Different dietary fats influence serum and tissue lipids and anti-cardiolipin antibody levels in autoimmune-prone NZB/W F1 mice

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(Received 10 April 1998 – Revised 12 November 1998 – Accepted 16 November 1998)

To investigate the influence of different dietary fats on lipids and anti-cardiolipin antibody levels, autoimmune NZB/W F1 mice were fed on diets containing 200 g dietary fat as palm oil, lard–soyabean oil (1 : 1, w/w), soyabean oil, rapeseed oil or fish oil/kg. In addition, each dietary fat group was divided into an early-feeding group with feeding from 2 months of age, and a late-feeding group with feeding from 5 months of age. Serum levels of triacylglycerol, phospholipid, cholesterol and anti-cardiolipin antibody were measured at regular intervals, and mice were killed at the age of 7 months for analysis of hepatic lipid and fatty acids. The results showed that hepatic triacylglycerol and cholesterol contents were lower in mice fed on fish oil than in those fed on palm oil. In contrast, hepatic phospholipid content was higher in mice of the fish oil group than in those of the other four dietary fat groups. Composition profiles for both hepatic and renal oleic acid (18 : 1*n*-9), linoleic acid (18 : 2*n*-6) and eicosapentaenoic acid (20 : 5*n*-3) were similar to those of the dietary fats in mice of both early-feeding and late-feeding groups. Fish oil intake decreased arachidonic acid (20 : 4*n*-6) concentration in kidney tissue but not in liver tissue. Serum triacylglycerol, cholesterol and phospholipid levels were lower in mice fed on fish oil than in those fed on palm oil. Immunoglobulin (Ig) M anti-cardiolipin antibody was lower for the fish oil group than for the other groups. The IgG anti-cardiolipin antibody level was significantly lower in mice fed on fish oil compared with that of the palm oil group only in the early-feeding group. There was a positive correlation between serum IgM anti-cardiolipin antibody and phospholipid levels (early-feeding group r 0·902, $P < 0·05$; late-feeding group r 0·894, $P < 0·05$). These findings suggest dietary fish oil may affect both lipid levels and anti-cardiolipin antibody, contributing to alleviation of the autoimmune process in autoimmune-prone NZB×NZW F1 mice.

Systemic lupus erythematosus: Dietary fat: Fish oil: Cardiolipin: Autoimmunity

Systemic lupus erythematosus (SLE) is characterized by the presence of a hetergenous group of antibodies cross-reactive with cardiolipin, phospholipid, DNA, nucleoprotein and certain surface antigens (Theofilopoulos *et al*. 1989). Anti-cardiolipin antibody has been demonstrated in the sera for both human and murine lupus, and its presence has been closely related to venous and arterial thrombosis, thrombocytopenia, neurological diseases and recurrent fetal loss (Lockshin *et al*. 1985; McNeil *et al*. 1991). Cardiolipin is a mitochondrial dianionic and tetra-acylated phospholipid, and anti-cardiolipin antibody has been found to cross-react with negatively-charged phospholipids, such as phosphotidylserine and phosphotidylinositol (Rauch *et al*. 1984; Eilat *et al*. 1986; Harris *et al*. 1987).

Autoimmune-prone NZB/W F1 mice fed on a high-fat diet, which was composed of equal amounts of lard and soyabean oil, developed more severe disease and had a shorter lifespan (Lin *et al*. 1996). In addition, higher amounts of dietary fat can also affect the level of anti-cardiolipin antibody and fatty acid concentrations in NZB/W F1 mice (Lin *et al*. 1997*b*). However, the role of anti-cardiolipin antibodies in the pathogenesis of this disease has not been clearly elucidated. Studies have shown that an increase in vascular diseases is noted only in SLE patients with high serum triacylglycerol levels, suggesting that serum triacylglycerol level is closely related to production of anti-cardiolipin antibodies and subsequent vascular diseases (Hashimoto *et al*. 1992; MacGregor *et al*. 1992).

Abbreviations: DHA, docosahexaenoic acid; Ig, immunoglobulin; SLE, systemic lupus erythematosus.

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Dietary *n*-3 fatty acids have been shown to affect spleen and macrophage fatty acid composition (Chapkin *et al.* 1991; Robinson *et al.* 1993*a*). Plasma cholesterol level was found to be affected by the degree of saturation of dietary fat (Fernandez *et al.* 1997). Dietary fats with different degrees of saturation or their *n*-3 : *n*-6 fatty acid value might have a critical effect on the serum levels of triacylglycerol, cholesterol and phospholipid, and subsequent anti-phospholipid antibody production, which may result in further pathological damage in patients with SLE.

Although dietary fish oil has been shown to reduce the level of immunoglobin (Ig)G anti-double-stranded DNA antibody levels by regulation of cytokine expression (Fernandes *et al.* 1994) and to reduce proteinuria and prolong lifespan in autoimmune-prone NZB/W F1 mice (Robinson *et al.* 1993*b*), only a few studies concerning the effect of fat nutrition on anti-cardiolipin antibody production in autoimmune-prone NZB/W F1 mice have been documented (Lin *et al.* 1997*b*). The present study investigated the effect of different dietary fats on hepatic lipid and hepatic and renal fatty acid composition, and further on serum levels of triacylglycerol, cholesterol, phospholipid and anti-cardiolipin antibody in autoimmune-prone mice.

Materials and methods

Animals and diets

Female NZB×NZW F1 (B/W F1) mice (6 weeks old) were purchased from the Jackson Laboratory (Bar Harbor, ME, USA). The mice were housed individually in stainless-steel wire cages and fed on a non-purified diet (Lab Rodent Chow; Ralson Purina, St Louis, MO, USA). The animal room was maintained on a 12 h light–12 h dark cycle and at constant temperature ($25 \pm 2^{\circ}$) and humidity. Each diet group comprised twelve mice. Animal care and handling conformed to the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1985).

