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Effect of n-6, n-3 Fatty Acids and Vitamin A Levels on Humoral Immunity in Rats

Kim, Suh Hae · Lee, Lilha

Department of Home Economics, Chung Ang University, Seoul, Korea

ABSTRACT

This study was carried out to observe the effects of dietary n-6, n-3 fatty acids and vitamin A levels on humoral immunity in rat. Sixty eight male Sprague-Dawley rats were fed 6 different experimental diets for 6 weeks.

The diets were composed of 10% of either corn oil or fish oil with various levels of vitamin A ; deficient(1240 IU/kg diet), adequate(4000 IU/kg diet), and excess(400,000 IU/kg diet).

The weight of spleen from the excess vitamin A-fish oil group showed the lowest value of all the groups when spleen weight was expressed/100g body weight. The number of PFC to SRBC was not affected by dietary fat type and vitamin A levels. Hemagglutination titers were significantly lower in fish oil groups compared to corn oil groups and the values of vitamin A deficient groups were lower than the ones of adequate and excess vitamin A groups. IgM contents in serum were significantly lower in fish oil groups than in corn oil groups. IgG contents were higher in fish oil groups than in corn oil groups and the highest level was recorded in excess vitamin A-fish oil group, which showed the smallest spleen size. Light microscopical examination showed that spleen tissues of fish oil groups were well developed than those of the corn oil groups and vitamin A deficient and excessive groups showed poor development than the adequate groups.

Therefore, it is suggested that adequate amounts of vitamin A consumption is necessary for healthy individuals and fish oil intake along with excess vitamin A should be avoided in order to maintain immune function properly.

KEY WORDS : Excess vitamin A · n-6, n-3 fatty acids · Humoral immunity · PFC · Hemagglutination titer · IgM, IgG.

Introduction

Vitamin A either deficiency or excess have been shown to affect a number of immune functions[1~3]. Vitamin A deficiency has been reported to impair the cellular and humoral immune responses. Vitamin A deficiency in animals leads to atrophy of lymphoid organs[4~7], decrease in circulatory lymphocytes[5] and mitogenic responses to various mitogens[6][7], and depression in antibody production to various antigens[8~10]. On the other hand, excess vitamin A has been shown to enhance immune responses. High vitamin A intake was reported to enhance immune functions suppressed by

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burns in mice[11]. High vitamin A treatment in mice that were induced tumor resulted in inhibition of tumor development[12]. Also high level of vitamin A increased the hemagglutination response to sheep red blood cell(SRBC) and enhanced the production of plaque forming cell(PFC) in rat and mice[6][13]. Conversely, several studies have reported that excess vitamin A suppressed immune functions. Some very high doses of vitamin A caused depletion of spleen and thymus lymphocytes[14]. Acute hypervitaminosis A did not alter the titer of specific IgM antibodies in guinea pigs[15][16].

Many studies have also reported the effect of dietary fats on the immune function. High PUFA diets were shown to depress the immune responses. Unsaturated fatty acids, in particular, n-6 fatty acid caused atrophy of thymus and spleen[17][18], suppressed the immune responses such as mitogenic responses of lymphocytes[18][19], delayed hypersensitivity[18][19], and antibody responses[17][18][20]. Dietary n-3 fatty acid, especially eicosapentaenic acid(EPA) of fish oil also has been known to prevent or suppress the autoimmune disease and cancer[21][22].

As described above, since vitamin A and dietary fat seem to affect the immune function differently by the intake levels, it seems to be important to observe interactions between dietary fat and vitamin A on immune function. Thus we examined the effect of dietary n-3, n-6 fatty acids at different levels of vitamin A on humoral immunity in rats.

Material and Methods

Animals and diets

Sixty-eight male rats of Sprague-Dawley strain weighing 103± 2.2g were divided into 6 groups and fed experimental diets for 6 weeks. Experimental groups were consisted of vitamin A deficient corn oil or fish oil group(DeA-CO, DeA-FO), vitamin A adequate corn oil or fish oil group (AdA-CO, AdA-FO) and vitamin A excess corn oil or fish oil group(ExA-CO, ExA-FO). The experimental diets were based on AIN-76 rodent diet with slight modifications[23]. Vitamin A was added to be 1240 IU/kg diet(31% of adequate level), 4000 IU/kg diet(adequate level), and 400, 000 IU/kg diet(100 times of adequate level) for the deficient, adequate, and excess diets, respectively. Corn oil and fish oil concentrate were used as a source of n-6, n-3 fatty acid, respectively. The fatty acid composition of two dietary fats was given in Table 1. Diets of fish oil groups were contained approximately an additional 0.005mg of retinoic acid per day since 0.003mg of retinoic acid/mililiter of fish oil concentrate was determined.

