Original Article

The effect of n–3 PUFA/γ-cyclodextrin complex on serum lipids in healthy volunteers - a randomized, placebo-controlled, double-blind trial

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Objectives: This study was carried out to examine whether serum triglyceride concentrations were decreased by administration of n–3 polyunsaturated fatty acid (PUFA)/γ-cyclodextrin (γ-CD) complex-containing capsules as reported previously with n–3 PUFA without γ-CD.

Study Design: A placebo-controlled double-blind study with healthy subjects (n=35) and hypertriglyceridemic subjects (n=7) of 35-66 years of age was performed. The subjects were randomized to a group (n–3 group) supplemented with n–3 PUFA/γ-CD-containing capsules (660 mg EPA + 280 mg DHA/day) or a control group supplemented with capsules containing essentially no n–3 PUFA for 8 weeks with stratification by sex, age, and serum triglyceride levels in a double blind manner. Fasting blood samples were obtained at the start of administration and 4 and 8 weeks afterward.

Results: EPA concentrations in the total phospholipid fraction of red blood cells increased significantly in all subjects in the n–3 group, whereas no changes were seen in the control group. Triglyceride levels were significantly decreased (–17%) in the n–3 group compared with the control group at week 8. The following serum lipids did not significantly change over time: total-cholesterol, low-density lipoprotein-cholesterol and high-density lipoprotein-cholesterol. Only two subjects in the n–3 group guessed at the end of the study that their capsules were active.

Conclusion: n–3 PUFA/γ-CD complex lowered triglyceride levels in normal and slightly hypertriglyceridemic subjects. There was a possibility that γ-CD might at least partly cover the smell and aftertaste of fish oil.

Key Words: EPA, n–3 polyunsaturated fatty acids, γ-cyclodextrin, fatty acid composition, triglycerides

INTRODUCTION

Many intervention trials with fish oils have been performed, and the effects on serum triglyceride levels are now well-known.[1] However, after-taste of fish oil and/or fish smell are not acceptable to some people.

γ-Cyclodextrin (γ-CD) is a cyclic compound made of 8 alpha-(1,4)-linked glucose molecules. Because the inner side of the molecule is lipophilic compared with the outer, γ-CD may be used as a carrier/stabilizer for polyunsaturated fatty acids (PUFA).[2] A double-blind, placebo-controlled, cross-over study in 24 healthy human volunteers showed that a single dose of 8g γ-CD did not show any difference in gastrointestinal tolerance from 8g maltodextrin.[3] Studies even with β-CD, a non-digestible 7-glucosed cyclodextrin, have shown that the bioavailability of fat-soluble vitamins is not impaired in animals.[4,5] It is, therefore, expected that the bioavailability of n–3 PUFA is not reduced with γ-CD. In the present study, we compared the effects of γ-CD-treated n–3 PUFA with placebo in a double-blind manner.

MATERIALS AND METHODS

Subjects

Fifty-two apparently healthy employees working at a local company were invited to the present study. Those subjects were excluded whose triglyceride levels were not less than 3.4 mmol/L or total cholesterol levels were not less than

