Effects of Tuna Fish Oil on Hyperlipidemia and Proteinuria in Childhood Nephrotic Syndrome

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Abstract

This is a randomized, double-blind, placebo-controlled, cross-over study to determine whether tuna fish oil decreased hyperlipidemia and proteinuria in children with steroid-resistant nephrotic syndrome. Five boys were supplemented with both 4 grams of tuna fish oil and placebo in a randomized order for 8 weeks of each treatment separated by 6-week wash-out period. The results showed no statistically significant difference in serum creatinine, triglyceride, cholesterol, urine protein and creatinine clearance between fish oil supplemented group and placebo group. Small sample size, low dosage, short duration of supplementation and wash-out period are among the important limitations in this study. Further study should be performed to identify the effects of fish oil on this entity in nephrotic syndrome.

Key word : Fish Oil, Hyperlipidemia, Nephrotic Syndrome

Nephrotic syndrome (NS) is a disorder of glomerular permeability. It is defined as any condition with proteinuria over 40 mg/m^2/h (2^+ or more from albustix), serum albumin less than 2.5 g/dl and edema. Hyperlipidemia and hypercholesterolemia in patients with NS associate with the increase of lipoprotein produced by liver to compensate urinary albumin loss as well as the abnormal lipoprotein metabolism[1,2]. Hyperlipidemia also accounts for the glomerular and tubulointerstitial injuries leading to the progressive deterioration of renal function[3,4].

The most common primary NS found in children are minimal change nephrotic syndrome (MCNS) and steriod-responsive nephrotic syndrome (SRNS)[5]. Most of patients with MCNS and SRNS

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respond to corticosteroids, and are more likely to be free from the abnormalities after treatment. However, transformation of SRNS to steroid resistance can occur. Another entity of steroid-resistant NS is focal segmental glomerulosclerosis (FSGS), which has high tendency to progress to end-stage renal failure. The complications such as hypovolemia, infection, thrombosis, hyperlipidemia and chronic renal failure are among the causes of morbidity and mortality in patients with NS[6,7]. In addition, long-term use of corticosteroids has adverse effects including immunosuppression, growth failure and osteoporosis. The alternative treatments, such as cyclophosphamide and cyclosporin A, not only are expensive but also have serious side effects requiring close monitoring. There are very few data regarding the effects of lipid-lowering drugs as adjuvants in childhood NS. No specific dietary advice is completely beneficial.

Fish oil such as Menhaden oil may be used either as another alternative treatment or as adjuvant therapy with corticosteroids to reduce proteinuria and hyperlipidemia. De Caterina et al[8] showed the significant reduction of proteinuria and hypertriglyceridemia in patients with either membranous glomerulonephritis or FSGS when taking 7.7 g of n-3 polyunsaturated fatty acids (PUFA) daily for 6 weeks. Donadio et al[9] reported that supplementation of Menhaden oil for 2 years in adult patients with IgA nephropathy retarded the deterioration of renal functions significantly compared with placebo.

To date, there is no reported study of the effects of fish oil in the pediatric-aged group of nephrotic patients. We performed a double-blind, placebo-controlled, cross-over trial to demonstrate the effects of tuna fish oil on hyperlipidemia and proteinuria in children with NS. Tuna fish oil has high amount of n-3 PUFA especially docosahexaenoic acid (DHA). Our hypothesis is that daily supplementation of 8 capsules of tuna fish oil containing 1.45 g of eicosapentaenoic acid (EPA) and DHA for 8 weeks decreases proteinuria, serum triglyceride and cholesterol in pediatric patients with steroid-resistant NS. The dose chosen in this study contributed to the compliance. The results did not support our hypothesis and will be discussed.

MATERIAL AND METHOD

Pediatric subjects with NS enrolled in this randomized, double-blind, placebo-controlled, cross-over study at Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Mahidol University. Subjects who did not respond to corticosteroids and cyclophosphamide were eligible to be enrolled in the study. The additional inclusion criteria for enrollment were normotension, albustix 3^+ or over, fasting serum triglyceride $\geq$ 150 mg/dl and cholesterol $\geq$ 200 mg/dl, serum creatinine $\leq$3 mg/dl, and creatinine clearance > 15 ml/min/1.73 m^2 to confirm that subjects did not have the end-stage renal disease at the time of enrollment. Subjects with severe infection, diarrhea, hemostatic disorder, or taking lipid-lowering drugs were excluded from the study. The study protocol was approved by the faculty review board. The protocol was explained to both parents and subjects; informed consents were obtained before the beginning of the study. During the study, all subjects continued taking the medications given by their nephrologists.

