Physiological Activity of ω3 Polyunsaturated Fatty Acids in Dark Fleshed Fishes

I. The Effects on Protein and Phospholipid Contents, and Cholesterol Levels in Rats

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The effects of ω3 polyunsaturated fatty acid (PUFA) on protein and phospholipid contents, and cholesterol level were studied in rats fed with diets of different fat composition. Body weights of fish oil groups were decreased to 11.1%~14.4% compared with lard group (control), and also 16.4%~23.3% compared with corn oil group, respectively. Protein contents of ω3 PUFA and sardine oil groups in liver were increased to 6.78%~8.51% compared with control group, but were no significant difference in brain and serum. ω3 PUFA and sardine oil slightly repressed the phospholipid in microsome of liver. Moreover they effectively reduced the serum cholesterol levels compared with control group.

INTRODUCTION

The elevated level of plasma cholesterol has been shown to correlate with the degree of atherosclerosis and the incidence of coronary heart disease (1). In addition, hyperlipidemia and conditions such as coronary heart disease and insulin-independent diabetes are frequently associated with obesity in humans (2-3). Sterol balance also have been established that, on an ideal body weight basis, obese subjects produce up to twice as much cholesterol per day as lean individuals (4-5).

A number of studies have provided equivocal data on the relationship between diet and cholesterol. Plasma and tissue cholesterol levels were affected by dietary composition such as protein (6), fat (7), zinc (8), manganase (9), vitamin E (10-11), steroid (12), tyrosine (13) and fiber (14-15). It is also reported that marine algae have hypocholesterol effects on plasma and tissue cholesterol (16-17).

Recently, hypercholesterolemic patients (Type III) given a salmon diet obtained a decrease in plasma cholesterol and triglyceride levels (18). Bang and Dyerberg (19-20) reported that intake of ω3 polyunsaturated fatty acid resulted in low incidence of coronary disease, reduced platelet aggregation by thromboxane B_2 (TX B_2), and elevated high density lipoprotein (HDL) cholesterol in Greenland Eskimos.

No data are available on cholesterol-related changes of fish oil containing with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in serum, liver and brain tissues compared with other plant oil and animal fat. To compare the effect on cholesterol levels by dietary oils, we examined body weight, feed efficiency, protein and phospholipid contents, and cholesterol levels in serum, liver and brain of rats.

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MATERIALS AND METHODS

Animal and Diets: Male Sprague-Dawley rats (160-180 *g*) were maintained in plastic cages with wire mesh floors in airconditioned room (24~26℃, 65~70% RH) with a 12 hours light-dark cycle. The rats were divided into six groups of 8 each, and were fed various diets for 6 weeks. Body weight and amount of diets remained were weighed every morning (10 a.m.), and water was given freely. The semi-synthetic diets were prepared to be the same calorie level, and diet composition are shown in Table 1. All fat components were incoporated into diets except for squalene and ω3 PUFA which were orally administered every morning.

Body weight and amount of food intake were measured every mroning, and feed efficiency was calculated by body weight gain (*g*)/food intake (*g*)× 100.

Isolation of ω3 PUFA: ω3 polyunsaturated fatty acid (PUFA) was isolated from sardine(*Sardinops melanosticta*). Sardine oil(1.0 *kg*) was saponified by adding 2.0 *l* of 6N NaOH and 2.0 *l* of 75% EtOH in shaking water bath (60℃) for 1 hour. Reaction mixture was extracted with 5.0 *l* of hexane in shaking water bath for 1 hour, and conc-HCl was added to lower phase until pH 1.0. Hexane layer was evaporated with nitrogen gas in water bath (40℃) to obtain ω3 PUFA. Purity of ω3 PUFA was identified with GLC, and yields of ω3 PUFA were 74.41% including 35.54% of EPA and 23.84% of DHA. Squalene was supplied by Kyungdong Special Co. (Pusan).