All rats were housed indiviually in metabolic cage, and provided with food and water ad libitum.

Immunological analysis

For measurement of antibody responses, rats were immunized with 0.5ml of 10% SRBC intraperitoneally on five days before the termination of the experiment. Blood samples were obtained via cardiac puncture under ether anesthesia and

Table 1. Fatty acid composition of dietary oils[24]

(%)

	Corn oil	Fish oil
linoleic acid	55.2	–
α-Linolenic acid	1.6	–
EPA	–	25
DHA	–	12
Saturated fatty acid	13.0	25.4
Monounsaturated fatty acid	25.5	28.3
Saturated fatty acid	13.0	25.4
Monounsaturated fatty acid	25.5	28.3
Polyunsaturated fatty acid	56.8	37.0
P/S ratio	4.37	1.46

used as a source of rat serum. The spleen was removed, weighed and rinsed, and blotted cut into halves by aseptic techniques. One half of the spleen was used to assay the PFC and the other half was transferred to 10% neutral buffered formalin(NBF) for histologic analysis.

The response of spleenic PFC to SRBC was determined by Jerne-Nordin plaque assay[25]. The number of PFC per million nucleated spleen cells was determined. Serum hemagglutination responses were determined by semi-quantitative technique. The test expressed the agglutination of erythrocytes by increasing dilution of anti-erythrocyte sera[26]. Serum IgM and IgG levels were determined by enzyme-linked immunoabsorption method of Voller et al[27]. Anti-rat IgM or IgG and alkaline-phosphatase conjugate were purchased from Zymed lab(USA).

For light microscopic examination, the spleen tissues from rats were fixed in 10% NBF, and then paraffin sections were prepared, and stained with hematoxylin-eosin[28].

Statistical analysis

All data were initially subjected to a two way analysis of variance and comparision were made by using Tukey's test and Duncan's multiple range test.

Results and Discussion

Spleen weight

As shown in Table 2, there were no significant differences in spleen weight by dietary fat types and vitamin A levels. However, the spleen weight of vitamin A excess-fish oil group was the lowest among all the dietary groups when it was expressed per 100g body weight, which indicates interactions between fish oil and excess vitamin A. These results may suggest that vitamin A levels used in this study and/or n-6, n-3 fatty acid alone does not affect spleen weight.

Table 2. Weight of spleen[1)]

Group	Weight(g)	Weight/100g B.W.
DeA−CO	1.01± 0.11[NS]	0.36± 0.03[ab]
DeA−FO	1.08± 0.06	0.42± 0.03[ab]
AdA−CO	1.14± 0.13	0.38± 0.03[ab]
AdA−FO	1.20± 0.14	0.47± 0.07[a]
ExA−CO	1.15± 0.15	0.42± 0.06[ab]
ExA−FO	0.84± 0.09	0.30± 0.03[b]
Significant factor	−	AB

1) Mean± S.E.
NS : Not significant by Tukey's and Duncan's multiple range tests at $p<0.05$.
Values with different superscripts within the same column differ at $p<0.05$ by Duncan's multiple range test.
AB : There are interactions between dietary fat type and vitamin A levels at $p<0.05$ by a two-way analysis of variance.

Considering the effect of vitamin A levels on the weight of spleen, Krisnan et al[7], Nauss et al[5] reported that spleen were atrophied in rats fed vitamin A deficient diet. But in the present study no significant difference was found in vitamin A deficiency. These discrepancies may be resulted from the difference in the degree of vitamin A feeding level. In other experiments vitamin A free diets were used for vitamin A deficiency, but in the present study it was prepared with vitamin A at 31% of the recommended value. In the meantime, very high dose of vitamin A was reported to cause depletion of spleen lymphocytes[14]. Therefore, in the present study the lowest spleen weight observed in the excess vitamin A-fish oil group(ExA-FO) was resulted probably due to the interaction between fish oil and excess vitamin A.

