

Dietary fat affects lipids and anti-cardiolipin antibody levels in autoimmune-prone NZB/W F1 mice

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Studies in autoimmune-prone NZB/W F1 mice have demonstrated that the amount of dietary fat can affect autoantibody production and the disease course of autoimmune diseases. Anti-cardiolipin antibodies have been found to play a major role in thrombus formation and the increase of abortion rate in both human lupus patients and murine lupus. The present study investigated further the effect of dietary fat on lipid and anti-cardiolipin antibody production in autoimmune-prone mice. Two groups of NZB/W F1 mice were fed on diets containing 200 g dietary fat/kg and 50 g dietary fat/kg respectively, the fat being composed of equal amounts of lard and soyabean oil. Serum levels of lipids, immunoglobulin (Ig) anti-single stranded DNA and anti-cardiolipin antibodies were followed regularly every month and mice were killed for *in vitro* experiments after 5 months on the experimental diets. The results showed that serum triacylglycerol concentration was lower in mice fed on the high-fat diet than in those fed on 50 g fat/kg. There was no significant difference in hepatic lipid contents; however, the fatty acid contents were different between these two groups. Hepatic linoleic acid (18:2n-6) and arachidonic acid (20:4n-6) concentrations were higher in mice fed on the high-fat diet. There were no significant differences in serum IgM concentrations or IgM anti-cardiolipin antibody levels between these two groups. However, IgG anti-cardiolipin antibody levels were higher in mice fed on the high-fat diet at the age of 3–4 months. Total serum IgG concentration was noted to be higher, but in contrast, serum IgA was lower, in the high-dietary-fat group. These findings suggest that high dietary fat may affect lipid metabolism and autoantibody levels in autoimmune diseases.

Systemic lupus erythematosus: Autoimmunity: Cardiolipin

Murine lupus models such as NZB × NZW F1 (NZB/W F1), NZB.H-2^{bm12}, NZB × SWR F1 (SNF1), MRL.*lpr/lpr* and BXSB mice develop a wide spectrum of autoantibodies against erythrocytes, DNA, ribonucleoproteins and phospholipids (Theofilopoulos *et al.* 1989). Among them, the anti-cardiolipin antibody 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, which may contain up to 85 g linoleate/100 g in mammals (Berger *et al.* 1992). Anti-cardiolipin antibody is a heterogeneous group of autoantibodies which may cross-react with negatively charged phospholipids, such as phosphatidylserine and phosphatidylinositol (Rauch *et al.* 1984; Eilat *et al.* 1986; Haeis *et al.* 1987).

A low-fat and low-energy diet can reduce anti-DNA antibodies and prolong life span in autoimmune NZB/W F1 mice (Fernandes *et al.* 1976; Kubo *et al.* 1984; Gajjar *et al.* 1987). A previous study showed that mice fed on a high-fat diet, which contained equal amounts

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of lard and soyabean oil, developed more severe disease and had a shortened life span (Lin *et al.* 1996). However, the metabolism of dietary fat in NZB/W F1 mice still needs to be investigated. It has been reported that phospholipids, especially cardiolipin, can compete with the DNA binding activity of monoclonal anti-DNA antibodies (Lafer *et al.* 1981). Polyspecific anti-DNA antibodies in sera from patients with systemic lupus erythematosus (SLE) have been demonstrated to cross-react with cardiolipin (Koike *et al.* 1982, 1984). However, the role of anti-cardiolipin antibodies in the pathogenesis of SLE has not been clearly elucidated. Two studies showed that an increase in vascular diseases was found only in lupus patients with increased 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). A high-fat diet might have a critical effect on the serum levels of triacylglycerols, cholesterol and phospholipids, and subsequent anti-phospholipid antibody production which may result in further pathological damage in SLE patients.

No study concerning the effect of fat nutrition on anti-cardiolipin antibody production in autoimmune-prone NZB/W F1 mice has been documented. The present study investigates further the effect of high dietary fat on serum levels of triacylglycerol, cholesterol, phospholipid and immune responses, including anti-cardiolipin and anti-single-stranded DNA (anti-ssDNA) antibody levels, serum total immunoglobulin (Ig)G, IgM and IgA concentrations and mitogen responses.

MATERIALS AND METHODS

Experimental animals

Four-week-old female NZB/W F1 mice were purchased from Charles River Japan (Tokyo, Japan). BALB/c (*Mus musculus*) mice were obtained from the Animal Centre, College of Medicine, National Taiwan University. The mice were housed and fed individually in an animal room (12 h light and 12 h dark) with constant temperature ($25 \pm 2^\circ$) and humidity. Each group consisted of sixteen mice. Each mouse was fed on the experimental diet starting from 1 month old, and six to eight mice were killed for *in vitro* experiments after 5 months' feeding. The rest of the mice were maintained for proteinuria and lifespan follow-up to confirm the pathogenic changes of these mice. Animal care and handling conformed to the *NIH Guide for the Care and Use of Laboratory Animals* (National Research Council, 1985).