Table 1. Animal diet compositions (%)

Starch	50.0
Sucrose	10.0
Casein	20.0
Fat*	10.0
Cellulose	4.0
Salt**	3.6
Vitamin***	2.0
Choline chloride	0.2
DL-Methionine	0.2
Total	100.0

* Fat contents in diets: A group, lard 10%; B group, corn oil 10%; C group, perilla oil 10%; D group, sardine oil 10%; E group, corn oil 7% + squalene 3%(p.o.); F group, corn oil 7% + ω3 PUFA 3%(p.o.).

** Salt composition: $CaCO_3$, 29.29%; $CaHPO_4$. $2H_2O$, 0.43%; KH_2PO_4, 34.31%; NaCl, 25.06%; $MgSO_4$. $7H_2O$, 9.98%; $FeCl_2$, 0.623%; $CuSO_4$. $5H_2O$, 0.156%; $MnSO_4$. $2H_2O$, 0.121%; $ZnCl_2$, 0.0005%; KI, 0.0005%; $(NH_4)_6Mo_7O_{24}$. $4H_2O$, 0.0005%.

*** Vitamin composition: B_1; 0.059%, B_2; 0.059%, B_6; 0.029%, B_{12}; 0.0002%, nicotinic acid; 0.294%, pantothenic acid; 0.235%, biotin; 0.001%, folic acid; 0.002%, inositol acid; 1.176%, ascorbic acid; 0.588%, lactic acid; 97.556%.

Preparations of Serum and Membranes: Six group rats were orally administered with 1.0 *ml* of ethanol prior to 12 hours of sacrifice for induction of lipid peroxidation according to the previous paper(21). These rats were anesthetized with ethyl ether, and blood was collected by heart puncture, and liver and brain were excised.

Serum was isolated by centifugation (600× *g*, 10 min), and liver and brain tissues were homogenized with isolation buffer (10% w/v; in 10 mM HEPES pH 7.4 containing 10 mM KCl and 280 mM sucrose), and follwed by centrifugation at 600× *g* for 10 min. The supernatants were centrifuged at 9,000 × *g* for 15 min, and the resulting pellets were used as mitochondrial fraction. The remaining supernatants were centrifuged at 105,000× *g* for 60 min to yield microsomal fraction. Each fractions were washed twice with the same buffer, and were made up to be 1.0 *ml*/*g* pellet.

Purity of the membranes isolated was assessed with the following marker enzymes; glucose-6-phosphatase (22), citrate synthase (23) and NADPH cytochrome C (P 450) reductase (24).

Protein content was determined by the method of Lowry et al. (25).

Determination of Cholesterol and Phospholipid: Cholesterol content was determined by the method of Rudel et al.(26) using o-phthalaldehyde. 0.3 *ml* of 33% KOH and 3.0 *ml* of 95% ethanol were added

to 0.1 *ml* of serum, and were heated in water bath (60℃) for 15 min. 5.0 *ml* of hexane and 3.0 *ml* of distilled water were forcefully added and completely mixed. 1.0 *ml* of hexane layer was evaporated under nitrogen gas, and 2.0 *ml* of o-phthalaldehyde reagent was added to each tubes. After 10 min, 1.0 *ml* of conc-H_2SO_4 was carefully added, and immediately mixed well, and measured at 550 nm.

Phosphorus content for phospholipid was determined by the method of Baginski et al. (27).

Data were statististically analyzed by Student t-test.

RESULTS

Changes in Body Weight and Feed Efficiency: As shown in Table 2, body weight was in the ranges of 164.1 *g* to 213.8 *g* , and body weights of ω3 PUFA and sardine oil groups were remarkably decreased to 11.1%～14.4% compared with lard control group, and 16.4%～23.3% compared with corn oil ($p<0.001$). In reducing body weight, ω3 PUFA was the most effective, followed by sardine oil ($p<0.001$), squalene ($p<0.05$) in order. But there was no significant difference in the other oil groups.

On the other hand, feed efficiency was decreased in order of squalene, sardine oil ($p<0.001$), and perilla oil ($p<0.05$), and also showed a similar trend in body weight except for ω3 PUFA group.

