DOCTOR'S FORMULAS

ΦΟΡΜΟΥΛΕΣ ΥΓΕΙΑΣ

βιβλιογραφία

LIPOPROTECT

περιεχόμενα

LIPOPROTECT

ΦΟΡΜΟΥΛΑ
λιποπρωτεϊνών

για τη διατήρηση των υγιών
επιπέδων των λιποπρωτεϊνών

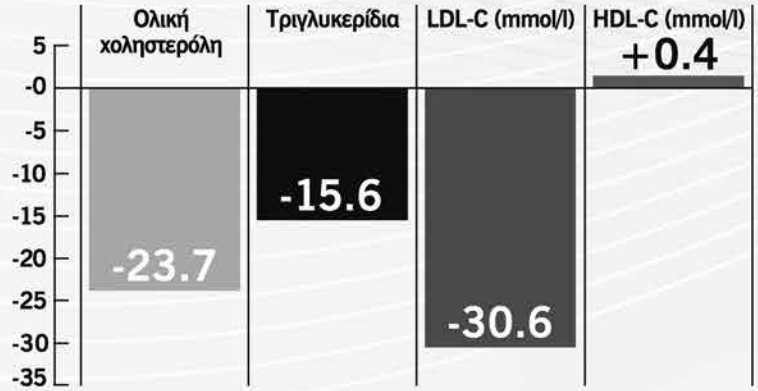
60 δισκία

LIPOPROTECT: επαναφέρει την ισορροπία των λιποπρωτεϊνών

Αυξήστε το LIPOPROTECT Μειώστε την LDL

MONASCUS PURPUREUS

Ποσοστιαίες διακυμάνσεις λιπιδικών παραμέτρων μετά 4 εβδομάδες με Monascus Purpureus.

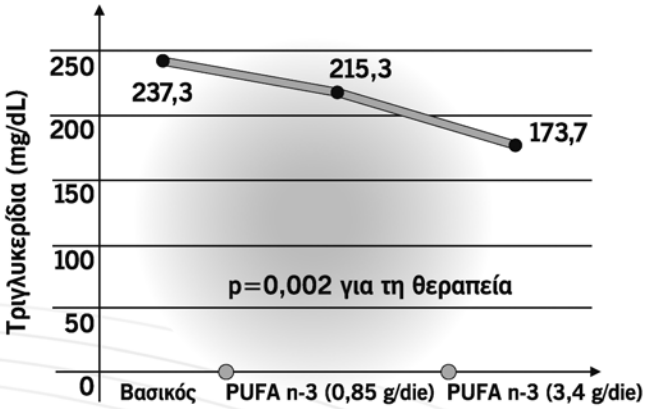


Το Monascus Purpureus ελάττωσε την ολική χοληστερόλη κατά 23,7% και τα τριγλυκερίδια κατά 15,8%.

Το Monascus Purpureus προσδιόρισε μία ελάττωση της LDL-C κατά 30,6% των τριγλυκεριδίων.

OMEGA 3

Η εξέλιξη σε ασθενείς με υπερβολικά τριγλυκερίδια σε διάστημα 8 εβδομάδων με PUFA n-3 (0,85 g/μέρα ή 3,4 g/μέρα).



LIPOPROTECT

***Χρήσιμο** για τον **έλεγχο** της **χοληστερόλης** και των **λιπιδίων** του **πλάσματος**.*

ΣΥΝΙΣΤΑΤΑΙ ΣΕ

- **Υπερχοληστεριναιμία**
- **Υπερτριγλυκεριδαιμία**
- **Αυξημένα λιπίδια**

ΔΟΣΟΛΟΓΙΑ

'Ενα έως δύο δισκία ημερησίως ή όπως συνιστά ο γιατρός σας.

ΔΙΑΤΡΟΦΙΚΕΣ ΠΛΗΡΟΦΟΡΙΕΣ	ανά δισκίο
Red Yeast Rice (Monascus purpureus) (std. 3% [5mg] total monacolins)	167mg
Fish oil providing	250mg
Total Omega-3 Fatty Acids	162,50mg
EPA (Eicosapentaenoic Acid)	68,25mg
DHA (Docosahexaenoic Acid)	44,85mg
Coenzyme Q10	25mg

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CASE SERIES

Powdered Red Yeast Rice and Plant Stanols and Sterols to Lower Cholesterol

Joseph Stefon Feuerstein, MD[1,2] & Wendy Sue Bjerke, ABD[3]

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ABSTRACT. Elevated low-density lipoprotein (ldl) cholesterol is a significant risk factor for cardiovascular disease. It is estimated that 42% of females and 34% of males in the USA have elevated total cholesterol. The current mainstay of lipid-lowering therapy utilizes 3-hydroxy-3-methylglutaryl-coezyme A (HMG-CoA) reductase inhibitor (i.e., statin) medications that lower total cholesterol and ldl cholesterol by an average of 20% and 28%, respectively. However, due to the significant side effects of statin medications, many patients seek alternative therapies to help manage their hypercholesterolemia. Red yeast rice (*Monascus purpueus*) has been used as a food and as an herbal medication in China for centuries. Phytosterols are foods that are similar in structure and function to animal cholesterol. Both of these compounds have been shown in clinical studies to significantly lower ldl cholesterol. We report on a case series of 18 patients with hypercholesterolemia despite therapeutic lifestyle change through diet and exercise who took a proprietary product combining red yeast rice and phytosterols as a powdered shake in an effort to improve their cholesterol indices. Statistically significant reduction ($p < .05$) in the following mean variables was seen: total cholesterol 19% (46 mg/dl) and ldl 33% (53 mg/dl) after 6 weeks using the blend. There was no significant difference in body mass index (BMI), triglyceride, high-density lipoprotein (hdl) cholesterol levels, or systolic and diastolic blood pressure over the same period. This magnitude of reduction in ldl cholesterol is significantly greater than the 28% reduction observed in the 1999 *Journal of the American Medical Association* (*JAMA*) meta-analysis on the average effectiveness of statin medications in lowering cholesterol levels. None of the participants in our study reported any muscle pains, and no abnormal liver function tests were seen while taking the product. Though this case series is limited by small sample size, study duration, and lack of control group, the product's significant reduction in ldl cholesterol without severe side effects indicates that this product may be a clinically effective and well tolerated alternative treatment to using statin medications to treat hypercholesterolemia.

KEYWORDS. hypercholesterolemia, red yeast rice, plant stanols and sterols

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INTRODUCTION

Elevated low-density lipoprotein (ldl) cholesterol is a significant risk factor for cardiovascular disease (Gordon, Castelli, Hjortland, Kannel, & Dawber, 1977). It is estimated that 42% of females and 34% of males in the USA have elevated total cholesterol (Bates, 1982).

The current mainstay of lipid-lowering therapy utilizes 3-hydroxy-3-methylglutaryl-coezyme A (HMG-CoA) reductase inhibitor (i.e., statin) medications which lower serum cholesterol levels. A 1999 *Journal of the American Medical Association* (*JAMA*) meta-analysis of over 30,000 participants from five different trials using either simvastatin, lovastatin, or pravastatin, demonstrated that the average HMG-CoA reductase inhibitor (i.e., statin) lowers total cholesterol by 20% and ldl cholesterol by 28% over a 5.4 years mean duration of treatment (Larosa, He, & Vupputuri, 1999).

Side effects of this class of drugs, which include muscle pain and elevated liver enzyme levels, have been well documented in the literature, and the risk of these adverse effects increases with age (Golomb & Evans, 2008). Due to these significant side effects, many patients seek alternative therapies to help manage their hypercholesterolemia. Two compounds that are commonly used for this purpose are red yeast rice and phytosterol supplements.

Red yeast rice (*Monascus purpueus*) has been used as a food and as a herbal medication in China for centuries. Clinical studies have shown its effectiveness in lowering ldl cholesterol, with its principal mechanism of action being through inhibition of the HMG-CoA reductase enzyme in the liver (Becker et al., 2009), though additional mechanisms of action for its cholesterol-lowering properties have also been reported (Herber et al., 1999).

Red yeast rice has been used to lower ldl cholesterol levels in patients who have had to discontinue the use of statin medication due to muscle pains.