The five diets used in the experiment all contained (g/kg): 200 casein (Sigma Chemical Co., St Louis, MO, USA), 3 methionine (Sigma Chemical Co.), 250 sucrose (Taiwan Sugar Co., Taipei, Taiwan), 250 maize starch (Roquatte, Paris, France), 50 α-cellulose (J. Bettenmaier & Söhne, Ellwangen-Holzmühle, Germany), 2 choline (Sigma Chemical Co.), 35 AIN-76 mineral mixture (American Institute of Nutrition, 1977), 10 AIN-76 vitamin mixture (American Institute of Nutrition, 1977), 200 fat. The fat sources used in the five experimental diets were (g/kg): 200 palm oil (President Co., Taipei, Taiwan), 200 soyabean oil (Taiwan Sugar Co.), 100 lard (Ya-Sern Co., Taipei, Taiwan) and 100 soyabean oil (lard–soyabean oil), 200 rapeseed oil (Cheil Industries Corp., Taipei, Taiwan), or 180 fish oil (President Co.) and 20 safflower oil (President Co.). DL-α-Tocopherol (100 mg/kg diet; 50 mg tocopherol equivalents/kg) was added to each diet and the final vitamin E contents of the diets were 80, 62, 74, 96 and 120 mg tocopherol equivalents/kg diet for palm oil, lard–soyabean oil, soyabean oil, rapeseed oil and fish oil respectively. The differences in vitamin E contents of the dietary fats were not adjusted because of their different oxidation potentials. The hepatic vitamin E content for the fish oil group was not found to be higher than that of the other four groups (Lin *et al.* 1997*a*). The palm oil was decolourized and its β-carotene content was below the level of detection. The fatty acid pattern of the dietary fat is summarized in Table 1.

The mice were given free access to food. They were weighed twice weekly, and food consumption was measured every 2–3 d. Each of the five dietary fat groups was further divided into early- and late-feeding groups. For the early-feeding groups, the mice had been fed on the experimental diets from 2 months of age. For the late-feeding groups, the mice maintained on the non-purified diet, then changed to the experimental diets from the age of 5 months. Six mice from each diet group were killed at 7 months of age, and the rest were maintained on the diets for determination of serum lipids and autoantibody levels, proteinuria and lifespan follow-up to confirm the pathogenic changes of these mice.

Table 1. The fatty acid pattern (g/100 g total fatty acids) in the dietary fat

	Dietary fat				
Fatty acid	Palm oil	Lard–soyabean oil	Soyabean oil	Rapeseed oil	Fish oil
14 : 0	3·3	0·75	ND	ND	5·7
16 : 0	44·7	17·9	10·9	5·2	8·2
16 : 1*n*-9	ND	1·3	ND	ND	7·2
18 : 0	3·9	8·0	3·9	1·6	3·3
18 : 1*n*-9	40·4	33·1	22·2	53·4	11·5
18 : 2*n*-6	9·3	33·8	54·0	22·9	7·2
18 : 3*n*-3	ND	3·9	7·2	12·7	1·7
20 : 4*n*-6	ND	ND	ND	ND	1·8
20 : 5*n*-3	ND	ND	ND	ND	26·8
22 : 6*n*-3	ND	ND	ND	ND	12·6
SFA	51·9	26·7	14·8	6·8	20·2*
MUFA	40·4	34·4	22·2	53·4	21·9†
PUFA	9·3	37·7	61·2	35·6	50·1
n-3 : *n*-6	0	0·12	0·13	0·55	4·6

ND, not detectable; SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; *n*-3 : *n*-6, *n*-3 fatty acids : *n*-6 fatty acids.
* The trace amounts of other saturated fatty acids were included.
† The trace amounts of other monounsaturated fatty acids were included.

Serum and liver triacylglycerol, cholesterol and phospholipid level, and analysis of tissues for fatty acid composition

Mice were bled retro-orbitally every month and serum collected for determination of serum triacylglycerol, cholesterol, phospholipid and anti-cardiolipin antibodies. Serum triacylglycerol, cholesterol and phospholipid concentrations were measured by colorimetric procedures using assay kits as described previously (Lin *et al*. 1997*b*). To investigate further the effect of fat intake on fatty acid composition, liver and kidney fatty acid compositions were analysed. Six mice from each group were killed by cervical dislocation at the age of 7 months. Livers and kidneys were excised, weighed and an approximately 0·5 g portion was extracted with chloroform–methanol (2 : 1, v/v) and prepared for further analysis of triacylglycerol, cholesterol and phospholipid according to the method described by Folch *et al*. (1957). Hepatic triacylglycerol, cholesterol and phospholipid contents were measured by colorimetric methods (Soloni, 1971; Stewart, 1980; Guo *et al*. 1982). The fatty acid composition was analysed according to the method of Lee *et al* (1990).

Determination of anti-cardiolipin antibodies

Serum anti-cardiolipin antibodies levels were determined using ELISA (Rupin *et al*. 1991). Briefly, ELISA plates were pre-coated with 150 μl protamine sulfate (5 mg/l) per well and incubated at room temperature for 6 h. The plates were then washed three times with PBS and coated with 50 μl bovine heart cardiolipin (Sigma Chemical Co.; 100 μg/ml absolute ethanol) per well. The control well (cardiolipin-free) was treated with 50 μl absolute ethanol as a non-specific binding background. After overnight incubation at 4°, the plates were air-dried at room temperature and washed three times with PBS. Subsequently, the plates were blocked with 200 μl post-coat solution (1 g gelatin/l PBS) per well and incubated at 37° for 2 h. After three washes with PBS, sera to be analysed were diluted 1 : 100 or 1 : 400 in a post-coat solution and 50 μl was added to the appropriate wells. After 3 h incubation at room temperature, plates were then washed nine times with PBS and peroxidase (*EC* 1.11.1.7) -conjugated goat anti-mouse IgG and IgM (γ- and μ-chain-specific; Jackson Inc., West Grove, PA, USA) secondary antibodies were added and incubated at room temperature for 2 h. After nine washes with PBS, adequate substrate was added for colour development and the reaction was stopped with 50 g SDS/1. Absorbance was determined at 415 nm (Microplate; Bio-Tek Instrument, Inc., Winooski, VT, USA). The supernatant fraction of monoclonal antibody 9C1A4 isolated from our laboratory was used as a positive control for IgM assays. Sera from one NZB/W F1 mouse which had high anti-cardiolipin antibody levels was used as a positive control for IgG assays. All the values were assayed in triplicate and derived by subtracting the non-specific binding of each sample.

