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Effects of Dietary Fat with Various Ratios in n-6/n-3 and P/S on Immune Responses of Rats at Different Age

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ABSTRACT

Effects of age and dietary fatty acid composition on immune responses were investigated in Sprague-Dawley male rats. The animals weighing 88.6 ± 2.2g were fed 10% dietary fat (W/W, 20% of calorie) with P/S ratio of 0.5, 1 and 2, and in each group, there were three different levels of n-6/n-3 fatty acid ratio; 2, 4 and 8 (3×3). The experimental periods were 1 month, 6 months and 12 months. The results of this study were;1) Weights of thymus and spleen were significantly reduced with increasing age, moreover thymus weights were reduced with increasing the degree of unsaturation in dietarty fatty acid at 12 month. 2) The proportion of splenic lymphocyte in the total T-cell was increased with age. The proportion of helper T-cell was not changed, while the proportion of suppressor T-cell was decreased. Thus at 12 month, the ratio of helper/suppressor T-cell was appeared to increase significantly, and showed the tendency to increase by consuming the low amount of dieraty n-3 fatty acid. 3) Proliferation stimulated by Con A or PWM reduced significantly at 12 month in which are high in dietary P/S ratio, representing the similar pattern with decrease in thymus weight. 4) Natural killer cell activities were shown significantly higher at 1 month than those at 6 month or 12 month.

KEY WORDS : P/S ratio · n-6/n-3 ratio · lymphocyte composition · mitogen response · natural killer cell

Introduction

Immune responses are complicated systems associated with age, infection, stress, nutrition, and other various multicauses[1-4]. Immune deficiency is well recognized as one of the causes of aging, which can contribute to the increased incidence of cancer and infectious disease in the elderly. Age-related functional changes have been well characterized for changing of relative proportion or number of total T lymphocyte and subpopulation, changing of T-cell surface factor, reducing activities of T-cell and B-cell, increasing immune complex, declining functions of both cell mediated and hummoral immune responses, alterations of eicosanoids production and intracellular biochemical signaling pathways linking receptors to lymphoid cell in human and animals[5-11]. Especially the major alterations occur in T-cell mediated functions which are probably due to the thymic atrophy.

Many studies have been carried out to explain the

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effects of dietary fat on immune response. It has been demonstrated that polyunsaturated fatty acid(PUFA) diet decrease the immune response rather than saturated fatty acid diet [12)13)]. And the proliferation of lymphocyte against PHA or Con A stimulation was increased in mice fed fish oil or linseed oil(n-3 fatty acid) rather than in mice fed corn oil(n-6 fatty acid)[14)15)], which showed that diets rich in n-3 fatty acid rather than n-6 fatty acids increased the immune function. Mechanisms of effects of dietary fat on immune function were studied with prostaglandin E_2(PGE_2). PGE_2 has been reported to diminish the immune function; reduce the cytotoxic activity, suppress the macrophage activation and reduce the NK cell activity[18)]. Erickson and Schumacher[17)] showed that PGE_2 production was increased and immune function was declined in animals fed corn oil compared to that fed palm oil.

α-Linolenic acids(n-3) are converted into EPA or DHA through the reactions of desaturation or elongation in the liver, and incorporated EPA in membrane metabolites to various eicosanoids by the catalytic action of cyclooxygenase or lipooxygenase. N-3 fatty acids and n-6 fatty acids mutually compete at each step by the action of same enzymes in the liver or cell membrane. Therefore it is anticipated that not only the total amount of n-3 fatty acid but also n-6/n-3 ratio may significantly affect the metabolism of n-3 fatty acid in the body.

Many studies were executed to investigate the respective effects of either unsaturated fatty acids or n-3 fatty acids, but the combined comparisons of effects of both total amounts of dietary unsaturated fatty acid and the ratio of n-6/n-3 fatty acids are not yet elucidated. Therefore, this study was aimed to investigate the effects of age and dietary fatty acid composition on immune response in rats fed nine various diets containing different P/S ratio and n-6/n-3 ratio for up to 1 year.

