Ruminant and industrial sources of *trans*-fat and cardiovascular and diabetic diseases

Anne-Laure Tardy[1,2,3], Béatrice Morio[1,2,3]*, Jean-Michel Chardigny[1,2,3]
and Corinne Malpuech-Brugère[1,2,3]

[1]*INRA, UMR 1019 Nutrition Humaine, F-63122 Saint Genès Champanelle, France*
[2]*Clermont Université, UFR Médecine, UMR 1019 Nutrition Humaine, F-63 000 Clermont-Ferrand, France*
[3]*CRNH Auvergne, F-63 000 Clermont-Ferrand, France*

Abstract

The various positional isomers of oleic acid ($18:1\Delta9c$ or $9c\text{-}18:1$) may have distinct biological effects. Detrimental effects of consumption of industrial *trans*-fatty acids (TFA) (elaidic acid; $18:1\Delta9t$) from partially hydrogenated vegetable oils on CVD risk factors are well documented. In addition, epidemiological data suggest that chronic consumption of industrial sources of TFA could alter insulin sensitivity and predispose for type 2 diabetes. However, intervention studies on this issue have remained inconclusive. Moreover, very little information is available on the effect of natural sources of TFA (vaccenic acid; $18:1\Delta11t$) coming from dairy products and ruminant meat on the development of CVD and type 2 diabetes. The review focuses on the impact of the consumption of ruminant TFA in relation to cardiovascular risk factors, inflammation and type 2 diabetes.

Key words: *Trans*-fatty acids: Atherosclerosis: Cholesterol: LDL: CVD: Risk factors: Diabetes

Introduction

Evidence is now emerging that the incidence of metabolic disorders such as cardiovascular diseases and type 2 diabetes could be affected by dietary fatty acid composition. Among all the dietary lipids, the present review will focus on *trans*-fatty acids (TFA), since they are the object of a worldwide public health discussion, which requires solid and pertinent metabolic demonstrations in order to promulgate new nutritional recommendations. In fact, food safety authorities need clear, scientifically proven information to further legislate on the subject. TFA are MUFA or PUFA in which the hydrogen atoms are on opposite sides of the fatty acid double bonds, therefore changing their configuration and their chemical properties. TFA represent on average 1 to 2% of daily energy intake. The main food sources of TFA are partially hydrogenated oil (industrial products) and ruminant-derived foods (dairy products and meat). The isomers of $18:1$ (oleic acid, $18:1\Delta9c$ or $18:1$ *cis*-9) have been found to contribute 54 to 82% of the total TFA intake[1]. The major TFA formed by partial hydrogenation of vegetable oils (industrial TFA; I-TFA), derived from oleic acid, is elaidic acid ($18:1\Delta9t$ or $18:1$ *trans*-9), whereas the main TFA resulting from rumen biohydrogenation (ruminant-TFA; R-TFA) is

vaccenic acid ($18:1\Delta11t$ or $18:1$ *trans*-11)[2] (Fig. 1). In partial hydrogenation of vegetable oils, the *trans*-$18:1$ isomers usually vary from 4 to 16 carbons from a Gaussian distribution that centres about the d9 or d10 double bond. The distribution depends first on the starting vegetable oil and second on the extent of hydrogenation. For instance, in mildly hydrogenated soyabean oils, $10t\text{-}18:1$ and $11t\text{-}18:1$ are the major isomers whereas $9t\text{-}18:1$ is the major one in heavily hydrogenated soyabean oil[3]. The distribution is different in ruminant fats. The major isomer is always $11t\text{-}18:1$, reaching about 70% of the total *trans*-$18:1$, whereas $10t\text{-}18:1$ and $9t\text{-}18:1$ appear in small amounts[4].

The R-TFA term comprises total TFA (all the geometrical isomers of MUFA and PUFA) having non-conjugated, carbon–carbon double bonds in the *trans* configuration, except the conjugated linoleic acids, according to the definition of the French Food Safety Agency (L'Agence française de sécurité sanitaire des aliments; AFSSA, now known as ANSES)[5]. The health effects of both industrial and ruminant sources of TFA are an important issue, since both are widespread ingredients in the food industry. As will be discussed later, the metabolic effects of I-TFA have been widely studied, whereas the impact of R-TFA
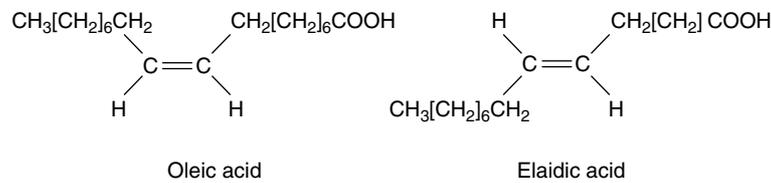
Fig. 1. Cis- and trans-MUFA: 18 : 1 (n-9) cis or oleic acid, and 18 : 1 (n-9) trans or elaidic acid, respectively.