Statistical analysis

The significance of difference among groups in the early-feeding groups and in the late-feeding groups was analysed statistically by one-way ANOVA and Duncan's multiple range test of the SAS program system (SAS/STAT version 6; SAS Institute Inc., Cary, NC, USA) throughout the study.

Results

Food intake and growth

There was no significant difference in feed intake and body-weight gain among the five different dietary fat groups of mice, either in the early-feeding or the late-feeding groups. The lifespan of mice fed on the fish oil diet (>450 d) was

Table 2. Hepatic lipids of 7-month-old female NZB/W F1 mice fed on different dietary fats*
(Mean values with their standard errors for the no. of mice per dietary group shown)

		Triacylglycerol (mg/g liver)		Cholesterol (mg/g liver)		Phospholipid (mg/g liver)	
Dietary fat	*n*	Mean	SE	Mean	SE	Mean	SE
Early-feeding group							
Palm oil	5	26·07	6·90	5·26^{a}	0·93	14·72^{c}	0·47
Lard–soyabean oil	6	22·75	3·85	4·06^{ab}	0·34	16·88^{b}	0·48
Soyabean oil	5	23·74	6·36	3·79^{b}	0·38	16·70^{b}	0·31
Rapeseed oil	6	21·77	3·55	4·63^{ab}	0·29	15·93^{b}	0·19
Fish oil	5	12·26	0·17	1·91^{c}	0·07	18·90^{a}	0·42
One-way ANOVA		$P=0·1171$		$P=0·004$		$P=0·0001$	
Late-feeding group							
Palm oil	5	16·35^{ab}	5·08	5·67^{a}	0·86	15·13^{b}	0·32
Lard–soyabean oil	4	20·86^{ab}	1·80	4·36^{a}	0·35	15·25^{b}	0·25
Soyabean oil	6	26·57^{a}	3·19	4·61^{a}	0·46	15·06^{b}	0·21
Rapeseed oil	4	23·13^{a}	5·19	5·65^{a}	0·51	15·29^{b}	0·39
Fish oil	6	11·48^{b}	1·18	2·62^{b}	0·17	16·94^{a}	0·53
One-way ANOVA		$P=0·0330$		$P=0·0023$		$P=0·0051$	

^{a,b,c} Mean values in the same column within feeding group with unlike superscript letters were significantly different ($P<0·05$; Duncan's multiple range test).

* Mice were fed for 5 months with a diet containing 200 g fat/kg in the early-feeding group, or fed for 2 months on a diet containing 200 g fat/kg in the late-feeding group. For details of diets and procedures, see pp. 332–333 and Table 1.

longer than those of mice fed on the other four diets both in the early-feeding group (palm oil 292 (SD 50) d, lard–soyabean oil 282 (SD 22) d, soyabean oil 327 (SD 72) d, rapeseed oil 366 (SD 56) d and the late-feeding group (palm oil 234 (SD 24) d, lard–soyabean oil 323 (SD 53) d, soyabean oil 293 (SD 50) d, rapeseed oil 258 (SD 66) d. Two-thirds of mice fed on fish oil survived longer than 16 months. However, there was no significant difference in the lifespans among the other four dietary fat groups either in the early-feeding or the late-feeding groups.

Triacylglycerol, cholesterol and phospholipid contents of liver

The effects of different dietary fats and feeding period on hepatic triacylglycerol, cholesterol and phospholipid contents of NZB/W F1 female mice are summarized in Table 2. Overall, the hepatic triacylglycerol content was lowest in the fish oil group in the late-feeding group. Although the hepatic triacylglycerol content of mice fed on the fish oil diet tended to be the lowest within early-feeding group, there was no significant difference between the five dietary fat groups. For both early-feeding and late-feeding groups, the hepatic cholesterol content of the fish oil-fed group was significantly lower than those of the other groups ($P < 0{\cdot}05$). Mice fed on palm oil had a higher hepatic cholesterol level compared with those of the soyabean oil and fish oil groups in the early-feeding groups ($P < 0{\cdot}05$). However, the hepatic phospholipid level was lowest in the palm oil-fed group of the early-feeding group. In contrast, phospholipid contents were significantly higher in mice fed on fish oil ($P < 0{\cdot}05$).

Liver and kidney fatty acid composition

The fatty acid composition of liver and kidney could be affected by dietary fat, as shown in Tables 3 and 4. The oleic acid (18 : 1*n*-9) compositions of both palm oil and rapeseed oil were higher than those of lard–soyabean oil, soyabean oil and fish oil (Table 1). The tissue fatty acid compositions reflected this dietary fat pattern for both liver and kidney oleic acid with palm oil > rapeseed oil > lard–soyabean oil >soyabean oil > fish oil. The linoleic acid (18 : 2*n*-6) compositions of liver and kidney was highest in the soyabean oil group and lowest in the palm oil and fish oil groups, reflecting the pattern in the dietary fat. Thus, the profiles for both oleic acid and linoleic acid composition in murine tissues reflected the composition profiles for these fatty acids in the dietary fats. For palmitic acid (16 : 0), palmitoleic acid (16 : 1*n*-9) and stearic acid (18 : 0), however, the tissue fatty acid composition profiles were not related to those of the corresponding dietary fats. The linolenic acid (18 : 3*n*-3) contents were low in the liver and kidney tissues. Although linolenic acid comprises up to 12 g/100 g total fatty acids in rapeseed oil, the linolenic acid contents in liver and kidney tissues were not significantly different between soyabean oil, rapeseed oil and fish oil groups. In addition, both eicosapentaenoic acid (20 : 5*n*-3) and docosahexaenoic acid (22 : 6*n*-3; DHA) were higher in the liver and kidney of mice fed on eicosapentaenoic acid- and DHA-rich fish oil.