Materials and Methods

1. Animals and diets

162 male Sprague-Dawley strain rats weighing 88.6 ± 2.2g were randomly divided into nine different dietary groups, eighteen animals in each group. At each point of 1 month, 6months or 12months experimental period, six animals in each group were randomly chosen and sacrificed for assay. The rats were fed one of nine diets containing 10% fat(wt/wt), which is equivalent to 20% of calories, with P/S ratio of 0.5, 1, 2, and in each P/S ratio group, there were three different levels of n-6/n-3 ratio of 2, 4, 8 respectively. These data were referenced from the recent survey of dietary fat consumption of Korean people, P/S ratio was 1 and n-6/n-3 ratio was 4~5[19)20)]. P/S ratio and n-6/n-3 ratio of diets determined based on the combination of four different fat sources, beef tallow, soybean oil, sesame oil, and perilla oil. Experimental diets are showed in Table 1 and the fatty acid compositions of fats are represented in Table 2. All diets contained milk casein for protein source, corn starch for carbohydrate source. And mineral and vitamin mixture were the american institute of nutrition method. Experimental animals were placed in individual cage and were provided with food and water ad libitum. Body weights of animals were weighed every two weeks.

2. Immunal Assay

1) Preparation of spleen cell suspensions

After sacrificing the animals at each experimental time points, spleen was excised aseptically and spleen single cell suspensions were prepared in RPMI 1640 culture medium(Gibco., Grand Island N.Y. : 200mM glutamin, 10mM Hepes, 1.4μl/ml gentamycin), supplemented with scapel. These were used for measuring mitogen response and partly used for lymphocyte separation to measure T cell subpopulation and NK-cell activity. Lymphocyte was separated by difference of density using ficoll-hypaque solution(Phamacia)[21)]. Four ml ficoll-hypaque solution were put in the 15ml test tube(Falcon oxnard, CA), 8ml single cell suspension were added carefully, covered and centrifuged at 1800 rpm for 30 minutes. Pale yellow band made between the single cell suspension and the ficoll-hypaque solution was the lymphocyte layer, which were collected with sterilized Pasteur pippete, washed with

Table 1. Composition of experimental diets

Ingredients \ Group	0.5 - 2	0.5 - 4	0.5 - 8	1 - 2	1 - 4	1 - 8	2 - 2	2 - 4	2 - 8
Corn starch	700	700	700	700	700	700	700	700	700
Casein[1]	150	150	150	150	150	150	150	150	150
Methionine[2]	3	3	3	3	3	3	3	3	3
Fat	100	100	100	100	100	100	100	100	100
Beef tallow	70	70	70	50	50	50	20	20	20
Soybean oil	12.5	17	18	30	30	30	30	42.5	30
Sesame oil	10	10	12	10	15	20	32.5	30	47.5
Perilla oil	7.5	3	0	10	5	0	17.5	7.5	2.5
Salt mixture[3]	35	35	35	35	35	35	35	35	35
Vitamin mixture[4]	10	10	10	10	10	10	10	10	10
Choline chloride	2	2	2	2	2	2	2	2	2
P/S ratio[5]	0.5	0.5	0.5	1	1	1	2	2	2
n-6/n-3 ratio[6]	2	4	8	2	4	8	2	4	8

1) Milk casein, New Zealand 2) 0.3% of diet weight
3) Salt mixture(g/Kg mixture):Calcium phosphate,dibasic 500, Sodium chloride 74, Potassium citrate, monohydrate 220, Potassium sulfate 52, Magnesium oxide 24, Manganous carbonate 3.5, Ferric citrate 6, Zinc carbonate 1.6, Cupric carbonate 0.3, Potassium iodate 0.01, Sodium selenite 0.01, Chromium potassium sulfate 0.55, Sucrose, finely powdered to make 1000
4) Vitamin mixture (mg/kg mixture):Thiamin.HCl 600, Riboflavin 600, Pyridoxine.HCl 700, Nicotinic acid 3000, D-Calcium pantothenate 1600, Folic acid 200, D-Biotin 20, Cyanocobalamin 1, Retinyl palmitate 400,000IU vitamin A activity, dl-α-Tocopheryl acetate 5000 IU vitamin E activity, Cholecalciferol 2.5, Menaquinone 5, Sucrose, finely powdered to make 1000g
5) P/S ratio = Polyunsaturated fatty acids/Saturated fatty acids of experimental groups
6) n-6/n-3 ratio = n-6 fatty acids/n-3 fatty acids of experimental groups

