

Linoleic and α -linolenic acids differently modify the effects of elaidic acid on polyunsaturated fatty acid metabolism and some immune indices in rats

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To explore whether the metabolic responses to *trans*, compared with *cis*, fatty acids depend on the source of dietary polyunsaturated fatty acids (PUFA), male Sprague-Dawley rats, 5 weeks old, were fed on diets containing 30 g oleic (*cis*) or elaidic (*trans*) acids/kg in combination with either 70 g perilla oil (α -linolenic acid) or safflowerseed oil (linoleic acid)/kg for 3 weeks in separate experiments. The dietary fats were adjusted to have the same level of total PUFA. The dietary manipulation did not influence the growth indices, but spleen weight was greater when the dietary PUFA source was perilla oil. The incorporation of *trans* fatty acid into liver phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine and adipose tissue lipids, particularly phospholipids, was significantly higher when rats were fed on safflowerseed oil compared with perilla oil. However, only limited differences were observed in the effects of *cis* and *trans* fatty acids on the proportions of PUFA in liver phospholipids. Splenic production of prostaglandin E₂ was reduced by *trans* fatty acid when safflowerseed oil was the PUFA source, but no *trans* effect was observed on leukotriene C₄ production. Dietary PUFA significantly influenced the concentration of plasma immunoglobulins (Ig) but the effect of geometry was only seen in IgG which was increased by *trans* acid. Dietary *trans* fatty acid increased the CD4⁺:CD8⁺ T-lymphocyte ratio in the spleen, reflecting a decreasing trend of the proportion of CD8⁺, when combined with perilla oil. These observations indicate that the type of PUFA simultaneously ingested specifically influences the effect that *trans* acid exerts on PUFA metabolism, eicosanoid production and some immune indices.

Elaidic acid: Oleic acid: α -Linolenic acid: Linoleic acid: Eicosanoids

The undesirable effects of *trans* octadecenoic acids on the concentration of plasma cholesterol and the profile of plasma lipoproteins have been reported for human subjects (Mensink & Katan, 1990; Mensink *et al.* 1992; Nestel *et al.* 1992; Troisi *et al.* 1992; Zock & Katan, 1992; Wood *et al.* 1993; Abbey & Nestel, 1994; Almendingen *et al.* 1995; Kris-Etherton, 1995), and the effects are apparently dose-dependent (Mensink & Katan, 1990; Zock & Katan, 1992). In addition, certain epidemiological studies have indicated a possible link between *trans* fatty acids and the development of coronary heart disease (Willet *et al.* 1993; Mann, 1994; Ascherio & Willet, 1995). In well-controlled animal studies, however, *trans* octadecenoic acids are not hypercholesterolaemic (Zevenbergen & Haddeman, 1989; Borlak & Welch, 1994). In fact, *trans* monoenes, compared with their *cis* counterparts, do not change plasma cholesterol concentrations in rats (Sugano *et al.* 1989)

or the LDL receptor activity in hamsters (Woollet *et al.* 1994), although these findings are still controversial (Hayashi *et al.* 1993). Since human diets are much more complex in their compositions than the purified diets used in animal studies, these discrepancies might, at least in part, be attributed to the influence of dietary components consumed simultaneously.

One such possibility is the nature of the polyunsaturated fatty acids (PUFA) consumed. PUFA of different families, *n*-3 and *n*-6, appear to influence plasma lipid levels differently (Harris 1989; Schmidt *et al.* 1993). Hence, in the present study we investigated how dietary *n*-3 and *n*-6 PUFA modify the effect that *trans* fatty acids exert on the various variables of lipid metabolism. Since *trans* fatty acids interfere with the metabolism of linoleic acid to arachidonic acid and, hence, the eicosanoid production from arachidonic acid (Kinsella *et al.* 1981; Sugano *et al.* 1989), we measured the production of prostaglandin E₂ and leukotriene C₄ which are both typical chemical mediators. Since dietary PUFA significantly influence immune functions (Yaqoob & Calder, 1993), the effects of these dietary manipulations on the serum levels of immunoglobulins and splenic T-lymphocyte subsets were also studied. Thus, male rats were given elaidic acid (*trans*) or oleic acid (*cis*) in combination with vegetable oils rich in either α -linolenic or linoleic acids.