Six subjects were eligible for the study; all of them were boys. They were randomly divided into 2 groups. The first group was supplemented with 8 capsules of Uni-E® (Unicord Public Company Limited, Bangkok, Thailand), which is tuna fish oil containing EPA 230 mg and DHA 1.12 g as well as 240 IU of D-α-tocopheryl acetate everyday for 8 weeks. The other was supplemented with placebo containing olive oil for 8 weeks. The placebo capsules (provided by Unicord Public Company Limited) had the same shape and color as Uni-E®. The washout period was 6 weeks; after that, the supplementation was switched in both groups for another 8 weeks. Both the doctor and subjects did not know the type of supplementation until the end of the study.

At the beginning of the study (week 0) and each visit (week 4, 8, 14, 18, 32), the physical examinations, weight and height measurements were performed by the same doctor. In addition, dietary advice were given to subjects by the doctor and the dietitian to reduce dietary fat intake. Food frequency questionnaires and 3-day dietary record were collected at each visit. Compliance was determined by counting the amount of capsules which remained in the containers. Blood was drawn for measuring blood urea nitrogen (BUN), creatinine, total protein, albumin, triglyceride, total cholesterol, high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol by using enzymatic kits (Wako, Dallas). Twenty-four hour urine samples were collected to analyze total protein and

creatinine by enzymatic kits (Boehringer Mannheim, France).

Statistical analysis

The comparisons of baseline data (week 0, week 14) to post-treatment (week 8, week 32) were performed using the two-tailed paired Student's *t*-test. The significance was considered at $P < 0.05$.

RESULTS

Only 5 patients completed the study. Clinical characteristics were summarized in Table 1. Three patients had focal segmental glomerulosclerosis. Four had short stature and one was malnourished according to Z-score by WHO[10]. None of them had hypertension but two had mild pitting edema at the end of the study without any specific cause or symptom. Caloric intake, dietary compositions (protein, fat and carbohydrate as percentage of total caloric intake) were not significantly different between the 2 periods for each subject (data not shown). The food frequency questionnaires and 3-day dietary record however were not validated at the time of the study. The compliance of most subjects was good (80% or more) except that in two subjects, one in fish oil period (66%) and the other in placebo period (69%), in the second period for each. Both subjects and parents did not report any side effects during the study.

As shown in Table 2, there was no significant difference in serum creatinine and lipid profile between fish oil supplementation and placebo. Likewise, the amount of urinary protein and calculated creatinine clearance[11] were not significantly different between the 2 groups (Table 3).

DISCUSSION

This study was done to demonstrate the effects of tuna fish oil on hyperlipidemia and renal functions of pediatric patients with NS. However, the results showed no statistically significant difference in both serum lipids and renal functions between 8-week fish oil supplemented group and placebo group.

There are many limitations in this study including small sample size, small dosage of fish oil and short duration of supplementation. Very small sample size is one of the major weaknesses in this study. We are unable to show any significant difference between 2 groups unless the difference is very large. Likewise, the dosage of tuna fish oil supplemented in this study was much lower than that in the study of De Caterina[8] although the duration in this study was 2 weeks longer. The supplemented dose per body weight per day in this study was close to that in Donadio's study[9]. However, the duration of supplementation in Donadio's study was much longer than this study; in addition, the glomerular lesions in subjects between two studies were totally different.

Regarding lipid-lowering effect of fish oil, Miller et al[12] studied the effect of 1 gram MaxEPA in hypertriglyceridemic patients and showed that serum triglyceride decreased significantly in patients supplemented with MaxEPA for 3 months compared to olive oil as a placebo. However, Miller's study did not focus on renal functions even though 33 subjects had hypertension. Moreover, the number of subjects in Miller's study was much more than in our study.