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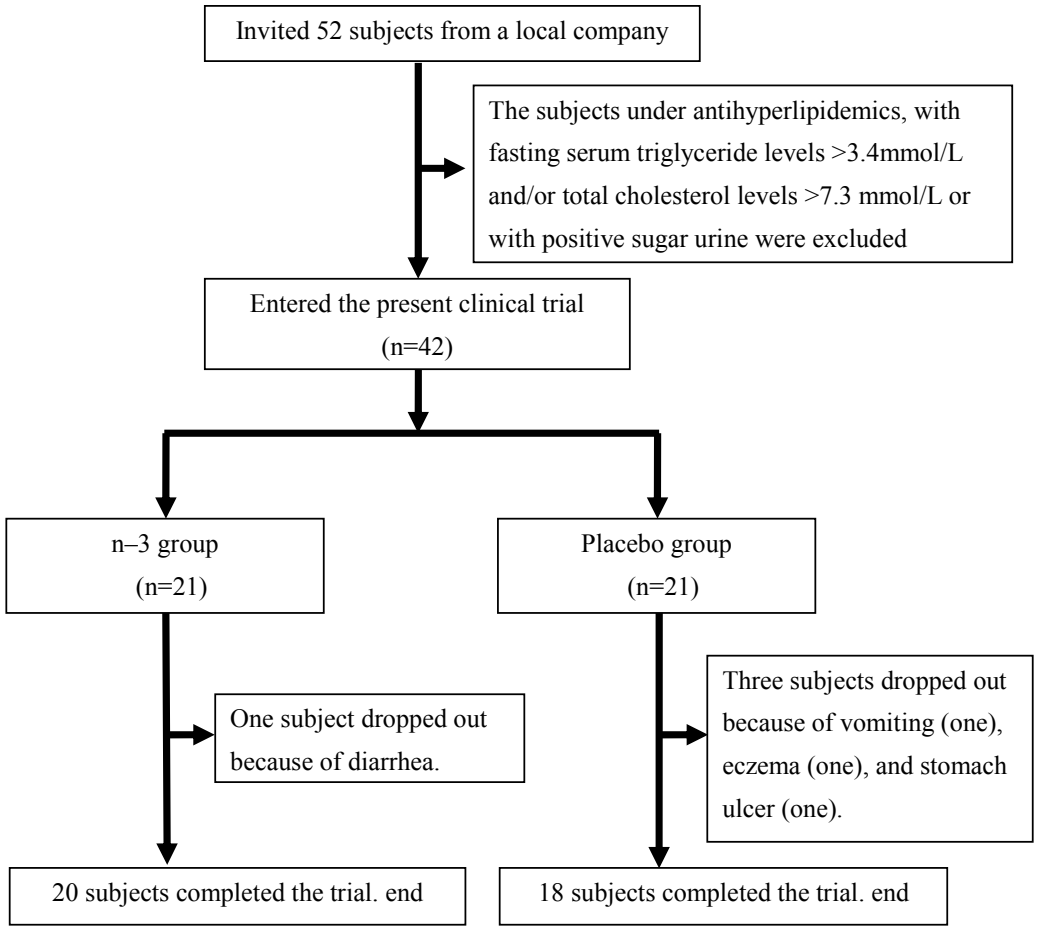

Figure 1. Flow chart of study subjects

7.3 mmol/L. Those with positive urine sugar or under hypolipidemic therapy were also excluded. Finally forty-two entered the study (Fig 1). They were 21 males and 21 females (49±8 years old).

Study design

The present study was a randomized, placebo-controlled, double-blind trial of 8 weeks. Subjects (n=42) were allocated either to a group consuming n–3 PUFA/γ-CD complex (n–3 group) or to a placebo group. Subjects in the n–3 group (n=21) consumed 12 active capsules/day. Twelve active capsules contained γ-CD-treated tuna-head meal fortified with 660mg EPA and 280mg DHA. The other subjects (the placebo group, n=21) consumed 12 placebo capsules containing a placebo oil mixture (47% olive oil, 25% soybean oil, 25% colza oil and 3% fish oil) and lactose in place of tuna-head meal. The ingredients of the two capsules are shown in Table 1. Subjects were asked to maintain their body weights and physical activity during the study period, and to take a usual meal. Fasting blood samples were collected before the start of administration and at weeks 4, 8. At the end of the study, they were asked to complete another questionnaire about any side effects of the supplements, and to guess the nature of their capsules by selecting one from the following three: placebo, hard to tell and n–3 PUFA/γ-CD complex. This study was approved by the ethics committee of One K Ltd., and written informed consent was obtained from each participant.

