, fourth edition' - *Trans* fatty acids in the diet and disease - *Trans* fatty acid levels in the Danish diet - Legislation relating to the level of industrially produced *trans* fatty acids in food - Conclusion - Recommendations	Includes sections on *trans* fatty acids and heart disease, epidemiologic studies, prospective studies, and *trans* fatty acids and CHD risk factors

APPENDIX 2.1: OVID search history

Ovid Technologies, Inc. Email Service

Search for: limit 18 to english language
Results: 1-1937

Database: Ovid MEDLINE(R) <1999 to June Week 4 2005>
Search Strategy:
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1 *trans* fatty acid.mp. or Trans Fatty Acids/ (138)
2 Cholesterol, Dietary/ or Fatty Acids/ or Dietary Fats/ (14865)
3 saturated fat.mp. (955)
4 hydrogenated fat.mp. (21)
5 MEAT/ or MEAT PRODUCTS/ (4701)
6 eggs.mp. or EGGS/ (7802)
7 animal fat.mp. (167)
8 1 or 2 or 3 or 4 or 5 or 6 or 7 (27327)
9 cardiovascular disease.mp. or Cardiovascular Diseases/ (25940)
10 coronary heart disease.mp. or Coronary Disease/ (23511)
11 cvd.mp. (2262)
12 chd.mp. (3599)
13 ldl.mp. (16107)
14 LIPOPROTEINS, LDL/ (4624)
15 Lipoproteins, LDL Cholesterol/ (5180)
16 low density lipoprotein.mp. (10237)
17 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 (61967)
18 8 and 17 (2052)
19 limit 18 to english language (1937)