

Narrative review: The relationship between dietary trans-fatty acids and adverse health outcomes

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Executive summary

Evidence from previous reviews suggests that dietary *trans*-fatty acid (TFA) intake is positively associated with risk of coronary heart disease and cardiovascular disease. TFA intake has also been linked to increased risk of other health conditions, however these relationships appear to be less consistent. This narrative review aimed to build on the risk assessment included in the 2009 FSANZ report of *Trans Fatty Acids in the New Zealand and Australian Food Supply*, by evaluating the recent evidence around TFA intake and associations with a variety of chronic disease outcomes, including CVD and CHD, cancer, and type 2 diabetes. We also briefly reviewed studies examining other health conditions including macular degeneration, asthma, and dementia.

We searched databases and reference lists to identify 46 studies suitable for inclusion in the review. Most associations reported by the studies were not significant (55%), but there were more positive associations reported (35%) than inverse associations (10%), indicating the balance leans towards a detrimental effect of TFA. Direction of association did not appear to differ by gender, TFA assessment type (dietary or serum) or study design (prospective vs case-control or cross-sectional). Total mortality, CVD/CHD and colorectal, pancreatic and prostate cancer demonstrated more positive or neutral associations with TFA, while results for type 2 diabetes and breast cancer were more evenly distributed. A category of other health conditions, including macular degeneration, dementia, asthma and eczema, also showed mixed results.

The differences observed between outcomes may be due to differences in study populations, whether different factors such as blood lipids or membrane fluidity are involved in disease progression, and potential differences in effects of specific TFAs. Future research is required to better separate out the effects of TFA from other dietary components within the context of different health conditions.

1. Purpose

To provide Food Standards Australia New Zealand (FSANZ) with a synthesis of available evidence on the relationship between dietary *trans*-fatty acid (TFA) intake and adverse health outcomes including coronary heart disease (CHD), cardiovascular disease (CVD), cancer and type 2 diabetes.

2. Background

This review builds on the risk assessment included in the 2009 FSANZ report of *Trans Fatty Acids in the New Zealand and Australian Food Supply (Food Standards Australia New Zealand, 2009)*. The 2009 risk assessment reviewed the scientific literature in the area of TFA intake and adverse health outcomes and found there was a well-established relationship between intake of TFA and increasing risk of cardiovascular disease. This was consistent with findings of the 2007 FSANZ report of the same name and the 2005 Booker and Mann report. However, it was concluded in the 2009 report that the relationship between TFA intake and other health outcomes is less well established as not many studies were available in these areas. Therefore, this review will focus on new research published from 2009-2014 in the area of CHD and CVD, as well as publications published earlier that report on risk of other adverse health outcomes.

2.1 Structure of *trans*-fats

In most naturally occurring unsaturated fats, the two carbon atoms either side of the double bond bind a hydrogen atom on the same side of the bond in a *cis*-isomer form (see Figure 1a). Having both hydrogens on the same side causes the fatty acid molecule to bend, with more double bonds creating more bends. Unsaturated fats can also occur in a *trans*-isomer form, with hydrogen atoms on opposite sides of the two carbon atoms either side of the double bond (see Figure 1b). Having one hydrogen on either side creates a straight, rather than bent molecule. Unsaturated *cis*-fats in membranes are bent, allowing fatty acids to pack loosely and make the membrane more fluid. In contrast, *trans*-fats pack into the membrane tightly more like saturated fatty acids, decreasing the fluidity (Mahan & Escott-Stump, 2008).

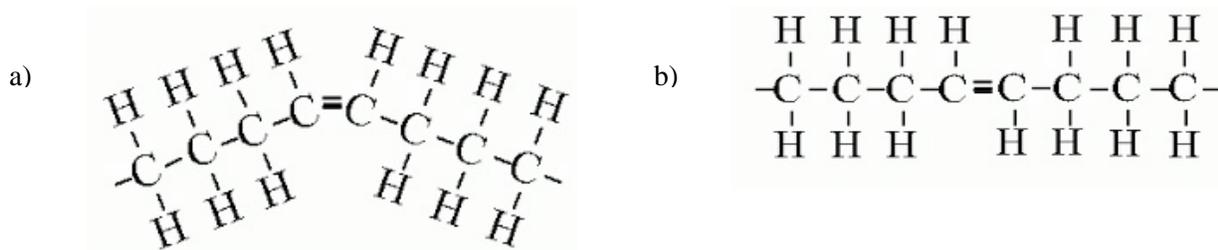


Figure 1 a) The *cis* configuration of fatty acids showing two hydrogen atoms on the same side, creating a bent molecule, b) the *trans* configuration of fatty acids showing a hydrogen atom on each side, creating a straight molecule.
Diagrams adapted from: <http://legacy.owensboro.kctcs.edu/>

2.2 Sources of TFA

Naturally occurring *trans*-fats are produced through bio-hydrogenation in ruminant animals. Cis-unsaturated fatty acids from feed, including grass and seeds, undergo partial hydrogenation and conversion to TFAs through a process of substrate oxidisation, with bacterial enzymes acting as catalysts. Products of ruminant animals including meat, milk, butter and cheese therefore contain naturally occurring TFAs. Artificially occurring *trans*-fats are produced by partial hydrogenation of unsaturated vegetable oils using hydrogen gas and a metal catalyst. Partially hydrogenated vegetable oils were originally developed to be “healthier” alternatives to saturated fats such as lard and butter. The process of hydrogenation creates a more stable, solid fat such as those used in some margarines, and can be used in baked goods, snacks and frying oils. Unsaturated fish oils can also be hydrogenated using the same process.