Diet

The compositions of the diets and sources of ingredients have been described previously (Lin *et al.* 1996). The experimental diets contained (g/kg): casein 200, DL-methionine 3, cellulose 50, choline bitartrate 2, vitamin mixture 10, mineral mixture 35. The high-fat group's feed contained 200 g fat/kg and the low-fat group's feed contained 50 g fat/kg. The fat was composed of equal amounts of lard and soyabean oil to mimic ordinary human dietary fat consumption. The difference in energy content was compensated for by carbohydrates. The high-fat diet contained 250 g maize starch/kg and 250 g sucrose/kg. The low-fat diet contained 325 g maize starch/kg and 325 g sucrose/kg. The mice were given free access to feed, weighed twice weekly, and feed consumption was measured every 2–3 d. The fatty acid pattern of the dietary fat is summarized in Table 1.

Table 1. *The fatty acid contents of the low-fat diet and the high-fat diet, and the fatty acid pattern in the dietary fat composed of equal amounts of lard and soyabean oil*

Fatty acid	Composition of fat (g/100 g total fatty acids)	Content in diet (g/kg diet)	
		50 g fat/kg	200 g fat/kg
14:0	0.75	0.375	1.5
16:0	17.90	8.95	35.8
16:1 n -9	1.30	1.65	6.6
18:0	8.00	4.0	16
18:1 n -9	33.05	16.53	66.1
18:2 n -6	33.75	16.88	67.5
18:3 n -3	3.90	1.95	7.8
20:4 n -6	ND	ND	ND

ND, not detectable.

Serum and liver triacylglycerol, cholesterol and phospholipid levels

Mice were bled retro-orbitally every month and serum was collected for determination of serum triacylglycerol, cholesterol, phospholipid, anti-cardiolipin and anti-ssDNA antibodies, and total serum immunoglobulins. Serum triacylglycerol, cholesterol and phospholipid concentrations were measured by colorimetric procedures. The triacylglycerols were determined after enzymic hydrolysis with lipase (*EC* 3.1.1.3), glycerol kinase (*EC* 2.7.1.30) and glycerol-3-phosphate oxidase (*EC* 1.1.99.5) to produce H₂O₂ (Fossati & Prencipe, 1982). Serum cholesterol was determined as free cholesterol, and as cholesterol released from its esters after cholesterol esterase (*EC* 3.1.1.13) hydrolysis, and oxidized by cholesterol oxidase (*EC* 1.1.3.6) to produce H₂O₂ (Richmond, 1973; Carlson & Goldfarb, 1979). The phospholipid was hydrolysed by phospholipase D (*EC* 3.1.4.4) and oxidized by choline oxidase (*EC* 1.1.3.17) to produce H₂O₂. The indicator, quinoneimine, of these assays is formed from H₂O₂, 4-aminoantipyrine, phenol or 4-chlorophenol, in the presence of peroxidase (*EC* 1.11.1.7; Takeyama *et al.* 1977), and was read at 500 nm using a spectrophotometer (U-3400 Hitachi, Tokyo, Japan). The triacylglycerol and cholesterol assay kits were purchased from Randox Laboratories Ltd (Antrim, N. Ireland). The phospholipid assay kits were purchased from Kyokuto Pharmaceutical Industrial Co., Ltd (Tokyo, Japan).

Serum and liver fatty acid composition analysis

To investigate further the effect of fat intake on fatty acid composition, serum and liver fatty acid compositions were analysed. Six to eight mice of each group were killed by CO₂ asphyxiation after 5 months of consuming the experimental diets. Livers were excised, weighed and a portion, of approximately 0.5 g, was extracted with chloroform-methanol (2:1, v/v) and prepared for further analysis of triacylglycerols, cholesterol, phospholipid and fatty acid composition according to the method described by Folch *et al.* (1957). Hepatic triacylglycerol, cholesterol, phospholipid and total lipids were measured by colorimetric methods with commercial assay kits as described earlier. The total lipid assay kits were purchased from Randox Laboratories Ltd. The fatty acid compositions were analysed according to the method of Lee *et al.* (1990).

