

Platelet *trans* fatty acids in relation to angiographically assessed coronary artery disease

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Abstract

Epidemiological and metabolic studies indicate that a higher intake of *trans* fatty acids (TFA) may be associated with increased risk of coronary heart disease (CHD). In a cross-sectional study of patients who underwent coronary angiography, the relationships between TFAs, measured in platelets, and the degree of coronary artery disease (CAD) were examined in 191 non-diabetic patients (134 men and 57 women). The degree of CAD was quantified by using an angiographic scoring system developed to provide an estimate of the extent of coronary atherosclerosis: an 'extent score'. The TFA composition of platelets, including palmitelaidic (16:1 ω 7t), elaidic (18:1 ω 9t), *trans*-10-octadecaenoic acid (18:1 ω 8t), *trans* vaccenic (18:1 ω 7t), *trans*-12-octadecaenoic acid (18:1 ω 6t) and linoelaidic (18:2 ω 6tt) acids, was measured by using gas chromatography and quantified as a percentage of total fatty acids. After adjustment for established CHD risk indicators, including age, gender, cigarette smoking, hypertension and serum total cholesterol concentration, elaidic acid ($P = 0.0300$) and *trans*-10-octadecaenoic acid ($P = 0.0434$) were positively associated with the extent score of CAD. The adjusted associations between other individual TFAs, including palmitelaidic acid ($P = 0.1189$), vaccenic acid ($P = 0.7651$), *trans*-12-octadecaenoic acid ($P = 0.0582$) and linoelaidic acid ($P = 0.8793$), and the extent score were not significant. The results of this study, therefore, provide evidence for an association between particular platelet TFAs and the degree of CAD in the patient population studied.

Keywords: *Trans* fatty acids; Elaidic acid; Coronary artery disease

1. Introduction

Many vegetable oils are partially hydrogenated to attain properties required for particular food uses. The process of hydrogenation produces a variety of *trans* and uncommon *cis* fatty acids. The other main source of *trans* fatty acids (TFAs)

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Table 1
Patient characteristics and descriptive statistics for non-diabetic patients

Characteristic	(%)	Characteristic	mean \pm S.D. (n)
Angina	98	Extent score (out of 10)	3.9 \pm 2.1 (191)
Previous myocardial infarction	37	Age (years)	59 \pm 11 (191)
Valvular heart disease	3.1	Total cholesterol (mmol l ⁻¹)	5.9 \pm 1.0 (191)
History of hypertension	47	HDL cholesterol (mmol l ⁻¹)	1.07 \pm 0.37 (187)
Smokers	15	Triglycerides (mmol l ⁻¹)	1.71 \pm 0.92 (191)
Ex-smokers	55	Smoking (years ^a)	22.8 \pm 18.2 (160)
Never smoked	30		

^aNumber of years as a cigarette smoker.

in the human diet is from products of ruminant animal origins [1,2]. There have been concerns about possible adverse effects of TFAs on human health, particularly in relation to coronary heart disease (CHD). Studies have shown that dietary TFAs can adversely alter blood lipid/lipoprotein concentrations [3–7], with higher TFA intake resulting in higher serum low density lipoprotein (LDL) cholesterol [3,4,7] and Lipoprotein(a) [5,6], and lower serum high density lipoprotein (HDL) cholesterol concentrations [3,4,7]. In addition, several studies have now examined the relationship between TFAs and CHD risk [8–13]. Most of these studies have found a positive association [9,11–13]. A higher intake of TFAs has been associated with increased risk of myocardial infarction (MI) [9] and CHD [13]. In both these studies [9,13] the increased risk was attributable to hydrogenated vegetable oil intake rather than ruminant sources. In the EURAMIC study [8], which was an international multicentre study in European and Israeli population groups, adipose tissue TFAs were not significantly related to the risk of MI across all populations. In this present study, the aim was to examine relationships between platelet TFAs and the degree of angiographically assessed coronary artery disease (CAD).

2. Methods

Consecutive patients who were on the routine cardiac catheterisation list for investigation of chest pain thought to be due to either CAD (~97%) or valvular heart disease (~3%) were

enrolled. At the time of enrolment, all patients were included in the study and underwent coronary angiography. However, only non-diabetic patients (134 men and 57 women aged 16 to 80 years) were included in the analyses. The project was presented to and approved by the Monash Medical Centre, Ethics and Research Committee.