Lipid analysis

Fasting blood samples were collected between 8 and 9 am. Serum concentrations of triglycerides, low-density lipoprotein (LDL)-cholesterol and high-density lipoprotein (HDL)-cholesterol were measured enzymatically.[6] Packed RBCs were obtained from EDTA-anticoagulated blood, washed twice with saline, added with butylated hydroxytoluene (0.05mg/mL), and frozen at –80°C until fatty acid analysis. The fatty acid composition of the total phospholipid fraction of RBCs (RBC PL) was determined as written elsewhere.[7] Briefly, the total lipids were extracted by the method of Bligh and Dyer; the total phospholipid fraction was separated by thin-layer chromatography and transmethylated with HCl-methanol. The fatty acid composition was analyzed by gas chromatography (GC14A Shimadzu Corporation, Kyoto) with a capillary column DB-225 (0.25mm, 30 m length id, 0.25 μM; J&W Scientific, Folsom, CA).

Statistical analysis

The results are expressed as means±SD. The fatty acid composition and serum lipids were analyzed by paired t test for intra-group comparison and analysis of covariance for inter-group comparison. Because in cases of serum lipids we were interested in differences from baseline, those differences were first analyzed by two-way analysis of variance (groups x sampling times). Further analysis was done only when two-way analysis of variance showed a significant difference. Chi-square test was used

for the comparison of subjects' guesses about the randomization status between two groups. Stat View-J 5.0 (Abacus Concepts Inc., Berkeley and CA) was used for the statistical analysis. A p value <0.05 was considered to indicate statistical significance.

RESULTS

Three subjects in the placebo group dropped out of the study because of vomiting, eczemas and stomach ulcer, respectively. Stomach ulcer appeared on day 2 of placebo administration. Therefore, it is unlikely that placebo capsules caused it. One subject in the n–3 group dropped out because of diarrhea. There were no significant differences at baseline between groups in age, sex ratio, BMI, blood pressure, and the ratios of smoker and hyperlipidemia; there were no diabetics (Table 2). At the end of the study the subjects guessed their allocated groups. Their guesses were essentially identical between groups (Table 3).

The changes in the fatty acid composition in RBC are shown in Table 4. EPA and n–3 docosapentaenoic acid concentrations increased significantly in the n–3 group, but there was no significant difference in DHA. Moreover, linoleic acid concentration decreased significantly in the n–3 group. On the other hand, significant changes were not observed in the control group.

Triglyceride levels decreased significantly at week 8 in the n–3 group compared with the changes in the control group although there were no significant changes at week 4 (Table 4). The net decrease of triglyceride levels (the difference observed between the placebo and n–3 groups) was –27% (–10–17= –27). Triglyceride levels decreased by 40% at week 8 in the n–3 subjects whose triglyceride levels were more than 1.1 mmol/L at baseline. There were no significant changes over time in the other serum lipids; namely, total cholesterol, HDL cholesterol and LDL cholesterol (Table 5).

Table 1. Macronutrient composition of capsules

	Group	
	n–3	Placebo
Energy (kcal/day)	25.6	25.2
Protein (g/day)	0.2	0.0
Carbohydrate (g/day)	0.5	1.0
Fat (g/day)	2.5	2.4
γ-Cyclodextrin (g/day)	0.38	0.0
Oleic acid (g/day)	0.05 (1.9%)	1.28 (54.2%)
Linoleic acid (g/day)	0.80 (31.5%)	0.64 (27.1%)
EPA (g/day)	0.66 (26.0%)	ND
DHA (g/day)	0.28 (11.0%)	ND

Subjects took 12 capsulels per day. ND: not detected.

Table 2. Baseline characteristics of the randomized subjects

	Group	
	n–3 (n=20)	Placebo (n=18)
Age (yr)	48.5 ± 7.8	48.4 ± 7.7
Men/Women (n)	10 / 10	9 / 9
Body mass index (kg/m^2)	22.3 ± 2.7	23.5 ± 3.2
Blood pressure		
Systolic (mmHg)	145 ± 22	153 ± 28
Diastolic (mmHg)	88 ± 11	92 ± 19
Total cholesterol (mmol/l)	5.71 ± 0.78	5.34 ± 0.65
Triglyceride (mmol/l)	1.05 ± 0.63	1.07 ± 0.52
Diabetes (n)	0	0

There were no significant differences between groups.