Table 3. The fatty acid composition (g/100 g total fatty acids) of the livers and kidneys from NZB/W F1 female mice fed on different dietary fats in the early-feeding group*

(Mean values with their standard errors for four to six mice per dietary group)

	Dietary fat									
	Palm oil		Lard–soyabean oil		Soyabean oil		Rapeseed oil		Fish oil	
Fatty acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver										
16 : 0	23·4^{b}	1·7	19·3^{c}	0·6	17·7^{c}	0·9	17·7^{c}	1·9	25·9^{a}	2·3
16 : 1*n*-9	3·9^{ab}	0·9	3·0^{bc}	0·3	2·3^{c}	0·4	4·6^{a}	1·1	3·7^{ab}	0·7
18 : 0	5·5^{c}	1·3	7·4^{b}	1·7	7·2^{b}	1·7	9·4^{a}	0·7	11·0^{a}	1·0
18 : 1*n*-9	47·9^{a}	3·7	24·7^{c}	1·7	18·2^{d}	1·7	38·3^{b}	2·9	11·0^{e}	0·2
18 : 2*n*-6	9·8^{d}	0·8	28·2^{b}	3·7	36·7^{a}	4·9	16·0^{c}	2·4	9·3^{d}	0·7
18 : 3*n*-3	ND		1·5	0·5	2·5	0·9	2·2	0·9	ND	
20 : 4*n*-6	7·6	2·1	8·4	2·8	8·7	3·1	5·6	2·3	7·3	1·1
20 : 5*n*-3	ND		ND		ND		0·6^{b}	0·3	7·5^{a}	2·8
22 : 6*n*-3	2·0^{c}	0·8	7·5^{b}	1·4	6·6^{b}	1·4	5·7^{b}	2·2	24·2^{a}	0·7
Kidney										
16 : 0	22·4^{a}	1·4	18·7^{bc}	1·2	17·5^{c}	1·2	16·5^{d}	0·7	19·9^{b}	1·0
16 : 1*n*-9	2·4^{ab}	0·9	1·6^{c}	0·4	1·3^{c}	0·3	1·8^{bc}	0·3	2·5^{a}	0·5
18 : 0	12·2^{b}	3·2	13·8^{b}	0·9	16·1^{a}	0·7	13·3^{b}	0·9	16·4^{a}	1·5
18 : 1*n*-9	23·5^{a}	6·0	16·8^{b}	3·7	11·3^{c}	1·4	21·1^{a}	2·3	11·4^{c}	1·3
18 : 2*n*-6	10·5^{d}	1·4	19·8^{b}	1·9	22·1^{a}	1·5	15·8^{c}	0·7	5·6^{d}	0·9
18 : 3*n*-3	ND		ND		ND		1·9	0·7	ND	
20 : 4*n*-6	20·2^{a}	3·8	16·2^{b}	1·8	18·9^{ab}	2·1	16·2^{b}	2·1	12·9^{c}	0·9
20 : 5*n*-3	ND		ND		ND		ND		11·7	0·6
22 : 6*n*-3	8·8^{c}	1·7	13·1^{b}	2·3	12·8^{b}	2·1	11·9^{b}	0·7	19·6^{a}	1·0

ND, not detectable.

^{a,b,c,d,e} Mean values in the same row with unlike superscript letters were significantly different ($P < 0{\cdot}05$; one-way ANOVA and Duncan's multiple range test).

* Mice were fed for 5 months on a diet containing 200 g fat/kg. For details of diets and procedures, see pp. 332–333 and Table 1.

Table 4. The fatty acid composition (g/100 g total fatty acids) of the livers and kidneys from NZB/W F1 female mice fed on different dietary fats in the late-feeding group*

(Mean values with their standard errors for four to six mice per dietary group)

	Dietary fat									
	Palm oil		Lard–soyabean oil		Soyabean oil		Rapeseed oil		Fish oil	
Fatty acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver										
16 : 0	24·4[a]	1·5	21·6[b]	0·9	19·0[c]	1·0	18·7[c]	1·6	24·8[a]	1·4
16 : 1*n*-9	2·9	0·8	3·0	0·6	2·7	0·3	3·4	1·4	3·4	0·3
18 : 0	13·0[a]	1·5	10·8[ab]	2·3	9·4[b]	1·5	11·7[ab]	3·9	11·9[ab]	1·4
18 : 1*n*-9	32·6[a]	4·5	24·6[b]	3·1	16·4[c]	3·3	32·5[a]	8·7	12·1[c]	1·5
18 : 2*n*-6	10·7[d]	1·4	22·0[b]	0·7	37·2[a]	4·2	14·7[c]	2·0	10·4[d]	1·1
18 : 3*n*-3	2·1[b]	1·2	2·1[b]	0·3	3·8[a]	0·5	2·5[a]	0·6	2·5[a]	0·5
20 : 4*n*-6	9·4	2·7	8·8	2·6	5·9	1·4	8·1	4·4	7·3	0·7
20 : 5*n*-3	ND		ND		ND		0·9[b]	0·5	8·9[a]	0·4
22 : 6*n*-3	4·9[b]	1·5	7·2[b]	1·6	5·5[b]	1·2	7·5[b]	3·7	19·7[a]	0·9
Kidney										
16 : 0	20·3[a]	1·5	19·0[ab]	0·8	17·2[cd]	1·3	15·9[d]	1·2	18·2[bc]	0·9
16 : 1*n*-9	1·7	0·3	1·5	0·1	1·4	0·5	1·8	0·4	1·8	0·2
18 : 0	15·6	2·1	14·9	1·9	15·2	1·2	14·8	1·2	15·4	2·3
18 : 1*n*-9	17·5[a]	1·5	12·9[b]	0·7	10·4[c]	0·6	17·8[a]	1·7	9·6[c]	0·7
18 : 2*n*-6	12·0[d]	0·9	19·3[b]	0·9	23·8[a]	1·2	15·9[c]	0·9	9·3[c]	2·3
18 : 3*n*-3	1·3[b]	0·2	2·2[a]	0·9	2·3[a]	0·3	2·2[a]	0·5	1·8[a]	0·4
20 : 4*n*-6	22·7[a]	1·3	16·7[b]	1·9	16·8[b]	0·7	17·0[b]	1·4	12·5[c]	0·7
20 : 5*n*-3	ND		ND		ND		1·8[b]	0·5	11·1[a]	0·9
22 : 6*n*-3	9·3[c]	1·4	13·5[b]	0·9	13·0[b]	0·5	12·8[b]	0·7	20·3[a]	0·4