Determination of anti-cardiolipin antibodies

Serum anti-cardiolipin antibody levels were determined using ELISA (Haeis *et al.* 1987; Rupin *et al.* 1991). Briefly, ELISA plates were pre-coated with 150 μ l protamine sulphate/well and incubated at room temperature for 2 h. Then, the plates were washed three times with phosphate-buffered saline (PBS) and coated with 50 μ l bovine heart cardiolipin/well (Sigma Chemical Co., St. Louis, MO, USA) in absolute ethanol at a concentration of 100 μ g/ml. The control well (cardiolipin-free) was treated with 50 μ l absolute ethanol as a non-specific binding background. The plates were air-dried at room temperature and washed once with PBS. Subsequently, the plates were blocked with 200 μ l adult bovine serum (100 g/l; ABS)/well and incubated at 37° for 1 h. After three washes with PBS, sera to be determined were diluted 1 : 50 or 1 : 100 in ABS (100 g/l) and 50 μ l was added to the appropriate wells. After 2 h incubation at room temperature, plates were then washed nine times with PBS and peroxidase-conjugated goat anti-mouse IgG or IgM (γ - and μ -chain specific, Jackson Inc., West Grove, PA, USA) secondary antibodies were added and incubated at room temperature for 1 h. After nine washes with PBS, adequate substrate was added for colour development and the reaction was stopped with 50 g/l SDS. Absorbance was determined at 415 nm (Microplate, Bio-Tek Instrument, Inc., Winooski, VT, USA). Sera of SLE patients were used as positive controls; procedures were the same except for secondary antibodies. All the values were assayed in triplicate and obtained by subtracting the non-specific binding of each sample.

Determination of anti-single stranded DNA antibodies

Levels of anti-ssDNA were quantified by ELISA on Immunolon I (Nunc Inc., Roskilde, Denmark). For the assay of anti-ssDNA antibodies, plates were initially coated with 100 μ l methylated bovine serum albumin (10 μ g/ml)/well. Stock calf thymus DNA (500 μ l/ml in PBS) was denatured by boiling for 15 min, then incubated on ice for 5 min with agitation. The denatured stock was diluted to 2.5 μ g/ml with PBS and added to the plates at 100 μ l/well. The subsequent procedure was the same as that for the determination of anti-double stranded DNA antibodies described previously (Lin *et al.* 1996).

Serum immunoglobulin levels

Serum IgG, IgM and IgA levels were quantified by radial immunodiffusion, and the assays were validated with standard mouse IgG, IgM and IgA. The assay kits were purchased from Serotec Ltd (Oxford, Oxon).

Mitogen responses

Single spleen cells were plated in ninety-six-well round-bottomed plates with a concentration of 1×10^6 cells/ml in RPMI 1640 medium supplemented with 100 ml fetal calf serum/l, 4 mM-L-glutamine, 25 mM-HEPES, 5×10^{-5} M-2-mercaptoethanol, 100 U penicillin/ml, 100 μ g streptomycin/ml and 0.25 mg amphotericin/ml in the absence or presence of mitogen. The optimal concentrations of the mitogens for this study were pretested and 5 or 25 μ g/ml lipopolysaccharide (LPS; Sigma), 5 or 10 μ g/ml concanavalin A (Con A; Sigma) were used in triplicate for the present study. The cells were incubated at 37° with 5 % CO₂ for 72 h. At 16 h before harvest, 37 KBq [³H]thymidine was added to each well. The cells were harvested and radioactivity was measured with a β -counter

(Beckman LS 5000 CE; Beckman, Los Angeles, CA, USA). The data were expressed as stimulation index.

Statistical analysis

The significance of difference between groups was analysed statistically by the two-sided non-paired Student's *t* test of the SAS program system (SAS/STAT, version 6, 1990; SAS Institute Inc., Cary, NC, USA) throughout this study. Differences were considered significant at $P < 0.05$.

RESULTS

Feed intake and growth

There was no significant difference in feed intake and body weight between mice fed on diets containing 50 and 200 g fat/kg before the onset of disease at 7–8 months old. The average daily feed intakes of mice were 3.7 (SD 0.4) g for the low-fat diet group and 3.2 (SD 0.5) g for the high-fat diet group. The daily energy intakes were similar between these two groups (60.5 (SD 6.3) kJ for the low-fat diet group and 61.6 (SD 9.9) kJ for the high-fat diet group). The body weights of mice were 46.7 (SD 3.8) g for the low-fat diet group and 46.4 (SD 3.6) g for the high-fat diet group at 6 months old. However, the average feed intake and body weight decreased in mice of the high-fat group starting from 8 months old as described previously (Lin *et al.* 1996). In addition, the life span of mice fed on the high-fat diet (285 (SD 60) d) was found to be shorter than that of mice fed on the low-fat diet (389 (SD 97) d, $P = 0.0439$).

Serum triacylglycerol, cholesterol and phospholipid concentrations

The serum lipid profiles of mice fed on diets containing different amounts of dietary fat are shown in Fig. 1. Serum triacylglycerol levels were lower in mice fed on 200 g fat/kg compared with those in mice fed on 50 g fat/kg diet ($P < 0.05$, $P = 0.0574$ at 4 months, Fig. 1(a)). In contrast, cholesterol and phospholipid levels between mice fed with different amounts of dietary fat were very similar (Fig. 1(b), 1(c)). However, it should still be pointed out that both cholesterol and phospholipid levels of mice fed on the high-fat diet were lower than those of the low-fat diet group at 6–7 months of age.