has thus far been ignored. Indeed, milk fat contains low amounts of TFA (3–7 % by wt) compared with partially hydrogenated oil (10–60 % by wt), making it difficult to compare their respective effects[6]. The study of specific effects of the TFA isomer of ruminant origin has become possible since the discovery that milk fat TFA content can be enhanced by modifying the cows' diet[7]. Understanding the specific effect of ruminant-sourced TFA is an important issue for two main reasons. First, in Europe, the contribution of TFA from ruminant fat ranged from about 30 to 80 % of total TFA, corresponding to 0·3–0·8 % of energy[8] and perhaps even more since food safety authorities incite food companies to decrease the amount of industrially sourced TFA in food formula[1,5]. Second, the average distribution of R-TFA intake is about 85 % from dairy products and 15 % from ruminant meat[9,10]; consequently, the intake of R-TFA is mainly determined by the intake of high-fat dairy products. In fact, the cows' diet is the major environmental factor regulating milk fat composition and many strategies have been developed to reduce medium-chain SFA, for instance fresh pasture modifies the SFA/MUFA-PUFA ratio, decreasing milk fat SFA and enhancing milk fat conjugated linoleic acids and TFA[1,11].

In the present review, to identify the most accurate papers, we used both PubMed databases and ISI Web of Knowledge[SM], using 'trans fatty acid' and 'cardiovascular' as keywords leading to 291 hits for the cardiovascular section, where the only inclusion criterion was human; for the diabetes part, 'trans fatty acid' and 'diabetes' were used as keywords, there were no inclusion or exclusion criteria, and 208 hits were recovered.

Trans-fatty acids and cardiovascular risk biomarkers

Impact of trans-fatty acids on the risk of CHD

A complete review on the effects of TFA on risks for CVD has been recently published by Micha & Mozaffarian in 2009[12,13]. In the present review, we will only focus on the studies that distinguished the origins of the dietary TFA (Table 1).

Few studies investigated the association between the total intake of TFA and the risk of CHD; moreover, to our knowledge, only five studies looked at the association between intake of R-TFA and CHD. Among them, three prospective studies[14–16] and one case–control study[15] showed no significant association between intake of R-TFA and risk of CHD and one no differences between the different TFA sources[17].

In the Nurses' Health Study, after 20 years of follow-up, total TFA intake was positively associated with CHD risk, as the multivariate relative risk for the highest v. the lowest quintile of intake was 1·33 (P for trend=0·01). However, when these authors did an analysis excluding women who in 1980 reported that their margarine intake had greatly changed in the previous 10 years, after adjustment for established risk factors and dietary lipids, the relative risk (highest v. lowest quintile of trans-isomer intake) of CHD with the intake of trans-isomers from animals was 0·59 (95 % CI 0·30, 1·17; P for trend=0·230), which means no significant associations between R-TFA intake and the risk of CHD[14].

In a case–control study conducted in the Boston area (MA, USA) with both men and women aged about 57 years, Ascherio et al.[15] observed no significant association between the intake of trans-isomers from animal fats (R-TFA) (about 0·7 % of energy in the highest quintile) and myocardial infarction: relative risk adjusted for established risk factors, dietary lipids and energy was 1·02 (95 % CI 0·43, 2·41; P for trend=0·57).

Recently, results obtained from 3686 men and women from four Danish cohort studies[16] suggest that an intake of R-TFA of about 1·1 % of total energy intake (TEI) (about 2·7 g/d for women and 3·4 g/d for men in the highest quintile) is not associated with a higher risk of CVD. No evidence of a higher risk associated with R-TFA intake within the wide range of intake among both women (90 % central range: 0·5–3·1 g/d) and men (90 % central range: 0·6–4·1 g/d) was found.

Moreover, in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study, which was a randomised, double-blind, placebo-controlled primary prevention trial, 21 930 men (aged 50–69 years) followed-up for 5–8 (median 6·1) years were analysed[18]. Men were grouped into quintiles of energy-adjusted intakes of nutrients as calculated from the dietary questionnaire. No association was reported between coronary death and dietary intake of TFA of animal origin (P for trend=0·857). The relative risk of coronary death after adjustment for age, smoking, BMI, blood pressure, intakes of energy, alcohol, and fibre, education (<7, 7–11, >11 years) and physical activity (<1, 1–2, >2 times per week) was 0·83 (95 % CI 0·62, 1·11) (P for trend=0·035) for animal TFA for the highest (2·5 g, about