ND, not detectable.

[a,b,c,d] Mean values in the same row with unlike superscript letters were significantly different ($P < 0·05$; one-way ANOVA and Duncan's multiple range test).

* Mice were fed for 2 months on a diet containing 200 g fat/kg. For details of diets and procedures, see pp. 332–333 and Table 1.

Although both eicosapentaenoic acid and DHA were hardly detected in the other four dietary fats, DHA can be synthesized in the tissue and comprised about 6 g/100 g total fatty acids in liver and 12 g/100 g total fatty acids in kidney, except in the palm oil group. The tissue DHA composition in the palm oil group was significantly lower than those of the other four dietary groups ($P < 0·05$).

Most of the fatty acid profiles for the early-feeding and late-feeding groups were very similar despite the difference in feeding period. The fatty acid composition profiles for the liver were similar to those for the kidney. Although there was a low level of arachidonic acid (20 : 4*n*-6) in the dietary fat (Table 1), the arachidonic acid composition of liver tissue remained constant in both the early- and late-feeding groups. However, there was a significant difference in arachidonic acid composition of kidney among the five diet groups. Renal arachidonic acid compositions were significantly lower in mice fed on fish oil ($P < 0·05$) and was higher in mice fed on palm oil ($P < 0·05$) in both early- and late-feeding groups.

Serum triacylglycerol, cholesterol and phospholipid concentrations

The serum lipids of mice fed on diets containing dietary fats varying in saturation is shown in Table 5. The average serum triacylglycerol concentration of each dietary group before diet treatment was 0·79–0·85 mmol/l at 2 months of age in the early-feeding group and 1·57–1·88 mmol/l at 4 months of age in the late-feeding groups. There were no significant differences between the five diet groups before diet treatment. However, when feeding different dietary fats serum triacylglycerol and cholesterol levels were the lowest in mice fed on the fish oil diet among the five dietary groups both in the early-feeding and late-feeding groups. For the late-feeding groups, serum triacylglycerol levels of mice fed on palm oil were highest among the five dietary groups. The effect of fish oil in lowering serum lipid level was observed after 1 month of feeding ($P < 0·0001$ for early-feeding group and $P < 0·01$ for late-feeding group). For the late-feeding group, serum cholesterol and phospholipid levels increased significantly ($P < 0·05$) at 6 months of age, after 1 month on the high-fat diet, except the mice fed on fish oil (Table 5 and Fig. 1(b)). There was a clear decrease in triacylglycerol, cholesterol and phospholipid levels in response to dietary fish oil. There was no significantly consistent effect among the other four dietary fats. However, the mice fed on palm oil, a relatively more saturated fat, tended to have elevated serum triacylglycerol, cholesterol and phospholipid levels in the late-feeding group (Table 5 and Fig. 1(b)).

Anti-cardiolipin antibodies

Mice were bled retro-orbitally every month, and sera were collected for determination of anti-cardioplipin antibody levels. In all five groups, both IgM and IgG anti-cardioplipin antibody tended to increase gradually with age, except the IgG titre of the late-feeding group (Fig. 2). Interestingly, IgG anti-cardiolipin antibodies in the fish oil group were significantly ($P < 0·05$) lower than those of the palm oil group in the early-feeding group (Fig. 2(a)). However, no

Table 5. Serum triacylglycerol and cholesterol level of female NZB/W F1 mice fed on different dietary fats*
(Mean values with their standard errors for ten to twelve mice per dietary group)

	Triacylglycerol (mmol/l)				Cholesterol (mmol/l)			
Age (months)...	6		8†		6		8†	
Dietary fat	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Early-feeding group								
Palm oil	1·09[a]	0·06	2·01	0·41	5·69[ab]	0·13	7·50[ab]	1·32
Lard–soyabean oil	1·10[a]	0·14	2·22	0·58	5·49[ab]	0·17	8·43[ab]	1·79
Soyabean oil	0·98[a]	0·07	2·56	1·06	4·93[b]	0·10	6·34[bc]	1·38
Rapeseed oil	0·73[b]	0·07	1·52	0·17	6·84[a]	0·97	11·09[a]	1·63
Fish oil	0·58[b]	0·04	0·98	0·07	2·66[c]	0·07	3·11[c]	0·12
One-way ANOVA	$P=0·0001$		$P=0·3170$		$P=0·0001$		$P=0·0009$	
Late-feeding group								
Palm oil	2·36[a]	0·61	3·72[a]	1·45	10·45[a]	1·56	15·91[a]	2·54
Lard–soyabean oil	0·98[b]	0·17	1·78[bc]	0·36	8·36[a]	1·51	11·68[a]	1·67
Soyabean oil	0·93[b]	0·15	3·03[ab]	0·83	7·07[ab]	1·16	11·17[a]	1·77
Rapeseed oil	0·88[b]	0·15	1·31[bc]	0·24	8·32[a]	1·34	13·68[a]	1·75
Fish oil	0·72[b]	0·07	0·83[c]	0·06	3·67[b]	0·25	3·43[b]	0·14
One-way ANOVA	$P=0·0017$		$P=0·0171$		$P=0·0061$		$P=0·0001$	

[a,b,c] Mean values in the same column within feeding group with unlike superscript letters were significantly different ($P<0·05$; Duncan's multiple range test).
* Mice were fed for 5 months on a diet containing 200 g fat/kg in the early-feeding group, or fed for 2 months on a diet containing 200 g fat/kg in the late-feeding group. For details of diets and procedures, see pp. 332–333 and Table 1.
† Data from 8-month-old mice were obtained from four to six surviving mice per dietary group.

