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REVIEW

Trans fat involvement in cardiovascular disease

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Coronary heart disease is becoming a worldwide epidemic and diet and lifestyle are well known contributing factors. Identifying the kinds of foods that may have a cardioprotective or cardiotoxic effect and understanding their molecular mechanisms of action has become of increasing importance. Through largely epidemiological evidence, trans fatty acid (TFA) intake has been associated with a variety of cardiovascular complications including atherosclerosis. Traditionally, industrial TFAs (iTFAs) have been associated with these deleterious cardiovascular effects. However, there is a current body of research that suggests that ruminant trans fats (rTFAs) may have a cardioprotective role within the heart. The molecular mechanisms whereby TFAs are delivering their effects are largely unknown. In the following review, we discuss recent *in vitro*, animal and epidemiological research to better understand the effect of TFAs in the diet on cardiovascular disease, particularly atherosclerosis.

Keywords:
Atherosclerosis / Fatty acid / Heart disease / Nutrition / Trans fat

1 Introduction

Coronary heart disease (CHD) is induced as an accumulation of atheromatous plaque in the arteries that deliver oxygen and blood to the working heart. The development of plaque in the arteries is a result of multiple risk factors including infection, diabetes, smoking, physical activity, increased body mass index and high triglyceride levels along with many other components [1]. Diet has also been identified as an important risk factor for CHD [2–7]. In recent years, the effect of trans fatty acids (TFAs) on CHD has become an area of great interest. TFAs are unsaturated fats that contain at least one double bond [8] (Fig. 1). A lipid molecule may contain a double bond in a cis or trans geometric configuration. In the more common and natural cis configuration, the carbon chain extends from the same side of the double bond. This results in a kink or bend in the molecule. In the trans configuration, the carbon chain extends from opposite sides of the double bond. This results in a much straighter molecule. Although the chemical composition of a cis and trans fat may be identical, this change in configuration will induce obvious effects on the packing of the lipid in, for example, a phospholipid bilayer and on the function of both lipids and proteins in a membrane structure. TFAs are usually associated with industrially produced trans fats (iTFAs) since many TFAs are found in partially hydrogenated vegetable oils that can be converted into semi-solid fats. These iTFAs are used in many baking and manufactured foods [6, 8, 9]. It is estimated that the North American population consumes an average daily intake of 5.8 g or 2.6% of calories from TFAs [10]. However, individual consumption can vary between 1 and 29 g daily [10]. TFAs are also produced within the gut of ruminant animals and are largely found in dairy products and meats. These TFAs are known as ruminant TFAs (rTFAs) and account for 1% or less of TFAs found within our diet [11].

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Abbreviations: **CHD**, coronary heart disease; **HDL**, high density lipoprotein; **iTFAs**, industrially produced trans fatty acids; **LDL**, low density lipoprotein; **LDLr$^{-/-}$**, mice-low density lipoprotein receptor knock out mice; **MUFAs**, monounsaturated fatty acids; **PUFAs**, polyunsaturated fatty acids; **SFAs**, saturated fatty acids; **rTFAs**, ruminant trans fatty acids; **TFAs**, trans fatty acids

2 Risk of coronary heart disease

On a per-calorie basis, the risk of coronary heart disease increases almost 1–3% with TFA consumption [12]. Within the Nurses' Health Study, those in the highest quintile of trans fat consumption had a higher risk of CHD (~1.5×) than those in the lowest quintile [13]. Epidemiological data have provided convincing evidence that the inclusion of TFAs in our diet is associated with an induction of cardiovascular

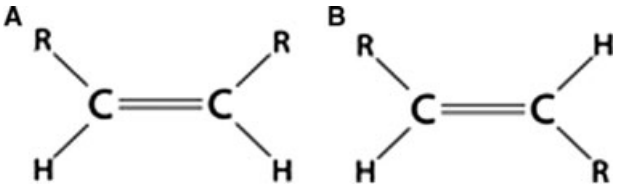

Figure 1. Differences between cis and trans fats. There are distinct differences in the configuration of cis and trans fats, specifically, the orientation of the R groups around the double bond will promote distinct differences both in chemical configuration and biological effects.

disease. Dietary TFAs have been correlated with significant coronary heart disease and an increased incidence of myocardial infarctions [14–19]. Individuals with high dietary TFA content possess elevated low-density lipoprotein (LDL) levels and significantly lower high-density lipoprotein (HDL) levels [20, 21]. Patients with CHD have elevated levels of TFAs in their adipose tissue [22, 23]. TFAs have also been identified within atherosclerotic plaques [24, 25].