Table 2. Fatty acids composition of different lipid resources;beef tallow, soybean oil, sesame oil, perilla oil

Fatty acids \ Dietary fat	Beef tallow	Soybean oil	Sesame oil	Perilla oil
14 : 0	4.8	-	-	-
16 : 0	21.7	9.6	11.9	9.2
16 : 1	3.3	0.3	0.07	-
18 : 0	24.3	4.5	4.7	1.2
18 : 1(n-9)	36.0	19.1	28.5	10.6
18 : 2(n-6)	7.1	52.9	50.5	29.2
18 : 3(n-3)	0.7	10.8	1.5	47.0
Unknown	2.1	2.8	2.8	2.8
P/S ratio[1]	0.15	4.5	3.1	7.3
n-6/n-3 ratio[2]	9.7	4.9	33.7	0.6

1) P/S ratio of each fat 2)n-6/n-3 ratio of each fat

RPMI 1640 solution and used for immunal assay.

2) T-cell subpopulation percentage[22]

Lymphocyte suspension separated from the spleen cell suspension was divided into three polystylene tube. 3μl of alpha/beta T-cell antibody(Serotec), or helper/macrophage antibody(Serotec), or suppressor/cytotoxic cell antibody(Serotec) were respectively add-

ed to the individual tube. After reaction at 4℃ for 30 minutes, these were washed with 0.01 M PBS, stabilized with 0.1% paraformaldehyde. The number of T-cell binding with antibody per 10,000 cell were measured with flow cytometry(Becton Pickinson) and expressed as percentage.

3) NK-cell activity

Seperated lymphocyte from spleen cell suspension was adjusted to 10.0×10^8 cells/ml in RPMI 1640 medium supplemented with 10% fetal bovine serum, aliquots of 100μl were added into 96 well round bottom microtitier plate(falcon). Splenocytes(effector cell) were mixed with 100μl $^{51}Cr(1.0\times10^5$ cells/ml) labeled Yac-1 (target cell, Microbiological section at Yonsei Medical School) in each well. Target cells to effector cells ratio was adjusted to 1 : 100. Plates were incubated at 37℃ for 4 hours in a humidified CO_2 incubator(Flow Lab, 5% CO_2). Count per minute(CPM) of ^{51}Cr release by incorporation of Yac-1 were measured with a gamma counter(Tackard akaberra Co). The spontaneous release was determined for target cells incubated in medium alone, and maximum ^{51}Cr release was determined by lysing target cells with one drop triton-X(Sigma. Co). Mean percentage of activity was calculated according to the formula,

$$\text{Activity}(\%) = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100$$

experimental release : cpm of solution incubated with lymphocyte and Yac-1

spontaneous release : cpm of solution incubated with Yac-1 only

maximal release : cpm of solution incubated with Yac-1 and triton-X

4) Mitogen response

Spleen single cell suspensions were adjusted to 2.5×10^8 cells/ml in RPMI 1640 medium supplemented with 10% fetal bovine serum. Aliquots of 100μl were cultured in 96 well round bottom microtiter plate in the presence of 0.75μg/μl Con A(Concanavalin A, Sigma) or 10μg/10μl PWM(Pokeweed mitogen, Gibco) except the control well, every sample was repeated three times. After 48 hours of incubation at 37℃ in humidified CO_2 atmosphere, cultures were pulsed with 0.5μCi methyl-^{3}H-thymidine(specific activity : 20ci/nmole, NEN) and cells were reincubated for 8 hours. After incubation, these were filtered and dried through glass fiber filter(FLOW) using a multiple automated sample harvester(Tiertek, flow lab). Dried disc was put in the scintillation fluid and cpm of ^{3}H-thymidine incorporation was measured by liquid scintillation β-counter(LKB, 1214 Lackbeta). Stimulation index was determined for cell proliferation by mitogen from the formula,

$$\text{stimulation index} = \frac{\text{cpm of sample with mitogen}}{\text{cpm of sample without mitogen}}$$

3. Statistical analysis

The results are expressed as mean ± standard error. Data were initially subjected to the test of three way analysis of variance at $\alpha = 0.01$, 0.05, 0.1, involving the factors, age, P/S ratio, and n-6/n-3 ratio in the dietary fatty acid and then to Duncan's multiple range test at $\alpha = 0.05$ in order to test the significance among the nine different diet groups in each experimental period.