A randomized controlled trial published in the *Annals of Internal Medicine* in 2009 showed a 21% ldl cholesterol drop and a 15% total cholesterol decrease after 24 weeks in 62 patients taking red yeast rice who had to discontinue statins previously due to muscle pains. This drop was statistically significant compared to placebo, and the intervention group reported no muscle pain or liver function abnormalities over the duration of the study.

Phytosterols are foods that are similar in structure and function to animal cholesterol. Though more than 40 different sterols have been identified, sitosterol, campesterol, and stigmasterol are the most abundant. Plant sterols are poorly absorbed in the intestine and thus compete with and block the absorption of animal cholesterol in the gut, thereby lowering ldl cholesterol levels (Katan et al., 2003). A 1998 *JAMA* meta-analysis of 41 trials published in *The Mayo Clinic Proceedings* concluded that supplementation with 2 g of stanols and sterols reduced ldl cholesterol by 10%.

In an effort to further study the synergistic effects of combining red yeast rice and phytosterols on reducing both total and ldl cholesterol, the principal investigator (PI) Joseph Stefon Feuerstein formulated a proprietary product that combined both compounds into a powdered shake taken twice daily before meals.

METHODS

All patients were seen by the PI during the period August 2010 through June 2011. Generalized inclusion criteria included age > 18, total cholesterol > 200 mg/dl, and ldl > 130 mg/dl despite therapeutic lifestyle change through diet, and exercise. Exclusion criteria were use of a HMG-CoA reductase inhibitor (i.e. statin) medication or any other pharmaceutical medication to lower cholesterol. The Institutional Review Board of Stamford Hospital, Connecticut, granted approval by expedited review before the chart review was undertaken. Patients who did not complete the repeat blood tests after 6 weeks on the powdered supplement or who were lost to follow-up were also not included in the chart review.

The powdered supplement used in the study contained 1,200 mg of citrinin free red yeast rice (*Monascus purpueus*) and 1,250 mg of phytosterol complex (sitosterol, campesterol, and stigmasterol) taken before breakfast and dinner as a 5 gm serving scoop of powder added to 2–3 ounces of almond, unflavored soy, or 1% fat milk. Patients were asked to measure the amount of almond, soy, or low-fat milk using a standard kitchen measuring cup and to use a blender to mix the powder for approximately 30–60 s in order to ensure adequate mixing. An inquiry at each office visit was made regarding their compliance with the twice daily dosing regimen and their tolerance of the product. The supplement also contained fructose (less then 1 g of carbohydrate per serving) and dark chocolate flavoring to make the product more palatable. The product was supplied by Rx Vitamins of Elmsford, New York, USA.

A list of all patients who used the powered blend to lower their cholesterol between 8/2/10 and 6/13/11 was kept by the principal investigator.

The chart review was undertaken between 9/1/11 and 10/28/11 by the principal investigator and the following baseline data were collected: age; body mass index (BMI) in kg/m^2; baseline level of exercise (high was defined as >150 min of aerobic activity per week and low was defined as less than that weekly level); whether the patient suffered from hypertension (defined as a systolic blood pressure of >140 mmHg and a diastolic blood pressure of >90 mmHg), diabetes (defined as a fasting blood glucose > 126 mg/dl), or hypothyroidism (defined by a thyroid stimulating hormone level > 4.5 mU/l); whether the patient was taking any antihypertensive medication or omega-3 fatty acid supplementation, was a smoker; or had a family history of hypercholesterolemia.

The demographics of the patients are shown below in Table 1. The average age and BMI of the patients were 57 years old and 25, respectively. Seventy-eight percent of the patients were male and 56% of them did greater than 150 min of aerobic activity per week, while the other 44% performed aerobic activity for less than 150 min a week. Twenty-eight percent of patients were hypertensive and were on antihypertensive medication. In addition, 89% of patients reported being on an omega 3 supplementation at baseline and they were instructed to continue using the same brand and dosing throughout the duration of the trial in order to limit confounding variables. Eleven percent of patients were diabetic and 6% were smokers, while 28% reported a family history of elevated cholesterol.

Baseline laboratory data included total cholesterol (mg/dl), ldl cholesterol (mg/dl), high-density lipoprotein (hdl) cholesterol (mg/dl), triglycerides (mg/dl)

TABLE 1. Patient Demographics

Variable	Mean Result ($n = 18$)
BMI (kg/m^2)	25
Age (years)	57
Sex	22% male, 78% female
Baseline level of exercise: high >150 min per week and low <150 min per week	56% high, 44% low
Hypertension (systolic blood pressure/diastolic blood pressure >140/80	28% yes, 72% no
Antihypertensive medication	28% yes, 72% no
Taking omega-3 fatty acid supplements	89% yes, 11% no
Diabetes	11% yes, 89% no
Hypothyroidism	17% yes, 83% no
Smoking	6% yes, 94% no
Family history of elevated cholesterol	28% yes, 72% no

aspartate amino transferase (ast in U/L), transaminase [Alanine transaminase (alt) in U/L] levels, and systolic and diastolic blood pressure (mmHg). The outcome data were collected at the end of 6 weeks and included BMI, total cholesterol, ldl cholesterol, hdl cholesterol, triglycerides, alt, ast levels, systolic, and diastolic blood pressures. All data were entered into an excel spread sheet with the age and sex of patient as the only identifiers and statistical analysis of student's *t* paired mean test using the SPSS program was performed.

RESULTS

Twenty-three patients met the inclusion criteria and were enrolled in the study. The most prevalent exclusion criterion was the use of prescription cholesterol-lowering medication. Two patients discontinued the product, one due to abdominal pain and the other due to the development of a rash. Three patients were lost to follow-up. A total of 18 patients completed the 6-week protocol and repeat laboratory testing and were thus included in the chart review.

Descriptive and inferential statistical analysis was performed using PASW statistical package software. Tests of normality relative to the variables were performed first. Both the Kolmogorov–Smirnov and Shapiro–Wilk tests of normality indicated that the variables total cholesterol, hdl, and ldl were normally distributed. As a result, paired *t* tests were selected to assess mean changes pre and post.

The baseline and outcome total cholesterol, ldl cholesterol, hdl cholesterol, triglycerides levels are shown in Table 2.

TABLE 2. Baseline, Outcome, and Average Change in Variables in mg/dl. Results Presented as Mean ± Standard Deviation

Variable	Baseline ($n = 18$)	Outcome ($n = 18$)	Average Change ($n = 18$)
Total cholesterol (mg/dl)	244 ± 29.9	198 ± 28.8	−46
ldl (mg/dl)	162 ± 30.5	109 ± 22.4	−53
hdl (mg/dl)	63 ± 15.3	63 ± 16.2	0
Tag (mg/dl)	99	87	−12

Note: Tag, triacylglycerol.

Statistically significant reductions ($p < .05$) in the following mean variables were seen: total cholesterol 244 mg/dl was reduced to 198 mg/dl (−46 mg/dl) and ldl cholesterol 162 mg/dl dropped to 109 mg/dl (−53 mg/dl) after 6 weeks using the product.

Statistically significant changes in hdl cholesterol and triglyceride levels were not observed at the end of this study. In addition, there was also no significant change in BMI, systolic, or diastolic blood pressure during the same period. Levels of ast and alt were within the normal range at baseline and remained normal at the end of the 6-week period.

DISCUSSION

Total cholesterol and ldl cholesterol levels were reduced 19% and 33%, respectively. This magnitude of change in ldl cholesterol levels is greater than the 28% reduction seen in the 1999 *JAMA* meta-analysis on the average effectiveness of statin medications in lowering cholesterol levels. The drop in total cholesterol seen in our study (19%) is comparable to the 20% reduction seen in the *JAMA* meta-analysis.

In our study, there were no significant changes in either triglyceride levels or hdl cholesterol levels, contrasting with the 13% reduction in triglyceride levels and 5% increase in hdl cholesterol levels seen in the 1999 statin study.

It is noteworthy that 89% of participants were taking omega-3 fatty acid supplementation during the study period, which could have potentially introduced a confounding variable into the study due to the triglyceride lowering effects of omega-3 fatty acid supplementation. However, all these participants were already on fish oil when their baseline laboratory indices were obtained at the start of the trial, and no fish oil doses were changed during the study period.