To compare with the lipid profile in female NZB/W F1 mice, serum lipid contents of three BALB/c and three male NZB/W F1 mice fed on laboratory chow diet at 3 months of age were measured. The serum triacylglycerol concentrations of BALB/c mice and NZB/W F1 male mice were higher (1.39 (SD 0.34) mmol/l and 2.75 (SD 0.35) mmol/l respectively). In contrast, the serum cholesterol concentrations of BALB/c mice and male NZB/W F1 mice were lower (2.04 (SD 0.34) mmol/l and 4.11 (SD 0.78) mmol/l respectively). The serum phospholipid concentrations of BALB/c mice and NZB/W F1 male mice were 1.88 (SD 0.13) mmol/l and 3.60 (SD 0.27) mmol/l respectively.

Liver and serum fatty acid pattern and fat content of liver tissues

The fat content of liver tissue is summarized in Table 2. There were no significant differences in hepatic triacylglycerol, cholesterol, phospholipid or total lipid concentrations between the low-fat diet group and the high-fat diet group. The liver and serum fatty acid composition patterns are summarized in Table 3. The results showed that palmitic acid (16:0), palmitoleic acid (16:1n-9) and oleic acid (18:1n-9) contents were significantly

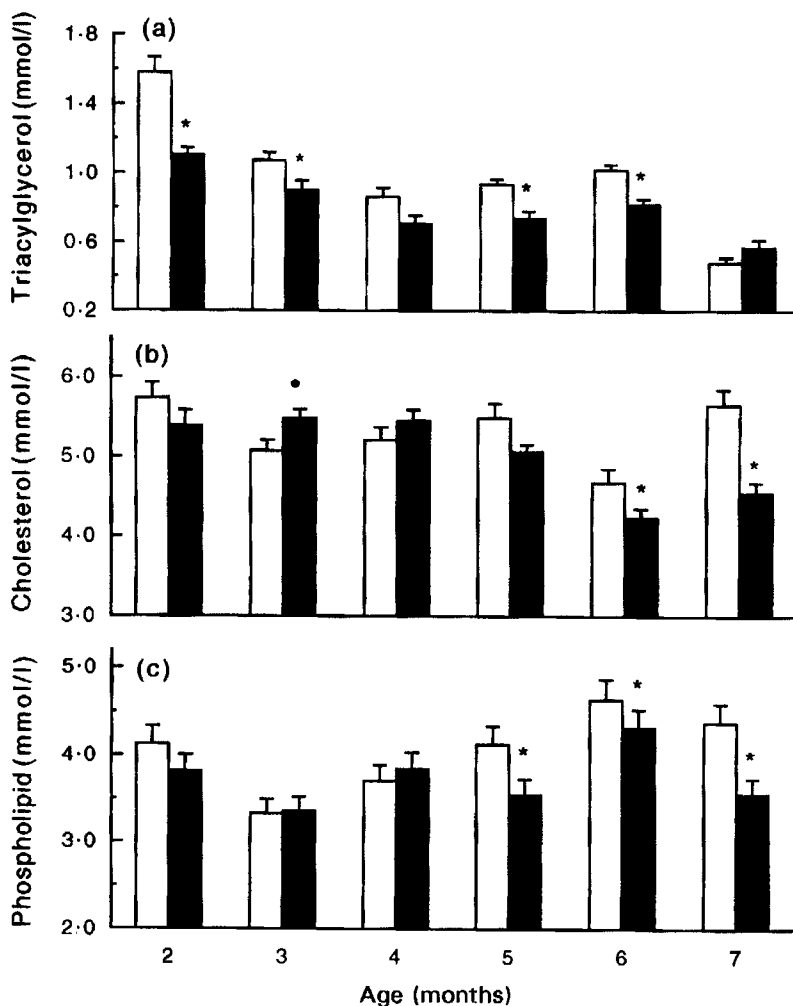


Fig. 1. (a) Serum triacylglycerol, (b) serum cholesterol and (c) serum phospholipid concentrations of NZB/W F1 female mice fed on diets containing 50 (□) or 200 (■) g fat/kg. Values are means with their standard errors for examples from twelve mice. * Mean values were significantly different for those for the 50 g fat/kg diet group ($P < 0.05$; Student's *t* test).

higher in mice fed on 50 g fat/kg diet compared with those of the 200 g fat/kg diet group. In contrast, linoleic acid (18:2n-6) and arachidonic acid (20:4n-6) contents in the high-fat group were significantly higher than those in the low-fat diet group. The serum fatty acid patterns are also listed in Table 3. Because a limited amount of serum was available for analysis, sera from six mice of each group were pooled for this measurement. Although statistical analysis was not possible, serum fatty acid patterns for low-fat and high-fat groups were similar to their hepatic fatty acid patterns.