The removal of TFAs from our diet has resulted in improvements in CHD. Replacing just 2% of energy that we obtain from TFAs in our diet with energy from un-hydrogenated unsaturated fats has been estimated to reduce the risk of coronary disease by 53% [17]. Leth and colleagues found a 60% decline in cardiovascular disease in Denmark after the Danish Government implemented legislation to limit the amount of iTFAs available in foods [26]. This has led many to be convinced that TFAs have a causal relationship with cardiovascular disease. However, the mechanism whereby dietary TFAs induce cardiovascular disease has remained less certain.

3 Atherosclerosis and trans fats

Since epidemiological data are largely correlative, it is important to conduct relevant animal studies to better understand the mechanistic relationship of TFAs with cardiovascular disease. However, the initial results produced in animal models of cardiovascular disease and atherosclerosis were surprising in view of the epidemiological data cited above. TFA ingestion did not affect metabolism and mitochondrial function in Vervet monkeys fed TFA compared to a cis fat diet [27]. Furthermore, the same laboratory also could not detect a significant effect of TFAs on atherogenesis in rabbits fed a high cholesterol diet [28]. The authors concluded that TFAs were not detrimental to cardiovascular health nor did it contribute to atherosclerosis in these animal models. More recent studies have established that TFAs contribute to hypercholesterolemia [29, 30]. This confusion of results had left researchers with little conclusion until more definitive studies were completed recently.

The development of transgenic animals has resulted in excellent models for mimicking atherosclerosis in humans. The LDL receptor deleted mouse is one of the best models for atherosclerosis since it has the ability to develop plaques only in the presence of lipid-rich diets [31]. This is unlike many other animal models where plaque development can be independent of cholesterol. The development of plaques was assessed in LDL receptor deleted mice fed a diet rich in elaidic acid [32]. They showed for the first time that a diet rich in TFAs could induce the development of atherosclerotic plaques. Furthermore, it was shown that mice fed the TFA diet together with a cholesterol-supplemented diet did not induce an additive effect. It was concluded that cholesterol was 'masking' the effect of TFAs on atherosclerosis [33]. Hence, previous studies [27, 28, 34] that could not detect an effect of TFAs on atherosclerosis likely could not show this due to the overwhelming atherogenic action of the dietary cholesterol. Just as importantly, subsequent work [11] conducted in the LDL receptor knockout mouse has detected differences between industrially produced TFAs such as elaidic acid and rTFAs such as vaccenic acid. Although the inclusion of the TFA elaidic acid in the diet induced atherogenesis, the inclusion of the TFA vaccenic acid in the diet induced a surprising inhibition of plaque formation [11]. This work may compel us to view TFAs in an entirely different light. We may have to change our current thinking of TFAs as an entire class of deleterious substances and instead pursue the structural characteristics that make some TFAs beneficial.

4 Lipids and lipoproteins

There is a positive correlation between plasma LDL and atherosclerosis and/or CHD [35]. Although epidemiological evidence suggests that there is a positive association between TFA intake and elevated plasma LDL [20, 21] (as well as triglycerides [36]), a clear mechanism has not been established. In the human hepatoblastoma (HepG2) cell line, TFAs have been associated with increased LDL: high-density lipoprotein (HDL) ratios, increased apolipoprotein B: apolipoprotein A (apoB:apoA) ratio and increased cholesterol content in both LDL and HDL particles in comparison to saturated fats [37]. All of these findings have in turn been associated with a higher risk of atherosclerosis and CHD. Similar findings were reported by Mitmesser et al. who suggested that TFAs altered the size and composition of apoB-100 containing lipoproteins [38]. These studies provide a basic mechanism whereby TFAs deposit cholesterol in arteries. However, it is important to recognize that these studies are primarily correlative and more concrete evidence is necessary. Furthermore, there is no distinction in these studies between rTFAs and iTFAs. These two types of TFAs are structurally different and, therefore, their biological effects may also be very different.

Epidemiological evidence has generated conflicting results with respect to an association of TFAs with serum lipid

levels. In a meta-analysis of 13 randomized controlled trials observing the effects of isocaloric replacement of polyunsaturated fatty acids (PUFAs), saturated fatty acids (SFAs) or monounsaturated fatty acids (MUFAs) with TFAs, a significant increase in low-density lipoprotein cholesterol (LDL-C) levels, total cholesterol:high-density lipoprotein cholesterol (HDL-C) ratio and the ratio of apo B:apo A was observed as well as a decrease in HDL-C levels [12]. Others have shown a decrease in LDL-C particle size with consumption of TFAs as opposed to unsaturated fatty acids [39]. It is important to recognize that these studies investigated a particular isomer of TFAs associated with the hydrogenation of vegetable oils (such as the trans isomer of oleic acid); therefore, more investigation with a wider variety of TFAs may be necessary to fully understand the effect of TFAs on lipoproteins.