MATERIALS AND METHODS

Animals and diets

Two experiments were carried out in succession using male 4-week-old Sprague-Dawley rats (Seiwa Experimental Animals, Fukuoka, Japan). The animals were caged individually in a room with controlled temperature (20–22°C) and lighting (06.00–18.00 hours), and freely accessible AIN-type purified diets (American Institute of Nutrition, 1977) for 3 weeks after 1 week of acclimation. The diet contained the following ingredients (g/kg): casein 200; fat 100, mineral mixture 35, vitamin mixture 10, choline bitartrate 2, cellulose 50, maize starch 150, and sucrose 453. The dietary fat was composed of octadecenoic acids, either oleic (*cis*) or elaidic (*trans*) acid (Wako Pure Chemicals, Osaka, Japan) and edible-grade vegetable oils in the proportions 3 : 7 (w/w). The elaidic acid corresponded to approximately 6 % energy. The vegetable oils used were perilla oil (from the seed of *Perilla frutescens*; Ohta Oil Co., Okazaki, Japan) in one experiment and a mixture of safflowerseed oil (Linol Oil Co., Nagoya, Japan) and palm oil (Fuji Oil Co., Osaka, Japan) (8.7 : 1.0, w/w) in the other experiment. Using this combination, the content of total PUFA, linoleic and α -linolenic acids, was comparable between the two experiments, and in each experiment the difference in the geometry (*cis* or *trans*) of octadecenoic acid was the sole

Table 1. *Fatty acid compositions (g/100 g total fatty acids) of dietary fats**

Dietary fat	Fatty acid					
	16:0	18:0	<i>trans</i> -18:1	<i>cis</i> -18:1	18:2 <i>n</i> -6	18:3 <i>n</i> -3
Perilla oil diet						
<i>cis</i>	4.4	1.3	–	46.5	9.0	38.7
<i>trans</i>	4.4	1.4	32.0	13.7	9.0	39.6
Safflowerseed oil diet						
<i>cis</i>	7.1	1.6	–	42.3	48.0	–
<i>trans</i>	7.1	1.6	30.0	12.3	47.9	–

* Three parts of oleic or elaidic acid were mixed with seven parts of either perilla oil or a mixture of safflowerseed oil and palm oil (8.7 : 1, w/w), respectively.

variable (Table 1). Body weight and food intake were recorded every other day. Rats were killed by withdrawing blood from the abdominal aorta under diethyl ether anaesthesia. Liver, spleen and abdominal adipose tissue were excised immediately. The experiments were performed under the Guidelines for Animal Experiments approved by Nakamura Gakuen University.

Analytical procedures

Liver and serum lipids were analysed for cholesterol, triacylglycerol and phospholipid as described elsewhere (Sugano *et al.* 1984). Liver phospholipids were separated into phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, phosphatidylserine and cardiolipin by TLC, and their fatty acid compositions were analysed as methyl esters by GLC (Koga *et al.* 1994). The adipose tissue lipids were also extracted and their fatty acid compositions analysed. A portion of spleen was homogenized in phosphate-buffered saline, pH 7.2, and the homogenate was incubated in the same buffer at 37° for 30 min. Leukotriene C₄ was extracted from the medium and measured by radioimmunoassay using a commercial kit (NEK-030, New England Nuclear, Boston, MA, USA) under the linear relationship with respect to the homogenate volume and incubation time (Gu *et al.* 1994). Splenic prostaglandin E₂ production was similarly measured (NEK 020, New England Nuclear) (Koga *et al.* 1994). Serum immunoglobulin (Ig) levels were measured by the sandwich ELISA as reported previously (Gu *et al.* 1994). In brief, mouse anti-rat IgA, rabbit anti-rat IgG (Fab')₂, goat anti-rat IgE and goat anti-rat IgM (Zymed Lab, San Francisco, CA, USA) were used to fix respective immunoglobulins. After blocking the bound IgA, IgG and IgM were measured by reacting with their respective peroxidase (POD)-conjugated anti-rat immunoglobulins (Zymed Lab). For measurement of bound IgE POD-conjugated avidin (Betyl, Montgomery, TX, USA) was used. Spleen lymphocytes were separated by applying Lympholyte-Rat (Cedarlane, Hornby, Canada) and CD4 and CD8 positive T-cells were counted by flow cytometry (Epics Plofile II, Coulter Electronics Ltd, Luton, Beds.) (Gu *et al.* 1994).

Statistical analysis

After confirming the similarity of the variances (*F* values) of all the data, the results were analysed by 2 × 2 ANOVA (Gomez & Gomez, 1984) followed by Duncan's new multiple range test (Duncan, 1955) to evaluate the statistical difference among the groups.