Anti-cardiolipin and anti-single-stranded DNA antibodies

Mice were bled retro-orbitally every month, and serum samples were collected for determination of anti-cardiolipin (Fig. 2) and anti-ssDNA antibodies (Fig. 3). In these two

Table 2. *Hepatic lipids of 7-month-old female NZB/W F1 mice fed on diets with different contents of fat**

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

Group	Triacylglycerol (mg/g liver)		Cholesterol (mg/g liver)		Phospholipid (mg/g liver)		Total lipid (mg/g liver)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
50 g fat/kg	29.9	4.9	6.52	0.70	6.27	0.67	94.7	5.5
200 g fat/kg	37.6	4.7	6.52	0.85	7.26	0.42	142.7	40.2
Student's <i>t</i> test	$P=0.2859$		$P=0.9941$		$P=0.2425$		$P=0.2890$	

* Mice were fed for 5 months on a low-fat diet containing 50 g fat and 650 g carbohydrate/kg or a high-fat diet containing 200 g fat and 500 g carbohydrate/kg. For details of diets see Table 1 and p. 658.

Table 3. *The fatty acid pattern in serum and liver of 7-month-old female NZB/W F1 mice fed on diets with different contents of fat**

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

Group ...	Liver (mg/g liver)				Student's <i>t</i> test	Serum (mmol/l)†	
	50 g fat/kg		200 g fat/kg			50 g fat/kg	200 g fat/kg
	Mean	SE	Mean	SE			
Fatty acid							
16:0	13.7	1.2	9.24	1.20	$P=0.0252$	1.33	1.05
16:1 _{n-9}	5.02	0.42	1.56	0.25	$P=0.0001$	ND	ND
18:0	1.86	0.40	2.58	0.28	$P=0.1750$	0.81	1.13
18:1 _{n-9}	35.6	3.5	11.1	1.4	$P=0.0001$	0.99	0.64
18:2 _{n-6}	7.22	0.88	14.6	2.22	$P=0.0117$	1.50	1.64
20:4 _{n-6}	1.05	0.18	4.08	1.07	$P=0.0370$	0.33	0.53

ND, not detectable.

* Mice were fed for 5 months on a low-fat diet containing 50 g fat and 650 g carbohydrate/kg or a high-fat diet containing 200 g fat and 500 g carbohydrate/kg. For details of diets see Table 1 and p. 658.

† The values for serum fatty acid composition were obtained from a pooled serum sample from six mice.

groups, IgM anti-cardiolipin antibody increased gradually with age and no statistical significance was noted between these two groups (Fig. 1(b)). Interestingly, IgG anti-cardiolipin antibodies (Fig. 1(a)) in the high-fat group were significantly higher than in the low-fat diet group at the early age ($P=0.0001$ at 3 months; $P=0.0146$ at 4 months) and remained at that level at the late age. IgG anti-ssDNA antibodies in these two groups increased from the age of 5 months (Fig. 2), with a higher value in the high-fat diet group, although not significantly ($P=0.0523$ at 6 months, $0.134 < P < 0.151$ at 4, 5 and 7 months). Increased IgM anti-ssDNA antibody levels were noted at the early age and increased markedly at the age of 7 months.

Serum immunoglobulin level

To investigate whether the high-fat diet affected total serum Ig contents, serum IgG, IgM and IgA levels were measured. Values for serum IgG, IgM and IgA for 6-month-old mice are shown in Table 4. The serum IgG concentration in the high-fat group was significantly higher than that of the low-fat diet group. The data shown here suggested that the serum IgM levels of these two groups were very similar. In contrast, the serum IgA concentration