The patient characteristics are presented in Table 1. Clinical details were gathered by using a questionnaire administered at the time of angiography, or were collected from medical records. Coronary angiography was performed according to the Judkins technique, and recorded on 35 mm film. The degree of CAD was quantified by using an angiographic score which has been constructed to reflect the proportion of coronary endothelial surface area affected by atheroma. The score was developed to provide an estimate of the extent of coronary atherosclerosis. The severity and location of coronary lesions were not taken into account. The proportion of each vessel of the coronary arterial tree with detectable atheroma, identified as luminal irregularity, was multiplied by a factor for each vessel [14]. The factor for each vessel is related to the size and amount of myocardium supplied. The scores for each vessel were added to give a total score out of a maximum of ten. A score of zero indicated that no coronary atheroma was detected and a score of ten meant that 100% of the coronary arteries visualised showed detectable atheroma. All assessments of the degree of CAD were performed by a single cardiologist in order to limit errors due to inter-observer variability. The cardiologist was blinded to the results of fatty acid measurements.

Fasting blood was drawn from the femoral artery, immediately prior to cardiac catheterisation, and placed into evacuated glass tubes. The untreated tubes were allowed to clot and the serum was separated by centrifugation. Total cholesterol, triglycerides, and HDL cholesterol were measured on fresh serum. Total cholesterol and triglycerides were measured enzymatically with commercial kits (Trace Scientific Pty Ltd., Clayton, Victoria, Australia, catalogue numbers 13225 and 22203, respectively). High density lipoprotein cholesterol was measured enzymatically, as for total cholesterol, following the precipitation of apolipoprotein B containing lipoproteins using equal volumes of 20% polyethylene glycol 6000 and serum. Cholesterol and triglyceride measurements were performed on a KONE Progress random access analyser (KONE Instruments Corp., Espoo, Finland).

The fatty acid composition of platelets was measured by using gas chromatography. Methods relating to platelet harvesting, extraction and methyl esterification, and measurement of platelet fatty acids (not including TFAs) by gas chromatography, and results showing relationships between platelet fatty acids (not including TFAs) and degree of CAD are presented elsewhere [15]. For the measurement of TFAs, improved separation of fatty acid methyl esters was required. To improve separation, two 50 m BPX70 fused silica capillary columns with a 70% cyanopropyl siloxane polar stationary phase (SGE Scientific Pty Ltd Melbourne, Australia. Part number 054603) were joined to produce a 100 m column. Temperature programming was used in the determination of TFAs. The starting temperature of 175°C was maintained for 35 min, raised at 2.5°C min⁻¹ until 220°C was reached then held at this temperature for 15 min. The temperature was then increased at 2.5°C min⁻¹ until 240°C was reached and held constant for 14 min. Standards for the identification of TFA peaks, including palmitelaidic (16:1 ω 7t), elaidic (18:1 ω 9t), *trans* vaccenic (18:1 ω 7t) and linoelaidic (18:2 ω 6tt) acids, were obtained from Alltech (Baulkman Hills, NSW, Australia, catalogue numbers ME0161T, ME0181T, ME0181TV, ME0182 respectively). Other 18:1 TFAs including *trans*-10-octade-

Table 2
Reproducibility of *trans* fatty acid measurement

<i>Trans</i> fatty acid	Coefficient of variation (%)
Palmitelaidic acid (16:1 ω 7t)	12.0
Elaidic acid (18:1 ω 9t)	13.8
<i>Trans</i> -10-octadecaenoic acid (18:1 ω 8t)	17.7
Vaccenic acid (18:1 ω 7t)	10.5
<i>Trans</i> -12-octadecaenoic acid (18:1 ω 6t)	20.2
Linoelaidic acid (18:2 ω 6tt)	18.6

caenoic acid (18:1 ω 8t) and *trans*-12-octadecaenoic acid (18:1 ω 6t) were tentatively identified. The reproducibility of TFA measurements is presented in Table 2.

The data analysis package used for the statistical analyses performed was SPSS (Michigan, USA). At a univariate level, Spearman's rank correlation coefficient (*r*) was used to determine the degree and direction of association between two variables and logistic regression was used to determine the associations of continuous variables with dichotomous variables. The relationships between individual TFAs and the CAD score were examined in non-diabetic patients by using multiple linear regression, with adjustment for recognised CHD risk factors.