Results and Discussion

1. Weight of lymphoid organ

Weight changes of lymphoid organs according to the experimental peroids were in Table 3. Thymus is the lymphoid organ which affects differentiation and maturation of T lymphocyte. Generally thymolymphatic atrophy starts from adolescence and thymus weight reduced 10% of maximum size at aged 40~50 years in human[23]. Thymic hormone secreated from thymus regulates the immune function, but starts to reduce from age of 20~30 years old and is hardly secreted at 60[24]. The spleen is the largest single lymphoid organ, containing about 70×10^9 lymphocytes which represent 15% of the lymphocytes present in the adult human body, and plays a critical role in host defence[23]. Therefore it is well known that

Table 3. Weight and weight index of thymus and spleen of experimental groups at differental feeding periods

Organ / Feeding period / Group	Thymus weight(g)			Thymus weight index (mg/g body weight)			Spleen weight(g)			Spleen weight index (mg/g body weight)		
	1 month	6 month	12 month	1 month	6 month	12 month	1 month	6 month	12 month	1 month	6 month	12 month
0.5-2	[1]0.53 ± 0.05[NS2]	0.18 ± 0.02[a3]	0.13 ± 0.02[ab]	1.57 ± 0.11[NS]	0.32 ± 0.03[ab]	0.22 ± 0.04[ab]	1.49 ± 0.12[NS]	1.42 ± 0.12[a]	1.06 ± 0.14[NS]	4.63 ± 0.39[ab]	2.46 ± 0.23[ab]	2.22 ± 0.32[NS]
0.5-4	0.46 ± 0.04	0.20 ± 0.01[ab]	0.11 ± 0.01[ab]	1.36 ± 0.12	0.37 ± 0.02[ab]	0.21 ± 0.03[abc]	1.44 ± 0.16	1.31 ± 0.12[ab]	1.06 ± 0.16	5.25 ± 0.82[a]	2.45 ± 0.12[ab]	2.19 ± 0.34
0.5-8	0.50 ± 0.04	0.17 ± 0.02[a]	0.12 ± 0.02[ab]	1.36 ± 0.15	0.30 ± 0.03[b]	0.17 ± 0.02[c]	1.17 ± 0.13	1.35 ± 0.11[ab]	1.08 ± 0.31	3.53 ± 0.42[b]	2.39 ± 0.17[ab]	2.22 ± 0.70
1-2	0.39 ± 0.04	0.22 ± 0.02[ab]	0.15 ± 0.03[a]	1.24 ± 0.12	0.43 ± 0.04[ab]	0.31 ± 0.04[a]	1.24 ± 0.12	1.17 ± 0.11[ab]	1.24 ± 0.19	3.87 ± 0.20[ab]	2.22 ± 0.22[ab]	2.53 ± 0.10
1-4	0.45 ± 0.04	0.26 ± 0.04[a]	0.09 ± 0.00[ab]	1.51 ± 0.20	0.46 ± 0.03[a]	0.21 ± 0.01[abc]	1.31 ± 0.13	1.04 ± 0.14[b]	1.02 ± 0.12	3.88 ± 0.36[ab]	1.89 ± 0.19[b]	2.36 ± 0.26
1-8	0.46 ± 0.03	0.17 ± 0.02[b]	0.09 ± 0.00[ab]	1.46 ± 0.14	0.35 ± 0.04[ab]	0.21 ± 0.00[abc]	1.35 ± 0.11	1.11 ± 0.04[ab]	0.69 ± 0.11	4.24 ± 0.41[ab]	2.30 ± 0.12[ab]	1.54 ± 0.15
2-2	0.40 ± 0.05	0.19 ± 0.02[ab]	0.09 ± 0.01[b]	1.28 ± 0.13	0.35 ± 0.03[ab]	0.21 ± 0.02[abc]	1.10 ± 0.06	1.24 ± 0.09[ab]	1.17 ± 0.20	3.54 ± 0.18[b]	2.25 ± 0.16[ab]	2.51 ± 0.47
2-4	0.45 ± 0.03	0.20 ± 0.04[ab]	0.12 ± 0.02[ab]	1.38 ± 0.08	0.38 ± 0.08[ab]	0.28 ± 0.02[ab]	1.43 ± 0.09	1.14 ± 0.09[ab]	1.02 ± 0.14	4.47 ± 0.60[ab]	2.08 ± 0.17[ab]	2.50 ± 0.12
2-8	0.41 ± 0.05	0.19 ± 0.01[ab]	0.09 ± 0.02[ab]	1.36 ± 0.14	0.37 ± 0.03[ab]	0.20 ± 0.03[bc]	1.27 ± 0.11	1.29 ± 0.08[ab]	0.94 ± 0.08	4.32 ± 0.36[ab]	2.50 ± 0.17[a]	1.86 ± 0.12
Significant factor[4]	Age***			Age***			PS* Age***			Age***		