One possible explanation for the high baseline hdl levels seen in our participants (63 mg/dl) is perhaps the high level of physical activity at baseline (>150 min of aerobic activity per week) that was reported by more than half of our subjects. In contrast, the average baseline hdl levels in the 1999 *JAMA* meta-analysis was only 40.4 mg/dl a significantly lower value than that of our own participants. This may explain why their participants hdl levels increased on medication, while our subjects much higher baseline levels did not further rise while using the product.

The 13% reduction in triglyceride levels observed in the statin study was not seen with our product. Again, the baseline triglyceride level seen in the statin study was 150 mg/dl, a level that is significantly greater that the baseline level of 99 mg/dl for our own participants. A possible explanation for our subjects' with extremely low baseline triglyceride levels may be the combination of a high level of baseline aerobic activity coupled with significant dietary modifications that all the participants had adopted prior to starting the product. Again, the significantly lower baseline triglyceride levels of our own subjects may explain why their levels did not further decrease while using the product.

None of the participants in our study reported any muscle pains and no abnormal liver function tests were seen after taking the blend. This can be contrasted with the side effects profile that is seen when using statin medication. A plausible explanation for this difference in side effect profile may be that the blend's mechanism of action is not solely through inhibition of the HMG-CoA reductase but also

through a reduction in cholesterol absorption from the gut due to its high phytosterol content. In addition, some of the other cholesterol-lowering properties of red yeast rice could be augmenting the blend's lipid-lowering effect.

Though this case series is limited by small sample size and study duration, as well as lack of a control group, the product's significant reduction in ldl cholesterol without appreciable side effects should be further investigated in a larger randomized controlled trial.

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We would like to thank all the patients who participated in the study.

Declaration of interest: Joseph Stefon Feuerstein sits on the medical advisory board of RX Vitamins and was involved in the original formulation of the product

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Safety and Efficacy of Red Yeast Rice *(Monascus purpureus)* as an Alternative Therapy for Hyperlipidemia

Matthew Klimek, PharmD, Shan Wang, PharmD, and Adeleye Ogunkanmi, PharmD

Key words: red yeast rice, *Monascus purpureus*, hyperlipidemia, myopathy, dietary supplement, lovastatin

ABSTRACT

Red yeast rice is a Chinese fermented rice product *(Monascus purpureus)* that some have claimed improves blood circulation by decreasing cholesterol and triglyceride levels in humans. The supplement contains naturally occurring monacolin K, the active ingredient found in Merck's prescription agent lovastatin (Mevacor). Lovastatin is associated with various adverse effects such as myopathy and abnormal liver function test results, which can lead to serious problems if patients are not monitored and treated. The inclusion of lovastatin in red yeast rice and the lack of dietary supplement regulation by the FDA raise safety concerns for health care professionals as well as for patients. Studies have shown that red yeast rice products can be beneficial in lowering serum cholesterol levels, but they are not without risk. Furthermore, product uniformity, purity, labeling, and safety cannot be guaranteed.

BACKGROUND

Despite the increase in FDA-approved prescription medications, alternative therapies have become more prevalent in the U.S. About 42% of Americans use alternative medicine, and the demand for these therapies continues to grow.[1,2] In 1997, patients paid approximately 629 million visits to alternative medicine practitioners, a rate that was 47% higher than in 1990. At approximately $27 billion, total out-of-pocket expenditures for alternative therapies exceeded total out-of-pocket expenditures for all hospitalizations in the U.S.[1]

Red yeast rice, a Chinese dietary supplement, has gained popularity because of its properties as a natural statin. This fermented rice product is used as a medicinal food to improve blood circulation by decreasing cholesterol and triglyceride levels.[3,4] The supplement contains varying amounts of natural monacolins as a result of the different strains of *Monascus purpureus* used in fermentation.[5] Monacolins lower cholesterol by inhibiting HMG–CoA (5-hydroxy-3-methylglutaryl-coenzyme A) reductase, the rate-limiting step for cholesterol synthesis in the liver. The primary monacolin in red yeast rice is monacolin K, which has the same chemical structure as lovastatin. Although levels of lovastatin vary in the product, 2.4 g of red yeast rice daily may contain about 4.8 mg of lovastatin, or 0.2% of the total dose. Red yeast rice supplements may also contain isoflavonoids, monounsaturated fats, and sterols that help to reduce cholesterol levels even further.[3]

The natural inclusion of low-dose lovastatin raises concerns for patient safety. In 2007, the FDA warned consumers to avoid red yeast rice supplements promoted on the Internet (Red Yeast Rice, Red Yeast Rice/Policosonal Complex, and Cholestrix) to lower cholesterol because of the possibility of myopathy, leading to kidney impairment.[6] The widespread use of evidence-based medicine has caused health care professionals to become skeptical about dietary supplement use. The lack of studies and regulations to ensure the safety of these products has the result of steering health care professionals away from herbal products and toward prescription medications that have been demonstrated to be safe and efficacious. Health care practitioners should become aware of herbal products that patients might be using in place of commonly prescribed medications. This article reviews the safety and effectiveness of red yeast rice as a "natural" alternative treatment to statins for hypercholesterolemia.

LITERATURE FINDINGS

Before performing a MEDLINE search, we identified the most appropriate search terms using the MeSH (medical subject headings) database provided by the National Library of Medicine (www.pubmed.gov). Instead of using this database to perform the search, we conducted a "text word" search (from 1966 to November 2008) using PubMed to include articles indexed for MEDLINE as well as those not yet indexed (those that do not yet have MeSH terms assigned).

When a relevant article was located, we reviewed the MeSH terms assigned to that article to identify other relevant search terms. To be as complete as possible, we did not use any limitations.

PubMed includes millions of citations from MEDLINE and other life science journals for biomedical articles. We reviewed the bibliographies of all relevant articles to identify any other pertinent articles that the previous searches might have missed. Our article includes some of the more provocative data about red yeast rice but might not include all available relevant literature.

Matthew Klimek and Adeleye Ogunkanmi are postdoctoral fellows at Rutgers University Ernest Mario School of Pharmacy in Piscataway, New Jersey. Shan Wang is a clinical pharmacist at Winthrop University Hospital in Mineola, New York.

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EFFICACY

Lu et al.[7]

Lu et al. conducted a randomized, double-blind, placebo-controlled clinical trial of 4,870 Chinese subjects over 4.5 years to evaluate the efficacy of Xuezhikang (XZK), an extract of cholestin and derived from fermented red yeast rice. The study medication consisted of 300-mg capsules of XZK. Each capsule contained 2.5 to 3.2 mg of lovastatin and a small amount of hydroxyl acid, ergosterol, and other components. To be included in the study, patients must have had a myocardial infarction (MI) within 60 months of enrollment.

Patients underwent a four-week initial period of a controlled diet beginning with the cessation of all lipid-lowering agents. At the end of four weeks, baseline lipid levels were measured. Baseline characteristics were similar for all treatment groups except for sex (3,986 men and 884 women).

Mean low-density lipoprotein-cholesterol (LDL-C) levels were 129 mg/dL in both groups at baseline. Patients treated with XZK showed a significant decrease in frequency of major coronary events such as nonfatal MI and death from coronary or cardiac causes when compared with those receiving placebo (–10.4% and –5.7%, respectively; $P < 0.001$). They also experienced a 33% decrease in the need for coronary revascularization compared with the placebo recipients ($P = 0.004$).

Within eight weeks after randomization, total cholesterol (–10.9%) and LDL-C (–17.6%) levels decreased significantly and were maintained over the duration of the study in the XZK-treated group ($P < 0.001$). The authors concluded that XZK demonstrated efficacy in decreasing cholesterol, recurrent coronary events, and mortality rates.

Lin et al.[8]

Lin and coworkers assessed the lipid-lowering effect and safety of *M. purpureus* in a randomized, double-blind, placebo-controlled study of 79 patients 23 to 65 years of age with hyperlipidemia. Subjects received *M. purpureus* 600 mg twice daily or placebo for eight weeks. The mean baseline LDL-C level was 203.9 mg/dL. At week eight, *M. purpureus* therapy significantly reduced concentrations of LDL-C by 27.7%, total cholesterol by 21.5%, triglycerides by 15.8%, and apolipoprotein B (apo-B) by 26%. High-density lipoprotein-C (HDL-C) and apolipoprotein A-I (apo A-I) levels were increased nonsignificantly by 0.9% and 3.4%, respectively.