RESULTS

Growth indices and tissue weights

There were no significant differences in food intake and weight gain between *cis* and *trans* fat groups irrespective of the source of dietary PUFA (results not shown). The weights of liver and spleen were also comparable between the *cis* and *trans* fatty acid groups, but the latter was heavier after feeding perilla oil than after feeding safflowerseed oil (0.71 (SE 0.02) and 0.70 (SE 0.03) g for the *cis* and *trans* fatty acid groups on perilla oil diets, and 0.59 (SE 0.04) and 0.59 (SE 0.03) g for the corresponding groups on safflowerseed oil diets respectively, *P* < 0.05).

Concentrations of serum and liver lipids

Table 2 summarizes the concentrations of serum and liver lipids. Statistically significant differences in serum lipids were observed for the concentrations of triacylglycerol and phospholipid. The concentration of serum triacylglycerol tended to be lower in rats fed on perilla oil than in those fed on safflowerseed oil, and it was lowest in the perilla oil–*trans* fatty acid combination. When *trans* fatty acid was fed, the serum phospholipid level was significantly lower in the perilla oil group than in the safflowerseed group, and there was a significant interaction with dietary PUFA. Although the source of dietary monounsaturated fatty acids did not influence the concentrations of serum cholesterol, they tended to be lower when rats were fed on perilla oil and there was a significant interaction in the concentration of serum cholesterol. In liver lipids, the only marked difference was observed for the concentration of phospholipid, which was significantly higher in rats fed on safflowerseed oil than in those fed on perilla oil. The concentration of liver phospholipid was significantly higher in the *trans* group than in the *cis* group when perilla oil was fed. These differences were not observed when rats were fed on safflowerseed oil. There was a PUFA-dependent difference in the concentration of liver phospholipid.

Fatty acid compositions of liver phospholipids

The fatty acid compositions of the liver phospholipid classes are summarized in Tables 3 and 4. Elaidic acid was incorporated at similar levels into phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol, while the extent of incorporation was lower in phosphatidylserine for each PUFA source. Apparently no *trans* fatty acid was incorporated into cardiolipin in either of the PUFA groups. However, the incorporation of *trans* fatty acid was always significantly higher in rats fed on safflowerseed oil than in those fed on perilla oil. In all phospholipid classes the compositions of PUFA were not substantially modified by the geometrical difference in the dietary octadecenoic acids,

Table 2. Effects of elaidic acid on concentrations of serum and liver lipids in rats fed on diets containing perilla oil or safflowerseed oil

(Mean values with their standard errors for eight rats)

Group	Serum lipids (mmol/l)						Liver lipids (μmol/g)					
	Cholesterol		Triacylglycerol		Phospholipid		Cholesterol		Triacylglycerol		Phospholipid	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Perilla oil diet												
<i>cis</i>	1.66	0.05	1.77 ^{ab}	0.22	2.29 ^{ab}	0.05	10.2 ^{ab}	0.6	39.8	2.7	36.0 ^a	0.8
<i>trans</i>	1.59	0.12	1.29 ^a	0.11	2.0 ^a	0.11	9.3 ^a	1.0	32.4	1.4	43.0 ^b	1.2
Safflowerseed oil diet												
<i>cis</i>	1.89	0.13	2.12 ^b	0.21	2.23 ^{ab}	0.11	11.8 ^b	0.4	37.5	3.1	59.9 ^c	1.5
<i>trans</i>	1.82	0.10	2.01 ^b	0.24	2.44 ^b	0.15	10.6 ^{ab}	0.7	37.5	5.0	56.6 ^c	3.1
Pooled SE		0.12		0.25		0.18		0.8		5.2		4.7
ANOVA <i>F</i> value												
PUFA		<i>P</i> < 0.05		<i>P</i> < 0.05		NS		NS		NS		<i>P</i> < 0.01
<i>cis</i> – <i>trans</i>		NS		NS		NS		NS		NS		NS
Interaction		NS		NS		<i>P</i> < 0.05		NS		NS		<i>P</i> < 0.01

^{a,b,c} Mean values within a column not sharing a common superscript letter were significantly different, *P* < 0.05 (df 28).

* For details of diets see Table 1 and p. 646.

