감사의 글

내과 전문의가 된지 10년이 되었지만 오메가 3 지방산 연구처럼 흥미로운 연구는 없었습니다. 이 연구는 그 동안 제가 기울이지 못했던 부분에 넓은 시각을 부여해 주었습니다. 이 연구 논문을 완성하면서 제가 앞으로 임상 의사로서 부족했던 부분을 채워나가고 새로운 연구를 진행하여 도움을 주신 많은 교수님들처럼 발전된 모습을 보여드리고 싶습니다. 이 연구 논문에 물심 양면으로 아낌없는 도움을 주시고 연구 진행 및 논문 작성의 모든 과정을 지도해 주신 내과 윤정한 교수님, 논문 내용을 세심히 검토해 주시고 교정을 해주신 약리학 최재원 교수님, 학위 논문의 의의 및 논문 내용에 대해 조언을 해주신 내과 김장영 교수님께 진심으로 감사 드립니다.

끝으로 가정 일을 도맡고 있으면서 두 아이들을 키우느라 고생하는 사랑하는 아내 박수정에게 진심으로 고마움을 전합니다.

2009년 7월

저자 최현민 씀

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Abstracts

Effects of N-3 Polyunsaturated Fatty Acid Supplement on Inflammatory Markers and Insulin Resistance in Patients with the Metabolic Syndrome

N-3 polyunsaturated fatty acids (PUFA) supplement may be beneficial in patients with the metabolic syndrome (MS) through reduction of serum triglyceride (TG), systemic inflammation and insulin resistance. Recommendation dose of N-3 PUFA are 1 g/day for secondary prevention of myocardial infarction and 2-4 g/day for serum TG reduction. In the MS, there are few data regarding N-3 PUFA effect on the systemic inflammation and insulin resistance. Also, dose-dependent effects of N-3 PUFA on systemic inflammation and insulin resistance have never been evaluated. The goal of this study is to evaluate whether N-3 PUFA supplement may reduce systemic inflammation and insulin resistance in patients with the MS. We also investigate dose-dependent effects of N-3 PUFA on systemic inflammation and insulin resistance by comparing

conventional (2 g/day) with high (4 g/day) dose of N-3 PUFA. Sixty patients with the MS were randomly enrolled in N-3 PUFA and placebo groups. Fifty-three (N-3 PUFA, N=26 and placebo, N=27) subjects completed this study. N-3 PUFA group received 2 g/day (conventional dose) for 6 weeks and 4 g/day (high dose) for another 6 weeks. We compared serum lipid and lipoprotein subclass profiles, inflammatory markers and insulin resistance between two groups. N-3 PUFA administration significantly reduced mean high sensitive C-reactive protein (hs-CRP; $p = 0.01$, repeated measures ANOVA) and homeostasis model assessment of insulin resistance (HOMA-IR; $p = 0.03$, repeated measures ANOVA) levels compared with placebo. N-3 PUFA mediated TG ($p = 0.001$, repeated measures ANOVA) and HOMA-IR ($p = 0.000$, repeated measures ANOVA) reduction were significantly related to dose-dependent effect of N-3 PUFA. However, there was no significant dose-dependent effect of N-3 PUFA on hs-CRP. In conclusion, N-3 PUFA administration in patients with the MS significantly reduced serum hs-CRP and insulin resistance compared with placebo. Especially, the

improvement of insulin resistance was significantly related to dose-dependent effect of N-3 PUFA.

Key Words: N-3 Polyunsaturated Fatty Acids, Metabolic Syndrome, Insulin Resistance, Triglyceride, C-reactive protein, tumor necrosis factor, interleukin.

Effects of N-3 Polyunsaturated Fatty Acids Supplement on Inflammatory Markers and Insulin Resistance in Patients with the Metabolic Syndrome

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1.Introduction

The metabolic syndrome (MS), which is cluster of multiple risk factors (hypertension, hypertriglyceridemia, low high-density lipoprotein cholesterol [HDL-C] and elevated fasting glucose) and insulin resistance,

has been considered to be an important predictor for type 2 diabetes and progression of cardiovascular disease. Increased fat tissue in whole body which is major pathogenesis of the MS may contribute to increase in inflammation and insulin resistance.[1, 2]

N-3 polyunsaturated fatty acid (PUFA) supplements have shown pleiotropic effects on a number of cardiovascular risk factors including blood pressure,[3] triglyceride (TG),[4] cardiac arrhythmia,[5] inflammation[6] and insulin resistance.[7] Among beneficial effects of N-3 PUFA, anti-inflammation[8, 9] and improvement of insulin resistance[10, 11] may play an important role in preventive or therapeutic insight for improvement of the MS components compared with life style modification. General recommendation dose of N-3 PUFA are 1 g/day for secondary prevention of myocardial infarction[12] and 2-4 g/day for triglyceride (TG) reduction.[13]

However, daily 6 g of N-3 PUFA supplement study in type 2 diabetes patients showed detrimental effects on blood glucose and insulin resistance.[14]

There are few studies in the MS regarding the effects of N-3 PUFA on systemic inflammation and insulin resistance. Furthermore, there are no studies to evaluate the effects by comparing two different N-3 PUFA doses on improvement of systemic inflammation and insulin resistance in the MS. We hypothesized that N-3 PUFA could reduce the systemic inflammation and insulin resistance in patients with the MS compared with placebo. We also evaluated the dose dependent effects of N-3 PUFA on improving systemic inflammation and insulin resistance by comparing 2 g/day (conventional dose) with 4 g/day (high dose) of N-3 PUFA.

2. Methods and Materials

2.1 Patients

Sixty patients (29 men and 31 women) in the outpatient clinic at the Department of Cardiology, Wonju Christian Hospital from October 1, 2007, to August 1, 2008 were requested for this randomized single-blind placebo-controlled trial. All patients were newly diagnosed the MS for the first time in our hospital. Exclusion criteria were known hypersensitivity to study drugs, the presence of diabetes mellitus, current infection, cancer, renal or liver disease and the use of lipid-lowering, anti-inflammatory, or glucose-lowering drugs. This study was approved by the Institutional Review Boards of Wonju College of Medicine, Yonsei University. Written informed consent was obtained from all subjects.