Gheith et al.[9]

Gheith and colleagues compared the efficacy and safety of *M. purpureus* Went rice (red yeast rice) with fluvastatin (Lescol, Novartis) in the management of nephrotic dyslipidemia. The investigators conducted an open-label study of 72 patients with dyslipidemia secondary to idiopathic persistent nephrotic syndrome. Patients were randomly divided into three groups: 20 patients received *M. purpureus* Went rice 600 mg twice a day for one month, then 600 mg once daily; 30 patients were treated with fluvastatin 20 mg daily; and 22 controls group received no therapy.

The fluvastatin group had average total cholesterol readings of 436, 333, 313, and 302 mg/dL at baseline, three months, six months, and one year, respectively. Similar reductions were observed in the Went rice group, with cholesterol averages of 457 mg/dL at baseline, 408 mg/dL at three months, 283 mg/dL at six months, and 303 mg/dL at one year. A significant reduction in proteinuria was noted in the fluvastatin group (8.3 g/day at baseline vs. 2.4 g/day at one year) and in the Went rice group (8.6 g/day at baseline vs. 3.2 g/day at one year) but not in the control arm.

Compared with baseline evaluations, there was no clinical evidence of myopathy or neuropathy in patients who received statins or Went rice. The authors concluded that *M. purpureus* Went rice was a safe and effective strategy for treating nephrotic dyslipidemia.

Summary

In each of these studies, *M. purpureus* provided beneficial effects in hyperlipidemic patients[7–9] and might have also positively affected cardiac outcomes.[7] Studies like these give confidence to patients seeking alternative cholesterol-lowering therapies in place of more conventional statin therapies.

SAFETY

All three controlled trials (Lu, Lin, Gheith) showed that *M. purpureus* was well tolerated with few safety concerns.[7–9] In the trial conducted by Lin et al.,[8] none of the subjects receiving *M. purpureus* experienced alanine aminotransferase (ALT), aspartate aminotransferase (AST), or creatine phosphokinase (CPK) measurements that were more than three times the upper limit of normal (ULN) at the fourth or eighth week. Gheithet al.[9] also found no evidence of significant adverse effects in neuromuscular function associated with *M. purpureus*.

Despite these findings, published case reports show potential safety problems with the use of red yeast rice. The following case reports of myopathy and rhabdomyolysis illustrate these potential dangers.

Myopathy

Vercelli et al.[10]

A 76-year-old man with type-2 diabetes received statins for four years; 20 mg of simvastatin (Zocor, Merck) daily for two years, followed by 20 mg of atorvastatin (Lipitor, Pfizer) daily for two years. After four years of therapy, atorvastatin was discontinued upon patient complaints of generalized muscle weakness and serum creatinine kinase (CK) levels of 3,000 U/L. Six months later, generalized muscle weakness improved slightly, but CK levels climbed to 3,700 U/L.

An open quadriceps muscle biopsy revealed muscular atrophy. The patient then admitted that three months after discontinuing atorvastatin, he had begun using a product derived from red yeast rice as an alternative therapy to lower cholesterol. Red yeast rice was discontinued at this point. Muscle weakness improved and CK levels fell to 1,000 U/L three months after he stopped taking red yeast rice. The authors concluded that patients with statin-induced muscle damage should not use red yeast rice as a way to lower cholesterol.

Smith et al.[4]

Smith and colleagues described a case of symptomatic myopathy associated with the use of Chinese red yeast rice.[4] A 50-year-old man visited his primary care physician, reporting joint pain and muscle weakness for two months. At pres-

entation, he had diffuse body aching, upper-extremity weakness, and lower-back stiffness along with a CK level of 358 U/L. He had no history of muscle diseases or problems. According to the patient, the only new medications in his regimen were ginseng, Chinese red yeast rice, and rofecoxib (Vioxx, Merck). The patient had started taking ginseng and Chinese red yeast rice four weeks before his symptoms developed and then started to take rofecoxib after the onset of symptoms. He was instructed to discontinue both of these products. At the three-week follow-up visit, his muscle weakness and joint pain resolved completely and the CK level fell to 179 U/L. Eight months later, the patient resumed taking Chinese red yeast rice, and his CK level increased again to 212 U/L.

Mueller[11]

A case of symptomatic myopathy was attributed to red yeast rice in a 61-year-old woman with hyperlipidemia.[11] She was started on simvastatin 20 mg daily along with estradiol from a transdermal patch at a dose of 0.05 mg/week, aspirin 81 mg daily, and a multivitamin. The patient was otherwise in good health; vital signs and laboratory parameters were within normal limits. After four months of therapy, simvastatin was increased to 40 mg daily. At this point, the CK level was 189 U/L. Within one month, diffuse myalgia and an elevated CK level of 451 U/L were reported.

After simvastatin was discontinued, symptoms resolved and CK levels returned to 170 U/L. Soon afterward, the patient began using red yeast rice 600 mg twice daily as an alternative treatment. Three months later, diffuse myalgia returned and CK levels increased to 475 U/L. Red yeast rice was then discontinued; symptoms resolved, and CK levels decreased to 122 U/L. Like simvastatin, red yeast rice caused diffuse myalgia and elevated CK levels. After these products were discontinued, symptoms resolved soon thereafter.

Summary

Although the effects of red yeast rice are mild, the product can cause muscle pain and weakness similar to that associated with conventional statins. These events can become serious if the patient's intake is not monitored appropriately. Awareness of the previous two cases can help health care professionals understand the potential for myopathy when red yeast rice is used as a natural alternative to statins.

Rhabdomyolysis

Prasad et al.[12]

Prasad and coworkers reported on a 28-year-old stable renal transplant recipient who developed rhabdomyolysis after therapy with red yeast rice.[12] The patient experienced an asymptomatic elevation of CK to 1,050 IU/L; a second assessment showed a CK level of 2,600 IU/L. After questioning, the patient stated she had taken red yeast rice for the previous two months to lower cholesterol naturally. After she stopped taking the supplement, her CK levels fell to 600 IU/L within two weeks and she remained clinically asymptomatic.

The patient was also taking cyclosporine to prevent transplant rejection. Cyclosporine, a known cytochrome P450 3A4 inhibitor, most likely elevated red yeast rice serum concentrations by inhibiting its metabolism. It is important to note the probable drug–herbal interaction between cyclosporine and red yeast rice. Cyclosporine is a commonly prescribed prescription medication, and taking concomitant red yeast rice can result in serious rhabdomyolysis.

Consistency and Content of Red Yeast Rice

Besides the adverse effects caused by *M. purpureus*, the composition of red yeast rice can cause patient harm if quality control is inadequate. Heber et al. conducted an analysis of nine proprietary Chinese red yeast rice dietary supplements: Cholesterex, Cholestene, Cholactive, Cholester-Reg, Beyond Cholesterol, Hongqu, Cholesterol Power, red yeast rice, and Cholestin.[13] The authors of this study aimed (1) to determine whether the cholesterol-lowering effect of red yeast rice was consistent among all red yeast rice products and (2) to detect impurities in the product. They measured monacolin concentrations in each supplement along with citrinin, a nephrotoxic by-product of fermentation (Table 1). Citrinin, a dangerous nephrotoxin, was measured by radioimmunoassay and served as an indicator of potential danger as a result of its contents other than the active ingredients.

Results showed a wide rage of monacolin K (0.15–3.37 mg) and monacolin L (less than 0.006–0.02 mg) content per capsule. Only one of the tested products included all 10 monacolin compounds that a quality red yeast rice product should contain. Citrinin was found at measurable concentrations in seven of nine preparations (0.47–64.7 mcg/capsule). The quality and contents varied between each product, indicating that not all red yeast rice products are equal. The authors concluded that standardized manufacturing practices and adequate labeling are needed to ensure the equivalence of active ingredients for efficacy and a low concentration of unwanted fermentation by-products to ensure safety.[13]

DISCUSSION

The efficacy of dietary supplements is usually questionable because of the lack of controlled clinical trials supporting their use. Two randomized, double-blind, placebo-controlled trials[7,8] and an open-label trial[9] demonstrated that red yeast rice might be effective in lowering LDL-C levels.