Table 3. Effects of elaidic acid on fatty acid compositions (mol/100 mol total fatty acids) of liver phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol of rats fed on perilla oil or safflowerseed oil diets*

Fatty acid	Phosphatidylcholine												Phosphatidylethanolamine												Phosphatidylinositol											
	Perilla oil				Safflower-seed oil				ANOVA				Pooled				Perilla oil				Safflower-seed oil				ANOVA				Pooled							
	cis		trans		cis		trans		PUFA	cis-trans	Inter-action	SE	SE	SE	SE	cis		trans		PUFA	cis-trans	Inter-action	SE	SE	SE	SE	cis		trans		PUFA	cis-trans	Inter-action			
	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol	mol/100 mol				
14:0	-	-	0.2	0.4	0.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16:0	30.3 ^a	29.0 ^a	27.0 ^b	25.4 ^b	0.4	<i>P</i> < 0.01	NS	21.7 ^a	21.5 ^a	20.3 ^a	18.1 ^b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16:1	1.3 ^a	1.7 ^b	-	-	0.1	<i>P</i> < 0.01	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18:0	16.6 ^a	13.2 ^b	19.3 ^c	11.4 ^b	0.6	NS	26.7 ^a	21.5 ^b	27.7 ^a	18.4 ^c	12.7 ^b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
trans-18:1	-	7.2 ^a	-	11.4 ^b	0.6	-	-	8.1 ^a	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cis-18:1	12.3 ^a	9.5 ^b	9.7 ^b	7.2 ^c	0.4	<i>P</i> < 0.01	NS	5.9 ^a	4.4 ^b	7.6 ^c	5.1 ^{ab}	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
18:2n-6	10.2 ^a	11.0 ^a	12.4 ^b	13.4 ^b	0.3	<i>P</i> < 0.01	<i>P</i> < 0.05	3.0 ^a	3.4 ^a	5.7 ^b	6.5 ^c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:3n-6	0.8 ^{ab}	1.0 ^a	0.7 ^b	0.8 ^{ab}	0.1	NS	NS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:4n-6	5.2 ^a	5.2 ^a	24.1 ^b	22.4 ^c	1.7	<i>P</i> < 0.01	NS	6.2 ^a	6.1 ^a	26.8 ^b	27.2 ^b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20:5n-3	12.1	11.6	-	-	0.3	-	-	13.3	12.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:5n-3	2.7	3.0	2.5	2.3	0.1	NS	NS	6.3	6.4	5.9	5.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:5n-6	-	-	0.7	0.7	0.0	-	-	-	-	1.9	2.0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22:6n-3	6.4 ^a	6.3 ^a	1.5 ^b	1.3 ^b	0.5	<i>P</i> < 0.01	NS	15.5 ^a	14.6 ^a	3.8 ^b	2.8 ^b	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Desaturation index†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
n-6	0.59 ^a	0.57 ^a	2.03 ^b	1.74 ^b	0.1	<i>P</i> < 0.01	<i>P</i> < 0.05	2.05 ^a	1.77 ^a	4.78 ^b	4.06 ^c	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
n-3	0.44 ^a	0.44 ^a	0.39 ^b	0.61 ^b	0.0	<i>P</i> < 0.01	NS	0.82	0.78	0.65	0.69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

PUFA, polyunsaturated fatty acid.

^{a,b,c} Mean values within a row and within a phospholipid category not sharing a common superscript letter were significantly different, *P* < 0.05 (28 df).

* For details of diets, see Table 1 and p. 646.

† Desaturation index for n-6 PUFA = (20:3n-6 + 20:4n-6)/18:2n-6; and for n-3 PUFA, 22:6n-3/(20:5n-3 + 22:5n-3).

Table 4. Effects of elaidic acid on fatty acid compositions (mol/100 mol total fatty acids) of liver phosphatidylserine and cardiolipin of rats fed on perilla oil or safflowerseed oil diets*
(Mean values for eight rats with their pooled standard errors)