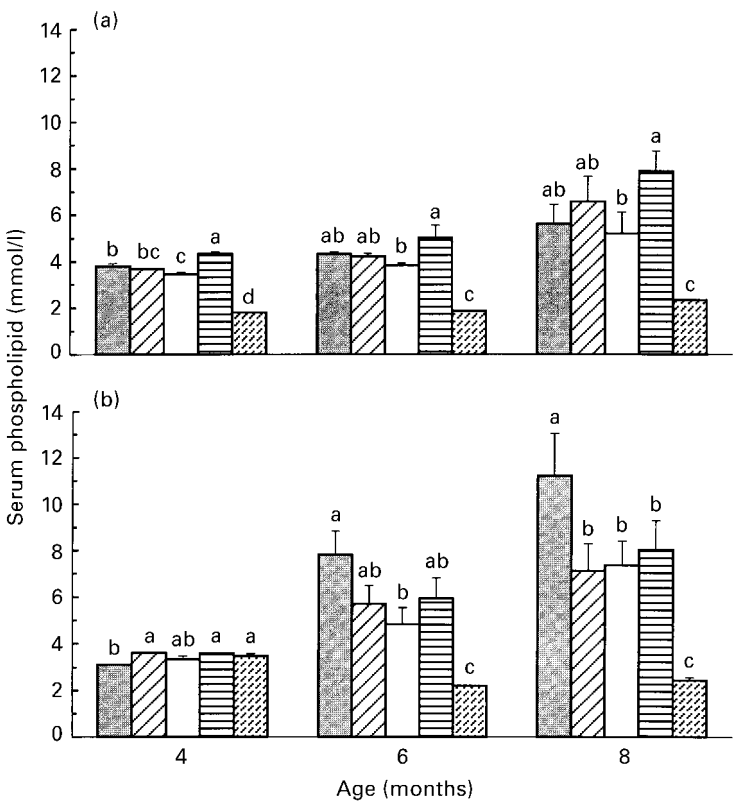

Fig. 1. Serum phospholipid levels of NZB/W F1 female mice in (a) the early-feeding group and (b) the late-feeding group, both fed on diets containing 200 g different fats/kg. The dietary fats were: palm oil (■), lard–soyabean oil (▨), soyabean oil (□), rapeseed oil (▤), and fish oil (▨). Results are mean values with their standard errors for twelve mice per group, except the rapeseed oil group where ten mice per group were used. Data from 8-month-old mice were obtained from four to six surviving mice per dietary group. [a,b,c] Means within age groups with unlike superscript letters were significantly different ($P<0·05$; Duncan's multiple range test). For details of diets and procedures, see pp. 332–333 and Table 1.

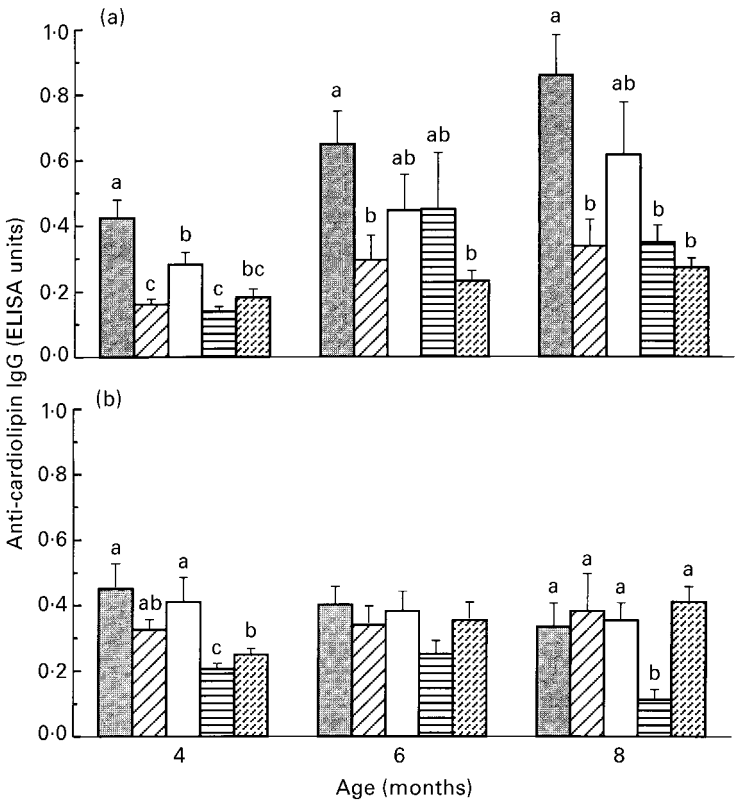

Fig. 2. Serum immunoglobulin (Ig) G anti-cardiolipin autoantibody levels of NZB/W F1 female mice in (a) the early-feeding group and (b) the late-feeding group fed on diets containing 200 g different fats/kg. The dietary fats were: palm oil (■), lard–soyabean oil (▨), soyabean oil (□), rapeseed oil (▤), and fish oil (▨). Each serum sample was diluted 100-fold. Results are mean values with their standard errors for twelve mice per group, except the rapeseed oil group where ten mice per group were used. Data from 8-month-old mice were obtained from four to six surviving mice per dietary group. [a,b,c] Means within age groups with unlike superscript letters were significantly different ($P < 0{\cdot}05$; Duncan's multiple range test). For details of diets and procedures, see pp. 332–333 and Table 1.