5 Cytokine and adipokine production

The binding of an agonist to a specific receptor protein which then affects a number of downstream signalling pathways is associated with the development of an inflammatory cascade which can then ultimately influence the progression of atherosclerosis, plaque rupture and cardiac failure and death [40,41]. The up-regulation of certain inflammatory proteins is one common but important example [41]. Adipose cells and the adipokines synthesized there are rapidly becoming recognized as important mediators of inflammatory pathways and associated recently with the atherosclerotic process. It is possible, therefore, that if TFAs are deposited within fat tissue, this change in fatty acid composition may influence adipokine synthesis, release, downstream signalling, and ultimately, inflammatory actions related to atherogenesis.

TFAs alter adipocyte size in *fa/fa* Zucker rats [42]. However, there were no significant effects on adipokine secretion and profile in these rats. The results were limited to the *trans* t-10, c12-conjugated linolenic acid (CLA) effects on adipocyte size and number in comparison to *cis* c-9, t-11-CLA-enriched diets. Although this effect may be subject to the animal model used and amount of TFA in the diet, this study does show an important effect of a specific isomer of TFAs. Obara et al. also analysed the effect of TFA consumption in non-alcoholic fatty liver disease in mice [43]. They found an up-regulation of TNF-α expression in mice fed a high TFA supplemented diet. Up-regulation of TNF-α expression is associated with alterations in inflammation, endothelial function and cardiotoxicity [44].

Bryk et al. demonstrated an increase in NF-κB expression suggesting that there was an increase in the inflammatory response in the cells after TFA administration [40]. Since inflammatory markers are a key risk factor for atherosclerosis [40, 41, 44], this study identifies a potential mechanism whereby TFAs induce an atherogenic process. Similar results were obtained in human umbilical vein endothelial cells (HUVECs) where a decrease in TNF-α and NF-κB with conjugated linolenic acid (CLA) administration was observed [45]. Again, studies on vaccenic acid as well as elaidic acid are necessary to fully define the effects of TFAs on inflammatory reactions that can be generated by endothelial cells.

The mechanism whereby this heart-healthy effect is achieved by vaccenic acid may involve an anti-inflammatory action. Blewett et al. observed a decrease in inflammatory markers such as IL-6, IL-2 and TNF-α in obese JCR:LA-cp mice fed a diet supplemented with vaccenic acid [46]. Vaccenic acid may share this anti-inflammatory action with other fatty acid species. Omega-3 fatty acids, for example, have an anti-inflammatory action [51]. An anti-inflammatory effect of omega-3 fatty acids and a pro-inflammatory effect of TFAs were reported in male Wistar rats that underwent coronary and femoral artery ligation [47]. The possibility exists that an inclusion of both omega-3 fatty acids in the diet with TFAs may negate the deleterious effects of iTFAs. Indeed, the atherogenic effects of dietary elaidic acid were recently prevented by inclusion of the omega-3 fatty acid alpha linolenic acid (ALA) in the diet [53], presumably through a complex interaction with the inflammatory/immune system.

As stated earlier, TFAs may also influence cardiovascular disease through an effect on HDL and LDL levels in animals. For example, iTFAs decrease the HDL:LDL ratio in rats fed a high TFA diet [33]. However, conflicting data have been reported that high TFA intake may decrease total plasma cholesterol [48]. These results may be difficult to explain in view of pro-atherogenic epidemiological and animal results identified above. Alternatively, these data suggest that TFAs may be stimulating atherosclerosis in a manner independent of cholesterol. This concept is supported by Bassett et al. who demonstrated that supplementation of the diet with elaidic acid will stimulate atherogenesis without an increase in plasma cholesterol [32]. Once again, the pro-inflammatory effects of the TFA diet are a logical alternative mechanism.

Adipokine regulation by TFAs has also been associated with the progression of atherosclerosis in animal models. Specifically, increased adiponectin levels have attenuated atherosclerosis progression [49]. Huang et al. showed that margarine-derived TFA diets fed to male Wistar rats decreased plasma levels of adiponectin, and increased resistin and leptin. TFA intake also affected adipocyte gene expression of peroxisome-proliferator-activated receptor (PPAR), resistin and lipoprotein lipase (LPL) in rats [50]. Increases in tumour growth factor (TGF-β) expression may be another potential mechanism whereby TFAs are affecting atherosclerosis [51–53]. Once again, these results have focused upon iTFAs. Pathways affected by rTFAs are largely unknown in relation to atherogenesis.