Fatty acid	Phosphatidylserine										Cardiolipin							
	Perilla oil			Safflowerseed oil			ANOVA				Perilla oil		Safflowerseed oil		ANOVA			
	cis	trans	Pooled	cis	trans	Pooled	PUFA	cis-trans	Inter-action	cis	trans	Pooled	cis	trans	Pooled	PUFA	cis-trans	Inter-action
14:0	1.9 ^a	2.1 ^a	0.2	3.7 ^b	2.9 ^{ab}	0.2	<i>P</i> < 0.01	NS	3.0 ^a	1.8 ^b	0.2	1.8 ^b	1.8 ^b	0.2	<i>P</i> < 0.05	<i>P</i> < 0.05	NS	
16:0	8.4	10.9	0.5	8.8	9.9	0.5	NS	NS	4.9 ^{ab}	5.7 ^a	0.2	4.4 ^b	4.0 ^b	0.2	<i>P</i> < 0.01	NS	NS	
16:1	-	-	-	-	-	-	-	-	4.6 ^{ac}	5.7 ^a	0.3	2.9 ^b	3.7 ^{bc}	0.3	-	-	-	
18:0	44.2 ^a	38.6 ^b	1.0	45.3 ^a	34.7 ^c	1.0	NS	<i>P</i> < 0.01	1.9	1.9	0.1	2.4	1.9	0.1	NS	NS	NS	
<i>trans</i> -18:1	-	4.8 ^a	0.4	-	7.1 ^b	0.4	-	-	-	-	-	-	-	-	-	-	-	
<i>cis</i> -18:1	3.9 ^a	3.3 ^a	0.2	5.1 ^b	4.0 ^a	0.2	<i>P</i> < 0.01	<i>P</i> < 0.05	30.2 ^a	26.6 ^b	1.1	20.6 ^c	16.3	1.1	<i>P</i> < 0.01	<i>P</i> < 0.01	NS	
18:2 <i>n</i> -6	1.7 ^a	1.9 ^a	0.1	2.3 ^{ab}	2.7 ^b	0.1	<i>P</i> < 0.05	NS	42.5 ^a	44.1 ^a	2.3	64.3 ^b	67.2 ^c	2.3	<i>P</i> < 0.01	NS	NS	
20:3 <i>n</i> -6	-	-	-	-	-	-	-	-	2.7	2.5	0.2	-	-	0.2	-	-	-	
20:4 <i>n</i> -6	5.8 ^a	5.3 ^a	1.9	23.7 ^b	25.7 ^b	1.9	<i>P</i> < 0.01	NS	3.2	2.4	0.1	-	-	0.1	-	-	-	
20:5 <i>n</i> -3	9.7	8.6	0.4	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-	
22:5 <i>n</i> -3	3.4	3.6	0.2	3.5	4.5	0.2	NS	NS	-	-	-	-	-	-	-	-	-	
22:5 <i>n</i> -6	2.0	1.9	0.2	-	-	0.2	-	-	-	-	0.2	2.4	4.6	0.2	-	-	-	
22:6 <i>n</i> -3	13.4	13.2	0.5	-	-	0.5	-	-	-	-	-	-	-	-	-	-	-	
Desaturation index†	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
<i>n</i> -6	3.61 ^a	3.04 ^a	0.9	10.7 ^b	10.9 ^b	0.9	<i>P</i> < 0.01	NS	0.12 ^a	0.10 ^a	0.0	0.04 ^b	0.03 ^b	0.0	<i>P</i> < 0.01	NS	NS	
<i>n</i> -3	1.03	1.10	0.1	-	-	0.1	-	-	-	-	-	-	-	-	-	-	-	

PUFA, polyunsaturated fatty acid.

^{a,b,c} Mean values within a row and within a phospholipid category not sharing a common superscript letter were significantly different, *P* < 0.05 (28 df).

* For details of diets, see Table 1 and p. 646.

† Desaturation index for *n*-6 PUFA = (20:3*n*-6 + 20:4*n*-6)/18:2*n*-6; and for *n*-3 PUFA, 22:6*n*-3/(20:5*n*-3 + 22:5*n*-3).

although they reflected the type of dietary PUFA. Thus, the proportion of arachidonic acid was significantly higher, while that of docosahexaenoic acid (22:6n-3) was significantly lower in rats fed on safflowerseed oil compared with those fed on perilla oil. Consequently, the desaturation indices for n-3 and n-6 PUFA (22:6n-3/(20:5n-3 + 22:5n-3) and (20:3n-6 + 20:4n-6)/18:2n-6 respectively) were not markedly influenced by the difference in the geometry of dietary monounsaturated fatty acids.

ANOVA showed a significant effect of the geometrical difference on the proportion of linoleic acid in phospholipids except for phosphatidylinositol and cardiolipin. The desaturation index for linoleic acid was also dependent on PUFA and/or the geometry in phospholipids except for phosphatidylinositol.

Fatty acid compositions of adipose tissue lipids

As shown in Table 5, the fatty acid composition of adipose tissue total lipids reflected that of the dietary fats. Slightly but significantly more elaidic acid was detected in rats fed on safflowerseed oil than in those fed on perilla oil. Although the type of dietary PUFA was reflected in the PUFA composition of adipose tissue lipids, the concentration of PUFA in adipose tissue was significantly lower when rats were given *trans* fatty acid compared with *cis* fatty acid. The decrease in the proportion of oleic acid by dietary elaidic acid was not totally compensated for by the incorporation of *trans* monoene fatty acid. Instead, the proportion of palmitoleic acid significantly increased on feeding *trans* fatty acid irrespective of the dietary PUFA.