such pattern was noted in the late-feeding group (Fig. 2(b)). IgM anti-cardiolipin antibodies in mice fed on fish oil were lower compared with those of the other groups (Fig. 3). For late-feeding groups, serum anti-cardiolipin IgM levels significantly increased after the start of feeding the high-fat diet at 5 months of age ($P = 0{\cdot}0001$ for each group), except in mice fed on the fish oil ($P = 0{\cdot}5585$). At the age of 8 months, surviving mice fed on fish oil had the lowest levels of IgM anti-cardiolipin autoantibodies among these five diet groups in both the early-feeding and late-feeding groups ($P < 0{\cdot}05$). The profile of changes in serum anti-cardiolipin IgM was similar to that of serum phospholipid, therefore, the correlations between serum triacylglycerol, cholesterol and phospholipid levels and anti-cardiolipin antibody level were determined. It was found that IgM anti-cardiolipin antibody levels were correlated with serum phospholipid levels (Fig. 4); in contrast, no such correlation was found between IgG anti-cardiolipin antibody levels and phospholipid levels.

Discussion

Both human and murine SLE exhibit a characteristic spectrum of autoantibodies such as anti-erythrocyte, anti-DNA, anti-phospholipid antibodies (Theofilopoulos & Dixon, 1981). Among these autoantibodies, antibodies to phospholipid are a heterogeneous group of autoantibodies found in the sera of patients with SLE or mice with lupus. Anti-cardiolipin antibodies have been shown to be closely related to anti-phospholipid syndrome, which includes venous and arterial thrombosis, thrombocytopenia, and recurrent fetal loss (Harris *et al*. 1983; Koike *et al*. 1984; Lockshin *et al*. 1985; McNeil *et al*. 1991). Studies suggest the level of IgG anti-cardiolipin antibodies is more closely related to the development of thrombosis and thrombocytopenia. In contrast, the anti-cardiolipin antibody of the IgM isotype has been linked to a haemolytic anaemia and livedo reticularis (Mackworth-Young, 1990).

In our previous study, levels of both IgG anti-cardiolipin and anti-ssDNA antibodies in the group of mice receiving 200 g fat/kg were higher than those in the group receiving 50 g fat/kg at 3 months of age (Lin *et al*. 1997*b*). The present study investigated the effect of different fats varying in their extent of saturation on the level of anti-cardiolipin antibody in autoimmune NZB/W F1 mice. The results suggested that the IgG anti-cardiolipin antibody level was lower in mice in the early-feeding group fed on fish oil compared with that of

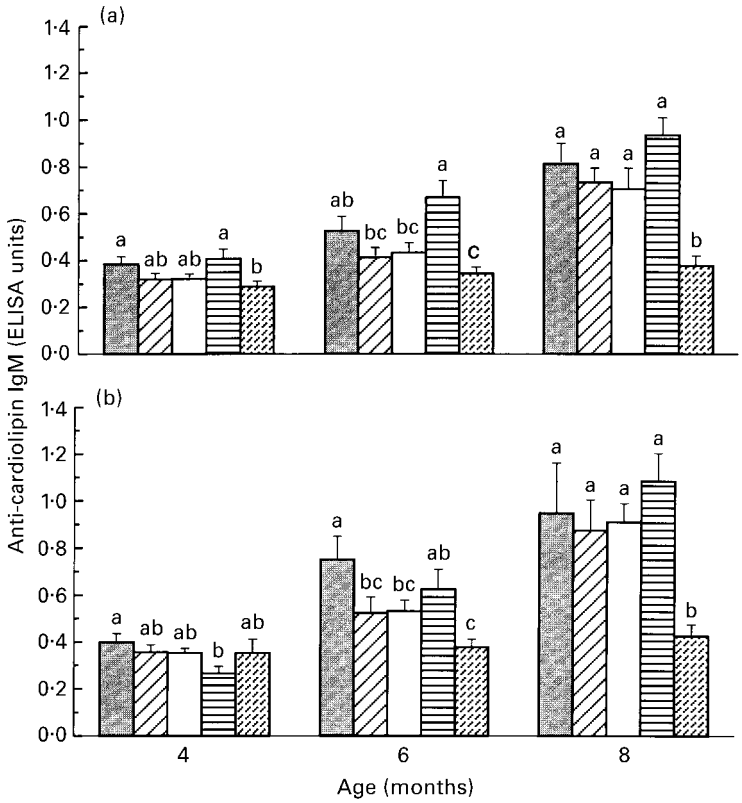

Fig. 3. Serum immunoglobulin (Ig) M anti-DNA autoantibody levels of NZB/W F1 female mice in (a) the early-feeding group and (b) the late-feeding group fed on diets containing 200 g different fats/kg. The dietary fats are palm oil (■), lard–soyabean oil (▨), soyabean oil (□), rapeseed oil (▤), and fish oil (▨). Each serum sample was diluted 400-fold. Results are mean values with their standard errors for twelve mice per group, except the rapeseed oil group where ten mice per group were used. Data from 8-month-old mice were obtained from four to six surviving mice per dietary group. [a,b,c] Means within age groups with unlike superscript letters were significantly different ($P < 0{\cdot}05$; Duncan's multiple range test). For details of diets and procedures, see pp. 332–333 and Table 1.

mice fed on palm oil. However, this effect was not observed in the late-feeding group. Furthermore, IgM anti-cardiolipin antibody level was lower in mice fed on fish oil in both the early- and late-feeding group.