The research studies synthesised in this review focus on TFAs in the form of conjugated linoleic acid (CLA), elaidic acid, linolelaidic acid, palmitelaidic acid and vaccenic acid. **Table 1** shows the name, class, main sources and predominant type of TFAs (both ruminant and industrial forms of the same TFA may be found in some cases).

Table 1: Common TFA investigated in the studies reviewed

Name	Class	Main source/s	Predominant type
Conjugated linoleic acid	18:2, trans-10 18:2, trans-11	Ruminant meat and dairy fat, supplements	Ruminant
Elaidic acid	18:1, trans-9	Hydrogenation of vegetable oils	Industrial
Linolelaidic acid	18:2, trans-9, 12	Hydrogenation of vegetable oils	Industrial
Palmitelaidic acid ^a	16:1, trans-9	Ruminant dairy fat & hydrogenation of vegetable oils	Ruminant & industrial
Vaccenic acid	18:1, trans-11	Ruminant meat and dairy fats	Ruminant

^aPalmitelaidic acid is the *trans*-isomer of the 16:1 fatty acid palmitoleic acid.

2.3 TFA in the New Zealand and Australian food supply

The 2009 FSANZ report estimated mean TFA intakes to be 1.3 g/day and 1.6 g/day for Australians (17 years and above) and New Zealanders (15 years and above), respectively (Food Standards Australia New Zealand, 2009). This equates to approximately 0.5% and 0.6% of total energy intake, respectively (Food Standards Australia New Zealand, 2009). In comparison to the World Health Organisation goal of no more than 1% of total daily energy intake from TFA (Uauy *et al.*, 2009), more than 90% of Australians and 85% of New Zealanders had intakes below this level in 2009 (Food Standards Australia New Zealand, 2009). In terms of TFA intake from specific food groups, dairy products, pastry products, fats, and oils, meat and poultry, and cereal based foods were identified as the main contributors for both countries in 2009, with the majority of TFA coming from foods with a food label (Food Standards Australia New Zealand, 2009).

Mean intakes of both ruminant and industrial TFAs have fallen since the 1990's, when total TFA intake in Australia was estimated to be closer to 2.0%-2.5% of total energy intake (Noakes & Nestel, 1994). Approximately 60% of TFA at this time was from industrial sources, with margarines being the major contributor (Noakes & Nestel, 1994). The drop in industrial TFA has been greater than for ruminant intake. The use of partially hydrogenated fats has been decreased or removed in many product categories including margarine, while for ruminant TFA,

TFA free palm oil has become more popular than tallow for frying foods and as shortening in baked goods (Shrapnel, 2012). One of the catalysts for change was a change in Heart Foundation Tick program for margarines to move from a limit on saturated fats to a limit on both saturated and *trans*-fats, introduced in 1996. Changes to food production techniques and novel ingredients have now occurred which allow for production of edible oil spreads with nil or minimal TFA content. This includes modification of the chemical hydrogenation process, production of oil seeds with modified fatty acid composition, use of tropical oils (eg, palm oils, palm kernel oil, and coconut oil) and interesterification of mixed fats (Chardigny *et al.*, 2008).

2.4 TFA and health outcomes

The majority of reviews of TFA and health outcomes have focused on CHD and CVD, and there is consistent evidence from both trials and prospective observational studies that TFA is positively associated with risk of CHD and CVD (Micha & Mozaffarian, 2009; Mozaffarian *et al.*, 2009; Teegala *et al.*, 2009). These associations may be due to TFA causing increases in low-density lipoprotein (LDL) cholesterol, decreases in high-density lipoprotein (HDL) cholesterol, increases in inflammation and changes in fluidity of cell membranes.

Despite evidence for the association between TFA and CHD and CVD, there is debate regarding whether ruminant TFA have the same effects as industrial TFA, given differences in fatty acid structure. The 2009 WHO Scientific update on TFA concluded that there is sufficient epidemiological and experimental evidence to recommend the need to significantly reduce or to virtually eliminate industrially produced TFA from the food supply (Uauy *et al.*, 2009). The focus was on industrial TFA rather than ruminant as industrial TFA are not present naturally in foods and have no known health benefits (Mozaffarian *et al.*, 2009).