Lu et al. further demonstrated that red yeast rice might be able to reduce cardiac events and provide positive effects on cardiovascular outcomes in a fashion similar to that of prescription statin therapy.[7] Although larger-scale trials are necessary to confirm these findings, red yeast rice seems promising in the treatment of hyperlipidemia. Such efficacy data influence patients to try natural remedies before using more common prescription drug therapies. The vast amount of data demonstrating the benefits of statin therapy in cardiovascular disease supports its continued use, but practitioners should be aware of alternative therapies being used by their patients.

Unlike prescription drugs, dietary supplements have not traditionally undergone extensive testing by the FDA. Without adequate testing, dietary supplements are not guaranteed to contain the quantity or quality of ingredients stated on the product label. As shown by Heber et al., red yeast rice is no exception.[13] Each of the nine products tested had different monacolin levels. Supplements with a lower monacolin content would be less effective in lowering cholesterol.

Table 1 Contents of Monacolin and Citrinin in Chinese Red Yeast Rice Products

Red Yeast Rice Supplements	Monacolin K (mg per Capsule)	Monacolin L (mg per Capsule)	Citrinin (mcg per Capsule)
Cholesterex[a]	1.35	<0.006	4.87
Cholestene[b]	2.87	<0.006	2.22
Cholactive[c]	1.80	<0.006	6.06
Cholester-Reg[d]	3.37	<0.006	3.23
Beyond Cholesterol[e]	0.15	0.02	No data available*
Hongqu[f]	2.86	<0.005	11.82
Cholesterol Power[g]	2.51	<0.007	0.47
Red Yeast Rice[h]	1.56	<0.006	64.7
Cholestin[i]	2.46	0.015	No data available*

* Limits of detectability = 0.04 mcg per capsule.

(a) Oralabs, Englewood, Colo.; (b) HPF, LLC, Hatboro, Pa.; (c) Herbscience, Windmill Health Products, West Caldwell, N.J.; (d) Nature's Sunshine, Provo, Utah; (e) TwinLab, Hauppauge, N.Y.; (f) Nature's Sunshine, Provo, Utah; (g) Nature's Herbs, Hauppauge, N.Y.; (h) Solaray, Park City, Utah; (i) Pharmanex, Brisbane, Calif.

Adapted with permission from Heber S, Audra L, Qing-Yi L, et al. *J Altern Complement Med* 2001;7(2):133–139. Copyright, Mary Ann Liebert Publishers, Inc.[13]

Seven of the tested samples contained citrinin, a mycotoxin produced by a variety of fungi in the production of foods intended for human consumption such as grain, cheese, and red pigments. Citrinin is a nephrotoxin in all animal species tested, but its acute toxicity varies.[14] Citrinin is genotoxic at high concentrations in cultured human lymphocytes; therefore, its concentration in supplements should be minimal.[15]

Inconsistencies such as these led the FDA to require current good manufacturing practices (CGMPs) for dietary supplements.[16] Final CGMPs were expected to become effective in June 2008 for large companies and are to be implemented in June 2009 for companies with fewer than 500 employees and in June 2010 for companies with fewer than 20 employees. Under this ruling, all domestic and foreign supplements must be processed in a consistent manner and to meet quality standards. To demonstrate quality and consistency, tests will be performed on all supplements to ensure their identity, purity, strength, and composition.

Statin drugs such as Merck's Mevacor (lovastatin) are associated with various side effects such as headache, dizziness, rash, upset stomach, and hepatic dysfunction. The most common adverse effect is muscle weakness, which can be a sign of more serious myopathy or, in rare cases, rhabdomyolysis.[17] Double-blind, controlled clinical trials have demonstrated that red yeast rice is effective and well tolerated in a wide range of patients;[7,8] however, case reports have linked it to muscular myopathy and rhabdomyolysis. In three cases described here,[4,10,11] red yeast rice caused or exacerbated myopathy marked by elevated serum CK levels. Rhabdomyolysis, the most severe adverse effect associated with statins, occurred in a renal transplant patient who used red yeast rice while concomitantly taking cyclosporine.[12] Although the more reliable controlled trials showed no need for safety concerns, case reports warn of the possibility of adverse effects with wider use. These reports should not be ignored for patients who are taking red yeast rice as an alternative to common prescription statins.

Lovastatin as a prescription drug is contraindicated in pregnancy and is a Category X agent. This labeling is a main reason for the FDA's rejection of the application submitted by Merck to sell lovastatin over the counter.[18] Red yeast rice, when used by pregnant women, places the fetus at unnecessary risk of central nervous system defects during the first trimester. Although red yeast rice contains a lower dose of lovastatin compared with the FDA-approved product, the risk posed may be similar.

CONCLUSION

Despite the growing interest in dietary supplements, red yeast rice *(M. purpureus)* is not recommended for patients with hypercholesterolemia. A lack of uniformity among products, the possibility of contamination, and the risk of severe adverse reactions pose a threat to individuals using this product. Overall, red yeast rice has not been shown to be a safe alternative to statins for patients with hyperlipidemia despite its demonstrated efficacy in controlled clinical trials. Physicians should be aware of its popularity as a "natural" way to lower serum cholesterol, and they should discuss the risks and benefits of this supplement with their patients.

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Monascus purpureus Went rice in nephrotic hyperlipidemia

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Abstract

Background:

Nephrotic dyslipidemia is a risk factor for the development of systemic atherosclerosis; and may aggravate glomerulosclerosis and enhance progression of glomerular disease. We aimed to assess the efficacy and safety of *Monascus purpureus* Went rice vs. fluvastatin therapy in the management of nephrotic dyslipidemia.

Materials and Methods:

Seventy-two patients with persistent idiopathic nephrotic syndrome (NS) with secondary dyslipidemia were included. They were randomly allocated into three age and sex-matched groups. The first group comprised 20 cases and were given *M. purpureus* Went rice in a dose of 600 mg twice/day for 1 month then once daily, the second group comprised 30 cases were given fluvastatin in a daily dose of 20 mg. The remaining 22 received no antidyslipidemic therapy and constituted a control group. All of these patients were subjected to thorough laboratory investigations including renal function tests and lipogram. Moreover, the neuromuscular status was evaluated with electromyography and nerve conduction velocity.

Results:

Our results showed that both fluvstatin and *M. purpureus* Went rice were well tolerated with no evidence of significant side effects including neuromuscular functions. Both of them significantly reduced cholesterol after 6 months and 1 year.

Conclusion:

Monascus purpureus Went rice is safe, effective, and economic treatment strategy for nephrotic dyslipidemia.

Keywords: Glomerulonephritis, *Monascus purpureus*, nephrotic syndrome, neurotoxicity, statin

Introduction

Nephrotic hyperlipidemia is a risk factor for the development of systemic artherosclerosis, and may aggravate glomerulosclerosis and enhance the progression of glomerular disease.[1]

Diet intervention should be the first-line of treatment, but it only partially corrects hypercholesterolemia in such patients.[2] The greatest and the most consistent reductions in low-density lipoprotein (LDL)-cholesterol is seen with 3-hydroxy-3-methylglutaryl-coenzyme A

reductase (HMG-CoA reductase) inhibitors. Many studies have shown that *M. purpureus* Went rice contained HMG-CoA reductase inhibitors, large quantities of unsaturated fatty acids, beta-sitosterol, campesterol, and stigmasterol.[3–5] These components are effective in reducing serum lipid.[6]

The lipid-lowering effects of *M. purpureus* - rice food flavor in China and Japan - have been shown in several animal models of hyperlipidemia.[7] One study showed that *M. purpureus* Went rice significantly reduced LDL-C, total cholesterol, triglycerides, and apolipoprotein B levels, and was well tolerated in patients with hyperlipidemia.[8,9]

However, the long-term safety and efficacy of these lipid-lowering strategies are lacking in patients with renal disease.[10] Treatments of nephrotic dyslipidemia in steroid resistant NS have resulted in a considerable benefit not only for the hyperlipidemia, but also for the nephrotic state.[11]

Prospective controlled studies are needed to evaluate the long-term safety of statins in a large patient population and assess whether reduction in cholesterol decreases the risk for atherosclerosis and inhibits the progression of glomerular disease in patients with NS.[12] Although statin therapy was considered to be safe in treating nephrotic dyslipidemia on the short-term follow-up studies considering electromyography (EMG),[12,13] long-term studies are lacking for this costly regimen.

We aimed to assess the efficacy and safety of *M. purpureus* Went rice vs. fluvastatin therapy in the management of nephrotic dyslipidemia.

