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Dietary *trans* α-linolenic acid from deodorised rapeseed oil and plasma lipids and lipoproteins in healthy men: the *Trans*LinE Study

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Trans isomers of α-linolenic acid, which are formed by deodorization of refined vegetable oils, can be found in significant amounts in edible oils. Effects of *trans* α-linolenic acid on plasma lipoproteins are unknown. We therefore investigated the effects of *trans* α-linolenic acid on plasma lipids and lipoproteins in healthy European men. Eighty-eight healthy men from three European countries (France, Scotland, UK and the Netherlands) first consumed for 6 weeks a diet with experimental oils 'free' of *trans* fatty acids (run-in period). For the next 6 weeks, they were randomly allocated to a diet with experimental oils 'high' or 'low' in *trans* α-linolenic acid. Daily total *trans* α-linolenic acid intake in the high *trans* group was 1410 (range 583–2642) mg. Experimental oils were provided as such, or incorporated into margarines, cheeses, muffins and biscuits. The high *trans* α-linolenic acid diet significantly increased the plasma LDL-:HDL-cholesterol ratio by 8·1 % (95 % CI 1·4, 15·3; $P = 0·02$), and the total cholesterol:HDL-cholesterol ratio by 5·1 % (95 % CI 0·4, 9·9; $P = 0·03$) compared with the low-*trans* diet. This was largely explained by an increase in LDL-cholesterol on the high-*trans* diet, while no change was observed in the low-*trans* group (mean treatment effect of 4·7 % (95 % CI −0·8, 10·5; $P = 0·10$). No effects were found on total cholesterol and HDL-cholesterol, triacylglycerols, apolipoprotein B and A-1, and lipoprotein(a) concentrations. In conclusion, *trans* α-linolenic acid may increase plasma LDL-:HDL-cholesterol and total cholesterol:HDL-cholesterol ratios. Whether diet-induced changes in these ratios truly affects the risk for CHD remains to be established.

***Trans* α-linolenic acid: Lipids: Total cholesterol:HDL-cholesterol ratio: Rapeseed oil**

Trans-monounsaturated fatty acids from industrially hydrogenated oils increase cholesterol concentrations in the atherogenic serum LDL and decrease those of the anti-atherogenic HDL. Therefore, a reduction in the intake of hydrogenated oils has been advocated (Food and Agriculture Organization/World Health Organization, 1993). However, not only *trans* monounsaturated fatty acids, which represent most of the intake of *trans* fatty acids, are found in processed fats. *Trans* isomers of polyunsaturated fatty acids are also formed during oil processing (Ackman *et al.* 1974). In particular, α-linolenic acid is easily converted into *trans* α-linolenic acid.

Soyabean oil and canola oil (low-erucic acid rapeseed oil) are important sources of α-linolenic acid (Hunter, 1990) and *trans* isomers may represent up to 40 % α-linolenic acid (Ackman *et al.* 1974; Ratnayake *et al.* 1991;

Abbreviations: Apo, apolipoprotein; Lp, lipoprotein.

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Wolff, 1992, 1993). There is an increasing recognition that the intake of *n*-3 fatty acids (such as α-linolenic acid and fish oil fatty acids) should increase. The growing consumption of *n*-3 fatty acid containing vegetable oils over the last few years (Food and Agriculture Organization, 2000) may therefore result in an increased intake of *trans* α-linolenic acid from refined oils. Although such *trans n*-3 polyunsaturated fatty acids are absorbed and incorporated into tissue lipids by man (Chardigny *et al.* 1993, 1995; Wolff, 1995), their health effects have hardly been investigated. Therefore, we have studied the effects of a high-*trans* α-linolenic acid diet *v.* a low-*trans* α-linolenic acid diet on plasma lipid and lipoprotein concentrations in healthy men.

Subjects and methods

Experimental design

The study was designed as a controlled, parallel intervention trial, which was carried out under standardised conditions in three different centres: (1) Université d'Auvergne, Laboratoire de Nutrition Humaine, Clermont-Ferrand, France; (2) Cardiovascular Research Unit, University of Edinburgh, Scotland, UK; (3) Department of Human Biology, Maastricht University, The Netherlands. The study was a part of the *Trans*LinE project (*trans* α-Linolenic acid in Europe) in which the health impact of *trans* polyunsaturated fatty acids in European populations was examined.