Evidence around the effects of ruminant TFA remains less certain. A recently published systematic review and meta-analysis of evidence from observational studies assessing intake of TFA and CHD with emphasis on distinguishing between industrial and ruminant sources not only found no association between ruminant TFA intake and CHD but also found no association

between intake of industrial TFA and CHD, although there was a trend towards a positive association for the latter (Bendsen *et al.*, 2011). A number of review articles have concluded that observational studies do not support adverse health effects of TFA intake in the amounts consumed in the usual diet of populations studied (Motard-Belanger *et al.*, 2008; Mozaffarian *et al.*, 2009).

TFA intake has also been linked to increased risk of other chronic conditions including cancer and type 2 diabetes, however these relationships are less consistent. A review in this area concluded that although emerging evidence suggests TFA consumption may promote insulin resistance, further research is required to clarify the effects of dietary TFA on insulin resistance, adiposity and type 2 diabetes, especially in high risk populations (Micha & Mozaffarian, 2009). The relationship between dietary TFA intake and cancer is also poorly understood. A mini-review of experimental and human studies of colon, breast and prostate cancer found inconsistent and insufficient evidence to link development with TFA intake (Smith *et al.*, 2009).

We aimed to undertake a narrative review to evaluate the recent evidence around TFA intake and associations with a variety of chronic disease outcomes, including CVD and CHD, cancer, and type 2 diabetes. We also aimed to briefly review studies examining other health conditions including macular degeneration, asthma, and dementia.

3. Methods

This review is narrative by nature and while comprehensive it is not systematic. We searched EMBASE for relevant material. Our search terms were: (Trans fat or TFA or trans MUFA or trans PUFA or hydrogenated oil or vegetable oil or ruminant or vaccenic acid or vaccenyl or elaidic acid or octadecenoic acid or conjugated linoleic acid or conjugated linoleyl or CLA or hexadecenoic acid or palmitoleic acid or palmitelaidic) AND (cancer or cardiovascular disease or stroke or coronary heart disease or Type 2 diabetes or macular degeneration or asthma or dementia or alzheimer's disease or birth weight or fetal blood)). In addition, reference lists of key papers were hand searched to identify additional material for inclusion in the review.

Inclusion criteria were a medically diagnosed health outcome, original research (not reviews), human studies and English language studies. Exclusion criteria were self-reported diagnosis of health outcome, and studies of CHD/CVD published prior to 2009.

We accepted any form of TFA status assessment, including dietary intake and serum TFA levels.

For each eligible study, information on study design, subjects, methods, confounding factors adjusted for and results were extracted. This included type of TFA investigated, method of assessment of TFA, and dietary assessment.

4. Results

Our search generated 109 results. Of these, 46 were review articles and 17 did not meet our inclusion or exclusion criteria. We identified five studies on CVD/CHD, four on type 2 diabetes, 24 on cancer and one on all-cause mortality. In addition, we identified 12 studies in other health conditions. Studies had differing ways of assessing TFA. The most common was dietary assessment via a food frequency questionnaire, followed by serum TFA used as biomarkers of intake. In one study both these techniques were employed.

Study details are summarised in **Appendix 1**. Some studies reported more than one association between the health outcome and TFA, for example, if results differed by gender or by assessment type (dietary or serum). Where data on TFA were lacking for conditions classified in the 'other' category, we included a small number of studies on margarine intake as a proxy for TFA (**Appendix 1**). We identified a total of 58 reported associations between adverse health outcomes and TFA— 20 (35%) showed a significant positive association, 32 (55%) showed a neutral/no significant association and six (10%) showed a significant inverse association (**Appendix 2**). Results across both serum TFA and dietary TFA studies appear to be relatively consistent. Both serum TFA studies and dietary TFA studies were more likely to report no association with adverse outcomes than a positive or inverse association (**Appendix 2**). Where gender was split or the study only investigated one gender, more findings were reported in

females than males. In females the distribution over positive, neutral or negative associations was approximately equal to the overall distribution, while in males the majority of associations were neutral. Most studies identified in this review were of a prospective design, generally considered to be a stronger study design than case-control or cross-sectional studies. The distribution of findings in prospective studies were similar to the overall findings.

4.1 TFA and CVD or CHD

This review updates the 2009 FSANZ risk report (Food Standards Australia New Zealand, 2009) with five new studies investigating TFA in relation to CHD or CVD. These five studies reported seven associations between TFA and CHD or CVD. Four of these studies measured TFA through dietary intake and one through serum (**Appendix 1 and 2**).

Two of the five studies reported varying results as a result of sub-analysis. One prospective study found no association with sudden cardiac death and TFA in women without a history of CHD, but a positive association in those women who did (Chiuve *et al.*, 2009). However, the interaction effect was not significant and the cohort was generally healthy with only small numbers of women with a prior history of CHD. Likewise, the second found a positive association with ischaemic stroke, but only in women who were non-aspirin users; those who used aspirin showed no association (Yaemsiri *et al.*, 2012). Of the additional three studies that did not report varying results, two were case control studies. One reported a significant positive association between dietary TFA and CHD/CVD (Mashal *et al.*, 2012), while the other reported no significant associations in levels of TFA in the blood and CHD (Khaw *et al.*, 2012). The third study was prospective and reported a variety of associations from partially hydrogenated vegetable and fish oils and ruminant TFA (Laake *et al.*, 2012).