Splenic production of leukotriene C₄ and prostaglandin E₂

Dietary *trans* fatty acid did not influence the splenic production of leukotriene C₄ in rats fed on either the n-3 or the n-6 PUFA diet (Table 6). However, perilla oil markedly reduced the production compared with safflowerseed oil, and there was a significant interaction of dietary PUFA on this variable. The production of prostaglandin E₂ was measured only in the experiment with safflowerseed oil, and it was significantly reduced by elaidic acid.

Table 5. Effects of elaidic acid on fatty acid compositions (mol/100 mol total fatty acids) of adipose tissue of rats fed on perilla oil or safflowerseed oil diets*

(Mean values with their standard errors for eight rats)

Fatty acid	Perilla oil				Safflowerseed oil				Pooled SE	ANOVA F value		
	<i>cis</i>		<i>trans</i>		<i>cis</i>		<i>trans</i>			PUFA	<i>cis-trans</i>	Interaction
	Mean	SE	Mean	SE	Mean	SE	Mean	SE				
14:0	1.8 ^a	0.1	1.9 ^a	0.0	1.2 ^b	0.0	1.3 ^b	0.0	0.1	P < 0.01	NS	NS
16:0	21.0 ^a	0.7	23.5 ^b	0.3	20.9 ^a	0.5	21.6 ^a	0.8	0.4	NS	P < 0.05	NS
16:1	5.2 ^a	0.2	7.3 ^b	0.2	4.1 ^c	0.3	5.9 ^a	0.3	0.2	P < 0.01	P < 0.01	NS
18:0	2.1 ^a	0.0	1.8 ^b	0.1	2.3 ^a	0.1	1.6 ^b	0.0	0.1	NS	P < 0.01	P < 0.01
<i>trans</i> -18:1	-	-	11.6 ^a	0.2	-	-	13.4 ^b	0.2	0.3	-	-	-
<i>cis</i> -18:1	41.2 ^a	0.4	24.3 ^b	0.5	40.1 ^c	0.2	20.5 ^d	0.2	1.7	P < 0.01	P < 0.01	P < 0.01
18:2n-6	8.2 ^a	0.4	7.7 ^a	0.3	30.0 ^b	0.7	34.1 ^c	0.8	2.2	P < 0.01	P < 0.01	P < 0.01
18:3n-3	20.0 ^a	0.5	22.0 ^b	0.5	0.3 ^c	0.0	0.5 ^c	0.1	2.0	P < 0.01	P < 0.01	P < 0.05

PUFA, polyunsaturated fatty acid.

^{a,b,c}Mean values within a row not sharing a common superscript letter were significantly different, P < 0.05 (28 df).

* For details of diets, see Table 1 and p. 646.

Table 6. *Effects of elaidic acid on splenic production of leukotriene C₄ and prostaglandin E₂ in rats fed on perilla oil or safflowerseed oil diets**

(Mean values with their standard errors for eight rats)

	Perilla oil diet				Safflowerseed oil diet				Pooled SE	ANOVA <i>F</i> value		
	<i>cis</i>		<i>trans</i>		<i>cis</i>		<i>trans</i>			PUFA	<i>cis-trans</i>	Inter-action
	Mean	SE	Mean	SE	Mean	SE	Mean	SE				
Leukotriene C ₄ (ng/g spleen)	49.7	2.3	52.4	10.5	432	63	326	25	33.2	<i>P</i> < 0.01	NS	NS
Prostaglandin E ₂ (ng/g spleen)	ND		ND		3.54 ^a	0.94	1.31 ^b	0.51	0.64	-	-	-

PUFA, polyunsaturated fatty acid; ND, not determined.

^{a,b} Mean values within a row with unlike superscript letters were significantly different, *P* < 0.05 (28 df).

*For details of diets, see Table 1 and p. 646.

Table 7. *Effects of elaidic acid on splenic T-lymphocyte subsets in rats fed on diets containing perilla oil or safflowerseed oil**

(Mean values with their standard errors for eight rats)

Group	T-lymphocyte					
	CD4 ⁺ (%)		CD8 ⁺ (%)		CD4 ⁺ :CD8 ⁺	
	Mean	SE	Mean	SE	Mean	SE
Perilla oil diet						
<i>cis</i>	25.2	1.1	21.9 ^a	1.3	1.17 ^a	0.06
<i>trans</i>	25.4	1.0	17.0 ^a	0.7	1.50 ^b	0.04
Safflowerseed oil diet						
<i>cis</i>	25.1	1.3	14.5 ^b	0.8	1.75 ^c	0.09
<i>trans</i>	26.0	1.1	15.6 ^b	0.6	1.57 ^{bc}	0.05
Pooled SE		0.53		0.67		0.05
ANOVA <i>F</i> value						
PUFA		NS		<i>P</i> < 0.01		<i>P</i> < 0.01
<i>cis-trans</i>		NS		<i>P</i> < 0.05		NS
Interaction		NS		<i>P</i> < 0.01		<i>P</i> < 0.01

PUFA, polyunsaturated fatty acid.