2.2 Methods

2.2.1. Study group and treatment

The MS was defined using the criteria of the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III,[14] with three or more of the following risk factors: waist circumference more than 90 cm in men or 80 cm in women, TG levels above 150 mg/dL, high-density lipoprotein cholesterol (HDL-C) levels lower than 40 mg/dL in men and 50 mg/dL in women, blood pressure higher than 130/85 mmHg, and fasting blood glucose levels higher than 110 mg/dL.

All subjects were randomly enrolled in N-3 PUFA (N=30) and placebo (N=30) groups that received an N-3 PUFA capsule (Omacor® eicosapentanoic acid, EPA 460mg + docosahexaenoic acid, DHA, 380mg, ProNova, Norway) or a matching placebo (corn oil capsules/day) for 12 weeks, respectively. We planned to treat our patients with daily dose of 2 and 4 g N-3 PUFA because daily N-3 PUFA dose ranging from 2 to 4 g

affected inflammatory markers and insulin sensitivity. Therefore, N-3 PUFA group consumed 2g/day (conventional dose) from baseline to 6 weeks and 4g/day (high dose) from 6 to 12 weeks (Fig. 1). Both groups were recommended to follow the diet and exercise program recommended by the standard protocol modified ATP-III guideline.[15]

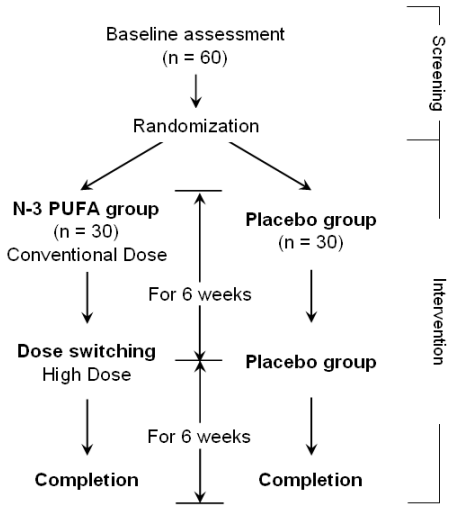

Fig. 1. Study flow diagram

PUFA: polyunsaturated fatty acid.

A complete physical examination, including weight, height, body mass index (BMI), waist circumference, blood pressure and pulse rate, was done at each period. Fasting blood was sampled at each period to assess serum levels of lipid and lipoprotein subclass profiles, inflammatory markers, fasting insulin and glucose. Insulin resistance was evaluated by the homeostasis model assessment of insulin resistance (HOMA–IR) equation.[15]

2.2.2. Anthropometric measurements

Body weight was measured to the nearest 10 g using a digital scale. Height was measured to the nearest 0.5 cm using a wall-mounted stadiometer. Waist circumference was taken as the smallest circumference between the lower costal margin and the pelvic brim measured to the nearest 0.5 cm.

Obesity was defined as BMI ≥ 25 according to the classification of obesity developed by the Japan Society for the Study of Obesity.[17] Systolic and diastolic blood pressure was determined as the mean of 2 consecutive measurements after a 5 minutes rest, with use of an automatic blood pressure monitor (Omron Corp. Kyoto, Japan) in the seated position.

2.2.3. Blood sampling and biochemistry

All subjects fasted for ≥12 h and were instructed to avoid exercise and alcohol consumption the day before blood sample collection. Venous blood samples were collected between 6 and 10 a.m. Blood samples were immediately processed and derived serum samples were either stored temporarily at 8 ◦C or stored at -80◦C: ultracentrifugation was performed to measure serum levels of HDL-C, low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), TG, blood urea nitrogen and creatinine,

while serum samples for analysis of fasting glucose, insulin, apolipoprotein (Apo)-AI and -B, hs-CRP, IL-6, TNF-α and small density LDL-C particle (SDLDLP) size were kept frozen at -80 ◦C. Frozen samples were transported to one of the local clinical laboratories (Seoul Clinical Laboratories, a College of American Pathologist certified laboratory in Seoul, Korea) and analyzed simultaneously.

Serum fasting insulin levels were measured using an immunoradiometric assay (Cobra II gamma counter, Packard, IL, USA) and fasting serum glucose levels using an enzymetric method (ADVIA 1650, Siemens, USA). The inter-assay coefficients of variation (CV) were 6.5 % at 14.4 μIU/mL and 6.1 % at 100.4 μIU/mL for insulin, and 2.2 % at 74.7 mg/dL for glucose. Fasting insulin and glucose concentrations were used to calculate homeostasis model assessment values. Apo-AI and Apo-B levels were

measured using an immunoturbidimetric method (Cobas integra 800, Roche, Swiss). The inter-assay CVs were 2.4 % at 88 mg/dL for Apo-AI and 2.9 % at 80 mg/dL for Apo-B. We calculated the Apo-B/AI ratio because the MS is associated with a high Apo-B/AI ratio.[18] Serum IL-6 and TNF-α levels were measured using enzyme immunoassays (Molecular Devices V-Max 220 VAC ELISA reader, USA). The inter-assay CVs were 6.5 % at 5.53 pg/mL for IL-6 and 7.4 % at 20.3 pg/mL for TNF-α. SDLDLP size was measured using a polyacrylamide gel electrophoresis based method (LIPOPRINT SYSTEM^{TM}, Quantimetrix, USA).