4.2 TFA and type 2 diabetes

We identified a total of four studies that reported four associations between risk of Type 2 Diabetes and TFA. Three measured TFA through diet and one used serum TFA (**Appendix 1 and 2**).

Two of these studies found no association between TFA intake and type 2 diabetes (Papantoniou *et al.*, 2010; van Dam *et al.*, 2002). These studies all used dietary TFA assessment. A third dietary study found a positive association (Salmeron *et al.*, 2001) while the fourth study found an inverse association between TFA as assessed using serum and type 2 diabetes (Mozaffarian *et al.*, 2013). Three of these studies were prospective (Mozaffarian *et al.*, 2013; Salmeron *et al.*, 2001; van Dam *et al.*, 2002) and one cross-sectional (Papantoniou *et al.*, 2010).

4.3 TFA and cancer

We identified a total of 24 studies that reported 33 associations between cancer risk and TFA as measured through serum or in the diet (**Appendix 1 and 2**). Overall, 20 reported associations were neutral, 9 were positive and three associations were inverse.

One extensive study by Laake and colleagues (2013) reported total cancer along with 19 specific types; the remainder reported on one type of cancer only. The Laake *et al* study (2013) reported varying cancer risk by type of TFA as assessed by food frequency questionnaire, either as partially hydrogenated vegetable oil, partially hydrogenated fish oil, or ruminant. For total cancer, an inverse association was observed with partially hydrogenated vegetable oil, while ruminant TFA showed a positive association (both $P < 0.01$). No significant association was observed with partially hydrogenated fish oil and total cancer. For specific cancers, five significant inverse associations (pancreatic in men, malignant melanoma in men, non-melanoma skin cancer, central nervous system cancer in women, non-Hodgkin lymphoma) and no significant positive associations were observed with partially hydrogenated vegetable oil. Both partially hydrogenated fish oil and ruminant TFA were positively associated with three types of cancer (stomach, multiple myeloma, lung in men who never smoked; and mouth/pharynx, non-Hodgkin lymphoma and breast cancer in postmenopausal women, respectively) and inversely associated with two types (lung in women and prostate; and malignant melanoma in women and multiple myeloma, respectively) (Laake *et al.*, 2013).

The most common type of cancer investigated in the other studies was breast cancer. Of the 11 studies investigating breast cancer, two found inverse associations with TFA (Aro *et al.*, 2000; Holmes *et al.*, 1999), with the case-controlled Aro *et al.* (2000) study reporting inverse associations for both serum and dietary TFA. One study reported both a positive association for serum *trans*-palmitoleic acid, and a neutral association for elaidic acid and breast cancer (Chajes *et al.*, 2008). A further two studies found positive associations (Kohlmeier *et al.*, 1997; Voorrips *et al.*, 2002) and six did not detect a significant association (Byrne *et al.*, 2002; Chajès *et al.*, 1999; McCann *et al.*, 2004; Rissanen *et al.*, 2003; Saadatian-Elahi *et al.*, 2002; Sczaniecka *et al.*, 2012).

Colorectal cancer, including colorectal adenomatous polyps, was the next most common type of cancer investigated with six studies. Two studies found a significant positive association in women, but not in men (Slattery *et al.*, 2001; Theodoratou *et al.*, 2007) (**Appendix 1**). Of the remaining four studies, two prospective (Limburg *et al.*, 2008; Lin *et al.*, 2004) and two case-controlled studies (McKelvey *et al.*, 1999; Vinikoor *et al.*, 2009) did not detect a significant association. However the Lin *et al.* study reported a positive association was seen between intake of fried foods away from home and colorectal cancer, in which TFA from partially hydrogenated vegetable oils may be a contributing factor. No studies identified any inverse associations for this type of cancer, and none used serum TFA (**Appendix 1**).

Three prospective studies investigated pancreatic cancer and dietary intake of TFA. Two (Heinen *et al.*, 2009; Michaud *et al.*, 2003) reported neutral associations, while the third reported a significant positive association with palmitelaidic acid but no significant associations with total TFA (Thiébaud *et al.*, 2009).

A further three studies investigated prostate cancer. One prospective study (Schuurman *et al.*, 1999) found no significant association, and two case control studies (Chavarro *et al.*, 2008; King *et al.*, 2005) both found significant positive and neutral associations. Both studies which found positive associations used serum TFA assessment rather than dietary intake. The King *et al.*

study reported positive associations between C:18 TFA but not C:16 TFA, whereas the Chavarro *et al.* study reported no association for total TFA and prostate cancer but a positive association between total TFA 18:2 and non-aggressive prostate tumours.