Table 2. Body weight gains, food intake and feed efficiency

Diet group	Body weight gain (*g*)	Food intake (*g*)	Feed efficiency
Lard (control)	191.7± 28.2*	833.5± 86.4	23.0± 3.3
Corn oil	213.8± 18.5	913.7± 45.2	23.4± 2.0
Perilla oil	204.0± 31.9	1,004.9± 76.5	20.3± 3.2b
Sardine oil	170.5± 23.9a**	852.5± 38.2	20.0± 2.9b
Squalene	189.8± 17.0b**	1,004.2± 58.3	18.9± 2.8a
ω3 PUFA	164.1± 20.7a**	749.3± 28.6b	21.9± 5.7

* Mean ± S. E. of 8 rats/group. a: $p<0.001$, b: 0.05.

** Significance on body weight analyzed by corn oil.

Effect of Dietary Lipid on Protein Content: As shown in Table 3, protein contents were in the ranges of 176.90 to 191.96 *mg/g* in liver, 42.56 to 51.08 *mg/g* in brain and 54.88 to 61.21 *mg/ml* in serum. Protein content of ω3 PUFA group in liver was the most effective, followed by corn oil group ($p<0.05$), sardine oil group ($p<0.01$) in order. Especially, protein content of ω3 PUFA group was increased to 8.51% compared with that of lard control group.

On the other hand, protein contents of brain showed a similar trend in the ranges of 42.56 to 48.11 *mg/g* except for lard control group, and protein content of these groups were decreased to the ranges of 5.81 to 16.68% compared with lard control group. It is observed that protein content in serum was no significant difference in all groups.

Table 3. Protein contents in serum, liver and brain

Diet group	Liver (*mg/g*)	Brain (*mg/g*)	Serum (*mg/ml*)
Lard (control)	176.9± 15.9	51.1± 5.5	59.0± 3.8
Corn oil	190.1± 12.0b	42.6± 4.9c	54.1± 3.7
Perilla oil	181.7± 15.3	43.7± 3.7c	61.2± 4.6
Sardine oil	188.9± 16.1c	47.6± 4.6	54.9± 4.0
Squalene	178.3± 17.4	48.1± 5.1	59.7± 4.0
ω3 PUFA	192.0± 8.9b	45.0± 3.9c	59.0± 2.7

b: $p<0.05$, c: $p<0.01$.

Effects of Dietary Lipid on Phospholipid and Cholesterol Levels: As shown in Table 4, lipid phosphorus contents (*μg* Pi/*mg* protein) were in the ranges of 11.7 to 14.7 *μg* for microsome, and 5.61 to 6.88 *μg* for serum. In lipid phosphorus content of microsome, ω3 PUFA group was remarkably decreased to 20.4% compared with lard control group ($p<0.001$), and sardine and corn oil groups were also decreased to 16.3% and 15.0% compared with lard control group ($p<0.05$), respectively. Therefore, it was effective to reduce the phospholipid in microsome of liver in order of fish oil, plant oil, animal fat.

In phosphorus content of serum, perilla oil, squalene and ω3 PUFA groups were remarkably decreased to 18.5% ($p<0.001$), 12.5% and 12.1% ($p<0.05$) respectively, compared with lard control group, whereas sardine and corn oil groups were not changed significantly compared with lard control group.

Table 4. Phosphorus contents of microsome and serum (*μg*/*mg* protein)

Diet group	Liver microsome	Serum
Lard (control)	14.7± 2.4	6.88± 0.40
Corn oil	12.5± 2.1b	6.59± 0.39
Perilla oil	13.2± 1.3	5.61± 0.48a
Sardine oil	12.3± 1.6b	6.55± 0.55
Squalene	12.9± 1.1	6.02± 0.35b
ω3 PUFA	11.7± 0.5a	6.05± 0.28b

a: $p<0.001$, b: $p<0.05$.

It is observed that serum cholesterol levels in rats fed with sardine oil, ω3 PUFA and perilla oil were remarkably decreased to 22.3%, 31.7% and 33.9% compared with control group ($p<0.001$), respectively (Table 5). But there were no significant differences in serum cholesterol levels among corn oil, squalene and lard.