The experiment consisted of two consecutive periods of 6 weeks. During the first period (run-in), all subjects received experimental products made from oil free of *trans* isomers of oleic, linoleic and α-linolenic acid (1 g *trans* fatty acids/kg) (*trans* 'free' diet). Thereafter, subjects were randomly allocated to a 'high'- or 'low'-*trans* diet. For the next 6 weeks, subjects in the high-*trans* group received on average 1·4 g *trans* α-linolenic acid/d (assessed by food diaries), which was provided by an oil containing 45 g *trans* α-linolenic acid/kg product and a margarine containing 34 g *trans* α-linolenic acid/kg product. In addition, foods prepared from the oil or margarine were provided (cheese, muffins, pies and biscuits). Identical experimental products free of *trans* α-linolenic acid were given to the low-*trans* group. The high-*trans* α-linolenic acid products had a reduced all *cis*-α-linolenic acid content, but in all other aspects the high- and low-*trans* products did not differ (Sébédio *et al.* 2000). During the preparation of the *trans* α-linolenic acid-rich oil, formation of *trans* linoleic acid could not be avoided (Hénon *et al.* 1999). Therefore, for the low-*trans* group a *trans*-free rapeseed oil was mixed with an isomerized sunflower oil (50:1, v/v), in order to compensate for the unavoidable formation of 5 g *trans* isomers of linoleic acid/kg during deodorization of the rapeseed oil for the high-*trans* group. All products were colour coded so as to blind the subjects.

For the duration of the study, subjects were advised to avoid consuming *trans* fatty acids: intake of ruminant meat, cheese (except for goats' cheese, which is low in *trans*), and foods containing hydrogenated fats were avoided. The main part of the *trans* fatty acid intake was, however, reduced by replacing subjects' usual oil and margarine by experimental products. Before and during week 5 and week 11, subjects recorded their food intake for 4 d, of which at least 1 d was in the weekend, to allow us to estimate their energy and nutrient intakes. Energy and nutrient composition of the diets were calculated separately for the three centres using country-specific food tables and the composition of the experimental fats, oils and products. Unfortunately, no reliable information on the *trans* fatty acid content of French and Dutch products was available. Therefore, it was not possible to estimate the amount of *trans* fatty acid in the background diet of subjects from Clermont-Ferrand and Maastricht. *Trans* 18:1 intake of the Scottish subjects was about 400 mg (Sébédio *et al.* 2000). Overall, 30 % fat in the habitual diet was replaced by foods prepared with the experimental fats and oils. Subjects visited the dietitian of the department once per week to receive a new supply of products and to measure their body weights.

Subjects recorded in diaries any signs of illness, medication used and any deviations from the protocol. In addition, except for avoidance of consumption of *trans* fatty acids, subjects were urged not to change their background diets, level of physical exercise, smoking habits, or use of alcohol during the study. The protocol and the aim of the study were fully explained to the subjects, who gave their written informed consent. The study had been approved by the local Medical Ethical Committees.

Subjects

A total of ninety-one men, recruited from a panel of volunteers (Clermont-Ferrand *n* 32), university staff and students (Edinburgh *n* 28) and from the general population (Maastricht *n* 31), entered the study. Volunteers were aged between 18 and 55 years, non-obese, clinically healthy, normotensive and normolipidaemic. During the study, one subject from Clermont-Ferrand dropped out because of a deviation from the protocol interfering with another aspect of this study, and two subjects from Edinburgh because of the constraints of the study. A detailed description of the study design has been reported elsewhere (Sébédio *et al.* 2000).

Blood sampling and analyses

Free-flowing blood was sampled after an overnight fast at weeks 0, 3, 5, 6, 9, 11 and 12 for lipid and lipoprotein analyses, and at weeks 0, 6 and 12 for measurement of fatty acid composition of plasma cholesteryl esters. Additional measurements will be reported elsewhere. In each centre, all punctures were performed by the same technician, at the same location, and as far as possible at the same time on the same day of the week. All blood samples were taken before 11.00 hours.