4.4 TFA and all-cause mortality

The study we identified investigating TFA intake and all-cause mortality (Kiage *et al.*, 2013) was conducted in the US where CVD, CHD, diabetes and cancer are among the leading causes of death, as they are in Australia and New Zealand. This study reported a positive association between dietary TFA intake and mortality, but it was only significant at higher intakes of TFA (defined as 3.45% of energy or above) (**Appendix 1**).

4.5 TFA and other conditions

In our search for literature on other health conditions potentially relevant to TFA, we looked for research on macular degeneration, asthma, dementia and Alzheimer's disease (see **Appendix 1** and **2**.)

We identified two studies in the area of age-related macular degeneration (AMD). A large Australian cohort study examined the relationship between AMD and dietary fats including TFA (Chong *et al.*, 2009). Higher TFA intake was associated with an increased prevalence of late stage AMD, with an odds ratio of 1.76 comparing the highest with the lowest quartile of TFA intake, although this result did not reach statistical significance (95% CI: 0.92-3.37). Cho *et al.* (2001) also reported a positive relationship between TFA intake and AMD among 567 AMD patients identified in the Nurses' Health Study, although additional adjustment for other fats attenuated this relationship.

The only cohort study we found investigating dementia or Alzheimer's disease was the Rotterdam Study, which found no association between TFA or other fat consumption and risk of any type of dementia (Engelhart *et al.*, 2002). A case-control study showed higher levels of

vaccenic acid in plasma of patients with Alzheimer's disease as determined via examinations and magnetic resonance imaging (Iuliano *et al.*, 2013).

We found one study examining a possible association between TFA intake and asthma or allergies. An ecological association was observed between rates of childhood asthma, allergic conjunctivitis and atopic eczema and consumption of TFAs, with higher rates observed in countries with higher average consumption of TFA, using data from the large International Study of Asthma and Allergies in Childhood (Wieland *et al.*, 1999). Although no other studies of TFAs were found in this category, Nagel and Linseisen (2005) reported a borderline positive association ($p=0.05$) between consumption of margarine and asthma in adults using data on 105 adult onset asthma cases from the European Prospective Investigation into Cancer and Nutrition. Similarly, Kim *et al.* (2005) reported a positive association between margarine consumption and asthma, along with a negative association between butter consumption and asthma in school children. Sausenthaler *et al.* (2006) reported a positive association between margarine intake and eczema and allergic sensitization to inhaled allergens.

Significant correlations have been reported between maternal dietary TFA and TFA in foetal plasma and erythrocytes, suggesting a potential role in birth outcomes (Enke *et al.*, 2011). With regards to birth weight, results are mixed. van Eijsden *et al.* (2008) reported lower birth weight of between 50 and 170 grams for babies of mothers in the highest quintile of TFA consumption in a prospective pregnancy cohort study. On the other hand, Dirix *et al.* (Dirix *et al.*, 2009) reported no association between dietary TFA and birth weight or foetal dimensions, and Cohen *et al.* (2011) reported greater foetal growth associated with higher second trimester consumption of TFA.

5. Discussion

5.1 Overall findings and study quality

Taken as a whole, our findings regarding TFA and risk of adverse health outcomes show inconsistent results. Most reported associations were not significant (55%), but there were more positive associations reported (35%) than inverse associations (10%). On balance, this indicates a detrimental effect of TFA is more likely overall. Findings by study types (case-control vs prospective) were relatively evenly distributed, despite the prospective type generally considered to be a stronger study design. Likewise, serum TFA and dietary TFA assessment both showed a similar distribution of positive, neutral and inverse associations. Fatty acids not synthesized endogenously such as TFA have been shown to correlate well with the diet (Hodson *et al.*, 2008), and serum levels of TFA seem to largely reflect dietary intakes (Vidgren *et al.*, 1998). The use of serum measures in conjunction with dietary intake would help validate dietary intake. This may be particularly useful where dietary intake assessment involves methods with a high risk of recall bias or portion size estimation error. Of the 34 studies reported in this review, only one used both methods to determine TFA intake in relation to cancer outcomes.

The dietary assessments used in the examined studies were all food frequency questionnaires, ranging from 61-item to 208-item. Many did not appear to be validated for TFA intake specifically and few used a nurse or other health professional to directly follow up with the subject regarding their intakes, which may have helped to reduce errors. Food frequency questionnaires are useful for large cohort studies as they are easy for researchers to code and administer. However, it can be difficult for subjects to estimate frequency of intake over a long period, or retrospectively, and estimate portion sizes from scratch or in relation to amounts given on the questionnaire (if it is a semi-quantitative questionnaire).