Table 1. Effect of industrial and ruminant *trans*-fatty acids (TFA) on CVD (adapted from Booker & Mann[35])*

Marker	Subjects and cases	TFA origin	1 (lowest)	2	3	4	5 (highest)	P for trend across groupings
Nurses' Health Study[14]: isomers from animal (R-TFA) and vegetable fat (I-TFA)								
CHD incidence	69 181 women 356 cases	R-TFA intake (% TEI)	0·43 % TEI	0·58 % TEI	0·70 % TEI	0·81 % TEI	1·03 % TEI	0·230
		RR	1	0·76 (0·51, 1·12)	0·69 (0·43, 1·10)	0·55 (0·31, 0·96)	0·59 (0·30, 1·17)	
		I-TFA intake (% TEI)	0·65 % TEI	0·86 % TEI	1·05 % TEI	1·22 % TEI	1·54 % TEI	0·009
		RR	1	1·43 (1·00, 2·04)	1·11 (0·74, 1·66)	1·39 (0·91, 2·13)	1·78 (1·12, 2·83)	
Zutphen Elderly Cohort Study[17]: total TFA: R-TFA and manufactured 18 : 1 TFA								
CHD incidence	667 men	R-TFA intake (% TEI)	2·36 % TEI	3·87 % TEI	6·38 % TEI			0·003
	98 events	RR	1·0	1·34 (0·78, 2·37)	2·00 (2·07, 3·75)			
Alpha-Tocopherol, Beta-Carotene Study[18]: total TFA, animal TFA (R-TFA) and elaidic acid (18 : 1Δ9t)†								
Coronary death	21 930 men 635 cases	Total TFA intake (% TEI)	0·81 % TEI	1·08 % TEI	1·31 % TEI	1·58 % TEI	2·79 % TEI	0·004
		RR	1·00	1·05 (0·81, 1·36)	1·12 (0·87, 1·45)	0·90 (0·59, 1·18)	1·39 (1·09, 1·78)	
		R-TFA intake (% TEI)	0·27 % TEI	0·50 % TEI	0·68 % TEI	0·86 % TEI	1·13 % TEI	0·035
		RR	1·00	0·97 (0·75, 1·25)	0·91 (0·70, 1·19)	0·90 (0·69, 1·17)	0·83 (0·62, 1·11)	
		18 : 1Δ9t intake (% TEI)	0·59 % TEI	0·77 % TEI	0·90 % TEI	1·22 % TEI	2·52 % TEI	0·02
		RR	1·00	1·03 (0·80, 1·34)	1·04 (0·80, 1·34)	0·90 (0·69, 1·18)	1·37 (1·07, 1·75)	
Scottish Heart Health Study[19]: I-TFA and R-TFA‡								
CHD (angina and/or myocardial infarction)	4490 men 369 cases	R-TFA intake (% TEI)	0·68 % TEI	0·99 % TEI	1·26 % TEI	1·53 % TEI	2·21 % TEI	0·79
		OR	1	0·74 (0·50, 1·11)	0·75 (0·48, 1·17)	0·81 (0·48, 1·39)	1·53 (0·75, 3·11)	
		I-TFA intake (% TEI)	0·45 % TEI	1·08 % TEI	1·67 % TEI	2·30 % TEI	3·96 % TEI	0·03
		OR	1	0·65 (0·45, 0·95)	0·88 (0·61, 1·27)	0·62 (0·41, 0·93)	0·58 (0·37, 0·91)	
	5170 women 235 cases	R-TFA intake (% TEI)	0·54 % TEI	0·86 % TEI	1·08 % TEI	1·35 % TEI	1·94 % TEI	0·27
		OR	1	1·03 (0·64, 1·67)	0·80 (0·46, 1·39)	0·74 (0·40, 1·40)	0·71 (0·30, 1·70)	
		I-TFA intake (% TEI)	0·41 % TEI	1·08 % TEI	1·58 % TEI	2·21 % TEI	3·74 % TEI	0·52
		OR	1	0·82 (0·52, 1·28)	0·59 (0·35, 0·97)	0·81 (0·49, 1·34)	0·84 (0·49, 1·45)	
Study from four Danish cohorts[16]: R-TFA								
CHD events	1837 men 253 cases	R-TFA intake (% TEI)	0·36 % TEI	0·59 % TEI	0·81 % TEI	1·08 % TEI	1·37 % TEI	
		Number of events	50	48	58	48	49	>0·05
	1849 women 121 cases	R-TFA intake (% TEI)	0·32 % TEI	0·50 % TEI	0·68 % TEI	0·86 % TEI	1·22 % TEI	
		Number of events	27	28	16	22	28	>0·05

R-TFA, ruminant TFA; I-TFA, industrial TFA; TEI, total daily energy intake estimated as 2000 kcal/d (8370 kJ/d); RR, relative risk.
* Values are expressed as RR (95 % CI) except for the Scottish Heart Health Study, where they are expressed as OR (95 % CI), and the study from four Danish cohorts, where they are expressed as number of events.
† Adjusted for age, treatment group, smoking, BMI, blood pressure, intakes of energy, alcohol and fibre, education and physical activities.
‡ Adjusted for age, weight, height, smoking, physical activity, diastolic pressure, TEI, intakes of SFA, linoleic acid and antioxidant vitamins.