2.2.4. Statistical analysis

All data were analyzed using the SPSS statistical package, version 12.0 (SPSS Inc., Chicago, IL, USA). Continuous variables were compared by using Student's *t*-tests for independent samples. Differences in proportion

were compared using a Pearson's Chi-square test. Lipid and sub-fractions, inflammatory markers and insulin resistance profiles were expressed as mean ± standard error of mean (S.E.M.). A repeated measures analysis of variance (ANOVA) was used to analyze the intergroup differences between N-3 PUFA and placebo group and dose-dependent effects of N-3 PUFA in lipid and lipoprotein subclass, inflammatory markers and insulin resistance profiles. A p-value of < 0.05 was considered to indicate statistical significance.

3. Results

Fifty-three subjects (26 N-3 PUFA group and 27 placebo group) completed this study. Seven patients were dropped out from the study. Reasons for withdrawal were unwillingness to continue the study (3 in the N-3 PUFA group and 2 in the placebo group) and loss to follow-up (1 in

the N-3 PUFA group and 1 in the placebo group). Demographic findings showed no significant differences in physical profiles, obesity proportion and vital sign (Table 1).

Table 1. Baseline characteristics of study patients

	N-3 PUFA (n = 26)	Placebo (n = 27)	p
Age (years)	56.8 ± 2.0	59.2 ± 1.9	0.39
Male (%)	17 (65.4)	12 (44.4)	0.08
Obesity (≥ 25 BMI, %)	17 (65.4)	21 (77.8)	0.11
Weight (kg)	72.2 ± 2.4	72.4 ± 2.4	0.98
WC (Inch)	37.7 ± 0.6	37.9 ± 0.6	0.94
Body mass index (kg/m^2)	27.2 ± 0.7	28.1 ± 0.6	0.32
Systolic BP (mmHg)	137.5 ± 2.5	136.7 ± 2.1	0.98
Pulse rate (/min)	76.4 ± 2.6	79.3 ± 2.3	0.38

Data expressed as mean ± S.E.M. PUFA: polyunsaturated fatty acid. BMI: body mass index. WC: waist circumference. BP: blood pressure.

3.1. Changes of anthropometric profiles after N-3 PUFA administration

Compared to baseline, mean body weight between two groups (conventional dose vs. placebo: 71.9 ± 2.6 vs. 71.1 ± 2.5 kg, p = 0.82 / high dose vs. placebo: 71.8 ± 2.5 vs. 70.1 ± 2.1 kg, p = 0.54, Student's t-test) and BMI (conventional dose vs. placebo: 27.1 ± 0.8 vs. 27.9 ± 0.6 kg/m^2, p = 0.46 / high dose vs. placebo: 27.1 ± 0.8 vs. 27.7 ± 0.6 kg/m^2, p = 0.49, Student's t-test), were unchanged during study period.

Table 2. Mean lipid and lipoprotein subclass, TNF-α, IL-6, glucose and insulin levels during study periods

	Baseline	Conventional	High
TC (mg/dL)			
Placebo	200.3 ± 6.6	196.4 ± 2.7	203.2 ± 2.9
N-3 PUFA	196.3 ± 7.6	188.7 ± 3.3	185.7 ± 4.8
LDL-C(mg/dL)			
Placebo	118.1 ± 9.1	116.6 ± 6.4	113.2 ± 6.1
N-3 PUFA	110.2 ± 6.1	105.5 ± 7.3	110.6 ± 7.5
HDL-C (mg/dL)			
Placebo	43.2 ± 2.1	45.9 ± 2.4	44.3 ± 2.3
N-3 PUFA	43.6 ± 1.2	43.4 ± 3.2	40.3 ± 1.7

TG (mg/dL)			
Placebo	298.8 ± 12.9	285.4 ± 28.3	310.8 ± 32.3
N-3 PUFA*	335.1 ± 12.8	289.7 ± 27.9	254.4 ± 20.9
Apo-B/AI			
Placebo	0.78 ± 0.05	0.80 ± 0.06	0.80 ± 0.05
N-3 PUFA	0.82 ± 0.04	0.82 ± 0.05	0.83 ± 0.05
SDLDLP (nm)			
Placebo	25.92 ± 0.14	26.10 ± 0.12	25.95 ± 0.19
N-3 PUFA	25.81 ± 0.10	25.80 ± 0.13	25.79 ± 0.12
TNF-α (pg/dL)			
Placebo	0.95 ± 0.13	0.71 ± 0.10	0.74 ± 0.13
N-3 PUFA	0.96 ± 0.16	0.85 ± 0.12	0.92 ± 0.14
IL-6 (pg/mL)			
Placebo	1.15 ± 0.13	1.44 ± 0.19	1.60 ± 0.19
N-3 PUFA	1.93 ± 0.28	1.38 ± 0.23	1.11 ± 0.11
Glucose (mg/dL)			
Placebo	114.6 ± 4.5	110.5 ± 3.1	110.6 ± 3.7
N-3 PUFA	119.6 ± 5.3	117.7 ± 5.3	115.8 ± 5.2
Insulin (μIU/mL)			
Placebo	18.9 ± 3.2	13.6 ± 1.2	15.1 ± 1.3
N-3 PUFA*	19.7 ± 4.8	11.4 ± 0.7	9.9 ± 1.1

Data expressed as mean ± S.E.M. Significant intragroup differences (dose-dependent effect) are expressed as * ($p < 0.05$; repeated measures ANOVA). PUFA: polyunsaturated fatty acid. TC: total cholesterol. LDL-C: low-density lipoprotein cholesterol. HDL-C: high-density lipoprotein cholesterol. Apo: apolipoprotein. SDLDLP: small dense low-density

lipoprotein particle. TNF: tumor necrosis factor. IL: interleukin.

3.2. Effects of N-3 PUFA on serum lipid and lipoprotein subclass profiles

Mean serum TC, LDL-C, HDL-C, TG, Apo-B/AI and SDLDLP size during study period were not significantly affected by N-3 PUFA administration (Table 2). However, in N-3 PUFA group, TG reductions were significantly different between conventional and high dose of N-3 PUFA during study periods ($p = 0.001$, repeated measures ANOVA; Fig. 2).