The duration of wash-out period is another confounder. An insufficient wash-out period at time of cross-over can result in carry-over effect. Such effect takes account of whether subjects had been on treatment or placebo prior to the wash-out period. However, there were studies having less than 6-week wash-out period but showing the significant decrease of triglyceride in subjects supplemented with fish oil[13,14]. Nevertheless, Clark et al[15] studied the effect of MaxEPA in patients with lupus nephritis and demonstrated by comparing platelet membrane phospholipid levels that the 10-week wash-out period did not prevent a carry-over effect. The difference in dosage and underlying diseases of subjects between those studies and this study probably resulted in the inconsistent results. In addition, we did not analyze plasma phospholipid and fatty acid levels to determine the carry-over effect in our study.

We used olive oil as a placebo as other studies. In fact, olive oil has been used as a placebo in many studies with fish oil. One capsule of Uni-E® contains 15.64 per cent of fatty acid composition as olive oil. However, Clark showed that olive oil affects plasma viscosity and very-low-density lipoprotein (VLDL) cholesterol in the similar direction to fish oil; the use of olive oil as a placebo potentiates type II error in cross-over and parallel study. Olive oil has an effect on other cholesterols as well but to date there is no report regarding the effect of

Table 1. Characteristics of subjects.

Subject No.	Age	Histologic diagnosis	Medication during the study	Height (Ht) (cm)	Weight (Wt) (kg)	Z-score*	
						Ht/Age	Wt/Age
1	12 y	Inadequate tissue from biopsy	Prednisolone, dipyridamole, aspirin	138	37	-1.56	-0.43
2	7 y, 8 mo	Focal segmental glomerulosclerosis	Prednisolone, dipyridamole, coumadin, hydrochlorothiazide	108	19.5	-3.01	-1.51
3	15 y, 4 mo	Focal segmental glomerulosclerosis	Prednisolone, dipyridamole, coumadin	145	42.2	-3.33	-1.95
4	17 y	Mesangial proliferative glomerulonephritis (IgG deposit)	Prednisolone, dipyridamole, calcitriol	152	50	-3.69	-1.84
5	15 y, 2 mo	Focal segmental glomerulosclerosis with tubulointerstitial inflammation	Prednisolone	137	30.5	-4.14	-3.22

* Calculated with Epi Info software with regard to the following formula:

$$\text{Z-score} = \frac{\text{Individual's value - median value of reference population}}{\text{Standard deviation value of reference population}}$$

The interpretations of Z-score for Ht/Age, Wt/Age, and Wt/Ht are respectively as following: -2 or less = short, malnourished, and thin; +2 or more = tall, over-nutrition, and obese as recommended by WHO[10].

Table 2. Serum creatinine and lipid profiles of subjects during the study.

Parameter	Subject* No.	Placebo		Fish oil		P-value[t]
		0 wk	8 wk	0 wk	8 wk	
Creatinine (mg/dl)	1	1.7	1.7	2.2	1.7	
	2	0.6	0.5	0.5	0.7	
	3	1.1	1.1	1.2	1.1	NS[I]
	4	4.2	4.2	2.6	4.2	
	5	0.5	0.6	0.6	0.6	
Triglyceride (mg/dl)	1	241	165	88.5	100	
	2	295	177	336	124	
	3	165	112	106	88	NS
	4	195	271	455	200	
	5	354	236	224	270	
Cholesterol (mg/dl)	1	411	450	330	399	
	2	386	771	900	386	
	3	275	308	193	285	NS
	4	560	532	735	716	
	5	735	643	604	1295	
HDL cholesterol (mg/dl)	1	42.0	38.0	39.3	41.9	
	2	32.7	43.2	36.7	54.3	
	3	32.7	36.7	36.7	36.7	NS
	4	32.1	28.1	24.9	33.4	
	5	17.7	24.9	15.1	27.0	
LDL cholesterol[S] (mg/dl)	1	320.8	379.0	273.0	337.1	
	2	294.3	692.4	796.1	306.9	
	3	209.3	248.9	135.1	230.7	NS
	4	488.9	449.7	619.1	642.6	
	5	646.5	570.9	544.1	1214	

* Subject #1, 2, 3 and 5 started with placebo; subject # 4 started with fish oil supplementation.
t Compare the change of each parameter between placebo and supplemented period
I NS = not significant at 0.05 level.
S Calculated from the formula: LDL cholesterol = total cholesterol - (TG/5) - HDL cholesterol

Table 3. Urine protein and creatinine clearance (C_{Cr}) of subjects.