Plasma was obtained by centrifugation at 2000 ***g*** for 30 min at 4°C and stored at −80°C. Plasma total cholesterol and HDL-cholesterol (CHOD-PAP method, Monotest cholesterol; Roche, Mannheim, Germany) and triacylglycerols (GPO-Trinder; Sigma Diagnostics, St Louis, MO, USA) were analysed enzymatically. LDL-

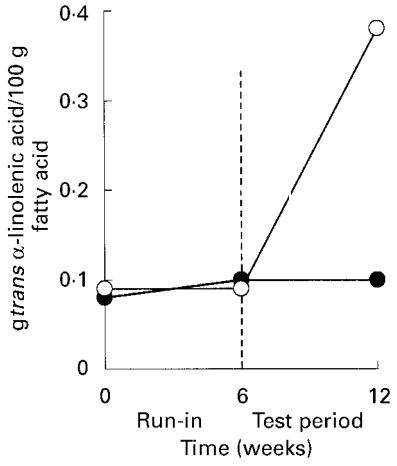

Fig. 1. Median proportions of *trans* α-linolenic acid in plasma cholesteryl esters during the run-in period (0–6 weeks) (*n* 43 in both groups at *t* 0; *n* 44 in both groups at *t* 6) and experimental period of low- (*n* 44;) or high- *trans* α-linolenic acid (*n* 42;) (6–12 weeks). For details of diets and procedures see p. 388. ● low *trans*; ○ high *trans*.

cholesterol was calculated with the Friedewald equation (Friedewald *et al.* 1972). Plasma apolipoprotein (Apo) A-1 and ApoB were measured using an immunoturbidimetric reaction (UNI-KIT ApoA-1 and UNI-KIT ApoB; Roche, Basel, Switzerland) with antiserum raised in sheep and rabbits respectively. Lipoprotein (Lp)(a) was measured using a kit from Biopool (TintElize; Umeå, Sweden). The technicians were blinded to treatment status. All lipid and Lp analyses were centralised in Maastricht in order to eliminate any possible systematic analytical bias between centres. All samples from one subject were analysed within one run, while each run contained samples from each centre.

To monitor dietary compliance, the fatty acid composition of plasma cholesteryl esters was determined by GC by the INRA (Dijon, France) (Sébédio *et al.* 2000)

Statistical analyses

Analysis of covariance was used using the Statistical Package for the Social Sciences (SPSS; SPSS Inc., Chicago, IL, USA) to examine the effect of a high-*trans* diet *v.* a low-*trans* diet (treatment effect) on the week 11+12 means, adjusted for the week 5+6 means, and to check whether results were consistent across the three centres. Because the distributions of triacylglycerols, Lp(a), LDL-:HDL-cholesterol ratio, total cholesterol:HDL-cholesterol ratio and ApoB:ApoA-1 ratio were skewed, a logarithmic transformation was applied to these measurements before analysis. Treatment effects were then expressed as percentage changes and their 95 % CI by taking antilogs. For the sake of uniformity, a similar procedure was used for the other lipid-related variables. All *P* values are two-tailed, and differences were considered statistically significant when $P < 0{\cdot}05$.

Results

For the high-*trans* group, the daily intake of *trans* α-linolenic acid, as estimated by food records, increased from 66 (range 19–585) mg during the run-in period to 1410 (range 583–2642) mg during the high-*trans* period (increase of 1344 (range 543–2612) mg). For the low-*trans* group, *trans* α-linolenic acid intake increased from 49 (range 16–310) during the run-in period to 60 (range 2–404) mg (increase of 10 (range −236–300) mg ($P < 0{\cdot}01$)) during the low-*trans* period. As expected, the proportion of *trans* α-linolenic acid (after adjustment for week 6 values, the mean treatment effect was 0·26 g/100 g fatty acid (95 % CI 0·21, 0·32; $P < 0{\cdot}001$)) in plasma cholesteryl esters increased significantly on the high-*trans* diet (from 0·11 (range 0–0·39) to 0·36 (range 0·02–0·74) g/100 g fatty acid) compared with the low-*trans* diet (from 0·11 (range 0·03–0·28) to 0·10 (range 0–0·21) g/100 g fatty acid) (Fig. 1). It was surprising that the men consuming a low-*trans* diet following the run-in period tended to increase the intake of dietary fat (2·1 (SD 5·7) % energy) whereas the opposite was true for the men in the high-*trans* group (−1·1 (SD 5·4) % energy). The difference in changes nearly reach statistical significance ($P = 0{\cdot}051$). Changes in the intakes of saturated fatty acids and monounsaturated fatty acids also differed (Table 1). The changes in protein and carbohydrate intake did not differ between the low-*trans* (−0·5 (SD 2·6) % energy and −1·7 (SD 6·3) % energy respectively) and the high-*trans* group (0·3 (SD 2·3) % energy and 0·9 (SD 5·6) % energy respectively). Further details of energy and nutrient intakes have been reported elsewhere (Sébédio *et al.* 2000).