PFC assay

The number of PFC to SRBC showed no significant differences among all dietary groups in the present study(Table 3). Although it was not signi-

Table 3. Plaque forming cell response[1)]

Group	PFC/1×10^6 spleen cells
DeA-CO	67.2± 14.03[NS]
DeA-FO	86.0± 15.00
AdA-CO	109.2± 14.21
AdA-FO	100.0± 15.82
ExA-CO	96.8± 13.84
ExA-FO	68.0± 5.50

1) Mean± S.E.
NS : Not significant by Tukey's and Duncan's multiple range tests at $p<0.05$.

ficant, type of fat at different vitamin A levels showed different aspects on PFC response. On deficient vitamin A feeding, the PFC response of the fish oil group tended to be higher than that of the corn oil group. Conversely, on an adequate or excess vitamin A feeding, the response tended to be higher in the corn oil groups(AdA-CO and ExA-CO) compared with the fish oil groups(AdA-FO and ExA-CO). These results may be explained by the fact that deficient or excess vitamin A feeding lowers PFC response[7)10)29)]. Thus, the abnormal PFC response observed in the fish oil groups of the present study, may be related to vitamin A levels rather than the fat types. And also, it is possible that interactions between fish oil and excess vitamin A may influence on the PFC response, since the spleen weight of the group was the lowest among all the groups.

Hemagglutination response

Hemagglutinin titers to SRBC were significantly different among the dietary groups(Table 4). It tended to be lower in the fish oil groups than in the corn oil groups. In the corn oil groups, hemagglutinin titers were increased as the level of vitamin A increased. In the fish oil groups, these were lower in both vitamin A deficiency and excess compared with the adequate level. It seemed that both, fish oil and vitamin A definitely influence on the immune function.

Table 4. Hemagglutination titers

Group	Hemagglutination titer	
	dilution factor	geographic means(log)
DeA−CO	1/2−1/16[1)]	2.6± 0.32[b2)3)]
DeA− FO	1/2−1/16	2.6± 0.39[b]
AdA−CO	1/8−1/32	3.8± 0.25[ab]
AdA− FO	1/4−1/16	3.5± 0.28[ab]
ExA−CO	1/8−1/32	4.1± 0.21[a]
ExA− FO	1/2−1/16	2.8± 0.42[b]
Significant factor		A, B

1) Values mean the range of the highest positive serial dilution.
2) Hemagglutination titers are expressed as geometric means(log) of the highest positive serial dilution.
3) Mean± S.E.
Values with different superscripts within the same column differ at $p<0.05$ by Tukey's and Duncan's multiple range tests.
A : Significantly different among vit A levels at $p<0.05$ by a two-way analysis of variance.
B : Significantly different between dietary fat type at $p<0.05$ by a two-way analysis of variance.

The effects of fish oil on immune function have been already reported that fish oil extract supplementation to healthy human depressed antibody response to tetanus toxoid[30)]. Vitamin A also influences hemagglutination response. Krisnan et al [7)] observed that serum antibody hemagglutinin titers in vitamin A deficient rats were lower than those of control rats. Jurin and Tannock[13)] also reported that injection of vitamin A led to a large increase in the production of hemagglutinin antibodies. The present study also showed similar result only in corn oil groups. On the other hand, it was also described that excess vitamin A did not alter the immune responses. Production of specific IgM antibodies to particular antigen in guinea pigs was not altered after vitamin A had been injected[16)]. Thus, decreased hemagglutinin titers in excess vitamin A-fish oil group(ExA-FO) in the present study seem to be related to the interactions among vitamin A and fish oil, and further related to the reduced spleen weight.

Serum IgM, IgG content

The values of serum IgM and IgG are shown in Table 5. Serum IgM contents of fish oil groups were significantly lower than those of corn oil groups and there were no significant differences by the vitamin A levels. These results are similar to many studies reported earlier. Virella et al[30] observed that reduced serum IgM contents in a normal person who ingested a commercial fish oil extract. Kelly et al[22] also reported that circulating retroviral antibodies were decreased in autoimmune mice fed fish oil. Smith et al.[10] reported that IgM contents were not different between the vitamin A deficient and vitamin A supplemented mice. Therefore, it is clear that fish oil is capable of lowering serum IgM contents regardless of vitamin A levels.

On the other hand, serum IgG contents of fish oil groups were influenced by both dietary vitamin A levels and fat types. The levels were significantly higher for the fish oils compared with the corn oils, and also, it tended to be higher as vitamin A level increases in fish oil groups. In the meantime, there was an interaction between vitamin A levels and types of fats, that the highest IgG content was recorded in ExA-FO group. These results were compatible to the studies reported earlier that IgG antibodies were increased with fish fat diet[31] and vitamin A supplementation[10]. But other studies revealed that fish oil consumption rather reduce IgG contents in human and mice[22][30].