Materials and Methods

Out of 450 patients with idiopathic nephrotic syndrome screened, 72 were recruited from the nephrology clinic of the Urology and Nephrology Center, Mansoura University for inclusion in this prospective, randomized controlled study for a planned duration of 1 year.

The patients were recruited with the following inclusion criteria: steroid resistant, steroid dependent, and frequently relapsing idiopathic nephrotic syndrome, hypercholesterolemia with no response to an appropriate diet for at least 4 weeks, serum creatinine < 2 mg/dl, recent renal biopsy proved focal segmental glomerulosclerosis (FSGS) or membranoproliferative glomerulonephritis (MPGN). We excluded patients with hepatic disease, muscle disease, history of familial dyslipidemia, diabetes mellitus and those on statins.

This protocol met the requirements of local ethical committee. Upon enrollment, patients were randomly assigned to one of the three treatment groups. The first group comprised 20 cases and was given *M. purpureus*, the second group comprising of 30 cases was given fluvastatin, and the third group (n=22) served as control.

Information regarding the randomized treatment was concealed in sequentially numbered, sealed opaque envelopes. These were opened in the absence of the patients immediately after obtaining informed written consent for participation in the study. The participant physicians were necessarily aware of the randomized treatment in all cases.

Patients were evaluated at start of treatment (control values) and monthly for 6 months (test values). The evaluation included thorough history taking and clinical examination, and following laboratory investigations: Complete urine analysis and 24-h urinary protein estimation, serum creatinine and creatinine clearance using Cockcroft and Gault formula; liver function tests; investigations to exclude secondary causes of glomerulonephritis such as blood sugar, anti-HCV, anti-CMV, anti-HIV, HbsAg, serologic tests for systemic lupus erythematosis rectal mucosal biopsy to exclude schistosomiasis; and total serum cholesterol.

Neurophysiological evaluation was done using the NIHON KOHDEN-evoked response recorder, model MEP-5200, for EMG[14] and nerve conduction velocity.[15]

Electromyography

The following muscles were tested:

1. The biceps brachii and the rectus femoris as representative of the proximal muscles of the upper

and lower limbs, respectively.

2. The abductor pollicis brevis of the thenar eminence and extensor digitorum brevis as representative of the distal muscles of the upper and lower limbs, respectively.

The electrical potentials were recorded by a bipolar EMG needle electrode (NW-120T). Resting and mild contraction activities were recorded at the interrupted speed and the interference pattern at continuous speed for each muscle.

The mean of at least 20 motor unit action potentials (MUPs), from different sites and depths, gave the duration and amplitude.

Nerve Conduction velocity

We chose the median nerve and the lateral popliteal nerve for estimating the conduction velocity. The median nerve was stimulated at the antecubital fossa and at the wrist, while the lateral popliteal nerve was simulated at the knee and ankle joints. A bipolar stimulating surface electrode (A-NM-4205) was applied over the nerve and the evoked potentials were recorded by a needle electrode from the abductor pollicis brevis in the upper limb and extensor digitorum brevis in the lower limb. The duration of the stimulus pulse was usually 0.5 ms and the stimulus voltage increased from zero to supramaximal to get the best M wave. A ground electrode was placed between the stimulating and the recording electrodes. Depending on the latency and duration of the evoked potentials, the time bases of the recordings were varied so that the whole pattern of potentials could be displayed on the sweep of the oscilloscope. Usually a time base of 10 ms/cm was needed.

The latency was measured from the start of the stimulus artifact to the onset of the muscle response. The same process was repeated twice, once at the proximal part of the nerve and the other at the distal part. The time difference between the two latencies in milliseconds was obtained.

The conduction velocity was calculated as follows:

Cv = Distance between the two points of stimulation in cm × 10.

Difference between the two latencies in milliseconds = m/sec (meters per second).

The first group received *M. purpureus* Went rice (600 mg twice/day for 1 month then once daily). The second group received oral fluvastatin in a daily dose of 20 mg. The third group received no additional therapy and ranked as a control. After 8 weeks, re-adjustment of the dose was performed according to patient's response (serum cholesterol) and patient's tolerance to the drug. Restriction of dietary cholesterol and proteins was illustrated to every patient by appropriate counseling and repeated reinforcement. Other supportive treatment including angiotensin converting enzyme inhibitor (ACEI) use was comparable in the two groups.

All patients were followed monthly for one year after which they were subjected to thorough clinical and laboratory evaluation.

Statistical analysis was done by an IBM compatible personal computer using the Statistical Package for Social scientists (SPSS) for windows 11.5 (SPSS Inc., Chicago, IL, USA). Qualitative data were displayed in cross tabulation and quantitative data were described in terms of arithmetic mean ± SD. A P-value of <0.05 was considered significant.

Results

The demographic characteristics of all patients were summarized in Table 1. There was no significant difference between the studied groups regarding patients' age, sex, body weight, smoking history, and renal histopathology.

Fig. 1 shows the serial value of serum cholesterol in different study groups.

In comparison to baseline values, fluvastatin produced a significant and progressive reduction of serum cholesterol by 35, 38, and 42% at 3 months, 6 months, and after 1 year, respectively ($P <$ 0.001). Similar reductions were observed in the *M. purpureus* Went rice group. After 1 year, we observed that serum cholesterol was significantly lower in statin and *M. purpureus* Went rice groups

compared to the control group ($P = 0.003$) [Table 2].

Serum albumin increased significantly in the statin, *M. purpureus* Went rice groups at 12 months compared to the control group and to the basal values ($P = 0.05$) [Table 2].

In our series, blood chemistry including liver enzymes, bilirubin, alkaline phosphatase, and creatine phosphokinase (CPK) were within normal ranges throughout the study. The mean of CPK showed no significant difference at the start of treatment, after 3 months and at the end of the study.

We observed no clinical evidence of myopathy or neuropathy in our patients who received statins or *M. purpureus* Went rice therapies compared to the basal evaluation.

The amplitude of the MUPs showed no significant changes in the distal muscles after 12 months of fluvastatin or *M. purpureus* Went rice therapies. On the other hand, there was a significant reduction in duration and amplitude of motor action potentials of proximal muscles. However, polyphasicity showed no significant change ($P > 0.05$) [Table 3]. Nerve conduction velocity and terminal latency of the median and lateral popliteal nerves showed no significant changes at 6 months compared to basal values ($P > 0.05$).

Discussion

Hyperlipidemia of the nephrotic syndrome is a risk factor for the development of systemic artherosclerosis, also it may aggravate glomerulosclerosis and enhance the progression of glomerular disease.[1,2,7]

Pattern of dyslipidemia in our cases is matching with that reported by Wheeler[16] and Warwick *et al.*[17] who showed that total plasma cholesterol; TG, VLDL, and LDL were elevated, with variable HDL concentration.

We observed significant reduction of serum cholesterol by 28.8% and 30.2% at 6 months and at 1 year of *M. purpureus* Went rice, possibly due to its HMG-CoA reductase inhibiting properties.[3–5] In contrast, there were no significant changes in serum cholesterol in the control group.

Similar findings were reported by Matzkies *et al.*[18] who showed a reduction of total cholesterol by 31% and LDL by 29% after 2 months of initiation of fluvastatin treatment (40 mg/day). The relatively small number of cases (10 cases) as well as the large dosage of fluvastatin they used might explain the earlier reduction in LDL and cholesterol they observed. Also, our observation goes hand in hand with the degree of reduction in total and LDL cholesterol reported by Olbricht *et al.*[19] on using simvastatin with nephrotic patients. Todd and Goa[20] achieved a 25-30% reduction in plasma LDL within 4 weeks which was maintained with continued treatment by a daily dose of 20-40 mg fluvastatin. Jokubaitis[21] reported similar findings by fluvastatin in a dose of 40 mg/day.

In this study, we succeeded in reducing cholesterol by the same degree after 6 months of fluvastatin (20 mg per day) and - for the first time in nephrotic patients - by *M. purpureus* (600 mg per day) therapies, without side effects and with relatively lower dosage.

In the same direction, Lin *et al.*[8] reported short-term efficacy and safety of *M. purpureus* went rice in treating hyperlipidemia.

Interestingly, a significant reduction in proteinuria was observed in the statin and *M.* Went rice treated patients, but not in the control group. Matzkies *et al.*[18] in a similar study - with statin failed to demonstrate such favorable effect. This may be explained by the fact that Matzkies' patients were of heterogeneous pathologic types while most of our patients were suffering from FSGS.