All except one study (Mashal *et al.*, 2012) utilised multiple confounding factors in their analysis, accounting for aspects such as physical activity, smoking and body mass index which can

potentially affect risk of chronic disease. However, less than half of the studies reviewed considered other dietary factors as potential confounding factors (**Appendix 1**). Adjustment for these factors, such as total energy intake, fibre, carbohydrate, sugars or food groups like fruit and vegetables, can help tease out TFA associations outside those due to dietary patterns. For example, the McKelvey et al study (1999) was the only one that appeared to adjust for sweetened baked goods, potentially an important source of industrial TFA. Interestingly, this study found a positive association with TFA, which then became non-significant after adjustment for sweetened baked goods. This indicates that either those foods were the source of TFA or the initial positive association observed was due to other aspects in the baked goods (for example, saturated fat and sugar).

5.2 TFA and CVD or CHD

Previously reported studies published up to 2009 suggest a positive association between TFA intake and increased risk of CVD, although there were sufficient inconsistencies to describe the association as “probable” rather than “convincing”¹ (Booker & Mann, 2005; Food Standards Australia New Zealand, 2009). Our findings contribute an additional five studies from 2009 to 2014. Again, inconsistencies in the data were evident, with different associations observed both between and within cohorts. TFA are likely to increase the risk of CVD through adverse effects on blood lipid levels (Mensink & Katan, 1990). Consistent with the previously published literature, our systematic review and meta-analysis summarised recent research to date to show that a small increase in total and LDL cholesterol, and decrease in HDL cholesterol can be attributed to increases in dietary TFA (Hafekost et al, 2014).

¹ The definition of ‘probable’ and ‘convincing’ evidence at this time were defined as follows:

Probable - a number of acceptable human studies, preferably including both observational and experimental studies. These studies show associations that are either not so consistent, with a number of studies not supporting the association, or the evidence base is insufficient to make a more definite judgement (for example, there are a limited number of studies or the studies are of limited duration, small sample size or with incomplete follow-up).

Convincing - consistent associations between the diet, food or component and the health effect, with little or no evidence to the contrary. There should be a substantial number of human studies of acceptable quality, preferably including both observational and experimental studies and preferably conducted in different population groups. Any intake–response relationships should be supportive of a causal relationship and the relationship should be biologically plausible. Supporting evidence sources should be consistent with the findings of human evidence.

In addition to the effect on lipids, markers of inflammation have been shown to be an independent risk factor for CVD (Uauy *et al.*, 2009). Associations differed by aspirin use and previous history of CHD, indicating that people who are at higher risk of a cardiac event may be more likely to be adversely affected by TFA intake. Guidelines for the prevention of ischemic stroke recommend use of aspirin as a form of antithrombotic therapy in patients (whose risk for stroke is sufficiently high for the benefits to outweigh the risks associated with aspirin use) (Goldstein *et al.*, 2006). Aspirin may help to mitigate the potential adverse effect of TFA on the overall CVD risk profile, potentially through decreasing and counteracting inflammation effects (Ridker *et al.*, 1997). Likewise, people who have already had a cardiac event may be more likely to suffer an additional event if detrimental changes in their LDL and HDL-cholesterol occur as a result of diet than people who have no prior history of CVD.

Studies in this category evaluating different types of TFA found no association with elaidic acid or CLA when these were separated out from total TFA (Chiuve *et al.*, 2009), or between ruminant and industrial TFA (Laake *et al.*, 2012). A randomised controlled trial investigating effects of ruminant and industrial TFA on CVD risk factors in healthy subjects found that the HDL cholesterol-lowering property of TFA seemed to be specific to industrial sources (Chardigny *et al.*, 2008). Although we did not observe any gender differences in our five studies, the trial also found that compared with industrial TFA, ruminant TFA significantly increased HDL cholesterol in women but not in men, suggesting gender or hormones may also play a role.

Overall the additional data obtained as a result of this review showed three positive associations and four neutral associations (from five studies) in relation to CHD/CVD. Future research may show the strength of the evidence depends on factors such as study population characteristics.

5.3 TFA and type 2 diabetes

Associations with TFA and type 2 diabetes were evenly spread. Two of the four studies found neutral associations with dietary TFA intake (Papantoniou *et al.*, 2010; Salmeron *et al.*, 2001; van Dam *et al.*, 2002), a third found a positive association (Salmeron *et al.*, 2001) while the fourth study by Mozaffarian *et al.*, (2013) found an inverse association with serum *trans*-palmitoleate in a large cohort of multi-ethnic US adults. Concentrations of *trans*-palmitoleate were positively correlated with dietary intake of dairy, butter, margarine and baked desserts, suggesting mixed industrial and ruminant sources within the study population. Dairy consumption has been linked with lower incidence of type 2 diabetes (Tong *et al.*, 2011), but there is debate over whether full fat or low fat dairy is more responsible for this association. Some studies suggest full fat dairy products show improved metabolic outcomes (Akter *et al.*, 2013; Liu *et al.*, 2005; Louie *et al.*, 2013), while others suggested that regular fat compared to low fat dairy may be detrimental for metabolic risk (Choi *et al.*, 2005; Yoo *et al.*, 2004).