^{a,b,c} Mean values within a column not sharing a common superscript letter were significantly different, *P* < 0.05 (28 df).

*For details of diets, see Table 1 and p. 646.

Splenic T-lymphocyte subsets

The results are shown in Table 7. Dietary manipulations did not influence the proportion of splenic CD4⁺ T-lymphocytes, but that of CD8⁺ cells decreased when rats were fed on safflowerseed oil rather than perilla oil. Dietary *trans* fatty acid did not influence these variables. However, the CD4⁺:CD8⁺ ratio was higher in the elaidic acid group than in the oleic acid group when perilla oil was fed, but not when safflowerseed oil was fed. ANOVA showed PUFA- and geometry-dependent differences in the proportion of CD8⁺.

Plasma concentrations of immunoglobulins

Table 8 shows the concentrations of plasma immunoglobulins. When the PUFA source was perilla oil, the concentration of IgA was significantly lower, while those of IgG and IgM

Table 8. Effects of elaidic acid on plasma immunoglobulin levels in rats fed on diets containing perilla oil or safflowerseed oil*

(Mean values with their standard errors for eight rats)

Group	Immunoglobulin							
	IgA ($\mu\text{g/ml}$)		IgE (ng/ml)		IgG (mg/ml)		IgM (ng/ml)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Perilla oil diet								
<i>cis</i>	1.87 ^a	0.14	17.8 ^a	1.1	5.23 ^a	0.56	93.1 ^a	8.5
<i>trans</i>	1.47 ^b	0.28	20.7 ^a	1.5	6.90 ^b	0.58	159 ^b	11
Safflowerseed oil diet								
<i>cis</i>	34.2 ^c	2.6	4.54 ^b	0.78	1.19 ^c	0.21	160 ^b	23
<i>trans</i>	33.4 ^c	4.8	4.84 ^b	0.84	1.40 ^c	1.10	188 ^b	18
Pooled SE		0.48		1.43		0.48		3.2
ANOVA <i>F</i> value								
PUFA		$P < 0.01$		$P < 0.01$		$P < 0.01$		$P < 0.01$
<i>cis-trans</i>		NS		NS		$P < 0.05$		NS
Interaction		NS		NS		NS		NS

PUFA, polyunsaturated fatty acid.

^{a,b,c} Mean values within a column not sharing a common superscript letter were significantly different, $P < 0.05$ (28 df).

* For details of diets, see Table 1 and p. 646.

were significantly higher on feeding *trans* acid than on feeding the *cis* counterpart. No such differences were observed when the PUFA source was safflowerseed oil. The concentration of IgA was significantly higher, while those of IgE and IgG were significantly lower, in the safflowerseed oil group than in the perilla oil group. ANOVA showed significant PUFA-dependent differences in the levels of all immunoglobulins, while a geometry-dependent difference was only evident for IgG.

DISCUSSION

There were detectable differences in the metabolic implications of elaidic acid with respect to the type of PUFA simultaneously ingested. Although the protocol of the present study (i.e. that two experiments were done separately) should be taken into consideration, the *F* values for various variables including growth indices and tissue weights were not statistically different. Thus, the comparisons were made on two experiments by two-way ANOVA followed by Duncan's multiple range test (Duncan, 1955).

The incorporation of *trans* fatty acid into liver phospholipids was approximately 1.5 times higher when linoleic acid was the source of PUFA than when α -linolenic acid was the major source of PUFA. This observation may simply indicate an alleviation by *n*-3 PUFA of the possible undesirable effect of *trans* fatty acid.

A study by Bernard *et al.* (1987) indicated that elaidic acid was absorbed to a lesser extent than oleic acid, and that the absorption rate appeared to be influenced by the type of fatty acid simultaneously infused through a duodenal cannula. In the present experiments, the incorporation of elaidic acid into adipose tissue lipids was slightly but significantly higher in rats fed on safflowerseed oil than in those fed on perilla oil. Since the fatty acid composition of adipose tissues routinely reflects that of dietary fats (Tjonneland *et al.* 1993), the different interference of individual PUFA with elaidic acid absorption seems

unlikely in the present study. It is also plausible that α -linolenic acid stimulates the metabolism of *trans* fatty acid. Elaidic acid caused a small but significant increase in the proportion of α -linolenic and linoleic acids in adipose tissue when the dietary fat supplied large amounts of these PUFA. Thus, elaidic acid may, alternatively, interfere with the utilization of PUFA, since in adipose tissue PUFA, particularly α -linolenic acid, appear to be mobilized most rapidly of all the fatty acids (Cunnane *et al.* 1991).