33 % of total TFA intake) compared with the lowest (0·6 g/d, about 33 % of total TFA) quintile[18].

Only Oomen *et al.*[17] found a similar effect between the different TFA sources. Indeed, in their prospective population-based study, 667 men of the Zutphen Elderly Study aged 64–84 years and free of CHD at baseline were studied. Dietary surveys were used to establish the participants' food consumption patterns[17]. The relative risks of CHD for an increase of 0·5 % in energy at baseline from TFA from different sources (R-TFA, manufactured 18 : 1 TFA and other manufactured TFA) was calculated. For a mean intake of 0·7 (SD 0·2) % of energy (about 1·22 g/d with TEI at 9·2 MJ) the relative risk was 1·17 (95 % CI 0·69, 1·98) and was similar to the others TFA[17]. It is important to note that during the follow-up, the food items have varied and the amount of TFA has decreased. For instance, after 10 years of follow-up, the mean contribution of TFA intake to TEI decreased from 1985 to 1990 and 1995 (4·3 (SD 2·2) *v.* 2·9 (SD 1·5) *v.* 1·9 (SD 0·6) %).

Impact of trans-fatty acids on markers of CHD

Effects of trans-*fatty acids on HDL- and LDL-cholesterol*. The story linking ruminant TFA intake or biomarkers of TFA intake with the serum lipid profile began about 15 years ago when Bolton-Smith *et al.* demonstrated that in between 4490 and 5170, respectively, for men and women, the total cholesterol varied significantly ($P < 0·001$) by fifths of the R-TFA for men (1·5 to 4·9 g R-TFA/d from the lowest to the highest fifths) and women (1·2 to 4·3 g R-TFA/d from the lowest to the highest fifths) (positive trend) and by fifths of I-TFA for men (1 to 8·8 g I-TFA/d from the lowest to the highest fifths) (negative trend) who participated in the Scottish Heart Health Study[19].

Recently, Chardigny *et al.*[20] compared the effects of an 8 weeks consumption of 5·4 % of TEI (about 11–12 g/d) as TFA from hydrogenated sources with the same amount of TFA from ruminant sources on lipoprotein metabolism in forty-six healthy men and women. This study was a monocentric, randomised, double blind, controlled, cross-over design. In women, but not in men, R-TFA beneficially increased HDL-cholesterol concentrations. However, an increase in LDL-cholesterol and TAG concentrations was also observed. For both sexes, the serum total cholesterol: HDL-cholesterol ratio did not change. In a second study published at the same time by Motard-Bélanger *et al.*[21], the effects of TFA from ruminant sources were studied in a double-blind, randomised, cross-over controlled study according to a Latin square design, in which each man was assigned to four different isoenergetic diets lasting 4 weeks each. In this study, diets were high in R-TFA (10·2 g/2500 kcal (10 460 kJ); 3·7 % of TEI), moderate in R-TFA (4·2 g/2500 kcal; 1·5 % of TEI), high in I-TFA (10·2 g/2500 kcal), or low in TFA from any source (2·2 g/2500 kcal) (control diet). It was found that at high intakes

(3·7 % of TEI), TFA from both ruminant and hydrogenated sources adversely affected cholesterol metabolism. At intakes of 1·5 % of TEI, no effects of TFA from ruminant sources on the serum lipoprotein profile were observed.

Relationship between trans-*fatty acids and markers of haemostatic function*. The story of the relationship between TFA intake and cardiovascular risk factors continues with the study of the effects of TFA on markers for platelet aggregation, coagulation and fibrinolysis, three main determinants of haemostatic function.

Tholstrup *et al.*[22] demonstrated no adverse effect of a daily intake of 3·5 g (about 1% of TEI) of vaccenic acid (18 : 1Δ11t) from enriched butter during 5 weeks on haemostatic risk markers (plasminogen activator inhibitor 1 and factor FVII coagulant activity) of forty-two healthy young men, compared with a control butter that provided 0·3 g vaccenic acid per d (18 : 1Δ11t)[22]. However, the authors concluded that these effects may have been partly attributable to the higher MUFA and lower SFA content of the high-vaccenic acid (18 : 1Δ11t) diet, rather than to the effects of vaccenic acid (18 : 1Δ11t) itself.