1) Mean ± S.E.

2) Not significant at $\alpha = 0.05$ by Duncan's multiple range test.

3) Values with different alphabet within the column were significantly different at α=0.05 by Duncan's multiple range test.

4) Statistical significance was calculated by 3-way ANOVA. PS:P/S ratio,N:n-6/n-3 ratio, Age:feeding period.

***:Significantat $\alpha = 0.01$, **:Significant at $\alpha = 0.05$, *:Significant at $\alpha = 0.1$

degeneration of these lymphoid organs alters the immune function.

In present study, the thymus weight reduced with aging as shown by other similar studies[23)25)], distinctively between 1 month and 6 month. And especially at 12 month, thymus weight reduced with increasing P/S ratio in dietary fatty acid, and thymus weight index decreased significantly with decreasing n-3 fatty acid in the same P/S ratio. Therefore it showed that thymic atrophy starts before maturation and may be affected by both the amounts and kinds of consumption of unsaturated fatty acid. Spleen weight also continuously decreased with age, and at 12 month splenic weight index reduced with decreasing consumption of n-3 fatty acid even though they were not significantly different.

2. Immune Response

1) T-cell composition

The number of T cell may be considered to reduce distinctively with thymic atrophy, but in this experiment relative proportion of total T-cell seemed to increase with age, helper T-cell did not change, suppressor T-cell decreased, and ratio of helper/suppressor T-cell significantly increased with age(Table 4). It was reported that the number of total T-cell in the blood or lymphoid organ in human body did not change with age , but these are the long-lived cell and can not execute the immune response[26)]. Therefore only the total number of T-cell could not be estimated to determine the immune responses.

Helper T-cells and suppressor T-cells regulate the immune response, that is, immune deficiencies are demonstrated if the helper T-cell reduced, and antibody overproductions are appeared if the suppressor T-cell decreased. This indicates that keeping the balance of both cells is important on regulation of immune response[27)]. In the present study, the ratio of helper/suppressor T-cell was demonstrated high in 0.5 – 8 group, 1 – 8 group, 2 – 8 group which contain low n-3 fatty acid in each dietary P/S ratio group. It was reported that incidence of hypertension by the autoimmune could be prevented by increasing consumption of n-3 fatty acid in SHR rat[28)]. Therefore it may be considered that consumption of n-3 fatty acid could maintain the helper/suppressor T-cell balance with increasing age.

2) Mitogen response

The results of mitogen responses were shown in Table 5. Spleen cell responses to T-cell mitogen(Con A) were significantly decreased at 12 month, especially in the 2 level of P/S ratio group, ie, 2 – 2 group, 2 – 4 group, 2 – 8 group which are high in dietary unsaturated fatty acid. Moreover, proliferation by mitogen demonstrated to be low in 0.5 – 8 group, 1 – 8 group, 2 – 8 group containing low n-3 fatty acid at each dietary P/S ratio group, which showed the similar pattern with reduction of thymus or spleen weight. Grossman[27)] examined the PHA responses on T-cell subsets in the blood lymphocytes of young and old men. Responses on total T-cell or helper T-cell did not show any differences with increasing age, but responses on suppressor T-cell decreased with age, which demonstrated that decline of immune function is expressed not by the total T-cell but by the alteration of specific subsets. In addition, PWM responses investigating the proliferation of T-cell dependent B-cell demonstrated significant decrease at 12 month. Therefore, effects of both T-cell and B-cell represented to decline with age.