Table 1. Effect of a diet low or high in *trans* α-linolenic acid on daily fat and fatty acid intakes†

(Mean values and standard deviations)

	Low *trans* (*n* 44)		High *trans* (*n* 44)	
	Mean	SD	Mean	SD
Fat (% energy)				
Run-in	32·4	6·3	35·3	5·9
Change‡	2·1	5·7	−1·1	5·4
SFA (% energy)				
Run-in	8·6	3·4	9·9	3·3
Change‡	1·1	2·2	−0·5**	1·9
MUFA (% energy)				
Run-in	14·1	2·8	15·1	2·5
Change‡	1·1	2·7	−0·5*	2·3
PUFA (% energy)				
Run-in	7·1	1·4	7·7	1·8
Change‡	0·1	1·9	−0·3	1·5

SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.
Mean values were significantly different from those of the low *trans* fatty acid group: *$P < 0{\cdot}05$, **$P < 0{\cdot}01$.
† Run-in (*trans*-'free' diet) and experimental periods each lasted 6 weeks. For details of diets and procedures see p. 388.
‡ Mean change in intake during experimental period.

The mean body weight of the subjects was increased by 0·2 (SD 1·1) kg in the high-*trans* group, which differed significantly from the decrease of 0·4 (SD 1·2) kg in the low-*trans* group (mean treatment effect of 0·6 (SD 2·4) kg (95 % CI 0·1, 1·1), $P = 0{\cdot}03$).

Changes in plasma lipids and lipoproteins were corrected for body weight changes and changes in the intakes of saturated and monounsaturated fatty acids. The consumption of *trans* α-linolenic acid did not change plasma total

Table 2. Plasma lipids and lipoproteins concentrations after 6 weeks of a diet low or high in *trans* α-linolenic acid*
(Mean values and standard deviations, and 95 % confidence intervals for treatment effect (high *v.* low *trans*))

	Low *trans* (*n* 44)		High *trans* (*n* 44)		Treatment effect (high *v.* low *trans*)		
	Mean	SD	Mean	SD	Mean	95 % CI	*P* value
Total cholesterol							
Run-in (mmol/l)	4·45	0·76	4·21	0·80			
Treatment effect (%)†	1·1		2·7		1·7	−2·2, 5·7	0·41
LDL-cholesterol							
Run-in (mmol/l)	2·79	0·65	2·59	0·71			
Treatment effect (%)†	−0·4		4·0		4·7	−0·8, 10·5	0·10
HDL-cholesterol							
Run-in (mmol/l)	1·27	0·28	1·22	0·26			
Treatment effect (%)†	3·8		0·3		−2·7	−6·8, 1·6	0·22
Triacylglycerol							
Run-in (mmol/l)	0·85	0·38	0·87	0·44			
Treatment effect (%)†	−1·4		−1·6		2·6	−9·1, 15·8	0·68
LDL-:HDL-cholesterol ratio							
Run-in	2·30	0·75	2·25	0·82			
Treatment effect (%)†	−4·0		3·6		8·1	1·4, 15·3	0·02
Total cholesterol:HDL-cholesterol ratio							
Run-in	3·63	0·91	3·60	0·98			
Treatment effect (%)†	−2·6		2·3		5·1	0·4, 9·9	0·03

* Subjects received a *trans*-'free' diet during the 6 week run-in period. For details of diets and procedures see p. 388.
† Treatment effects are expressed as percentage changes. Treatment effects did not differ between the three experimental centres (Clermont-Ferrand, France; Edinburgh, Scotland, UK; Maastricht, The Netherlands). Treatment effects (high *v.* low *trans*) were corrected for changes in dietary saturated and monounsaturated fatty acid intake and in body weight.