The consumption of a diet rich in vaccenic acid (18 : 1Δ11t) (about 3·5 g/d; about 1% TEI) for 5 weeks did not adversely affect blood pressure or isobaric arterial elasticity when measured in healthy young men by a volume-oscillometric method[23]. These authors compared the effect of a diet based on milk fat high in vaccenic acid (18 : 1Δ11t), and not vaccenic acid (18 : 1Δ11t) *per se*.

Relationship between trans-*fatty acids and markers of inflammation and endothelial dysfunction*. Markers of inflammation have been implicated as risk factors for several degenerative diseases. With respect to CVD, the data collected from cross-sectional and prospective studies provide evidence that some markers are independent risk factors, and several of them can be modifiable throughout lifestyle, by physical exercise, smoking and also diet.

No observational studies have reported a correlation between a high content of R-TFA and biomarkers of inflammation in healthy volunteers, and only a few interventional studies have followed the effect of the consumption of R-TFA on inflammatory parameters.

Kuhnt *et al.*[24] designed a study to investigate the effects of a 6-week dietary supplementation of 3 g of vaccenic acid (18 : 1Δ11t) and 3 g of 18 : 1Δ12t and endogenous conjugated linoleic acid synthesis on several biomarkers (IL-6 and IL-8, TNF-α, C-reactive protein, inter-cellular adhesion molecule-1, leptin and adiponectin) in young men and women. These markers belong to the systemic inflammation which has been reported as an independent risk factor for heart disease[25]. No significant differences in the plasma concentrations of any biomarker were observed during the 18 : 1Δ11t and 18 : 1Δ12t intervention; these results were in agreement with the results of Thosltrup *et al.*[22].

In healthy men, Motard-Belanger *et al.*[21] found that the circulating level of C-reactive protein was not statistically

different at the end of 4 weeks of experimental isoenergetic diets high in ruminant or industrial TFA (10·2 g/2500 kcal (10 460 kJ)). In our study published in 2008, we found no effect of consumption for 4 weeks of food items containing TFA (11–12 g/d) from ruminants and from industrial sources on the plasma high-sensitivity C-reactive protein concentration of forty-six healthy subjects[26].

These different results on the effects of the intake of R-TFA on the development of cardiovascular pathologies are still contradictory, even though they tend to underline isomer-specific effects. Weggemans *et al.*[27] concluded that epidemiological studies from Europe and the USA show that up to 2·5 g/d, the effects of total, ruminant or industrial TFA are similar. An intake of more than 3 g/d of total and industrial TFA is associated with increased CHD risk. However, there are insufficient data on the health effects of ruminant TFA intakes over 2·5 g/d to allow any comparison in this range. They therefore advised not to discriminate between these sources in legislation or dietary recommendations. However, recently, Jakobsen *et al.*[16], using data from the four Danish cohort studies, found no evidence of a higher risk associated with R-TFA intake within the wide range of intake among both women (90 % central range: 0·5–3·1 g/d) and men (90 % central range: 0·6–4·1 g/d). The authors concluded that a high intake of TFA from dairy and ruminant meat products is an issue of no concern to public health.

Trans-fatty acids and risks for type 2 diabetes

Type 2 diabetes is characterised by an increased blood glucose level due to impaired insulin sensitivity by peripheral tissues and decreased insulin secretion by pancreatic β-cells.

Effects of trans-fatty acids on glucose tolerance and type 2 diabetes

An important body of evidence relating total TFA intake to risk of type 2 diabetes came from the Nurses' Health Study that prospectively followed, during 14 years, 84 204 healthy women aged between 34 and 59 years[28]. The data suggested that total lipid intake, compared with equivalent energy intake from carbohydrates, was not associated with the risk of type 2 diabetes in women. By contrast, the study reported that high intakes of TFA (3 (SD 0·5) % of total daily energy intake, TEI) correlated positively with the risk of diabetes in age- and BMI-adjusted analyses. In addition, the authors estimated that replacing 2 % of energy from TFA isoenergetically with PUFA would lead to a 40 % lower risk of developing type 2 diabetes. Subgroup analyses revealed that effects were primarily observed in obese women, possibly due to the fact that these women were already more insulin resistant than non-obese women[28]. In addition, the Nurses' Health Study carried certain methodological limitations that may have affected the interpretation of TFA data. Notably, averaged food composition changed substantially over the 20-year experimental follow-up, in particular with regards to *trans*-fat content. In addition, foods rich in TFA were also rich in refined carbohydrates. Finally, and most tellingly, the techniques used to assess *trans*-fat content in foods have also improved[29]. In the epidemiological studies, the sources of TFA were not examined, so no conclusions could be brought regarding the respective effects of dairy and hydrogenated fats. In fact, differences in the relative abundance of each TFA isomer in food products could probably lead to different metabolic responses.