3) Natural killer Cell activities

The data of Natural killer(NK) cell activities were shown in Table 6. NK cell, a subpopulation of lymphocytes that differ from mature T or B-cells or macrophages, are thought to play a significant role in immune surveillance against microorganisms and tumors, viral infection, abnormal hematopoietic development and production of lymphokines such as interferon, and in resistance against metastates[29)]. In the present study, YAC-1 lymphoma cells were used as taget cells, the result showed significantly higher activities of NK cell at 1 month than 6 month or 12 month. Comparing with the result of Con A responses which slightly increased at 6 month and decreased at 12 month, immune responses of NK cell decreased ra-

Table 4. T-cell profile of splenic lymphocyte

Group \ Cell type / Feeding period	Total T-cell (%)			Helper T-cell (%)			Suppressor T-cell (%)			Helper/suppressor T-cell ratio		
	1 month	6 month	12 month	1 month	6 month	12 month	1 month	6 month	12 month	1 month	6 month	12 month
0.5 – 2	[1]59.4 ± 4.2[2]NS	65.7 ± 6.6[3]abc	64.7 ± 6.4NS	50.0 ± 2.6ab	52.5 ± 1.7NS	49.8 ± 2.1NS	43.7 ± 1.7ab	46.8 ± 4.2NS	42.2 ± 2.1NS	1.15 ± 0.07NS	1.14 ± 0.56NS	1.19 ± 0.06a
0.5 – 4	48.5 ± 1.6	52.4 ± 0.7cd	59.1 ± 4.2	48.3 ± 1.9ab	49.3 ± 2.0	51.0 ± 3.0	38.1 ± 6.8ab	43.6 ± 1.5	36.9 ± 3.9	1.37 ± 0.28	1.13 ± 0.03	1.43 ± 0.17B
0.5 – 8	52.0 ± 3.3	51.5 ± 1.3d	67.7 ± 2.2	57.9 ± 6.2a	46.3 ± 2.9	59.9 ± 3.7	47.3 ± 3.6a	45.7 ± 3.5	32.9 ± 2.4	1.24 ± 0.16	1.13 ± 0.18	1.86 ± 0.26ab
1 – 2	51.1 ± 8.6	53.9 ± 3.5cd	53.7 ± 4.6	40.4 ± 4.7b	51.7 ± 2.4	46.1 ± 1.3	38.4 ± 6.8ab	50.1 ± 3.5	27.1 ± 1.8	1.18 ± 0.35	1.05 ± 0.10	1.72 ± 0.15ab
1 – 4	59.4 ± 5.1	59.9 ± 2.3ab	62.6 ± 3.3	49.5 ± 3.3ab	53.6 ± 1.7	46.8 ± 4.6	33.0 ± 3.5ab	48.2 ± 1.8	39.1 ± 6.4	1.54 ± 0.16	1.12 ± 0.07	1.28 ± 0.17b
1 – 8	56.6 ± 2.0	58.6 ± 4.0bcd	62.6 ± 6.4	45.5 ± 2.0b	49.9 ± 2.2	47.9 ± 3.0	44.6 ± 1.7ab	43.8 ± 2.9	34.0 ± 2.9	1.02 ± 0.01	1.08 ± 0.07	1.45 ± 0.23b
2 – 2	44.3 ± 6.1	74.1 ± 3.4a	71.2 ± 6.2	39.9 ± 2.9b	50.8 ± 3.2	49.8 ± 10.3	28.9 ± 4.6b	49.1 ± 0.9	42.0 ± 9.3	1.42 ± 0.14	1.04 ± 0.08	1.46 ± 0.66b
2 – 4	44.0 ± 0.3	58.5 ± 7.6bcd	53.2 ± 1.2	44.0 ± 1.2b	49.3 ± 3.6	46.1 ± 3.0	36.7 ± 5.6ab	40.9 ± 1.8	37.8 ± 2.0	1.25 ± 0.17	1.21 ± 0.12	1.22 ± 0.02b
2 – 8	57.8 ± 2.7	61.3 ± 5.0abcd	69.2 ± 7.8	44.2 ± 3.5b	50.1 ± 2.3	62.2 ± 8.1	42.6 ± 7.8ab	46.5 ± 3.1	26.7 ± 6.7	1.11 ± 0.21	1.09 ± 0.11	2.87 ± 0.74a
Significant factor[4]	Age***			—			Age***			Age***		