Table 3. Guess of randomization status by subjects

	Guess		
Group	n–3 PUFA/γ-CD	Hard to tell	Placebo
n–3	2	4	14
Placebo	2	4	12

There was no significant difference between groups (p=0.98).

DISCUSSION

In the n–3 group, TG levels were decreased (the net effect = –27%) whereas the other lipid levels (total cholesterol and HDL-cholesterol) did not change. This was very similar to the combined results from the majority of the previous placebo-controlled, cross-over, or parallel design studies providing less than 7g n–3 fatty acids.[1] In our previous placebo-controlled, double-blind study with a very similar dose of n–3 fatty acids (600mg EPA and 260mg DHA), we found the net effect of TG reduction of –19% at week 8; namely, average TG levels were decreased from 1.8 to 1.5mmol/L (–20%), and 1.6 to 1.6mmol/L (–1%) in the active and control groups, respectively.[8] Those TG reducing effects of our present and previous studies were more or less similar to the net effect (–25%) obtained from the 281 subjects whose TG levels were less than 2.0 mmol/L.[1] The net increase in the percentage of EPA+docosapentaenoic acid+DHA in RBCs in the present study was +1.6% (Table 3). That of the previous study was +1.4% at week 12.[8] Combined together, it is unlikely that the addition of γ-CD to n–3 fatty acids decreased the bioavailability of those fatty acids, although our present study was not designed to see the differences in n–3 fatty acid increasing and TG decreasing effects between with and without γ-CD.

According to Artiss *et al*,[9] a group of rats fed a high-fat diet (40 % fat, wt/wt) with α-CD (10% of the fat, wt/wt) had significantly lower body weights, lower plasma lipid levels, including triglycerides and cholesterol, and higher fat contents in their feces than a control group of rats on a high-fat diet without α-CD. Is there a possibility that γ-CD used in the present study influenced the serum triglyceride levels? The answer is probably negative, because γ-CD is rapidly and completely digested by amylase, and will disappear from the digesta.[2] It is likely that the rapid clearance of γ-CD and reversible formation of inclusion complexes do not interfere with the absorption of fat and fat-soluble vitamins.[2]

The safety of γ-CD in the short, medium and long-term was well studied. The acute toxicity of γ-CD was examined in mice and rats receiving single doses. On oral administration, no deaths occurred at γ-CD levels of 15 g/kg [10] or 16 g/kg [11] body weight in mice and 8 g/kg body weight in rats.[11] Medium-term oral toxicity studies with γ-CD were conducted for 2-13 weeks in rats[12] and for 3 months in dogs.[13] Diets containing up to 20 weight % γ-CD or 20% lactose were added at the expense of starch.

Table 4. Changes in the fatty acid composition (area%) of phospholipids in red blood cells

Fatty acid	n–3 (n=20)		Placebo (n=18)		Intergroup comparison
	Week 0	Week 8	Week 0	Week 8	
16 : 0	25.9 ± 1.0	25.0 ± 0.7	25.7 ± 1.1	25.1 ± 1.1	NS
18 : 0	13.9 ± 0.6	13.4 ± 0.5	13.8 ± 0.9	13.3 ± 1.3	NS
18 : 1 n–9	14.0 ± 0.7	14.0 ± 0.8	14.1 ± 0.7	14.4 ± 0.8	NS
18 : 2 n–6	9.5 ± 1.2	8.9 ± 1.3 *	9.8 ± 1.2	9.5 ± 1.0	0.034
20 : 4 n–6 (AA)	9.7 ± 1.2	9.8 ± 1.2	10.2 ± 1.1	10.5 ± 1.0	0.12
20 : 5 n–3 (EPA)	2.1 ± 0.9	3.2 ± 0.9 **	1.9 ± 0.9	2.0 ± 0.8	< 0.0001
22 : 5 n–3	1.7 ± 0.3	2.3 ± 0.2 **	1.7 ± 0.3	2.0 ± 0.3	< 0.0001
22 : 6 n–3 (DHA)	7.0 ± 1.1	7.6 ± 0.7 †	7.0 ± 0.9	7.3 ± 1.0	0.057

There were no significant differences in baseline values between groups in any fatty acids. Intragroup comparison; †p<0.1, *p<0.05, **p<0.001. NS: not significant.