Short-term intervention studies in clinical or in animal models have not been very conclusive either. More recently, new data were obtained on the effects of TFA of dairy origin since it is now possible to produce experimental dairy vaccenic acid-enriched fat. A 5-week nutritional double-blind, randomised, parallel intervention study was performed in forty-two healthy young men stratified according to BMI (kg/m^2). The volunteers received either a diet high in vaccenic acid (3·5 g/d; about 1 % of TEI; 18 : 1Δ11t) or a control diet providing 0·3 g vaccenic acid per d (18 : 1Δ11t). The authors did not find any effects of vaccenic acid-rich dairy fat on fasting insulin and glucose[22].

In rat models, insulin and glucose responses to an intraperitoneal injection of glucose were not modified after 8 weeks of experimental diets enriched in TFA of either dairy or industrial origin at 4·1 % of total TEI[30]. These data were reinforced by recent findings by Wang *et al.*, given that, after 4 weeks of a control diet supplemented with 1·5 % (w/w) vaccenic acid (18 : 1Δ11t), insulin and glucose metabolism of both lean and obese rats was not affected in response to a meal tolerance test[31].

Effects of trans-fatty acids on insulin secretion by pancreatic β-cells

Long-term exposure of β-cells to high levels of fatty acids causes enhanced insulin secretion at low glucose (basal insulin release), while glucose-stimulated insulin secretion is decreased or unchanged. In isolated mouse islets, an acute incubation with the *trans* isomers *trans*-vaccenic (18 : 1Δ11t) and elaidic (18 : 1Δ9t) acids elicited a higher maximal insulin output than the *cis* isomer oleic acid[32]. The same authors compared in cultured INS-1 cells the effect of 3 d incubation with *cis*- (*cis*-18 : 1-11) and *trans*-vaccenic acid (*trans*-18 : 1-11), as well as oleic (*cis*-18 : 1-9) and *trans*-elaidic acid (*trans*-18 : 1-9), on basal and glucose-stimulated insulin secretion. All fatty acids tested increased basal insulin release; however, a significantly lower basal insulin release was demonstrated for cells cultured with 0·3 to 0·4 mM-*trans*-vaccenic acid compared with equimolar levels of the *cis* isomer. Glucose-stimulated insulin secretion was not changed by *cis*- or *trans*-vaccenic acid or by oleic acid, whereas it was stimulated by 0·3 to 0·4 mM-*trans*-elaidic acid[33]. Despite these

in vitro results, there are no available data on the impact of TFA of dairy origin on insulin secretion *in vivo*.

Effects of trans-fatty acids on peripheral insulin sensitivity

Little is known on the effects of TFA of both origins on insulin sensitivity in humans. An important step towards glucose intolerance and type 2 diabetes is peripheral resistance to insulin action.

A 4-week nutritional double-blind, randomised, parallel intervention study was performed in sixty-three healthy women with abdominal obesity (waist circumference > 88 cm and BMI > 28 kg/m^2). After a run-in period, the volunteers were randomly assigned to consume for 4 weeks one of the three following diets: 60 g low-TFA lipids/d (0·54 g/d; *n* 21), R-TFA-rich lipids (4·86 g/d; *n* 21), or I-TFA-rich lipids (5·58 g/d; *n* 21). Changes in peripheral insulin sensitivity were assessed by using hyperinsulinaemic–euglycaemic clamps. The results showed that TFA from dairy and industrial sources ingested at a nutritional level (2 % of total TEI) for 4 weeks do not further alter peripheral insulin sensitivity in overweight and obese women with impaired insulin sensitivity[26]. However, the principle of precaution required that these results are not extended to long-term effects of TFA.

To go further in the comprehension of the mechanisms, Tardy *et al.* established in C2C12 myotubes that insulin-stimulated Akt phosphorylation was similar to the control group (without fatty acid) after incubation with oleic, elaidic and vaccenic acids[30], suggesting no direct effect of TFA on muscle insulin pathways. Those results were reinforced by a recent study of Hommelberg *et al.*[34] which demonstrated in cultured murine skeletal muscle cells that insulin-stimulated glucose uptake and GLUT4 translocation were similar to those in the control group after incubation with vaccenic and elaidic acids.