In the same direction, Hattori *et al.*[22] reported that there was an improvement in renal function and proteinuria in drug resistant NS secondary to FSGS, by LDL apheresis combined with pravastatin. In placebo-controlled study performed in Hong-Kong over 2 years, it was suggested that despite no significant effect on proteinuria a decline in renal function was attenuated by lovastatin, particularly over the second year of the study.[23] Chan *et al.*[24] reported that in lovastatin-treated nephrotics with relatively good pretreatment renal function, glomerular filtration rate (GFR) increased at the end of 6 months treatment.

In contrast, Matzkies *et al.*[18] reported a significant rise in serum creatinine in their patients despite using fluvastatin in a dose of 40 mg/day. Again, the heterogeneity of pathologic types of their patients explains the difference in their results.

In this study, the significant reduction of creatinine clearance among control group 6 months onward compared to the nonsignificant change of creatinine clearance in *M. purpureus* Went rice and statin-treated groups, both suggested the protective effect of lipid-lowering agents on kidney function. Also, the electromyographic data showed a significant decrease in the amplitude and duration of MUP in the proximal muscles only with statin treatment compared to basal values. These changes were not observed in *M. purpureus*. However, this reduction was not excessive and there was no other electromyographic evidence of myopathy. In addition, there were no clinical findings of myopathy or elevated serum CPK.

Peters[25] similarly noted that drug-related myopathy and rhabdomyolysis have not been reported with fluvastatin on the basis only of clinical findings and elevated skeletal muscle enzymes. In the same direction, Jokubaitis[21] found no notable increase in serum CPK or liver enzymes, and no cases of clinically evident myopathy. On the other hand, Careless and Cohen[26] reported that statins and fibrates were associated with a variety of rheumatic problems including proximal myopathy, diagnosed on a clinical basis and confirmed by high serum CPK. Jacquet *et al.*[27] also reported that the frequency of severe side effects such as myopathy amounted to 1 per 1000 prescriptions with cholesterol-lowering drugs in current use. De Pinieux *et al.* ascribed the myopathic side effects of statins to mitochondrial dysfunction as the blood lactate/pyruvate ratio is high.[28] These reports regarding the safety of statins were based only on clinical data and muscle enzyme evaluation.

Jacobson *et al.*[29] reported increase in liver enzymes while Olbricht *et al.*[19] and Matzkies *et al.*[18] reported satisfactory tolerance of statin in their nephrotics.

In agreement with what was reported by Lin *et al.*[8], *M. purpureus* Went rice was well tolerated in patients with hyperlipidemia. Moreover, we found that *M. purpureus* Went rice was not only an efficacious modality of treatment that targeted the nephrotic dyslipidemia with the same potency like statin, but also achieved 50% cost reduction in comparison to fluvastatin. From this study, we can conclude that *M. purpureus* Went rice is safe, effective, and economic treatment strategy for nephrotic dyslipidemia.

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Footnotes

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Conflict of Interest: None declared.

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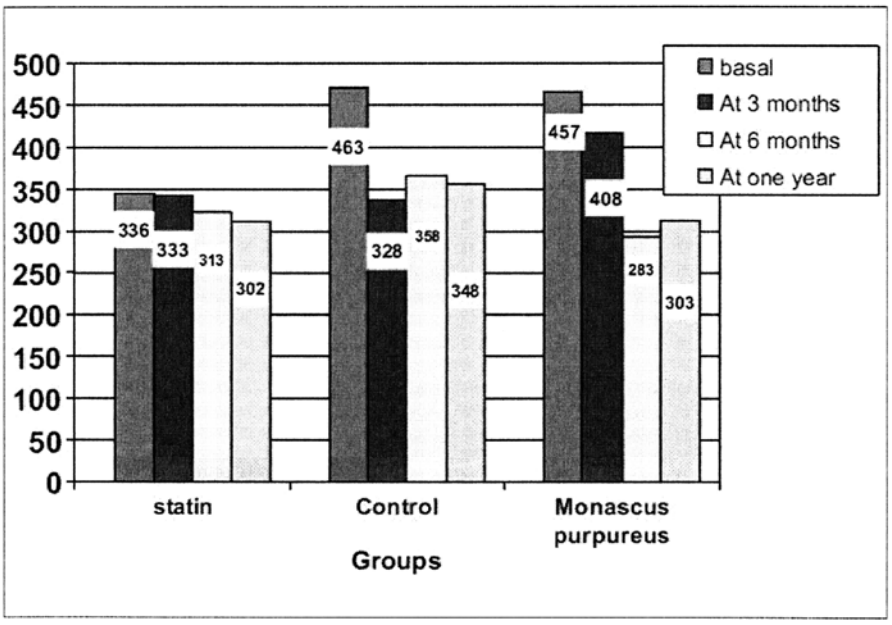
Figures and Tables

Table 1

Demographic characteristics of the studies nephrotic patients

	Fluvastatin ($N = 30$)	Control ($N = 22$)	*Monascus purpureus* ($N = 20$)	P value
Mean age (Y)	17.6 ± 7	19.7 ± 7	21.4 ± 14	0.19
Sex (male/female)	13/17	10/12	12/8	0.38
Smokers	3/27	4/18	3/17	0.92
Occupation				
Student	13	9	6	0.51
Worker	5	3	4	0.94
Professional	12	10	10	0.68
Body weight	51.3 ± 26	58.2 ± 25	55.2 ± 24	0.4
Renal pathology				
FSGS	24	14	18	0.58
MPGN	6	8	2	

Fig. 1



Showed serum cholesterol levels in different groups along follow-up period

Table 2

Biochemical characteristics of patients treated by fluvastatin and *Monascus purpureus* Went rice therapies at basal and at the last follow-up of the study

	Statin (*N* = 30)	Control (*N* = 22)	*Monascus purpureus* (*N* = 20)	*P* value, Control *vs* Other groups
Serum CR(mg/dl)				
Basal	1.07 ± 0.4	1.2 ± 0.3	0.85 ± 0.3	0.06
At 6 months	1.03 ± 0.8	0.9 ± 0.6	0.98 ± 0.5	0.53
At 1 year	1.2 ± 0.8	0.99 ± 0.5	0.96 ± 0.3	0.62
S. albumin (gm/dl)				
Basal	1.6 ± 0.7	1.3 ± 0.3	1.67 ± 0.7	0.06
At 6 months	2.1 ± 0.9	1.7 ± 0.8	2.09 ± 1.2	0.56
At 1 year	2.3 ± 0.9	1.7 ± 0.8	2.5 ± 1.6	0.05
Proteinuria (gm/day)				
Basal	8.3	8.8	8.6	0.06
At 6 months	5.2	6.6	5.5	0.055
At 1 year	2.4	7	3.2	0.04
Serum ALT (IU/L)				
Basal	16 ± 9	19 ± 8	15 ± 6	0.5
At 1 year	15 ± 5	17 ± 4.5	17 ± 4	0.9
S. cholesterol (mg/dl)				
Basal	436 ± 102	463 ± 169	457 ± 232	0.31
At 3 months	333 ± 155	328 ± 158	408 ± 239	0.55
At 6 months	313 ± 185	358 ± 178	283 ± 208	0.31
At 1 year	302 ± 171	348 ± 184	303 ± 178	0.003

Table 3

Electromyographic evaluation of *proximal* muscles of the nephrotic patients treated by fluvastatin and *Monascus purpureus* Went rice therapies at basal and at the last follow-up of study

	Fluvastatin (*N* = 30)	*Monascus purpureus* (*N* = 20)	*P* value
	Amplitude (mv)	Amplitude (mv)	
AMP biceps brachii			
Basal	1487 ± 1510	4250 ± 2500	0.031
Last	1257 ± 1075	3078 ± 1500	0.030
P within groups	0.078	0.078	
AMP Q femoris			
Basal	1813 ± 900	3971 ± 2030	0.075
Last	1245 ± 840	4100 ± 2300	0.025
P within groups	0.075	0.075	

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Antihyperlipidaemic effect of a *Monascus purpureus* brand dietary supplement on a large sample of subjects at low risk for cardiovascular disease: A pilot study

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Summary
Objectives: We planned to carry out a pilot study to evaluate the efficacy and safety as an antihypercholesterolemic agent of a brand dietary supplement made of *Monascus purpureus* titrated extract, octacosanols and niacin on 111 Caucasian patients with low cardiovascular disease risk (<20% by Framingham algorithms), comparing them with the antihypercholesterolemic effect of a low dosage of Pravastatin on 20 subjects with similar risk profile.
Results: In our study, the tested dietary supplement determined a significant decrease of Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), and Triglycerides (TG) in moderately hypercholesterolemic subjects without clinically relevant change in liver and muscular toxicity markers. The reduction of LDL-C reached the 20%, and it is similar to that obtained with a well-known effective statin like Pravastatin.
Conclusions: Further long-term and double blind evaluation have to be carried out before to infer the observed results, however it appears that the studied dietary supplements could be a safe and efficacious antihypercholesterolemic agent for patients at low risk for cardiovascular diseases.