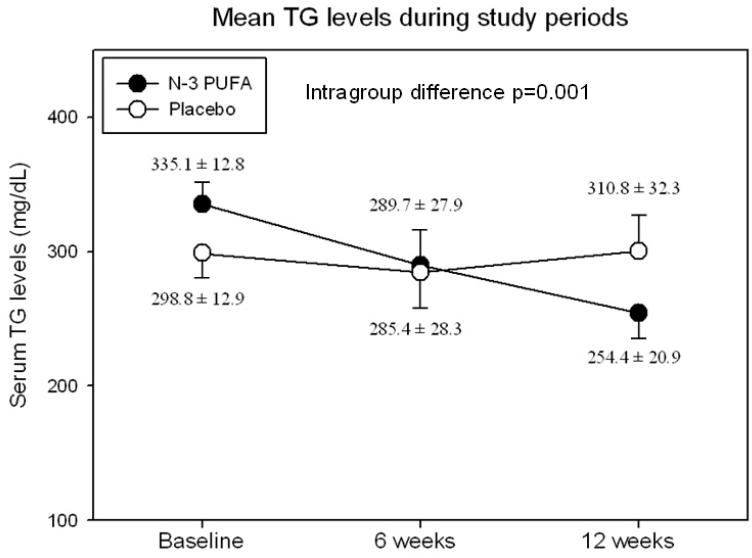

Fig. 2 Changes of TG levels during study periods. Data are mean ± S.E.M. Intragroup differences (dose-dependent effect) in N-3 PUFA group are analyzed by repeated measures ANOVA). PUFA: polyunsaturated fatty acid. TG: triglyceride.

However, mean percentage changes from baseline in TG after N-3 PUFA administration were significant compared with placebo (conventional dose: -27.4 ± 5.2 vs. 10.2 ± 12.1 %; p = 0.007, high dose: -35.3 ± 4.7 vs. 13.6 ± 10.1 %; p = 0.000, Student's t-test; Fig. 3).

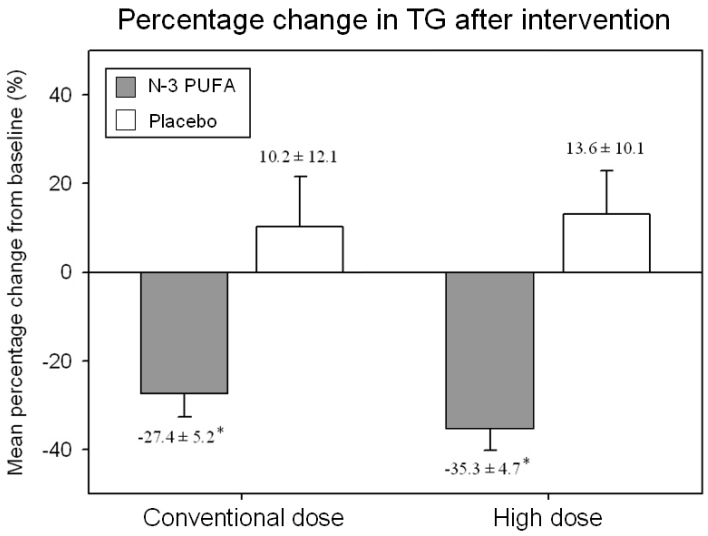

Fig. 3 Mean percentage change from baseline in TG. Data are mean ± S.E.M. Significant differences from placebo group are expressed as * ($p < 0.05$) using Student's t-test. PUFA: polyunsaturated fatty acid. TG: triglyceride.

3.3. Effects of N-3 PUFA on serum inflammatory markers

Mean hs-CRP levels were significantly reduced after N-3 PUFA administration ($p = 0.01$, repeated measures ANOVA; Fig 4) compared with placebo. However, there was no significant difference between conventional and high dose of N-3 PUFA in hs-CRP reduction in N-3

PUFA group (p = 0.33, repeated measures ANOVA). Mean TNF-α and IL-6 levels during study period were not significantly affected by N-3 PUFA administration compared with placebo (Table 2). Also, there were no significant TNF-α and IL-6 reduction irrespective of N-3 PUFA dose.

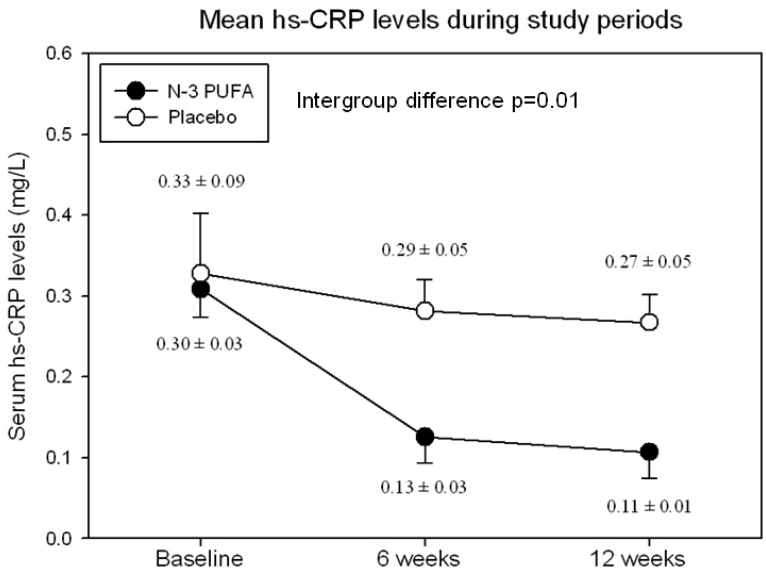

Fig. 4 Changes of hs-CRP levels during study periods. Data are mean ± S.E.M. Intergroup differences are analyzed by repeated measures ANOVA. PUFA: polyunsaturated fatty acid. hs-CRP: high-sensitive C-reactive protein.