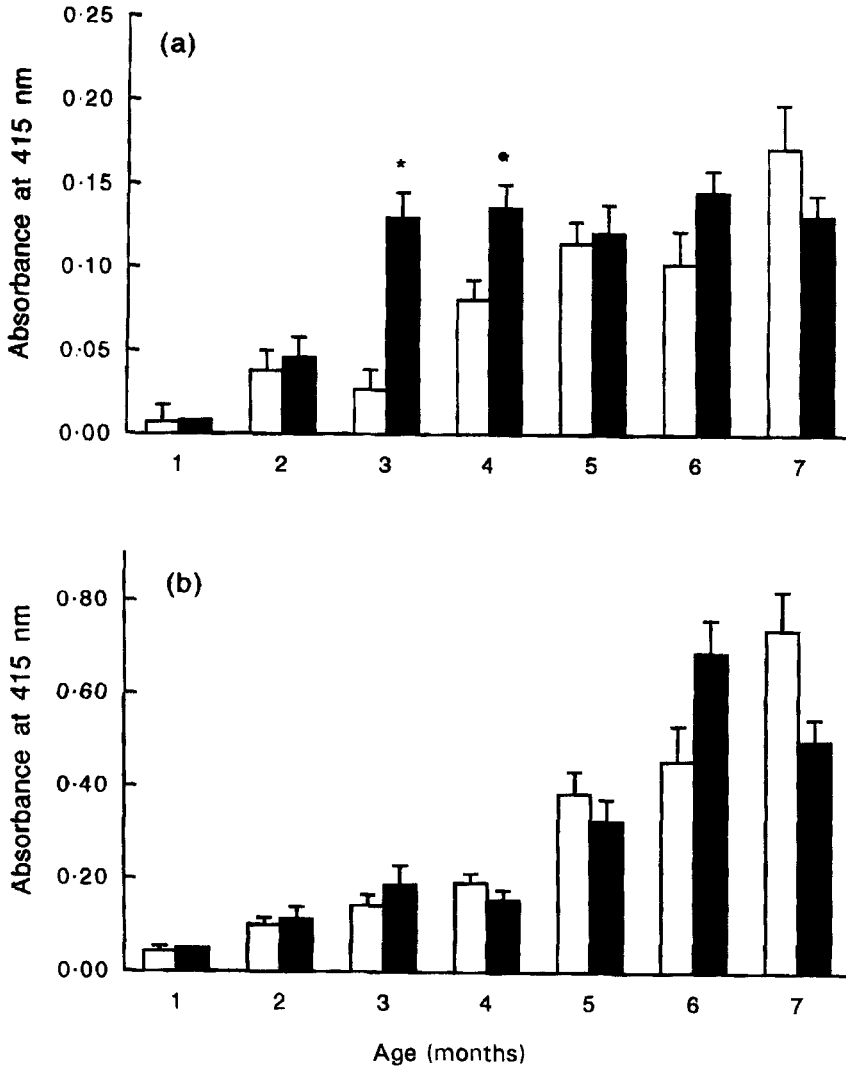


Fig. 2. (a) Serum anti-cardiolipin immunoglobulin G (IgG) levels and (b) serum anti-cardiolipin immunoglobulin M (IgM) levels of NZB/W F1 female mice fed on diets containing 50 (□) or 200 (■) g fat/kg. IgG levels were from 50-fold and IgM levels from 100-fold dilution of each serum sample. Values are means with their standard errors for examples from twelve mice. *Mean values were significantly different from those for the 50 g fat/kg diet groups ($P < 0.05$; Student's *t* test).

in mice of the high-fat diet group was lower than that in mice of the low-fat diet group ($P = 0.0002$).

Mitogen responses

Table 5 shows that spleen cells isolated from mice fed on either the low-fat diet or the high-fat diet had a higher proliferative response to LPS than to Con A. The spleen cells of mice fed on the high-fat diet showed higher responses to either 5 or 10 $\mu\text{g}/\text{ml}$ Con A stimulation compared with the low-fat diet group ($P < 0.05$ for 10 $\mu\text{g}/\text{ml}$ Con A). The responses of