In observational studies, TFAs have been associated with increased levels of specific inflammatory markers such as tumour necrosis factor (TNF) and its receptors, interleukin-6, C-reactive protein and NF-kappa B [46]. In a randomized cross-over trial of 50 healthy men, consumption of TFAs for

5 weeks increased plasma levels of IL-6 and CRP in comparison to consumption of oleic acid or carbohydrate intake [54]. However, Smit et al. reported that TFA consumption for 3 weeks did not severely effect markers of inflammation including tumour necrosis factor receptor-II (TNF-RII), TNF-α, C-reactive protein and IL-6 in participants consuming either conjugated linoleic acid, iTFAs or oleic acid [55]. This randomized study was performed on 61 healthy adults. The amount of TFAs consumed, the time-frame under investigation, the TFAs that were consumed and the characteristics of participants consuming the diets (i.e., males *vs*. females, etc.) may have participated in the conflicting results.

Many inflammatory markers are under genetic regulation by peroxisome-proliferator-activated receptors (PPAR), liver X receptor and sterol regulatory element-binding protein-1 (SREBP-1) [56]. In animal studies, TFA consumption alters PPAR-gamma activity, resistin and lipoprotein lipase activity suggesting a potential mechanism whereby TFAs are exerting their effects [57]. However, identifying a distinction between isomers of TFAs in human studies is still necessary to fully understand how TFAs are exerting their effects on cytokine regulation and systemic inflammation.

6 Endothelial dysfunction

Using fluorescence and mRNA measurements conducted by Bryk et al., an increase in vascular cell adhesion molecule-1 (VCAM-1) and intracellular cell adhesion molecule-1 (ICAM-1) expression was detected in human aortic endothelial cells (HAECs) after exposure to increasing doses of elaidic acid [58]. Elevated levels of E-selectin were detected in a randomized control trial of 50 men consuming elevated levels of TFAs [54]. Observational studies have also shown an increase in the expression of E-selectin, ICAM-1 and VCAM-1 [59]. These observations are consistent with findings in *in vitro* work and animal studies.

7 Membrane fluidity

Fatty acids are incorporated into phospholipids in all cell membranes of the body [60, 61]. The fatty acid composition of the membrane can strongly influence its biophysical characteristics. In particular, unsaturated and saturated fatty acids can act as potent regulators of membrane fluidity due to differential actions of phospholipids on cholesterol affinity and incorporation [62, 63]. This phenomenon can alter cellular activities such as the function of membrane proteins including membrane bound receptors [64]. It has been proposed that TFAs may alter cardiovascular integrity and function by incorporating into cell membranes and changing cellular membrane fluidity. Niu et al. demonstrated that TFA-derived phospholipids had significantly higher membrane affinity for cholesterol than cis analogues. Phospholipid membranes that contained TFA exhibited a higher acyl chain packing order. This effect was also correlated with reduced G-protein coupled receptor activation [64]. Because membrane cholesterol levels and membrane receptors are involved in the regulation of cholesterol homeostasis, the elevation in membrane cholesterol content and the lower receptor activation induced by the presence of TFA in the membrane could represent the mechanism responsible for the elevation of LDL cholesterol in TFA supplemented diets [64]. Engelhard et al. [65] found an increase in membrane fluidity with TFA administration in choline supplemented membranes. The TFA elaidic acid increased calcium incorporation into the cell [66] and decreased osmotic rupture of sheep and chicken erythrocytes in comparison to cis analogues [67]. Although there are clear differences between the effects of cis fatty acids and TFAs on membrane fluidity, studies have not investigated the effect of rTFAs on the membrane. These studies may be necessary since, as previously discussed; rTFAs have significantly different effects on atherosclerosis in animal studies.

8 Summary

There is now overwhelming evidence based upon retrospective clinical studies, animal data and *in vitro* experiments that TFAs have deleterious effects on our cardiovascular health when included in the diet in high amounts. The synergistic effect of TFAs and other dietary components, drugs and environmental factors also still needs to be investigated in order to better understand TFAs and CHD. The direct action of TFAs on cardiomyocyte function is also unclear and may represent yet another important mechanism for the deleterious effects of TFAs. Due to the impressive preliminary findings documented in Denmark, many other countries across the world are following their lead and creating legislation to limit the amount of iTFAs available for public consumption. However, restricting all TFA consumption may not be realistic or possible. Finding novel ways to block the atherogenic action of TFAs may be a more reasonable approach. Supplementation of the diet with flaxseed, for example, can prevent the atherogenic effects of dietary iTFAs in animal models [68]. Other dietary approaches remain to be discovered. It is also important to recognize that many of our concepts regarding TFAs do not consider other TFA isomers beyond iTFAs. Because of the intriguing positive effects of vaccenic acid consumption, the picture of the effects of TFAs as risk factors for atherosclerosis may require further study to fully reveal the effects of trans fats on cardiovascular disease. Ultimately, this may result in some changes in the enacted public legislation.

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