Table 5. Serum IgM and IgG levels[1]

Group	IgM (O.D.)	IgG (O.D.)
DeA−CO	0.682 ± 0.050^{ab}	1.089 ± 0.062^{b}
DeA−FO	0.702 ± 0.064^{ab}	0.990 ± 0.048^{b}
AdA−CO	0.700 ± 0.051^{ab}	1.118 ± 0.034^{b}
AdA−FO	0.605 ± 0.044^{b}	1.124 ± 0.082^{b}
ExA−CO	0.835 ± 0.045^{a}	1.007 ± 0.037^{b}
ExA−FO	0.621 ± 0.044^{b}	1.573 ± 0.109^{a}
Significant Factor	B	A, B, AB

1) Mean± S.E.

Values with different superscripts within the same column differ at $p<0.05$ by Tukey's and Duncan's multiple range tests.

A : Significantly different among vitamin A levels at $p<0.05$ by a two-way analysis of variance.

B : Significantly differnt between dietary fat type at $p<0.05$ by a two-way analysis of variance.

AB : There are interactions between vitamin A levels and dietary fat type at $p<0.05$ by a two-way analysis variance.

From these facts, it could be assumed that the increased serum IgG contents are induced by fish oil with excess vitamin A in this study. This result could be supported by the reports that vitamin A stimulates the antibody response, and EPA of fish oil reduces an immunosuppressive effect by arachidonic acid metabolites[6][13][31].

Histological changes

Histological changes of spleen tissues are shown in Fig. 1. In vitamin A deficiency, poorly developed germinal centers were observed in both corn oil(DeA-CO) and fish oil group(DeA-FO) (Fig. 1-A, a). On vitamin A adequate feeding, corn oil group showed poorly developed germinal centers while fish oil group showed well developed white pulp and germinal centers(Fig. 1-B, b). On vitamin A excess feeding, corn oil group showed poorly developed white pulp(Fig. 1-C) while fish oil group showed decreased germinal centers, depletion of lymphocytes, and lymphocyte infiltration in red pulp(Fig. 1-c). Histologic changes by vitamin A deficiency were examined by Walbach and Howe[1], and Krisnan et al.[7] They observed atrophic changes and lymphocyte depletion in spleen of vitamin A deficient rat, which were similar to the present study.

Thus, it could be assumed that type of dietary fat affects the development of spleen tissues in