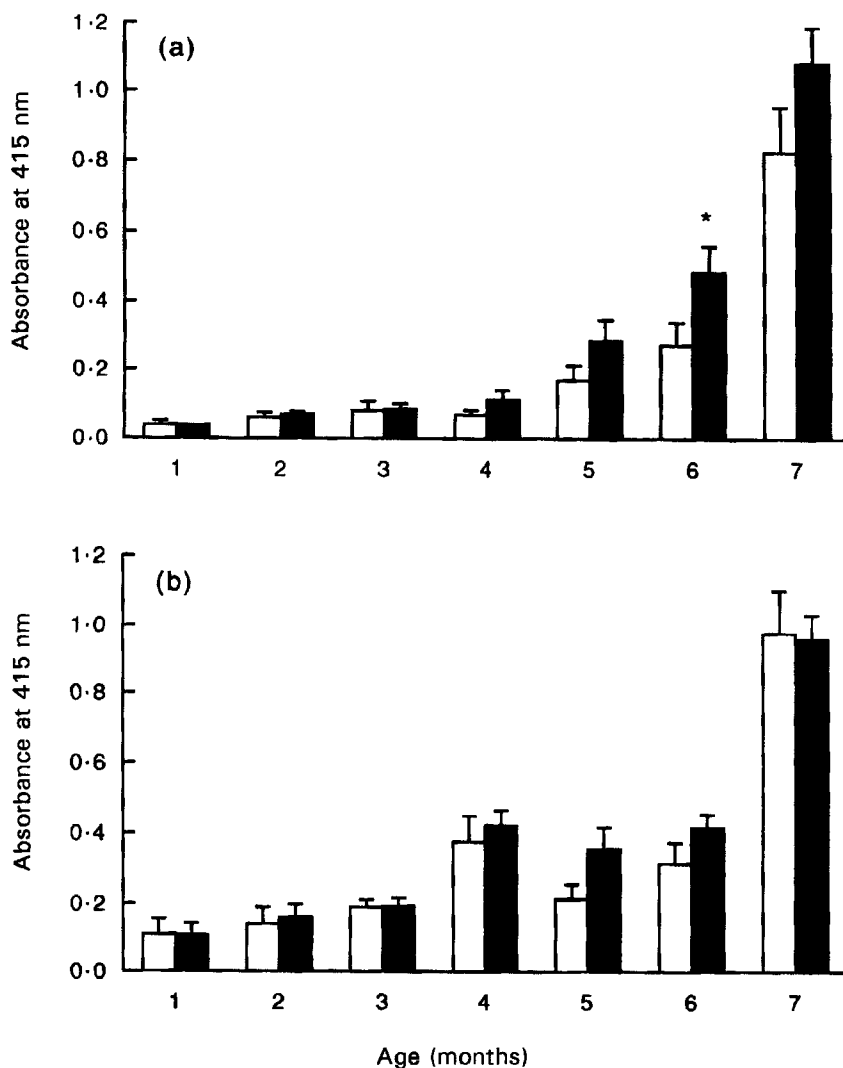


Fig. 3. (a) Serum anti-single-stranded DNA immunoglobulin G levels and (b) serum anti-single-stranded DNA immunoglobulin M levels of NZB/W F1 female mice fed on diets containing 50 (□) or 200 (■) g fat/kg. Each serum sample was diluted 400-fold. Values are means with their standard errors for examples from twelve mice. (* $P = 0.0523$, NS, Student's t test).

spleen cells to 5 $\mu\text{g/ml}$ LPS were higher in the high-fat diet group but this difference was not significant ($P = 0.1698$). The proliferative response to 25 $\mu\text{g/ml}$ LPS was very similar between the two groups.

DISCUSSION

It is noteworthy that dietary fat affected serum lipid contents but not hepatic lipid contents in autoimmune-prone NZB/W F1 mice. The mice fed on 200 g fat/kg diet had significantly lower levels of serum triacylglycerol. The serum cholesterol levels of mice fed on the high-fat diet were significantly higher at the age of 3 months but lower at the age of 6–7 months

Table 4. Serum immunoglobulin (Ig)G, IgM and IgA concentrations (mg/ml) in 6-month-old female NZB/W F1 mice fed on diets with different contents of fat*

(Mean values with their standard errors for twelve mice per dietary group)

Group	IgG		IgM		IgA	
	Mean	SE	Mean	SE	Mean	SE
50 g fat/kg	7.86	0.76	0.80	0.03	0.64	0.03
200 g fat/kg	10.26	0.53	0.79	0.02	0.48	0.02
Student's <i>t</i> test	<i>P</i> = 0.0165		<i>P</i> = 0.8956		<i>P</i> = 0.0002	

*Mice were fed for 5 months on a low-fat diet containing 50 g fat and 650 g carbohydrate/kg or a high-fat diet containing 200 g fat and 500 g carbohydrate/kg. For details of diets see Table 1 and p. 658.

Table 5. Mitogenic response of 7-month-old female NZB/W F1 mice fed on diets with different contents of fat*

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

Group	Stimulation index							
	ConA 5		ConA 10		LPS 5		LPS 25	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
50 g fat/kg	4.3	3.3	10.0	3.1	44.8	9.1	15.9	7.1
200 g fat/kg	11.8	5.1	25.6	4.5	72.8	16.6	16.0	5.8
Student's <i>t</i> test	<i>P</i> = 0.2916		<i>P</i> = 0.0217		<i>P</i> = 0.1698		<i>P</i> = 0.9934	

ConA, concanavalin A; LPS, lipopolysaccharide.

*Mice were fed for 5 months on a low-fat diet containing 50 g fat and 650 g carbohydrate/kg or a high-fat diet containing 200 g fat and 500 g carbohydrate/kg. For details of diets see Table 1 and p. 658. Spleen cells were incubated with medium only or in the presence of Con A at a final concentration of 5 or 10 µg/ml or LPS at a final concentration of 5 or 25 µg/ml for 72 h.

when disease developed. The serum phospholipid levels were also significantly lower in the high-fat diet group at the later age. One study reported that increased serum triacylglycerol levels were found in lupus patients who had been treated with 10 mg prednisolone/d (MacGregor *et al.* 1992). These lines of evidence suggest that a low triacylglycerol level and higher autoantibody levels may be closely related to the severity of disease in human and murine lupus. Dietary fat intake, as well as steroid treatment, might affect lipid metabolism in SLE patients although the relationship between lipid contents and autoantibody levels is not clear.