3. Results

Descriptive statistics for the study population are presented in Table 1. The mean concentrations of platelet TFAs, expressed as a percentage

Table 3
Trans fatty acid composition of platelets^a

<i>Trans</i> fatty acid	% total fatty acids
Palmitelaidic acid (16:1 ω 7t)	0.22 ± 0.04 (161)
Elaidic acid (18:1 ω 9t)	0.39 ± 0.17 (161)
<i>Trans</i> -10-octadecenoic acid (18:1 ω 8t)	0.20 ± 0.10 (161)
Vaccenic acid (18:1 ω 7t)	0.50 ± 0.15 (161)
<i>Trans</i> -12-octadecenoic acid (18:1 ω 6t)	0.30 ± 0.11 (161)
Linoelaidic acid (18:2 ω 6tt)	0.13 ± 0.07 (157)
Total <i>trans</i>	1.74 ± 0.49 (157)

^aMean (as percent of total fatty acids) ± standard deviation. *n* in brackets; includes non-diabetic patients.

Table 4
Correlations between individual *trans* fatty acids

	16:1 ω 7t	18:1 ω 9t	18:1 ω 8t	18:1 ω 7t	18:1 ω 6t
18:1 ω 9t	-0.09				
18:1 ω 8t	-0.08	0.84**			
18:1 ω 7t	0.10	0.55**	0.70**		
18:1 ω 6t	-0.06	0.83**	0.83**	0.57**	
18:2 ω 6tt	0.07	0.14	0.14	0.21*	0.12

* $P < 0.01$, ** $P < 0.0001$.

of total fatty acids, are presented in Table 3. Results showing the relationships between platelet fatty acids, other than *trans*, and CAD have been presented and discussed previously [15].

Correlations between each of the platelet TFAs are presented in Table 4. Palmitelaidic acid was not significantly associated with the concentration of any of the other TFAs and linoelaidic acid was significantly associated only with vaccenic acid. The 18:1 TFAs were, generally, strongly correlated with each other.

The univariate associations between individual TFAs and age, gender, hypertensive status, years as a cigarette smoker and serum lipids were assessed. Elaidic acid ($r = 0.16$ $P = 0.044$), *trans*-12-octadecaenoic acid ($r = 0.17$ $P = 0.034$) and linoelaidic acid ($r = 0.17$ $P = 0.038$) were positively associated with age. No significant associations between TFAs and gender, hypertensive status, years as a cigarette smoker, total cholesterol, HDL cholesterol or triglycerides were found.

A multiple linear regression model, which includes recognised CHD disease risk factors to predict the extent score of CAD, is presented in Table 5. Age, male gender and years as a cigarette smoker were significantly positively associated with CAD. Hypertensive status and total cholesterol did not reach significance in the model. Together, these variables were able to explain about 21% of the variance in the extent score of CAD, which was mostly accounted for by effects of age, gender and cigarette smoking.

Regression models to predict the extent score of CAD, with individual platelet *trans* fatty acids as the independent variables and with adjustment for established CHD risk factors, are presented in

Table 6. Two of the TFAs, namely elaidic acid and *trans*-10-octadecaenoic acid were significantly positively related to CAD. In addition, the adjusted relationship between *trans*-12-octadecaenoic acid and CAD approached significance ($P = 0.0582$). Other TFAs, including total *trans*, were not significantly associated with CAD.

4. Discussion

The extent score is an angiographic assessment which was designed to estimate the aggregate degree of coronary atherosclerosis [14]. Severity of coronary atheroma has been quantified previously in many ways. Methods that are of the greatest use clinically, such as those which estimate stenosis of individual vessels or provide an aggregate estimate of stenosis severity, are heavily weighted by severity of luminal narrowing and may not provide the best estimate of the degree of atherosclerosis. Episodic events, which underlie unstable angina and myocardial infarction, strongly influence angiographic stenosis severity and may not be related, as strongly, to the development of atheroma [14]. If the aim of the investigation is to determine the relationship of particular variables with the degree of coronary atherosclerosis, then an angiographic score which is not biased by stenosis severity and lesion location is probably more useful. This was the main reason for the development of the extent score, which was subsequently shown to be correlated more strongly, than either a vessel score or a stenosis score, with CHD risk factors [14].