Table 5. Changes in serum lipid concentrations

	Week 0	Week 4	Week 8
Triglycerides (mmol/l)			
n–3	1.05 ± 0.63	0.91 ± 0.34	0.88 ± 0.34 *
Placebo	1.07 ± 0.52	1.18 ± 0.64	1.16 ± 0.52
Total-cholesterol (mmol/l)			
n–3	5.71 ± 0.78	5.85 ± 0.66	5.56 ± 0.63
Placebo	5.34 ± 0.65	5.59 ± 0.67	5.33 ± 0.81
HDL-cholesterol (mmol/L)			
n–3	1.9 ± 0.5	1.9 ± 0.5	1.8 ± 0.5
Placebo	1.8 ± 0.5	1.9 ± 0.6	1.8 ± 0.6
LDL-cholesterol (mmol/L)			
n–3	3.4 ± 0.6	3.5 ± 0.5	3.3 ± 0.5
Placebo	3.0 ± 0.6	3.2 ± 0.7	3.0 ± 0.8

There were no significant differences between groups in baseline values of any lipids. *p<0.05 (between groups)

The ingestion of γ-CD was well tolerated. In long-term experiments it was also showed that diets including γ-CD up to 20 % were generally safe in rats.[12, 14] γ-CD was not embryotoxic/teratogenic up to 20 % in rats [15] or in rabbits.[16] The safety of γ-CD was summarized in a review written by Munro.[2] The dose of γ-CD in our study was only 380 mg/day, about 3 orders of magnitude lower than the doses used in the experiments written above. We, therefore, think that the dose used in our study was very safe.

One of the most interesting points of the present study was that the subjects who guessed their allocated capsules as those with n–3 fatty acids were only 10% of all the subjects in the n–3 group (Table 3). This finding suggests that γ-CD might have reduced fish smell and after-taste of the active food.

In conclusion, n–3 PUFA/γ-CD complex lowered triglyceride levels in normal and slightly hypertriglyceridemic subjects. There was a possibility that γ-CD might at least partly cover the smell and aftertaste of fish oil.

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Original Article

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n–3 PUFA/γ-Cyclodextrin 複合物對健康自願者血脂影響之隨機安慰劑控制雙盲試驗

目的：本研究測試是否給予 n–3 多不飽和脂肪酸(PUFA)/ γ 環糊精 (γ-CD)複合膠囊，會如同先前只有 n–3 PUFA 而沒有 γ-CD 的報告一樣，能降低血清三酸甘油酯濃度。

研究設計：一項安慰劑控制雙盲研究，以 35 位健康及 7 位高三酸甘油酯 35-66 歲受試為對象。以性別、年齡及血清三酸甘油酯分層後，受試者被隨機分配到 n–3 PUFA/γ-CD 複合膠囊(660 mg EPA + 280 mg DHA/day)組或是未含 n–3 PUFA 膠囊的控制組，進行 8 週的雙盲試驗。分別在開始、第 4 週及第 8 週取空腹血液樣本。

結果：n–3 組 受試者中的紅血球總磷脂質 EPA 濃度均顯著增加，但在對照組中則未見變化。在第 8 週時，n–3 組的甘油三酸酯比對照組顯著減少 (–17%)。以下血脂衛未時間顯著改變: 總膽固醇、低密度脂蛋白膽固醇 和高密度脂蛋白膽固醇。只有 2 個 n–3 組受試在研究終了猜測他們的膠囊含有效成分。

結論：n–3 PUFA / γ-CD 複合物降低正常及輕微高三酸甘油酯症受試者的三酸甘油酯。一個可能性是 γ-CD 至少部分掩蓋魚油的氣味和餘味。

關鍵字：EPA、N–3 多元不飽和脂肪酸、γ 環糊精、 脂肪酸組成、三酸甘油酯。