1) Mean ± S.E.

2) Not significant at $\alpha = 0.05$ by Duncan's multiple range test.

3) Values with different alphabet within the column were significantly different at α=0.05 by Duncan's multiple range test.

4) Statistical significance was calculated by 3-way ANOVA. PS : P/S ratio,N : n-6/n-3 ratio, Age : feeding period.

*** : Significantat $\alpha = 0.01$

Table 5. Mitogen response index

Group \ Mitogen / Feeding period	Con A(Concanavalin A)			PWM(pokeweed mitogen)		
	1 month	6 month	12 month	1 month	6 month	12 month
0.5 – 2	[1]11.2 ± 1.9[NS 2)]	21.5 ± 4.3[b 3)]	17.0 ± 1.3[a]	8.3 ± 0.9[NS]	11.5 ± 3.4[NS]	11.1 ± 2.5[a]
0.5 – 4	12.9 ± 3.7	17.0 ± 2.3[b]	15.6 ± 1.3[a]	8.8 ± 1.3	10.5 ± 3.5	7.9 ± 0.7[ab]
0.5 – 8	11.0 ± 1.9	17.6 ± 3.6[b]	12.3 ± 1.8[abc]	7.8 ± 1.5	17.1 ± 7.0	4.4 ± 0.5[bc]
1 – 2	11.5 ± 3.6	13.0 ± 3.0[b]	7.0 ± 2.1[bc]	7.2 ± 1.1	8.6 ± 1.2	3.2 ± 0.7[c]
1 – 4	12.9 ± 2.2	22.8 ± 11.9[b]	7.2 ± 1.4[bc]	7.3 ± 1.9	15.6 ± 8.9	3.8 ± 0.9[bc]
1 – 8	7.3 ± 2.5	11.9 ± 2.5[b]	13.3 ± 4.6[ab]	5.6 ± 0.6	4.3 ± 1.2	5.6 ± 1.8[bc]
2 – 2	13.0 ± 1.2	13.9 ± 8.0[b]	9.9 ± 5.7[ab]	7.5 ± 0.8	11.0 ± 3.7	4.9 ± 1.7[bc]
2 – 4	14.7 ± 5.1	24.1 ± 5.3[b]	4.9 ± 2.0[bc]	8.0 ± 2.9	12.8 ± 2.9	4.4 ± 0.5[bc]
2 – 8	13.3 ± 2.8	35.5 ± 11.9[a]	4.6 ± 1.3[c]	14.4 ± 3.5	10.3 ± 2.6	2.7 ± 0.5[c]
Significant factor[4)]	PS* Age***			PS* Age***		

1) Mean ± S.E.
2) Not significant at α=0.05 by Duncan's multiple range test.
3) Values with different alphabet within the column were significantly different at α=0.05 by Duncan's multiple range test.
4) Statistical significance was calculated by 3-way ANOVA. PS:P/S ratio, N:n-6/n-3 ratio, Age:feeding period.
***:Significant at α = 0.01, **:Significant at α = 0.05, *:Significant at α = 0.1

Table 6. NK-cell activity (%)