cholesterol or triacylglycerol concentrations (Table 2). LDL-cholesterol concentrations tended to increase in the men consuming the high-*trans* α-linolenic acid diet, whilst it remained unchanged in the group who consumed the low-*trans* diet following the run-in period. However, the treatment effect of 4·7 % failed to reach significance ($P = 0·10$). The reverse was observed for plasma HDL-cholesterol concentrations, where the concentrations tended to increase slightly in the low-*trans* group, whilst there was no change in the men consuming the high-*trans* α-linolenic acid diet. The treatment effect did not reach statistical significance. However, the total cholesterol:HDL-cholesterol and LDL-:HDL-cholesterol ratios increased significantly by 5·1 % ($P = 0·03$) and 8·1 % ($P = 0·02$) on the high-*trans* diet compared with the low-*trans* diet.

Trans α-linolenic acid did not affect plasma ApoB and ApoA-1 concentrations and their ratio (Table 3). Lp(a) concentrations tended to fall in men consuming the low-*trans* α-linolenic acid diet, whilst concentrations remained constant in the group that was switched to a high-*trans* α-linolenic acid diet. The treatment effect did not reach statistical significance.

Treatment effects did not differ significantly between the three centres.

Discussion

In this large multi-centre study with healthy normolipidaemic male volunteers, we did not find any unfavourable effects of high, but not unrealistic intakes of *trans* isomers from α-linolenic acid on plasma total cholesterol or HDL-cholesterol concentrations. LDL-cholesterol concentrations,

Table 3. Plasma apolipoprotein A-1 and B after 6 weeks of a diet low or high in *trans* α-linolenic acid*
(Mean values and standard deviations, and 95 % confidence intervals for treatment effect (high *v.* low *trans*))

	Low *trans* (*n* 44)		High *trans* (n 44)		Treatment effect (high *v.* low *trans*)		
	Mean	SD	Mean	SD	Mean	95 % CI	*P* value
ApoB							
Run-in (mg/l)	825	202	768	206			
Treatment effect (%)†	1·8		0·7		−1·8	−7·7, 4·4	0·56
ApoA-1							
Run-in (mg/l)	1382	242	1347	236			
Treatment effect (%)†	1·3		−2·1		−3·3	−8·2, 1·8	0·21
ApoB:ApoA-1 ratio							
Run-in	0·6	0·1	0·6	0·2			
Treatment effect (%)†	0·6		2·8		2·3	−4·4, 9·6	0·51

Apo, apolipoprotein.
* Subjects received a *trans*-'free' diet during the 6 week run-in period. For details of diets and procedures see p. 388.
† Treatment effects are expressed as percentage changes. Treatment effects did not differ between the three centres (Clermont-Ferrand, France; Edinburgh, Scotland, UK; Maastricht, The Netherlands). Treatment effects (high *v.* low *trans*) were corrected for changes in dietary saturated and monounsaturated fatty acid intake and in body weight.

however, tended to increase, while the LDL-:HDL-cholesterol and total cholesterol:HDL-cholesterol ratios were significantly increased on the high-*trans* diet compared with the low-*trans* diet. These effects were observed in each centre and were already evident after 3 weeks consumption of the experimental diets (data not shown). Results were corrected for differences in fatty acid intake and body weight between the two groups.

We found a difference in the total cholesterol:HDL-cholesterol ratio of 0·15 between the low- and the high-*trans* group. This ratio may predict the risk for CHD even better than total cholesterol or LDL-cholesterol concentrations (Castelli *et al.* 1986; Kinosian *et al.* 1994). It has been suggested that an increase of 1 unit serum cholesterol:HDL-cholesterol ratio may increase the risk of myocardial infarction by 53 % (Stampfer *et al.* 1991). This would suggest that the risk for CHD would decrease by 8 % when 1334 mg *trans* α-linolenic acid is replaced by dietary *cis* α-linolenic acid. It remains to be established, however, if diet-induced changes in HDL-cholesterol:total cholesterol ratio affect CHD risk.