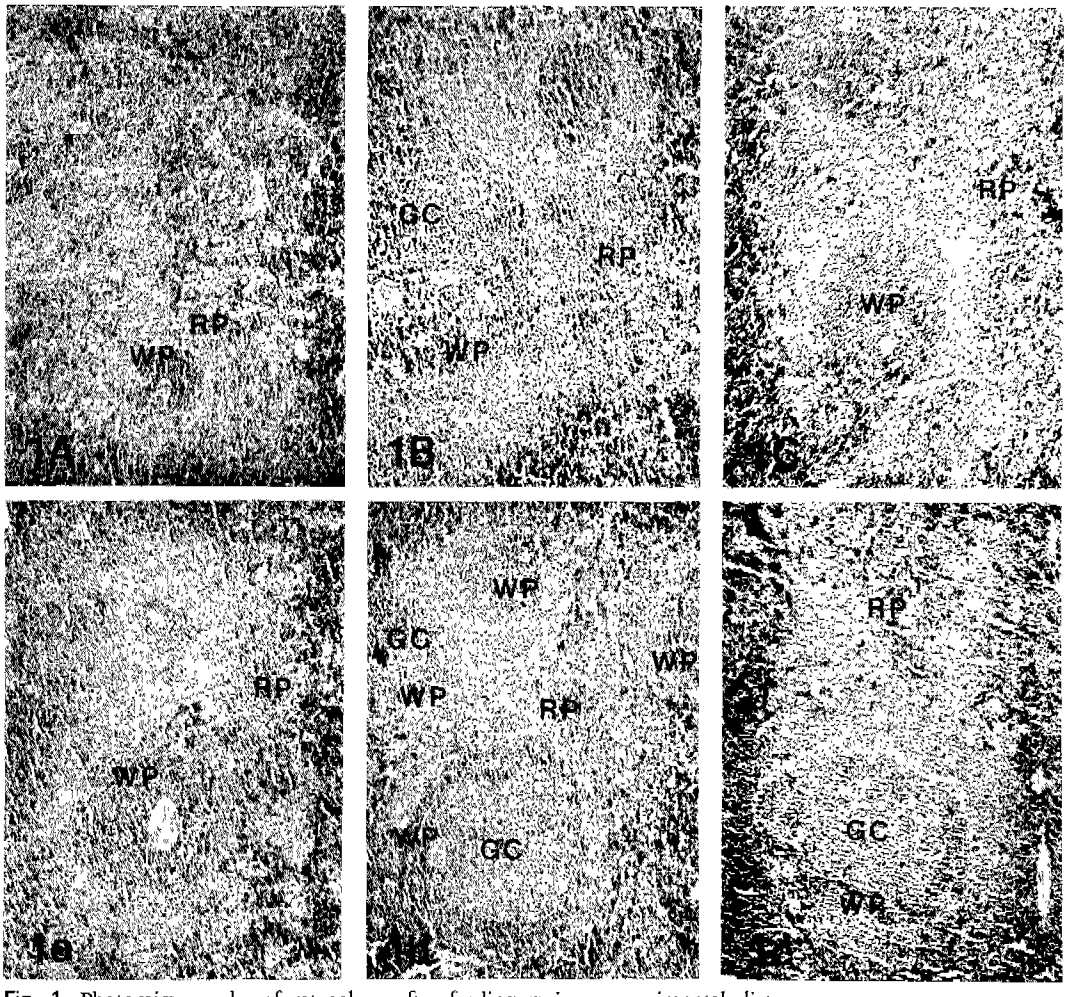

Fig. 1. Photomicrographs of rat spleen after feeding various experimental diets.

1A. DeA-CO ; Poorly developed white pulps(WP) are observed. RP : red pulp

1B. AdA-CO ; Germinal centers(GC) are rarely observed in the white pulps.

1C. ExA-CO ; The histological structures in this group are similar to those of the vitamin A deficient group.

1a. DeA-FO ; Poorly developed white pulps are observed.

1b. AdA-FO ; Well developed white pulps and germinal centers are observed.

1c. ExA-FO ; White pulps and germinal centers are rarely observed, but in existing cases, almost all of the white pulps have germinal centers.

different aspects at different vitamin A levels.

In summary, antibody responses of the fish oil tended to be lower than those of the corn oil. Both the vitamin A deficiency and excess suppressed antibody responses and spleen development compared with an adequate vitamin A ingestion.

Interactions were observed between fish oil and excess level of vitamin A.

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= 국 문 초 록 =

n−6, n−3 지방산과 비타민 A 수준이 흰쥐의 체액면역에 미치는 영향

김 서 혜 · 이 일 하

중앙대학교 가정학과

본 연구는 식이내 n−6, n−3 지방산과 비타민 A 수준이 흰쥐의 체액면역기능에 미치는 영향을 관찰하기 위해 평균 체중이 103±2.2g인 Sprague-Dawley종 수컷 흰쥐 68마리를 6군으로 나누어 6주간 사육하였다. 실험식이는 지방을 식이무게의 10%수준으로 하여 어유와 옥수수유를 사용하였고 비타민 A 수준은 결핍식이(1240 IU/kg diet), 적정식이(4000 IU/kg diet), 과잉식이(400,000 IU/kg diet)로 하였다.

비장무게는 체중 100g당, 비타민 A 과잉 어유섭취군이 유의하게 낮았으며 plaque-forming cell 반응은 지방종류와 비타민 A수준에 따른 차이가 없었다.

혈청의 혈구응집반응 결과는 어유군이 옥수수군에 비해 항체가가 낮았으며, 비타민 A 결핍군은 적정군과 과잉군에 비해 항체가가 낮았다. 혈청 IgM 농도는 어유군이 낮았으며, 비타민 A 수준에 따른 차이는 없었다. IgG 농도는 지방종류에 의한 차이가 있어 어유군이 옥수수군에 비해 높았으며, 비타민 A 과잉 어유군이 다른 식이군에 비해 현저하게 높았다. 비타민 A 과잉 어유군은 비장의 크기가 작고 혈청 IgG 농도가 높게 나타났으므로 비타민 A 과잉시 어유 섭취는 면역기능에 좋은 영향을 주지 못하였다. 광학현미경으로 살펴본 바에 의하면 비장조직은 어유군이 옥수수유군에 비해 발달이 좋았으며 비타민 A 결핍과 과잉시에 발달이 저해되었다.

그러므로 본 연구 결과로 건강한 사람의 경우 면역기능을 온전히 유지하기 위해서는 비타민 A를 적정량 섭취하는 것이 좋으며 비타민 A 과잉시에는 어유섭취를 피하는 것이 바람직하다는 것을 알 수 있다.