N-3 PUFA administration showed significant mean percentage change from baseline in hs-CRP (conventional dose: -20.4 ± 12.3 vs. -1.4 ± 8.8 %; p = 0.04, high dose: -25.9 ± 10.7 vs. -2.1 ± 12.6 %; p = 0.02, Student's t-test; Fig. 5).

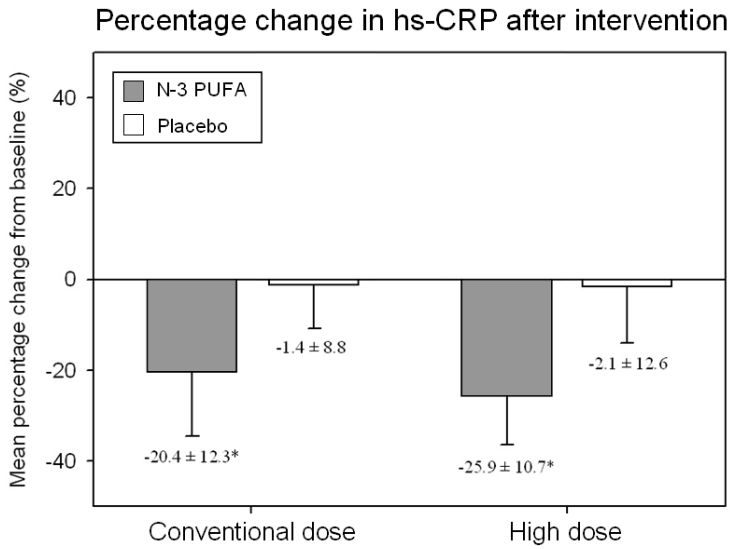

Fig. 5 Mean percentage change from baseline in hs-CRP. Data are mean ± S.E.M. Significant differences from placebo group are expressed as * ($p < 0.05$; Student's t-test). PUFA: polyunsaturated fatty acid. hs-CRP: high-sensitive C-reactive protein.

3.4. Effects of N-3 PUFA on insulin resistance

There was no significant change in fasting glucose during study periods (Table 2). However, mean HOMA-IR level (Fig. 6) were significantly reduced after N-3 PUFA administration ($p = 0.03$, repeated measures ANOVA) compared with placebo, and improvement of HOMA-IR in N-3 PUFA group significantly different between conventional and high dose of N-3 PUFA administration ($p = 0.000$, repeated measures ANOVA). Also, there was significant difference between conventional and high dose of N-3 PUFA on insulin reduction ($p = 0.01$, repeated measures ANOVA; Table 2).

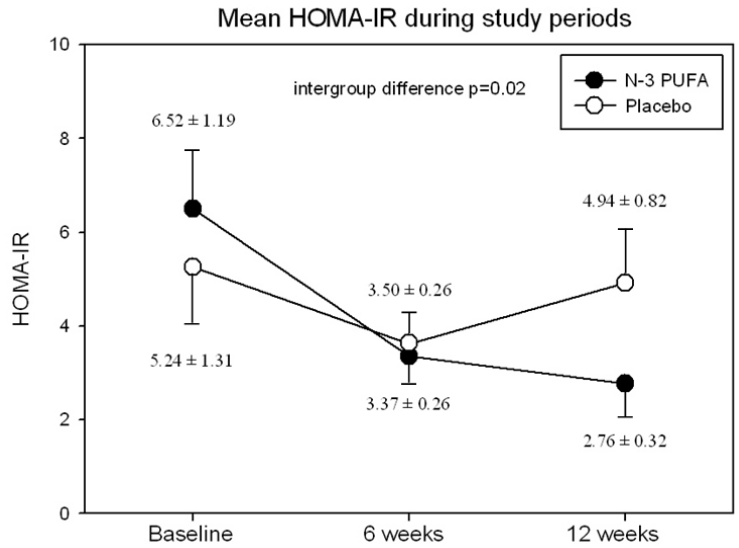

Fig. 6 Changes of HOMA-IR levels during study periods. Data are mean ± S.E.M. Intergroup and intragroup (dose-dependent effect) differences are analyzed by repeated measures ANOVA. PUFA: polyunsaturated fatty acid. HOMA-IR: homeostasis model assessment of insulin resistance.

3.5. Adverse drug reaction of N-3 PUFA

All adverse events were minor and equally distributed between the N-3 PUFA and placebo groups. Adverse events were reported by 5 patients in N-3 PUFA group (3 of dyspepsia, 2 of fishy smell eructation) and by 4

patients in the placebo group (4 of dyspepsia). These events were generally mild and resulted in none of discontinuation in the N-3 PUFA group.

4. Discussion

In this study, we investigated the effects of N-3 PUFA on improvement of systemic inflammation and insulin resistance compared with placebo in patients with the MS. Also we evaluated the dose-dependent effect of N-3 PUFA on systemic inflammation and insulin resistance by comparing conventional and high dose of N-3 PUFA. The major findings of present study have shown that N-3 PUFA administration reduces hs-CRP and HOMA-IR in patients with MS compared with placebo. Also, there were significant dose-dependent effects of N-3 PUFA on HOMA-IR reduction in N-3 PUFA group.

To our knowledge, this is the first study to evaluate the dose-dependent effect of N-3 PUFA on improvement of inflammatory markers and insulin resistance in the MS. Distinguished differences in our study, as compared to other studies as follows; first, we chose homogeneous subjects with frank MS who had abdominal obesity and high fasting serum TG. Second, we did not manipulate or enforce any weight reduction by physical exercise or diet restriction to investigate independent effects of N-3 PUFA in patients with the MS. Third, we performed N-3 PUFA dose switching at 6 weeks, which allowed us to assess the effects of treatment and time on inflammatory markers and insulin resistance.