In contrast, the compositions of PUFA in liver phospholipids were not essentially modified by the difference in the geometry of octadecenoic acids, and they apparently reflected those of dietary PUFA. It has been reported that *trans* fatty acids interfere with the desaturation of linoleic acid to arachidonic acid, and hence eicosanoid production (Kinsella *et al.* 1981; Sugano *et al.* 1989), but this effect disappears when the supply of essential linoleic acid is satisfactory (Zevenbergen & Haddeman, 1989; Koga *et al.* 1994). In the present study, the perilla oil diet supplied approximately 2 % energy as linoleic acid, which corresponds to the minimal requirement of this essential fatty acid for rats. Although a similar response pattern of the PUFA composition of spleen phospholipids is deduced between the *cis* and *trans* groups (Koga *et al.* 1994), this dietary level of linoleic acid may be border line in its ability to compensate for the effect of elaidic acid, since splenic production of prostaglandin E₂ decreased significantly in rats fed on *trans* fat in combination with safflowerseed oil. The observation that splenic leukotriene C₄ production was not influenced by the dietary fat sources indicates a possible difference in the sensitivity of the enzymic systems producing individual eicosanoids. A marked reduction of splenic leukotriene C₄ production after feeding perilla oil can be attributed to the incorporation of *n*-3 PUFA in phospholipids (Yaqoob & Calder, 1993). It is known that PUFA of the *n*-3 family interfere with the production of eicosanoids from arachidonic acid (Kinsella *et al.* 1990).

A PUFA-dependent difference in the concentrations of liver lipids was also observed, although the effects were not so marked. Previously, we reported that *trans* monoene fatty acids in partially hydrogenated vegetable oil were not hypercholesterolaemic in the rat model, compared with the *cis* counterparts and they simultaneously reduced the liver triacylglycerol level (Sugano *et al.* 1989). The hypocholesterolaemic effect was not reproduced in the present study with cholesterol-free diets, even when combined with α -linolenic acid (Drevon, 1992).

The different effects of *n*-3 and *n*-6 PUFA on the metabolic effects of *cis* and *trans* octadecenoic acid were also observed in some immune variables. Elaidic acid in combination with perilla oil increased the CD4⁺ helper T-cells : CD8⁺ suppressor T-cells ratio. However, this was not the case with safflowerseed oil. Huang & Fritsche (1992) observed an increase in spleen weight and immune cell yield in mice fed on diets high in high-oleic safflowerseed oil or menhaden oil compared with those fed on a high-linoleic safflowerseed oil diet, probably in response to the change in the fatty acid composition of phospholipids. It was suggested that the changes in the fatty acid compositions of membrane phospholipids of the immune cells might, directly or indirectly, influence their proliferation and/or migration (Novo *et al.* 1987). Although the current observation is difficult to interpret, it is at least likely that the immunological effect of elaidic acid may be modified by the source of dietary PUFA. Fly & Johnston (1990) showed that serum from previously immunized rats fed on *n*-3 PUFA-containing oils (Canola (rapeseed) and soyabean oils) contained significantly more antibody to bovine serum albumin than that from rats fed on maize oil, suggesting a difference in IgM production. Thus, *trans* fatty acid when fed with *n*-3 PUFA, compared with *n*-6 PUFA, may enhance the immune defence function. The observation that the concentrations of plasma IgG and IgM were

significantly higher in the elaidic acid group than in the oleic acid group in rats fed on perilla oil was not explained by the change in T-lymphocyte subsets in the spleen (Katz, 1980). Although the physiological significance of the current observation on the immune variables is not apparent at present, the results at least indicate diverse interactions of elaidic acid with different PUFA.

In conclusion, the present study indicated that the metabolic effects of elaidic acid are readily modified by the type of dietary PUFA, either *n*-3 or *n*-6, consumed simultaneously. α -Linolenic acid appeared to have more marked effects than linoleic acid in this respect. Thus, the present results emphasize the critical importance of the type of PUFA in evaluating the metabolic effects of *trans* fatty acid. However, due to the design of the present study it is difficult to draw definite conclusions about whether the observed differences are solely attributable to PUFA.

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