So far, most previous studies have focused on the effects of *trans* monounsaturated fatty acids on the serum lipoprotein profile (Katan *et al.* 1995). *Trans* monounsaturates have been found to increase LDL-cholesterol and to decrease HDL concentrations relative to its *cis* isomers (Katan *et al.* 1995). As a consequence, *trans* monounsaturated fatty acid increase the total cholesterol:HDL-cholesterol ratio as in our present study. Triacylglycerol concentrations were also increased on diets high in *trans* monounsaturated fatty acids (Katan *et al.* 1995). However, we did not observe effects of *trans* α-linolenic acid on triacylglycerols. Possibly, intakes of *trans* isomers were too low, resulting in a limited power of the present study to detect such effects. Alternatively, *trans* α-linolenic acid may act differently from *trans* monounsaturates. Lp(a) was also not affected by an intake of 1·4 g *trans* α-linolenic acid. Studies using *trans* monounsaturated fatty acids at high intakes, however, showed increased Lp(a) levels (Katan *et al.* 1995). One study has reported that partially hydrogenated fish oil, which contains *trans* isomers of the longer-chain polyunsaturated fatty acids, elevated plasma total cholesterol, LDL-cholesterol, and the LDL-cholesterol:HDL-cholesterol ratio relative to a partially hydrogenated soyabean oil rich in *trans* monounsaturated fatty acids (Almendingen *et al.* 1995). However, results from that study are difficult to interpret because of differences in the composition of other fatty acids between the two hydrogenated oil diets.

Studies with *trans*-monounsaturates found that effects were similar for women and men (Mensink & Katan, 1990; Mensink *et al.* 1992; Judd *et al.* 1994) and for subjects with normal and mildly elevated triacylglycerol levels (Nestel *et al.* 1992*a*,*b*). Whether this is also true for *trans* polyunsaturated fatty acids should be examined in future studies. Furthermore, it remains to be determined what would be the longer-term effects of a high intake of *trans* α-linolenic acid.

In our present study subjects consumed an average of 1410 mg *trans* α-linolenic acid. Based on the fatty acid composition of cholesteryl esters, we considered as a first reasonable estimate that the mean habitual diet in Edinburgh and Maastricht provided approximately 600 mg *trans* α-linolenic acid/d and in Clermont-Ferrand about 300 mg (Sébédio *et al.* 2000). Hulshof *et al.* (1999), however, have reported that for men the mean daily *trans* α-linolenic acid plus *trans* 20:1 intake (these two fatty acids could not be separated by GC) in fourteen Western European countries varied from 20 mg in Italy to 490 mg in Iceland. In addition, Voskuil *et al.* (1996) have estimated that in 1990 α-linolenic acid intake in the Netherlands was only 1·2 g. Although proportions of α-linolenic acid in serum cholesteryl esters from the Dutch cohort was about 1·7-times higher than in 1990 (0·55 % *v.* 0·32 %), suggesting that α-linolenic acid has increased considerably during the last years, these studies do not suggest that the average habitual intake of *trans* α-linolenic acid is as high as we have used. Thus, the amount of *trans* α-linolenic acid (1·4 g/d) seemed to be high but not unrealistic. Standard deviations and ranges in absolute *trans* α-linolenic acid intake were rather large. This was not only due to differences in energy intake, but also to inaccuracies inherent to the method used to estimate food intake and to differences in compliance. The oil for this study was deodorised especially for this study, but the composition of the different *trans* isomers (14 g *trans*, *cis*, *cis*-, 19 g *cis*, *cis*, *trans*- and 3·2 g *trans, cis, trans*-α-linolenic acid/100 g fatty acids) is typical for commercially available oils (Wolff, 1992; O'Keefe *et al.* 1994).

In conclusion, our present study suggests that a high, but not unrealistic intake of *trans* α-linolenic acid, which is formed during oil processing, may influence the total cholesterol:HDL-cholesterol ratio in an unfavourable way. Increasing the dietary intake of *cis, cis, cis*-α-linolenic acid has been recommended by an expert panel (De Deckere *et al.* 1998) and obviously it does not make sense to provide α-linolenic acid in the *trans* form. Careful deodorisation prevents the formation of *trans* α-linolenic acid (Hénon *et al.* 1999) and may help to improve the diet, deliver more *cis* α-linolenic acid and thereby possibly reduce ischaemic heart disease (De Lorgeril *et al.* 1994).

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