In addition, Tardy *et al.* also demonstrated that oxidative (*soleus*) and glycolytic (*tibialis anterior*) muscle mitochondria have the same capacity to oxidise *cis*-fatty acids and TFA (elaidic and vaccenic acids), suggesting that the geometrical MUFA configuration does not heavily influence their oxidation rate. Thus, any TFA-induced alteration in muscle metabolism is not due to differences in oxidation rates[30].

To conclude, there is a body of evidence showing an absence of TFA effects on skeletal muscle insulin sensitivity. However, additional studies are required, especially on adipose tissue, to further analyse metabolic responses to TFA, including bioenergetic, lipid and carbohydrate pathways.

Conclusion

While TFA from hydrogenated oils have been clearly implicated in the pathogenesis of CVD, those from dairy products may be less deleterious at a level lower than 2 % of total TEI. Furthermore, dietary intakes of TFA from dairy

origin are far from being high enough to constitute a serious threat for public health. As concluded by Booker & Mann[35], the advice would be to remove TFA from industrial sources from the food supply. Therefore, regular moderate consumption of dairy fats could be tolerated with respect to cardiovascular risks. Regarding the role of TFA from both origins on the incidence of type 2 diabetes, studies carried out on the topic are still controversial. Combined results suggest that TFA of dairy (R-TFA) and industrial (I-TFA) origins may not impair glucose tolerance at a physiological dose and during a short-term period. Mechanistic and intervention studies showed no significant effect of TFA on glucose tolerance or insulin resistance, suggesting that these fatty acids may not increase the risk for diabetes. The synthesis of these studies does confirm the food safety authority recommendations that their intake should be limited to 2 % of total TEI. Additional data are required to better understand possible long-term effect of TFA, especially to examine the impact of TFA in individuals at risk for the metabolic syndrome and type 2 diabetes.

References

1. Scientific Panel on Dietetic Products NaA (2004) Opinion of the Scientific Panel on Dietetic Products, Nutrition and Allergies on a request from the Commission related to the presence of *trans* fatty acids in foods and the effect on human health of the consumption of *trans* fatty acids (Request no. EFSA-Q-2003-022). In *EFSA Journal*, vol. 81, pp. 1–49. Parma, Italy: European Food Safety Authority.
2. Bauman DE, Mather IH, Wall RJ, *et al.* (2006) Major advances associated with the biosynthesis of milk. *J Dairy Sci* **89**, 1235–1243.
3. Wolff RL & Sebedio JL (1991) Geometrical isomers of linolenic acids in low-caloric spreads marketed in France. *J Am Oil Chem Soc* **68**, 719–725.
4. Ratnayake WM (2004) Overview of methods for the determination of *trans* fatty acids by gas chromatography, silver-ion thin-layer chromatography, silver-ion liquid chromatography, and gas chromatography/mass spectrometry. *J AOAC Int* **87**, 523–539.
5. Leger C-L & Razanamahefa L (2005) *Risques et bénéfices pour la santé des acides gras* trans *apportés par les aliments. Recommandations (Risks and Benefits of Food Trans Fatty*

Acids for Human Health. Recommendations). Maison-Alfort: AFSSA.

6. Jensen RG (2002) The composition of bovine milk lipids: January 1995 to December 2000. *J Dairy Sci* **85**, 295–350.

7. Bauman DE & Griinari JM (2003) Nutritional regulation of milk fat synthesis. *Annu Rev Nutr* **23**, 203–227.

8. Hulshof KF, van Erp-Baart MA, Anttolainen M, *et al.* (1999) Intake of fatty acids in western Europe with emphasis on *trans* fatty acids: the TRANSFAIR Study. *Eur J Clin Nutr* **53**, 143–157.

9. Chilliard Y, Ferlay A, Mansbridge RM, *et al.* (2000) Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, *trans* and conjugated fatty acids. *Ann Zootech* **49**, 181–205.

10. Chilliard Y & Ferlay A (2004) Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. *Reprod Nutr Dev* **44**, 467–492.

11. Jakobsen MU, Bysted A, Andersen NL, *et al.* (2006) Intake of ruminant *trans* fatty acids in the Danish population aged 1–80 years. *Eur J Clin Nutr* **60**, 312–318.

12. Micha R & Mozaffarian D (2009) *Trans* fatty acids: effects on metabolic syndrome, heart disease and diabetes. *Nat Rev Endocrinol* **5**, 335–344.

13. Micha R & Mozaffarian D (2008) *Trans* fatty acids: effects on cardiometabolic health and implications for policy. *Prostaglandins Leukot Essent Fatty Acids* **79**, 147–152.

14. Willett WC, Stampfer MJ, Manson JE, *et al.* (1993) Intake of *trans* fatty acids and risk of coronary heart disease among women. *Lancet* **341**, 581–585.