A major role of fatty acids in the body is as structural components of membranes. *Trans* fatty acids, measured in platelets, may provide an indication of membrane fatty acid composition, in addition to intake of these fatty acids. Apart from blood lipid and lipoprotein concentrations, TFAs might also influence CHD risk via effects on membrane function, prostaglandin synthesis and haemostasis. At the present time however there is little direct evidence to support this hypothesis.

The CV% for the measurement of individual TFAs in platelets was relatively high (> 10%) due to the low concentration of these fatty acids in platelets. The two TFAs found in highest concen-

Table 5
Multiple linear regression model to predict the extent score of CAD

Independent variables	Extent score		
	Estimated coefficient	S.E. of estimated coefficient	P-value
Age (years)	0.0560	0.0143	0.0001
Gender	-1.0059	0.3392	0.0035
Smoking (years)	0.0284	0.0084	0.0009
Hypertensive status	0.4000	0.3063	0.1938
Total cholesterol (mmol l ⁻¹)	0.1900	0.1488	0.2035
(constant)	-0.4650	1.2300	0.7059
Adjusted R ² = 0.2102			

trations were elaidic and *trans* vaccenic acids, both of which were at mean concentrations of well under 1% of the total fatty acids. The mean total TFA content of platelets was less than 2%. The relative mean contribution of TFAs to platelet fatty acid composition was, therefore, quite small. The incomplete chromatographic separation of *trans*-10-octadecaenoic acid from *trans* vaccenic acid, and of *trans*-12-octadecaenoic acid from oleic acid may also have contributed to poor reproducibility.

The pattern of TFAs formed in the partial hydrogenation of vegetable oils differs from that found from ruminant sources. Much of the TFA from ruminants is *trans* vaccenic acid, and the main TFA found in hydrogenated vegetable oils is elaidic acid, although other TFAs are also present. Given the evidence for adverse effects of TFAs from hydrogenated vegetable oils on blood lipid and lipoprotein concentrations [3–6] and the results of studies indicating that a higher intake of TFAs from hydrogenated vegetable oils is associated with increased risk of MI [9] and CHD [13], a positive association between elaidic acid and other TFAs found in hydrogenated vegetable oils, and CAD might be expected. It has been suggested that the combined results of metabolic and epidemiological studies provide strong evidence for a causal relationship between TFA intake and CHD risk [16].

In this current study the intake of TFAs was not assessed. It has been estimated that the mean TFA intake in Australia is likely to be less than

2–2.5% of energy, with approximately half of the TFAs derived from hydrogenated vegetable oils (1–1.5%) [17]. It is likely, however, that a wide range of TFA intake levels is present in the Australian population. The mean intake of TFAs in general, and those derived from hydrogenated vegetable oils, in the USA is likely to be considerably higher than in the Australian population, probably greater than 5% of energy [17–19]. Much of the epidemiological evidence linking hydrogenated vegetable oil intake with CHD risk comes from studies done in USA populations [9,13]. *Trans* fatty acids derived from hydrogenated vegetable oils may be a less important CHD risk indicator in populations with lower intakes. This suggestion is supported by results of the EURAMIC study [8] which did not find that TFAs increase the risk of heart disease significantly across all populations. However, when relationships were assessed in each population group separately, significant positive associations between TFAs in adipose tissue and risk of MI were found in Norway and Finland [8].

In this present study the relationships between individual platelet TFAs and the degree of CAD were assessed. Significant positive associations of two of the TFAs, elaidic acid and *trans*-10-octadecaenoic acid, with CAD were found. Although significant, the inclusion of these TFAs in regression models to predict CAD did not greatly improve the prediction of the extent score. However, total cholesterol and hypertensive status, which are established CHD risk factors, were not significantly associated with the degree of CAD.