Our data showed that a high-fat diet increased hepatic linoleic acid (18:2n-6) and arachidonic acid (20:4n-6) contents in autoimmune-prone NZB/W F1 mice. It has also been reported that NZB/W F1 mice fed on a diet containing 200 g safflowerseed oil/kg (high in 18:2n-6 fatty acid) showed earlier and higher anti-DNA antibody responses and shorter life spans compared with those fed on a 200 g coconut oil/kg diet (Hurd *et al.* 1981). Mice fed on 250 g beef tallow/kg diet were reported to have significantly higher serum 18:2n-6 and 20:4n-6 fatty acid contents and shorter life spans compared with mice fed on menhaden oil (Prickett *et al.* 1981). The data do not simply reflect the fatty acid composition of the diet because certain fatty acids such as 16:1n-9 and 18:1n-9 were higher in mice fed with only 50 g dietary fat/kg. In the present study, the serum fatty acid composition could not be analysed statistically due to the limited amount of sample

available. Nevertheless, the serum fatty acid profile between the two dietary fat groups was noted to be similar to the hepatic fatty acid profile. Increases of hepatic 18:2n-6 and 20:4n-6 as seen in the high-fat diet group may affect lipid metabolism and prostaglandin production, which may subsequently affect the immune response, for example by inhibition of interleukin 2 production and macrophage Ia expression (Snyder *et al.* 1982; Santoli & Zurier, 1989). A higher Con A-stimulated proliferative response of spleen cells was seen in mice fed on 200 g dietary fat/kg than in those fed on 50 g fat/kg. These findings are different from previous reports by other groups (Levy *et al.* 1982; Morrow *et al.* 1985); however, the difference in the quantity (12 and 90 v. 50 and 200 g fat/kg diet) and quality of dietary fat used in these studies may have contributed to the different results.

Although the aetiology of SLE remains elusive, it has been suggested that constitutional, environmental and immunological factors are all involved (Steinberg *et al.* 1981; Theofilopoulos & Dixon, 1981). Both human and murine forms of SLE exhibit a characteristic spectrum of autoantibodies such as anti-erythrocyte, anti-DNA and anti-phospholipid antibodies (Theofilopoulos *et al.* 1989). Among these autoantibodies, IgG anti-double stranded DNA (anti-dsDNA) antibody is thought to be pathogenic and T-cell dependent (Datta *et al.* 1987; Carteron *et al.* 1989). Antibodies to phospholipid are a heterogeneous group of autoantibodies found in the serum of SLE patients or murine lupus. Anti-cardiolipin antibodies have been closely related to antiphospholipid 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 that the level of IgG anti-cardiolipin antibodies is more closely related to the development of thrombosis and thrombocytopenia. In contrast, anti-cardiolipin antibody of the IgM isotype has been linked to haemolytic anaemia and livedo reticularis (Mackworth-Young, 1990).

Cross-reactivity of anti-cardiolipin antibodies with autoantigens such as negatively charged phospholipids and ssDNA has been well documented (Lafer *et al.* 1981). Pathogenic anti-DNA and anti-cardiolipin antibodies in both murine and human lupus are of the IgG isotype (Datta *et al.* 1987). In the present study, both IgG anti-ssDNA and anti-cardiolipin antibody titres increased with age. In NZB/W F1 mice, isotype switch of anti-ss, dsDNA antibodies has been well documented which suggests an antigen-driven process in the development of anti-DNA antibodies. A similar finding in the present study was noted in the serum level of anti-cardiolipin antibody, suggesting antigen involvement in this process. Because of obvious cross-reactivity between anti-cardiolipin antibody and anti-ssDNA antibody, both autoantibodies might be a result of response to a similar foreign antigen (Shoenfeld *et al.* 1983; Carroll *et al.* 1985). In the present study, IgG anti-cardiolipin antibodies in the high-fat diet group were higher than those in the low-fat group at the early age and IgG anti-ssDNA antibodies showed a higher value in the high-fat diet group at that time. Our previous study showed increased IgG anti-dsDNA antibody level in mice fed on high dietary fat (Lin *et al.* 1996). However, further investigation of cross-reactivity between anti-DNA antibodies and anti-cardiolipin antibodies is necessary to elucidate this problem.

The present study showed that the production of IgG anti-cardiolipin antibodies increased in mice fed on a high-fat diet at an earlier age than the level of IgG anti-DNA antibody. This suggested that a high-fat diet might play a critical role in increased abortion rate in SLE by increasing anti-cardiolipin antibody production. Our data also showed that high dietary fat increased 18:2 and 20:4 fatty acids in autoimmune-prone NZB/W F1 mice. However, more studies are needed to clarify the real mechanisms involved.

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