Group \ Feeding period	1 month	6 month	12 month
0.5 – 2	[1]23.6 ± 5.7[NS 2)]	21.2 ± 4.0[ab 3)]	14.1 ± 5.0[NS]
0.5 – 4	20.5 ± 2.9	18.7 ± 4.5[ab]	14.4 ± 4.2
0.5 – 8	15.5 ± 2.9	14.7 ± 2.7[b]	18.8 ± 2.5
1 – 2	25.8 ± 2.4	14.3 ± 2.3[b]	14.4 ± 2.5
1 – 4	16.6 ± 3.4	26.7 ± 5.1[a]	17.0 ± 4.0
1 – 8	21.6 ± 6.1	14.4 ± 1.5[b]	16.1 ± 1.9
2 – 2	19.8 ± 0.7	20.5 ± 2.2[ab]	14.0 ± 1.7
2 – 4	22.9 ± 3.4	16.6 ± 3.4[ab]	14.2 ± 1.6
2 – 8	21.4 ± 2.5	14.2 ± 2.2[b]	15.3 ± 2.7
Significant factor[4)]	Age***		

1) Mean ± S.E.
2) Values with different alphabet within the column were significantly different at α = 0.05 by Duncan's multiple range test.
3) Not significant at α=0.05 by Duncan's multiple range test.
4) Statistical significance was calculated by 3-way ANOVA. PS : P/S ratio, N : n-6/n-3 ratio, Age : feeding period. ** * : Significant at α=0.01

pidly rather than T-cell, suggesting that decreasing time point of immune function is different with various cell type. NK-cell activity was not affected by diet compositions of fatty acids in this study, which indicate that NK-cell activity might be considered to response more sensitively by feeding period than by diet composition.

In conclusion, proliferation activity was influenced by reducing lymphoid organ, especially thymic atrophy with increasing age. Consumption of dietary unsaturated fatty acid may reduce immune response and cell proliferation activity with age. Moreover, atrophy of lymphoid organ and increase in the ratio of helper/suppressor T-cell with age were reduced by consumption of n-3 fatty acid. Therefore consumption of n-3 fatty acid might be considered to play some beneficial role on alteration of immune responsiveness with age. However immune responses are very complex and interactive system involving many kinds of cells and their metabolites at even one reaction. Therefore, subsquent studies to perform nutritional and dietary manipulation of the immune system should be needed.

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Effects of Dietary Fat on Immune Responses of Rats at Different Age

= 국문 초록 =

나이가 다른 흰쥐에서 n-6/n-3 비율과 P/S 비율이 다른 식이지방이 면역능력에 미치는 영향연구

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본 연구는 나이와 식이지방의 지방산구성이 흰쥐의 면역능력에 어떠한 영향을 미치는지 알아보고자 시도되었다. 실험동물로 이유직후의 Sprague-Dawley종 수컷을 이용하였으며, 실험식이는 식이지방을 식이무게의 10%로 고정하고, 참기름, 들기름, 콩기름, 우지를 사용하여 P/S비율을 0.5,1,2 세수준으로 하고 각각의 P/S 비율에서 n-6/n-3비율이 2, 4, 8이 되는 9가지 실험식이를 준비하였다.(3X3) 실험기간은 12개월간 사육하면서 1개월, 6개월, 12개월되었을때 각 실험식이군에서 8마리씩 희생하여 시료를 얻었다. 실험결과는 다음과 같다. 1) 흉선 및 비장의 무게는 12개월에서 유의적인 감소가 있었으며, 특히 흉선무게는 P/S 비율이 높을때 많은 감소가 있었다. 2) 비장의 T-림프구조성은 나이가 증가함에 따라 total- T cell 은 증가하며 helper T-cell은 변화없고, suppressor T-cell은 감소하여 12개월에는 helper/suppresor T-cell의 비율이 유의적으로 증가하였다. 3) Con A와 PWM을 사용한 증식실험은 12개월에 유의적인 감소를 했으며 이는 P/S비율이 높을때 더 심한 감소의 경향이 나타났다.4) NK cell 활성은 6개월이후에 유의적인 감소가 나타났다. 결론적으로 나이가 증가함에 따라 흉선 퇴화와 mitogen에 대한 증식능력, NK cell 활성 감소등 면역능력이 감소되었으며 식이지방의 영향은 P/S ratio가 높을때 mitogen 대한 반응이 감소하고 n-6/n-3이 높을때 helper/suppressor T-cell 비율이 증가하여 식이 불포화지방산은 양과 종류가 면역능력에 영향을 주었다.