Table 5. Changes in serum cholesterol content

Diet group	Serum (*mg/dl*)	% Inhibition
Lard (control)	70.4± 9.1	—
Corn oil	65.1± 15.8	7.5
Perilla oil	46.5± 4.7a	33.9
Sardine oil	54.7± 3.4a	22.3
Squalene	68.6± 11.2	2.6
ω3 PUFA	48.2± 7.2a	31.7

a: $p<0.001$

DISCUSSIONS

We focused on the effects of dietary lipid on body weight, protein, phospholipid contents, and cholesterol levels. It is known that cholesterol content in blood and tissues were to be concerned with various physiological and pathological states, and increases of cholesterol content in blood and liver were to be related with senile disease such as obesity, atheriosclerosis, thrombosis and cardiac infarction etc.

In reducing body weight, ω3 PUFA group was the most effective (14.4%), followed by sardine oil group (11.1%) compared with control group ($p<0.001$). It is believed that body weight of fish oil groups decreased due to ω3 PUFA such as EPA and DHA, but partially concerned to feed efficiency. This result by marine products may be applied for prevention of obesity (28).

Proein contents of ω3 PUFA and sardine oil groups in liver were increased to 6.78%～8.51% compared with control group. Protein content may also be related to ω3 PUFA such as EPA and DHA in fish oil. It is found that protein synthesis and yield as physiological activated substance are very important in living cells in our previous paper (29), and the heme-protein acts as cofactor for prostaglandin synthesis (30).

It is worthy point out that microsome in liver consistently showed higher peroxidative activity than mitochondria by Laganiere et al. (31). Therefore, it is believed that this fact may be related to the higher lipid contents in microsome. Phospholipid contents of fish oil group such as ω3 PUFA and sardine oil were remarkably lower(20.4 % and 16.3%) than that of control group($p<0.001$). It is proved that fish oil was more effective than plant oil and/or animal oil for decrease of phospholipid which may be related to lipid peroxidation in liver. Therefore, it is believed that higher phospholipid content in control group may be related to lipid peroxidation in microsome.

Cholesterol levels of ω3 PUFA and sardine oil groups were remarkably decreased to 31.7% and 22.3% compared with control group. Therefore, ω3 PUFA in fish oil prevents the elevation of serum cholesterol. There is a possibility that incorporation

of fish oils into diets might affect the decrease of serum lipid and triglyceride levels in human subjects and animals (18, 32). Another possibility may be speculated that ω3 PUFA in fish oils affected the increase of high density lipoprotein (HDL) which concerned to decrease of serum cholesterol (19, 33). However, there are reports of no change in total cholesterol after the fish diet (34-35). It is possible that the amount of fish administered to subjects had too small an effect on total cholesterol to be detected.

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ω3 고도불포화지방산의 생리활성에 관한 연구

I. 단백질, 인지질 및 콜레스테롤 함량에 대한 연구

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요 약

ω3 고도불포화지방산의 투여가 체중변화, 단백질, 인지질 및 콜레스테롤 함량에 미치는영향을 비교하기 위하여 정어리유에서 분리한 ω3 고도불포화지방산, 식물유 및 동물성지방 (대조군)을 10%가 되도록 첨가한 사료로써 실험동물에 6주간 투여하였다.

체중변화는 어유 투여군이 대조군에 비해 11.1%～14.4%로 감소하였고, 또 식물유인 옥수수 기름 투여군에 비해서는 16.4%～23.3%로 감소하였다. 간장에서의 단백질 함량은 ω3 고도불포화지방산과 정어리 기름 투여군에 비해 6.78～8.51%로 약간 증가하였지만, 뇌 및 혈청에서는 유의성이 없었다. 간장의 마이크로좀에서의 인지질함량은 ω3 고도불포화지방산과 정어리 기름 투여군이 대조군에 비해 감소하는 경향을 나타내고 있었다. 더우기 이들 어유 투여군은 대조군에 비해 혈청 콜레스테롤 함량이 효과적으로 감소되었다.

따라서 ω3 고도불포화지방산을 포함한 어유는 단백질 합성을 촉진함은 물론 체중 증가와 인지질 함량을 감소시킬 뿐만 아니라 콜레스테롤 함량을 효과적으로 감소시킴을 알 수 있었다.