The MS was originally proposed a set of clinical risk factors that could be potentially associated with development of cardiovascular disease and type 2 diabetes. Interventions consisting of either lifestyle modification or

pharmacological treatment, aimed at reducing the incidence of the MS, would provide a major therapeutic insight for the MS. In therapeutic strategies for the MS, life-style modifications including dietary restriction and physical activity encouragement are preferred at first rather than drug intervention. Recently, emerging evidences from animal and human studies suggest that N-3 PUFA can show beneficial effects on serum TG, inflammatory markers and insulin resistance which are pathophysiologic components of the MS.

Fasting TG-lowering effects are among the best established in clinical studies of N-3 PUFA. N-3 PUFA can reduce TG levels through not only promotion of hepatic fatty acid oxidation but also increase in endogenous lipoprotein lipase activity.[19] It can also inhibits two key enzymatic activities involved in TG synthesis, such as diacylglycerol acyltransferase

and phosphatidate phosphohydrolase.[20] At doses of 1–2 g/day, N-3 PUFA significantly lowered plasma TG levels approximately 26 %.[21] And this reduction in TG levels was markedly greater in hypertriglyceridemic than in normal subjects. A review of placebo-controlled human studies concluded that an average intake of daily 2-4 g of N-3 PUFA decreased serum TG levels by 25–30 % in a dose-dependent manner.[21] N-3 PUFA administration has been demonstrated to slightly elevate TC and LDL cholesterol levels.[22] Likewise previous studies, TG-lowering effect of N-3 PUFA in our study showed significant difference between conventional and high dose of N-3 PUFA. However, there were no significant changes on serum TC, LDL-C, HDL-C and TG after N-3 PUFA administration in our study despite significant percentage change from baseline in TG.

Chronic inflammation in the MS is characterized by the increased

production of inflammatory cytokines (IL, TNF-α and CRP), arachidonic acid-derived eicosanoids (prostaglandins, thromboxanes and leukotrienes) and other inflammatory mediators (cyclooxygenase-2 and NF-kB).[23] Anti-inflammatory effects of N-3 PUFA have been widely studied for several chronic inflammatory diseases. N-3 PUFA can demonstrate anti-inflammatory effects directly by replacing arachidonic acid as an eicosanoid substrate and inhibiting arachidonic acid metabolism and indirectly by altering the expression of inflammatory genes through effects on transcription factor activation.[8] Some N-3 PUFA studies in healthy or overweight subjects,[24] in healthy postmenopausal women[25] and in overweight women[26] have demonstrated beneficial effects of N-3 PUFA on improving circulating concentration of inflammatory markers such as CRP, TNF-α and IL-6. However, several randomized controlled studies in

obesity men,[27] in overweight women[28] and in hypertensive type 2 diabetes[29] have failed to show beneficial effects on inflammatory markers. Our findings in inflammatory markers showed significant reduction in mean hs-CRP levels after N-3 PUFA administration compared with placebo group. On the other hand, there were no significant reduction in TNF-α and IL-6 levels.

In insulin resistance, increased deposit and hypertrophy of adipose tissue is a possible key mechanism that initiates insulin resistance in the MS. Hypertrophy of adipocyte leads to progressive accumulation of fat tissue to other organ, and excess of fat tissue activates glucose uptake by hyperinsulinemia.[30] N-3 PUFA supplement can normalize or improve fat tissue storage and promote glucose utilization by modulating insulin action and secretion.[31] This mechanism may be explained by activation of

peroxisome proliferator-activated receptors-γ2 (PPAR-γ2) isoform expression and pyruvate dehydrogenase complex activity by N-3 PUFA. This PPAR-γ2 activation in adipose tissue can promote hepatic glucose storage and glucose utilization in peripheral tissues. This fact suggests that N-3 PUFA may play an important role in insulin action. However, the most of N-3 PUFA intervention studies in healthy individual,[32] in type 2 diabetes women,[33] and in overweight women[25] failed to provide clear evidence for improvement of insulin resistance However, our results demonstrated that N-3 PUFA administration significantly reduced HOMA-IR compared with placebo. Also improvement of HOMA-IR was related to dose-dependent effect of N-3 PUFA.

We can consider several possibilities to account for our study findings in the MS patients. First, our study population is consisted of frank MS

patients and the most of them have abdominal obesity and hypertriglyceridemia. Increased abdominal fat tissue and circulating free fatty acid levels can stimulate inflammatory cytokine production by adipose tissue.[33] Because N-3 PUFA administration can reduce a further fat tissue accumulation as well as free fatty acid release in the MS, these effects of N-3 PUFA may result in significant reduction of inflammatory markers in our study. Second, increased fat tissue can trigger insulin resistance through increased glucose uptake by hyperinsulinemia in the MS. Thus, we speculate that the effects of N-3 PUFA on improvement of insulin resistance in the MS may be associated with N-3 PUFA mediated hyperinsulinemia reduction. However, it is not clear whether improvement of insulin resistance is associated with anti-inflammatory effects of N-3 PUFA in our study.

This study was prospective, randomized, placebo controlled and single-blind designed study. Limitations of our study include the relatively small sample size.

5. Conclusions

We evaluated not only N-3 PUFA mediated reduction of systemic inflammation and insulin resistance but also dose-dependent effects of N-3 PUFA by comparing conventional with high dose of N-3 PUFA in patients with the MS.

Main findings of this study as follows,

1. Effect of N-3 PUFA on mean TG reduction in the MS was not significant compared with placebo group. However, TG reductions in N-3 PUFA group showed significant dose-dependent effect of N-3 PUFA ($p = 0.001$, repeated measures ANOVA).

2. N-3 PUFA administration significantly lowered mean hs-CRP compared with placebo group ($p = 0.01$, repeated measures ANOVA). There was no significant dose-dependent effect of N-3 PUFA on mean hs-CRP reduction.