Table 6
Multiple linear regression models with the extent score of CAD as the dependent variable and individual platelet *trans* fatty acids as the independent variable, with adjustment for established CHD risk factors

Independent variables	Extent score		
	Estimated coefficient	S.E. of estimated coefficient	P-value
<i>Palmitelaidic acid</i> (16:1 ω 7t)	-5.4170	3.4486	0.1189
Age (years)	0.0454	0.0155	0.0040
Gender	-0.8516	0.3724	0.0240
Smoking (years)	0.0314	0.0092	0.0009
Hypertensive status	0.2736	0.3310	0.4102
Total cholesterol (mmol l ⁻¹)	0.0796	0.1645	0.6293
(constant)	1.8210	1.6241	0.2644
Adjusted R ² = 0.1981			
<i>Elaidic acid</i> (18:1 ω 9t)	2.2251	1.0129	0.0300
Age (years)	0.0425	0.0154	0.0069
Gender	-1.0006	0.3685	0.0076
Smoking (years)	0.0315	0.0091	0.0007
Hypertensive status	0.3027	0.3279	0.3578
Total cholesterol (mmol l ⁻¹)	0.1672	0.1606	0.3001
(constant)	-0.4003	1.3315	0.7642
Adjusted R ² = 0.2134			
<i>trans-10-Octadecaenoic acid</i> (18:1 ω 8t)	3.6193	1.7728	0.0434
Age (years)	0.0453	0.0153	0.0038
Gender	-1.0500	0.3732	0.0057
Smoking (years)	0.0313	0.0091	0.0008
Hypertensive status	0.3102	0.3289	0.3475
Total cholesterol (mmol l ⁻¹)	0.1594	0.1608	0.3236
(constant)	-0.3124	1.3304	0.8148
Adjusted R ² = 0.2092			
<i>Vaccenic acid</i> (18:1 ω 7t)	0.3378	1.1280	0.7651
Age (years)	0.0474	0.0156	0.0029
Gender	-0.9222	0.3741	0.0151
Smoking (years)	0.0307	0.0093	0.0012
Hypertensive status	0.3053	0.3420	0.3739
Total cholesterol (mmol l ⁻¹)	0.1346	0.1635	0.4122
(constant)	0.0983	1.4848	0.9473
Adjusted R ² = 0.1821			
<i>trans-12-Octadecaenoic acid</i> (18:1 ω 6t)	2.7756	1.4512	0.0582
Age (years)	0.0436	0.0155	0.0057
Gender	-0.9527	0.3687	0.0110
Smoking (years)	0.0315	0.0092	0.0008
Hypertensive status	0.2930	0.3293	0.3755
Total cholesterol (mmol l ⁻¹)	0.1716	0.1619	0.2915
(constant)	-0.4948	1.3639	0.7174
Adjusted R ² = 0.2059			
<i>Linoelaidic acid</i> (18:2 ω 6tt)	-0.3294	2.1459	0.8793
Age (years)	0.0499	0.0159	0.0022
Gender	-0.9475	0.3856	0.0155
Smoking (years)	0.0281	0.0094	0.0035
Hypertensive status	0.2712	0.3384	0.4245
Total cholesterol (mmol l ⁻¹)	0.1217	0.1647	0.4616
(constant)	0.3788	1.3507	0.7796
Adjusted R ² = 0.1762			
<i>Total trans</i>	0.5371	0.3553	0.1334
Age (years)	0.0467	0.0157	0.0037
Gender	-1.0160	0.3840	0.0093
Smoking (years)	0.0283	0.0093	0.0030
Hypertensive status	0.3079	0.3360	0.3614
Total cholesterol (mmol l ⁻¹)	0.1579	0.1647	0.3397
(constant)	-0.5827	1.4581	0.6902
Adjusted R ² = 0.1921			

The measurement of TFAs in biological samples, such as plasma, platelets and adipose tissue, has several advantages, in relation to measurement errors, over estimation of TFA intake from dietary assessment. However, the use of platelets as an estimate of fatty acid intake can have several problems. Platelet TFA concentrations may not provide an indication of absolute intake. What is measured is fatty acid concentration as a percentage of total fatty acids. This may serve as an indicator of the relative functional importance of individual fatty acids in membranes, in relation to the degree of CAD. If absolute intake of TFA is a more important CAD risk determinant than their relative proportions in the diet, or their relative composition in membranes, then relationships between platelet TFAs and CAD may be weak or not present. However, mechanisms for the proposed relationship between TFAs and CHD, other than those which operate through effects on lipoprotein profile, are yet to be investigated in any detail. Effects of particular TFAs on membrane function, prostaglandin synthesis and haemostasis may be equally important and, at least partially, account for the observed positive association between certain TFAs and CAD found here.

In conclusion, the results of this study provide evidence for an association between two of the platelet TFAs, which are likely to be derived predominantly from dietary hydrogenated vegetable oils, and the degree of CAD in the patient population studied.

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