15. Ascherio A, Hennekens CH, Buring JE, *et al.* (1994) *Trans*-fatty acids intake and risk of myocardial infarction. *Circulation* **89**, 94–101.

16. Jakobsen MU, Overvad K, Dyerberg J, *et al.* (2008) Intake of ruminant *trans* fatty acids and risk of coronary heart disease. *Int J Epidemiol* **37**, 173–182.

17. Oomen CM, Ocke MC, Feskens EJ, *et al.* (2001) Association between *trans* fatty acid intake and 10-year risk of coronary heart disease in the Zutphen Elderly Study: a prospective population-based study. *Lancet* **357**, 746–751.

18. Pietinen P, Ascherio A, Korhonen P, *et al.* (1997) Intake of fatty acids and risk of coronary heart disease in a cohort of Finnish men. The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. *Am J Epidemiol* **145**, 876–887.

19. Bolton-Smith C, Woodward M, Fenton S, *et al.* (1996) Does dietary *trans* fatty acid intake relate to the prevalence of coronary heart disease in Scotland? *Eur Heart J* **17**, 837–845.

20. Chardigny JM, Destaillats F, Malpuech-Brugere C, *et al.* (2008) Do *trans* fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in healthy subjects? Results of the *Trans* Fatty Acids Collaboration (TRANSFACT) study. *Am J Clin Nutr* **87**, 558–566.

21. Motard-Bélanger A, Charest A, Grenier G, *et al.* (2008) Study of the effect of *trans* fatty acids from ruminants on blood lipids and other risk factors for cardiovascular disease. *Am J Clin Nutr* **87**, 593–599.

22. Tholstrup T, Raff M, Basu S, *et al.* (2006) Effects of butter high in ruminant *trans* and monounsaturated fatty acids on lipoproteins, incorporation of fatty acids into lipid classes, plasma C-reactive protein, oxidative stress, hemostatic variables, and insulin in healthy young men. *Am J Clin Nutr* **83**, 237–243.

23. Raff M, Tholstrup T, Sejrsen K, *et al.* (2006) Diets rich in conjugated linoleic acid and vaccenic acid have no effect on blood pressure and isobaric arterial elasticity in healthy young men. *J Nutr* **136**, 992–997.

24. Kuhnt K, Kraft J, Vogelsang H, *et al.* (2007) Dietary supplementation with *trans*-11- and *trans*-12-181 increases *cis*-9, *trans*-11-conjugated linoleic acid in human immune cells, but without effects on biomarkers of immune function and inflammation. *Br J Nutr* **97**, 1196–1205.

25. Libby P (2002) Inflammation in atherosclerosis. *Nature* **420**, 868–874.

26. Tardy AL, Lambert-Porcheron S, Malpuech-Brugere C, *et al.* (2009) Dairy and industrial sources of *trans* fat do not impair peripheral insulin sensitivity in overweight women. *Am J Clin Nutr* **90**, 88–94.

27. Weggemans RM, Rudrum M & Trautwein EA (2004) Intake of ruminant *versus* industrial *trans* fatty acids and risk of coronary heart disease – what is the evidence? *Eur J Lipid Sci Technol* **106**, 390–397.

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

29. Clandinin MT & Wilke MS (2001) Do *trans* fatty acids increase the incidence of type 2 diabetes? *Am J Clin Nutr* **73**, 1001–1002.

30. Tardy A-L, Giraudet C, Rousset P, *et al.* (2008) Effects of *trans* MUFA from dairy and industrial sources on muscle mitochondrial function and insulin sensitivity. *J Lipid Res* **49**, 1445–1455.

31. Wang Y, Lu J, Ruth MR, *et al.* (2008) *Trans*-11 vaccenic acid dietary supplementation induces hypolipidemic effects in JCR:LA-cp rats. *J Nutr* **138**, 2117–2122.

32. Alstrup KK, Gregersen S, Jensen HM, *et al.* (1999) Differential effects of *cis* and *trans* fatty acids on insulin release from isolated mouse islets. *Metabolism* **48**, 22–29.

33. Alstrup KK, Brock B & Hermansen K (2004) Long-term exposure of INS-1 cells to *cis* and *trans* fatty acids influences insulin release and fatty acid oxidation differentially. *Metabolism* **53**, 1158–1165.

34. Hommelberg PP, Langen RC, Schols AM, *et al.* (2010) *Trans* fatty acid-induced NF-κB activation does not induce insulin resistance in cultured murine skeletal muscle cells. *Lipids* **45**, 285–290.

35. Booker CS & Mann JI (2008) *Trans* fatty acids and cardiovascular health: translation of the evidence base. *Nutr Metab Cardiovasc Dis* **18**, 448–456.