3. Improvements of mean HOMA-IR after N-3 PUFA administration were significant compared with placebo group ($p = 0.03$, repeated measures ANOVA). Also there was significant dose-dependent effect of N-3 PUFA on mean HOMA-IR in N-3 PUFA group ($p = 0.000$, repeated measures ANOVA).

In conclusion, N-3 PUFA administration in patients with the MS showed significant reduction in serum hs-CRP and insulin resistance compared with placebo. In addition, the improvement of insulin resistance was significantly related to dose-dependent effect of N-3 PUFA.

References

1. Rossi R, Nuzzo A, Origliani G, Modena MG. Metabolic syndrome affects cardiovascular risk profile and response to treatment in hypertensive postmenopausal women. Hypertension 52:799–800, 2008.

2. Kressel G, Trunz B, Bub A, Hülsmann O, Wolters M, Lichtinghagen R, Stichtenoth DO, Hahn A. Systemic and vascular markers of inflammation in relation to metabolic syndrome and insulin resistance in adults with elevated atherosclerosis risk. Atherosclerosis 202:263–71, 2009.

3. Taouis M, Dagou C, Ster C, Durand G, Pinault M, Delarue J. N-3 polyunsaturated fatty acids prevent the defect of insulin receptor signaling in muscle. Am J Physiol Endocrinol Metab 282:E664 –71, 2002.

4. Holness MJ, Greenwood G, Smith N, Sugden M. Diabetogenic impact of long-chain ω-3 fatty acids on pancreatic β-cell function and the

regulation of endogenous glucose production. Endocrinology 144:3958–68, 2003.

5. Geleijnse JM, Giltay EJ, Grobbee DE, A.Donders RT, Kok FJ. Blood pressure response to fish oil supplementation: metaregression analysis of randomized trials. J. Hypertens 20:1493–9, 2002.

6. Harris WS. N-3 Fatty acids and lipoproteins: comparison of results from human and animal studies. Lipids 31:243–52, 1996.

7. von Schacky C. Omega-3 fatty acids: antiarrhythmic, proarrhythmic or both? Curr Opin Clin Nutr Metab Care 11:94–9, 2008.

8. Calder PC. N-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases. Am J Clin Nutr 83:1505S–19S, 2006.

9. Delarue J, Couet C, Cohen R, Bréchot JF, Antoine JM, Lamisse F. Effects of fish oil on metabolic responses to oral fructose and glucose

loads in healthy humans. Am J Physiol 270:E353–62, 1996.

10. Robinson LE, Buchholz AC, Mazurak VC. Inflammation, obesity, and fatty acid metabolism: influence of n-3 polyunsaturated fatty acids on factors contributing to metabolic syndrome. Appl Physiol Nutr Metab 32:1008–24, 2007.

11. Schubert R, Kitz R, Beermann C, Rose MA, Baer PC, Zielen S, Boehles H. Influence of low-dose polyunsaturated fatty acids supplementation on the inflammatory response of healthy adults. Nutrition 23:724–30, 2007.

12. Van de Werf F, Bax J, Betriu A, Blomstom-Lundqvist C, Crea F, Falk V, Filippatos G, et al. Management of acute myocardial infarction in patients presenting with persistent ST-segment elevation. Eur Heart J 29:2909–45, 2008.

13. Simopolous AP, Leaf A, Salem N. Essentiality and recommended dietary intakes for omega-6 and omega-3 fatty acids. Ann Nutr Metab 43:127–30, 1999.

14. Dunstan DW, Mori TA, Puddey IB, Beilin LJ, Burke V, Morton AR, Stanton KG. The independent and combined effects of aerobic exercise and dietary fish intake on serum lipids and glycemic control in NIDDM. A randomized controlled study. Diabetes care 20:913–21, 1997.

15. Third report of the national cholesterol education program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (adult treatment panel III) final report. Circulation 106:3143–421, 2002.

16. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-

cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–9, 1985.

17. Matsuzawa Y. New Criteria for 'Obesity Disease' in Japan: The Examination Committee of Criteria for 'Obesity Disease' in Japan, Japan Society for the Study of Obesity. Circ J 66:987–92, 2002.

18. Sierra-Johnson J, Somers VK, Kuniyoshi FHS, Garza CA, Isley WL, Gami AS, Lopez-Jimenez F. Comparison of apolipoprotein-B/apolipoprotein-AI in subjects with versus without the metabolic syndrome. Am J Cardiol 98:1369–73, 2006.

19. Harris WS. N-3 fatty acids and serum lipoproteins: human studies. Am J Clin Nutr 65: 1645S–54S, 1997.

20. Harris WS, Bulchandani D. Why do omega-3 fatty acids lower serum triglycerides? Curr Opin Lipidol 17:387–93, 2006.

21. Ryan AS, Keske MA, Hoffman JP, Nelson EB. Clinical overview of algal-docosahexaenoic acid: Effects on triglyceride levels and other cardiovascular risk factors. American Journal of Therapeutics 16:183–92, 2009.

22. Mori TA, Watts GF, Burke V, Hilme E, Puddey IB, Beilinet LJ. Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. Circulation 102:1264–9, 2000.

23. Bastard JP, Maachi1 M, Lagathu1 C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B. Recent advances in the relationship between obesity, inflammation, and insulin resistance. Eur Cytokine Netw 17:4–12, 2006.

24. Robinson LE, Buchholz AC, Mazurak VC. Inflammation, obesity, and fatty acid metabolism: influence of n-3 polyunsaturated fatty acids on

factors contributing to metabolic syndrome. Appl Physiol Nutr Metab 32:1008–24, 2007.

25. Browning LM, Krebs JD, Moore CS, Mishra GD, O'Connell MA, Jebb SA. The impact of long chain n-3 polyunsaturated fatty acid supplementation on inflammation, insulin sensitivity and CVD risk in a group of overweight women with an inflammatory phenotype. Diabetes, Obesity and Metabolism 9:70–80, 2007.