There are several possible mechanisms for the effects observed with dietary fish oil. One study has suggested that increased serum triacylglycerol levels in patients with SLE might be closely related to production of anti-cardiolipin antibodies (MacGregor *et al*. 1992). Our findings showed that serum cholesterol, triacylglycerol and phospholipid levels were lower in mice fed on fish oil compared with those of the other groups. On the other hand, both the amount and content of dietary fatty acids can influence the composition of the cell membrane, and its recognition by the anti-phospholipid antibody (McNeil *et al*. 1991). Fish oil fatty acids may have just such an effect. The actual mechanism involved in the positive correlation between serum phospholipid levels and anti-phospholipid syndrome, therefore, needs to be further clarified. Although more studies are needed, the acyl composition of cardiolipin, which may play a critical role in the formation of antigenic determinants of B-cell (Levy *et al*. 1990; Qamar *et al*. 1990), can be influenced by various dietary fats (Berger *et al*. 1992). Thus, different dietary fats can affect the production of IgM anti-cardiolipin antibody and pathological damage in murine lupus. In the present study we have demonstrated a positive correlation between phospholipid level and IgM anti-cardiolipin antibody level, but not IgG anti-cardiolipin antibody. However, IgG is T-cell dependent and the antigenic determinants recognized by the T-cells are predominately protein in nature. It is suggested that IgG anti-cardiolipin antibody level is not related to serum phospholipid levels because the same protein source was used in the present study.

It is noteworthy that different dietary fats affected both serum and hepatic lipid contents in autoimmune-prone NZB/W F1 mice. Mice fed on a diet containing 250 g beef tallow/kg were noted to have significantly higher serum 18 : 2*n*-6 and 20 : 4*n*-6 fatty acid compositions and a shorter lifespan compared with those of mice fed on menhaden oil (Prickett *et al*. 1981). The lower levels of hepatic 18 : 2*n*-6 and kidney 18 : 2*n*-6 and 20 : 4*n*-6 fatty acid in the fish oil group may affect lipid metabolism and result in lower prostaglandin levels, which may subsequently alleviate the immune response (Synder *et al*. 1982; Santoli & Zurier, 1989). Furthermore, higher levels of eicosapentaenoic acid

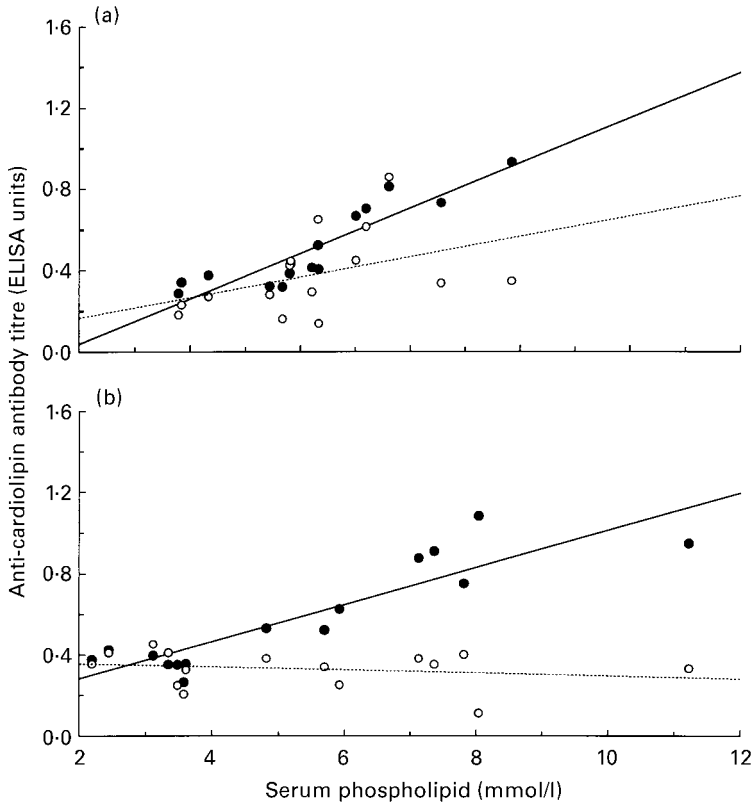

Fig. 4. Correlation between serum phospholipid level and immunoglobulin (Ig) G (○- – ○) or IgM (●—●) anti-cardiolipin antibodies level of NZB/W F1 mice in (a) the early-feeding and (b) the late-feeding groups. Values for the serum phospholipid levels and anti-cardiolipin antibodies titres were means for ten to twelve mice per dietary group from each age group. For IgM anti-cardiolipin antibody in the early-feeding group r 0·902, $P < 0·05$; for IgM anti-cardiolipin antibody in the late-feeding group r 0·894, $P < 0·05$ (Pearson correlate test).

and DHA in mice fed on fish oil might also contribute to a lowering of inflammatory mediators during the autoimmune process (Prickett *et al*. 1983; Kelley *et al*. 1985; Robinson *et al*. 1985). The results show lower cholesterol and triacylglycerol levels in mice fed on fish oil in both the early- and late-feeding groups. This finding is consistent with reports of other groups (Ikeda *et al*. 1994; Geelen *et al*. 1995). In contrast, the hepatic phospholipid content was significantly higher in fish oil-fed mice in both the early-feeding and late-feeding groups. This finding contrasts with that of the serum phospholipid level in mice fed on dietary fish oil, which was lower compared with those of the other groups.

The present study investigated the influence of different dietary fats on both serum and tissue fatty acid composition and anti-cardiolipin antibody production. Lower serum cholesterol, triacylglycerol and phospholipid levels were found in mice fed on fish oil compared with those of the other groups. The composition of both liver and kidney fatty acids, such as oleic acid (18 : 1*n*-9) and linoleic acid (18 : 2*n*-6), can be affected by dietary fats with different fatty acid compositions. The effect on lipid metabolism of the age of feeding and the duration of feeding the experimental diet was not obvious. Dietary fish oil was also found to decrease the level of anti-cardiolipin antibody in autoimmune NZB/W F1 mice. The findings demonstrated that the different dietary fats did exert different effects on lipid composition and anti-cardiolipin antibody level in autoimmune NZB × NZW F1 mice.

Acknowledgements

The authors are grateful for the helpful advice and for technical assistance from Professor Min-Hsiung Lee in fatty acid composition analysis. This study was supported by a grant from the National Science Council of the Republic of China; NSC 84-2321-B-002-026.

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