In the Mozaffarian *et al.*, (2013) study, higher *trans*-palmitoleate concentrations were associated with higher LDL-cholesterol. However an inverse association was observed with triglycerides, fasting insulin and blood pressure. The authors propose that *trans*-palmitoleate may improve peripheral insulin resistance through similar features to *cis*-palmitoleate, which was shown to stimulate muscle insulin action and suppress hepatosteatosis in animal models (Cao *et al.*, 2008). This potentially beneficial effect of *trans*-palmitoleate may not translate to other forms of TFA, based on the findings of the other three studies identified. However, it may not be detrimental for risk of type 2 diabetes either. A meta-analysis of randomised controlled trials concluded that although higher TFA intake leads to an increase in total and LDL-cholesterol and a decrease in HDL-cholesterol, it does not result in changes to glucose or insulin concentrations.

Overall, findings of this review suggest there is little evidence to support the notion that a reduction in dietary TFA would decrease risk of type 2 diabetes. More research is required to determine whether *trans*-palmitoleate may have a role in improving metabolic outcomes.

5.4 TFA and cancer

Breast cancer, colorectal cancer, prostate cancer and pancreatic cancer were the cancer types investigated in studies identified in this review, along with one study investigating total cancer and additional different subtypes. Most cancer studies in this review were in the area of breast cancer, which is the most common cancer in women in both Australia and New Zealand (Australian Institute of Health and Welfare, 2014; Cancer Society of New Zealand, 2011). Different types of cancers are likely to have differing etiologies, with both genetic and environmental aspects contributing to risk. Our results found varying associations between cancer risk and TFA. Although TFA appeared to increase risk for some types of cancer, particularly colorectal cancer, some studies showed a beneficial association with other cancer types, for certain TFAs or in certain groups. Aro et al. (2000) was the only study identified in the review that utilised both serum and diet measures of TFA, in this instance *trans*-vaccenic acid and CLA. It was a comprehensive study that adjusted for a wide range of confounding factors and had the food frequency questionnaire checked by a nurse at interview. Both intake and serum levels of CLA were inversely associated with breast cancer risk, while serum *trans*-vaccenic acid (precursor to CLA) was inversely associated (intake was not significant). At dietary intakes similar to human consumption levels, CLA has been demonstrated to have some anticarcinogenic activity in animal models (Ip *et al.*, 1994), and *in vitro* CLA has demonstrated cellular growth suppression of human breast and colon cancer cells (Eynard & Lopez, 2003). However, some dietary trials indicate that consumption of CLA can have mixed effects on markers of inflammation and immune function, which could affect cancer risk (Chardigny *et al.*, 2008). Both the type of TFA and the type of cancer may influence results.

Although currently uncertain, TFA may be linked to cancer development through effects on inflammation and oxidative stress. A substantial body of evidence supports the concept that chronic inflammation can increase risk of cancer, for example, inflammatory bowel disease is linked with increased risk of colon cancer (Shacter & Weitzman, 2002). The risk increases the longer the inflammation exists. Long term exposure to inflammatory mediators can lead to increased cell proliferation, blood vessel growth, mutation of genetic information, and

activation of genes that have the potential to cause cancer (Shacter & Weitzman, 2002).

Incorporation of TFA into cell membrane phospholipids creates a straight, rather than bent molecule and can result in reduced membrane fluidity. This has been associated with an increase in free radical activity, resulting in oxidative stress (Smith *et al.*, 2009). Oxidative stress, occurring when reactive oxygen species outbalance the body's antioxidant defences, may interfere with mechanisms involved in cell growth and regulation. Although reactive oxygen species are formed naturally as a by-product of oxygen metabolism and have important roles in the body, they have also been associated with DNA mutations which could lead to cancer (Smith *et al.*, 2009)

Our findings indicate that overall, there is inconsistent evidence around whether TFA may have a role in cancer development, although plausible biological mechanisms exist. The effects may vary depending on the type of TFA, the type of cancer, and factors such as gender.

5.5 TFA and all-cause mortality

We identified one study investigating TFA intake and all-cause mortality (Kiage *et al.*, 2013) (**Appendix 1**). This US study of over 18,000 subjects reported a positive association between dietary TFA intake as assessed by a food frequency questionnaire and mortality. However, the association between TFA and all-cause mortality observed was only significant at higher intakes of TFA, defined as 3.45% of energy or above. The study used portion size pictures alongside the food frequency questionnaire to assist in quantification of foods and the questionnaire had been previously shown to reliably estimate TFA intake. Another strength of the study was that the statistical models were adjusted for a wide range of factors including medical history, medications, physical activity, smoking, education, waist circumference, and dietary aspects outside TFA including saturated fats and carbohydrate.