Measurement	Subject* No.	Placebo		Fish oil		P-value[t]
		0 wk	8 wk	0 wk	8 wk	
Urine protein (g/day)	2	1.57	0.86	0.90	0.11	
	3	0.34	0.11	0.21	0.26	NS[I]
	4	6.23	8.82	6.93	2.99	
C_{Cr}[S] (ml/min/1.73 m^2)	1	44.65	45.29	35.75	45.94	
	2	99.00	122.1	123.2	89.34	
	3	72.50	72.25	66.92	73.50	NS
	4	19.87	19.90	32.15	19.90	
	5	150.70	126.5	126.5	127.42	

* Subject No.1, 2, 3 and 5 started with placebo; subject No. 4 started with fish oil supplementation; data from some subjects were not analyzed due to incompleteness.
t Compare the change of each parameter between placebo and supplemented period.
I NS = not significant at 0.05 level.
S $C_{Cr} = \frac{0.55 \times \text{height (cm)}}{\text{Serum Cr}}$ ml/min/ 1.73 m^2 (from reference 11)

olive oil on proteinuria. A further investigation is required to determine an appropiate oil used as a placebo in a fish oil study.

The compliance in this study was evaluated by capsule counts. As mentioned earlier, plasma fatty acid composition or other indirect assays such as malondialdehyde production was not measured to assess the accurate compliance.

In conclusion, this study was unable to show any beneficial effects of tuna fish oil on either hyperlipidemia or proteinuria in children with steroid-resistant nephrotic syndrome in our study. With regard to many limitations in this study, a further research taking these limitations into consideration should be performed to determine the effects of tuna fish oil on renal functions and lipid profile in this entity of nephrotic syndrome.

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ผลของน้ำมันปลาทูนาต่อระดับไขมันในเลือดและโปรตีนในปัสสาวะของผู้ป่วยเด็กที่เป็นกลุ่มอาการเนโฟรติค

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ได้ทำการศึกษาแบบ randomized, double-blind, placebo-controlled, cross-over study เพื่อศึกษาว่าน้ำมันปลาทูนาช่วยลดระดับไขมันในเลือดและโปรตีนในปัสสาวะของผู้ป่วยเด็กที่เป็น nephrotic syndrome หรือไม่ มีผู้ป่วยเด็กชาย 5 รายในการศึกษานี้ที่ได้รับน้ำมันปลาทูนาสลับกับ placebo อย่างละ 8 สัปดาห์ โดยมีช่วงที่ไม่ได้รับทั้งสองอย่างเป็นเวลา 6 สัปดาห์ ไม่พบความแตกต่างทางสถิติของระดับครีอาตินีนและระดับไขมันในเลือดรวมถึงระดับโปรตีนในปัสสาวะและค่า creatinine clearance ในช่วงที่ได้รับน้ำมันปลาทูนาเทียบกับ placebo ปัญหาในการศึกษาครั้งนี้ประกอบด้วยจำนวนผู้ป่วยที่เข้าร่วมในการศึกษาน้อยมาก ขนาดของน้ำมันปลาทูนาที่ให้อาจต่ำเกินไปและระยะเวลาให้ยารวมถึงช่วงว่างก่อนเปลี่ยนยาสั้นเกินไป

ควรมีการศึกษาเพิ่มเติมโดยคำนึงถึงปัจจัยต่างๆที่เป็นปัญหาในการศึกษาครั้งนี้มาพิจารณาปรับปรุงเพื่อให้ทราบถึงผลที่แท้จริงของน้ำมันปลาทูนาต่อระดับไขมันในเลือดและการทำงานของไตในผู้ป่วยกลุ่มนี้ต่อไป

คำสำคัญ : น้ำมันปลา, ภาวะไขมันในเลือดสูง, กลุ่มอาการเนโฟรติค

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