26. Ciubotaru I, Lee YS, Wander RC. Dietary fish oil decreases C-reactive protein, interleukin-6, and triacylglycerol to HDL-cholesterol ratio in postmenopausal women on HRT. J Nutr Biochem 14:513–21, 2003.

27. Jellema A, Plat J, Mensink RP. Weight reduction, but not a moderate intake of fish oil, lowers concentrations of inflammatory markers and PAI-1 antigen in obese men during the fasting and postprandial state. Eur J

Clin Invest 34: 766–73, 2004.

28. Krebs JD, Browning LM, McLean NK, Rothwell JL, Mishra JD, Moore CS, Jebb SA. Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinemic women. Int J Obes 30:1535–44, 2006.

29. Mori TA, Woodman RJ, Burke V, Puddey IB, Croft KD, Beilin LJ. Effect of eicosapentaenoic acid and docosahexaenoic acid on oxidative stress and inflammatory markers in treated-hypertensive type 2 diabetic subjects. Free Radic Biol Med 35: 772–81, 2003.

30. Laclaustra M, Corella D, Ordovas JM. Metabolic syndrome pathophysiology: The role of adipose tissue. Nutrition, Metabolism & Cardiovascular Disease 17:125–39, 2007.

31. Neschen S, Moore I, Regittnig W, Yu CL, Wnag Y, Pypaert M, et al.

Contrasting effects of fish oil and safflower oil on hepatic peroxisomal and tissue lipid content. Am J Physiol Endocrinol Metab 282:E395–401, 2002.

32. Giacco R, Cuomo V, Vessby B, Uusitupa M, Hermansen K, Meyer BJ, Riccardi G, Rivellese AA. KANWU Study Group. Fish oil, insulin sensitivity, insulin secretion and glucose tolerance in healthy people: Is there any effect of fish oil supplementation in relation to the type of background diet and habitual dietary intake of n-6 and n-3 fatty acids? Nutrition, Metabolism & Cardiovascular Diseases 17:572–80, 2007.

33. Kabir M, Skurnik G, Naour N, Pechtner V, Meugnier E, Rome S, et al. Treatment for 2 mo with n 3 polyunsaturated fatty acids reduces adiposity and some atherogenic factors but does not improve insulin sensitivity in women with type 2 diabetes: a randomized controlled study. Am J Clin Nutr 86:1670–9, 2007.

34. Lionetti L, Mollica MP, Lombardi A, Cavaliere G, Gifuni G, Barletta A. From chronic overnutrition to insulin resistance: The role of fat-storing capacity and inflammation. Nutrition, Metabolism & Cardiovascular Diseases 19:146–52, 2009.

35. de Roos B, Mavrommatis Y, Brouwer IA. Long-chain n-3 polyunsaturated fatty acids: new insights into mechanisms relating to inflammation and coronary heart disease. British Journal of Pharmacology doi:10.1111/j.1476–5381 (Published online) 2009.

국문 요약

대사 증후군 환자에서 N-3 다중 불포화 지방산 투여가 염증 표지 인자와 인슐린 저항성에 미치는 영향에 대한 연구

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최 현 민

대사 증후군 환자에서 N-3 다중 불포화 지방산 투여는 전신 염증과 인슐린 저항성의 호전을 통하여 유익한 효과를 나타낼 수 있다. N-3 다중 불포화 지방산의 하루 용량은 심근경색증의 이차예방에서 1 g, 중성지방 감소에서 2-4 g 이 권장되고 있다. 대사 증후군 환자에서 N-3 다중 불포화 지방산의 염증 표지 인자와 인슐린 저항성에 대한 효과에 관한 자료는 거의 없으며

용량 의존 효과에 대한 연구도 시도된 적이 없었다. 이번 연구는 대사 증후군 환자에서 N-3 다중 불포화 지방산이 전신 염증과 인슐린 저항성에 줄일 수 있는지 평가하였고 일반용량 (하루 2 g)과 고 용량 (하루 4 g)의 N-3 다중 불포화 지방산 효과를 비교하여 용량 의존 효과도 평가하였다.

60명의 대사 증후군 환자들이 무작위로 N-3 다중 불포화 지방산 투여 군과 위약 투여 군으로 배정되었다. 53명 (N-3 다중 불포화 지방산 투여군 26명, 위약 투여군 27명)이 연구를 완료하였다. N-3 다중 불포화 지방산 투여 군은 6주 동안 하루 2 g (일반 용량)을 복용하고 이후 나머지 6주 동안 하루 4 g (고 용량)을 복용하였다. 우리는 각 약물 치료 시점 동안 두 군간 혈청 지질 및 하위 지단백 지표, 염증 표지자 그리고 인슐린 저항성 지표를 비교하였다. 위약 군과 비교하여 평균 고 민감도

C-반응 단백질 (p = 0.01, 반복측정 분산분석)과 HOMA-IR (p = 0.03, 반복측정 분산분석) 수치가 N-3 다중 불포화 지방산의 투여 후 유의한 감소를 보였다. N-3 다중 불포화 지방산에 의한 중성지방 (p = 0.001, 반복측정 분산분석)과 HOMA-IR (p = 0.000, 반복측정 분산분석)의 감소는 유의한 용량 의존 효과를 보여주었다. 하지만 고 민감도 C-반응 단백질에서는 N-3 다중 불포화 지방산에 의한 용량 의존 효과는 없었다.

결론적으로 N-3 다중 불포화 지방산의 투여는 대사 증후군 환자에서 고 민감도 C-반응 단백질과 인슐린 저항성을 감소시키는데 효과적이었다. 특히, N-3 다중 불포화 지방산에 의한 인슐린 저항성의 호전은 유의한 용량 의존 효과를 보여주었다.

핵심 되는 말: N-3 다중 불포화 지방산, 대사 증후군, 인슐린 저항성, 중성 지방, C-반응 단백질, 종양 괴사 인자, interleukin.

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