Given that reports indicate more than 90% of Australians and 85% of New Zealanders had TFA intakes below the 1% energy level in 2009 (Food Standards Australia New Zealand, 2009) it is

unlikely that many Australians or New Zealanders would have a TFA intake as high as 3.45% of energy. Levels of TFA in the US food supply have been decreasing since the baseline data in the study were collected, meaning that Americans are also less likely to reach that level of intake now. The authors of the study note that their level of 3.45% of energy is noticeably higher than the 2% of energy reported in cardiovascular disease mortality studies (Chardigny *et al.*, 2008). This suggests that TFA may be more strongly linked to CVD than other causes of death. Again, more studies are required to further evaluate the contribution of TFA to death from all causes (Kiage *et al.*, 2013).

5.6 TFA and other conditions

Work with animal models has suggested that, at least in mice and rats, dietary TFA leads to incorporation of TFA in membranes throughout the body, including membranes within the retina of the eye, and neuronal membranes. This could provide a mechanism for TFA influencing changes in vision and brain neurochemical functions.

To date, there have been limited human studies to investigate if these mechanisms cause any health effects in humans. Breillon *et al.*, (2008) reported detecting deposition of TFA within the retina, and associated membranes and adipose tissue in 27 human donors. Despite this, neither of the two studies we found investigating AMD were able to directly link TFA intake with significantly increased risk (Cho *et al.*, 2001; Chong *et al.*, 2009). However, findings indicate further research with a larger sample size could be valuable.

Mouse and rat models have suggested the deposition of TFA in neuronal membranes may possibly affect mood, behaviour and memory. We found mixed results, with patients with Alzheimer's disease showing higher TFA levels (Iuliano *et al.*, 2013) but no association between TFA and risk of dementia in a cohort study (Engelhart *et al.*, 2002).

Studies suggest a genetic link with asthma and eczema, which are both characterised by an overactive immune response. Dietary fat has been previously suggested to play a contributing

role to these conditions. Fats can effect proinflammatory and immunologic pathways, and it has also been suggested that atopic dermatitis is associated with an enzyme defect in lipid metabolism (Devereux & Seaton, 2005). Omega-6 fatty acids are thought to be related to an increase in allergic sensitization, while omega-3 fatty acids are thought to have the opposite effect (Black & Sharpe, 1997). Although our results suggest that little is known about whether TFA specifically may have an effect, studies examining margarine intake in European countries consistently found a positive or borderline positive association with asthma or eczema (Kim *et al.*, 2005; Nagel & Linseisen, 2005; Sausenthaler *et al.*, 2006).

While there is some uncertainty as to the extent of passage through the placenta, it appears that at least some maternal dietary TFA is transmitted through the placenta to the developing foetus, and TFA is represented in proportion to maternal dietary intake in breast milk. Although dietary TFA intake during pregnancy has been hypothesised to be associated with foetal growth, we identified contradictory results showing lower growth (van Eijsden *et al.*, 2008), greater growth (Cohen *et al.*, 2011) or no association (Dirix *et al.*, 2009). Further work is required to elucidate these associations.

6. Conclusions

In our narrative review of the association of TFA with adverse health outcomes, we observed differing results depending on the health outcome. Total mortality, CVD/CHD and colorectal, pancreatic and prostate cancer demonstrated more positive or neutral associations with TFA, while results for type 2 diabetes and breast cancer findings were more evenly distributed. A category of other health conditions, including macular degeneration, dementia, asthma and eczema, also showed mixed results, although positive findings with margarine suggest TFA has a potential role with allergic conditions. The differences observed between outcomes may be due to differences in study populations, whether different factors such as blood lipids or membrane fluidity are involved in disease progression, and potential differences in effects of specific TFA.

Overall there was a wide variation in the degree of adjustment for potential confounding factors, with other dietary factors sometimes not considered. It is difficult to separate out the effects of TFA from foods that are important sources of TFA, but also contain potentially detrimental nutrients for health. An example would be the sugar and saturated fat also present in baked goods.

Studies included in this review covered a wide range of TFA intakes, although most subject intake means were noticeably higher than the average of 1.3 g/day (0.5% total energy) for Australians and 1.6 g/day (0.6% total energy) reported previously (Food Standards Australia New Zealand, 2009). Although we did not observe a tendency for studies of higher intakes to show more significant associations than no associations (**Appendix 2**), it was interesting to note that the study with one of the lowest reported subject intakes (TFA 0.5% total energy) reported a significant inverse association (Aro *et al.*, 2000). The relatively low levels of TFA in Australia and New Zealand may reduce the risk of adverse health outcomes observed with TFA in other countries. Studies in this review were most commonly conducted in the United States. The United States has traditionally had higher intakes of TFA compared to Australia and New Zealand, potentially due to the widespread use of partially hydrogenated soybean oil for frying (Shrapnel, 2012). In contrast, prior to the move away from saturated fat, the major frying fats used in Australia were beef and lamb tallow and palm oil, which have substantially less TFA (Shrapnel, 2012).

Although we observed more positive than inverse findings with TFA and adverse health outcomes, much of the literature is inconsistent and the majority of findings reported no significant associations. Future research is required to better separate out the effects of TFA from other dietary components within the context of different health conditions.

7. References

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