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Introduction

Hypercholesterolemia is a well-known risk factor for coronary artery disease, cerebrovascular disease and peripheral artery disease.[1] Moreover, reducing plasma cholesterol level coincides with a reduced incidence of cardiovascular complication (myocardial infarction, stroke, peripheral obstructive arterial disease) both before the first clinical event (primary prevention) and the following ones (secondary prevention).[2] The treatment of hypercholesterolemia with a specific drug is highly cost-effective when treating patients in secondary prevention,[3] while in primary prevention life-style change and dietary habits appear to be more cost-effective than any pharmacological treatment.[4] During the past decade, nutritionists have focused their knowledge on consensus guidelines[5] aimed at reducing dietary saturated fatty acids, cholesterol, and excess body weight.

However, recent researchers are looking at other ways in which diet may influence the progression of cardiovascular disease, including lipoprotein oxidation, thrombosis progression, cardiac arrhythmia, and medication interaction.[6] Some areas of investigation include the role of various fatty acids and supplements—in the form of vitamins, minerals, herbs, and functional foods—-as well as traditional foods and diets from other parts of the world. Some of the new and relevant nutritional approaches include: specific fatty acids (omega 3, monounsaturated and trans fatty acids), dietary supplements (herbs, antioxidants, vitamins C and E, Coenzyme Q10, B vitamins and homocysteine, l-arginine, Chinese red yeast rice, octacosanols, garlic, soy, flax seed, and dietary fibre), food and drink (tea, nuts, plant-sterol and stanol-ester-containing spreads, alcohol, and grapefruit juice), and the Mediterranean diet.[7] When the efficacy and safety of specific products would have been clearly tested by specific researches, their chronic assumption could be a useful way to reduce the public health costs of antihyperlipidaemic treatment of patients of an actual low global cardiovascular risk profile.[8]

In this context, we planned to carry out a pilot study to evaluate the efficacy and safety as an antihypercholesterolemic agent of a brand dietary supplement made of *Monascus purpureus* titrated extract, octacosanols and niacin on a wide sample of Caucasian patients with low cardiovascular risk, comparing them with a low dose pravastatin.

Materials and methods

For this study, we selected five specialized ambulatory physicians who had the possibility to recruit the kind of patients needed for this protocol.

The inclusion criteria were the following:

- Estimated 10-years cardiovascular disease risk <20% (by Framingham algorithm)
- Moderate hypercholesterolemia (TC < 300 mg/dL)
- Normal/border-line triglyceridemia (TG < 250 mg/dL)
- Normal HDL-C (>40 mg/dL)
- Patients in primary prevention for cardiovascular disease
- No family history of severe dyslipidemias nor previous cardiovascular events
- No high cardiovascular risk independently from plasma lipid values
- No liver and/or muscle disease

One hundred eleven Caucasian subjects were recruited for the study: 38 men aged from 33 to 73 years and 67 aged from 20 to 78 years concluded the study protocol. The main baseline characteristics of the studied sample are resumed in Table 1. These subjects have then been treated with the study.

A further sample of 20 control patients (10 men and 10 women) to be treated with pravastatin 20 mg with comparable baseline characteristics have also been recruited.

Before starting the study, all patients underwent an initial screening assessment that included a medical history, physical examination, vital signs, measurements of height and body weight, calculation of body mass index (BMI = weight in kilograms divided by the square of height in meters). The selected subjects received behavioural and dietetic suggestions, following the latest guidelines of the National Cholesterol Educational Program.[1] After 1 month, a 12-h fasting sample was taken from the basilic vein for ematochemical analyses. A second sample was taken after 2 months of treatment.

The following laboratory parameters have been monitored: Total Cholesterol (TC), Low Density Lipoprotein Cholesterol (LDL-C), High Density Lipoprotein Cholesterol (HDL-C), Total Triglycerides (TG), Glutammate Ossalacetate Transaminase (GOT), Glutammate Piruvate Transaminase (GPT), and Creatinin-Phosfo-Kinase (CPK). TC, HDL-C, TG. GOT, GPT and CPK have been measured by standard laboratory method, while LD-C plasma level was estimated by the Friedewald's formula (LDL-C = TC − HDL-C − TG/5).

Table 1 Baseline characteristics of the male and women subjects treated with the study product.

Parameter	Reference	Dietary supplement (mean ± S.D.)		Pravastatin 20 mg (mean ± S.D.)	
		Men ($N = 40$)	Women ($N = 71$)	Men ($N = 10$)	Women ($N = 10$)
Age (years)	–	54.3 ± 11.4	56.2 ± 12.6	55.1 ± 10.9	55.8 ± 11.8
BMI (m^2/Kg)	<25	26.1 ± 2.6	25.8 ± 4.1	26.0 ± 2.9	25.7 ± 4.2
SBP (mmHg)	<140	136.7 ± 13.1	141.3 ± 14.8	136.4 ± 11.5	140.4 ± 15.0
DBP (mmHg)	<90	80.5 ± 6.9	82.4 ± 8.7	80.6 ± 6.6	81.8 ± 7.3
TC (mg/dL)	<200	237.5 ± 38.7	244.5 ± 44.1	234.8 ± 35.5	246.1 ± 41.2
HDL-C (mg/dL)	>45 (M); >50 (W)	50.4 ± 14.9	62.0 ± 17.3*	50.6 ± 12.6	60.7 ± 14.5*
LDL-C (mg/dL)	<130	155.9 ± 38.2	150.4 ± 41.6	152.2 ± 35.4	154.4 ± 37.2
TG (mg/dL)	<200	155.0 ± 75.8	159.3 ± 66.9	158.7 ± 77.3	157.6 ± 69.5
GOT (mg/dL)	<30	21.9 ± 8.5	22.2 ± 6.7	22.3 ± 9.5	21.6 ± 5.9
GPT (mg/dL)	<30	27.2 ± 10.8	26.3 ± 20.3	27.8 ± 12.3	25.6 ± 17.8
CPK (mU/mL)	<200	76.9 ± 46.1	63.3 ± 48.0*	73.4 ± 48.3	64.3 ± 31.0*

* Significant difference between men and women ($p < 0.05$).

Treatment tolerability was assessed at each study visit interviewing patients on possible subjective side effects (headache, gastrointestinal disorders, asthenia, myalgia, others), and comparing of clinical and laboratory values with baseline levels.

Medication compliance was assessed by the investigators by counting the number of pills returned at the time of specified clinic visits.

The tested product is made of a patented dietary supplement association (*DIF1STAT®*) of *M. purpureus* titrated extract (1.5% monacolin K), linear aliphatic alcohols (60% octacosanol) and niacine, each capsule (daily dose) containing *M. purpureus* 340 mg (the maximal amount that can be used in a dietary supplement following Italian law), octacosanol 10 mg (extracted from derived from sugar cane wax), and Niacin 27 mg (150% RDA) (Difass S.r.l., San Marino Republic).

All data have been inserted by trained personnel in an appositely created database and statistically analysed with the help of SPSS 8.0, version for Windows.

A complete descriptive analyses of all studied parameters has been carried out (range, mean, standard deviation, mean standard error, Kurtosis, Skewness), followed by a Kolmogorov–Smirnov normality test. The comparative analysis was carried out applying the Student's *t*-test for paired samples to evaluate drug induced changes, while the *t*-test for unpaired samples to evaluate eventual differences as regards the two treatment groups. A linear regression analysis was also carried out to evaluate if the hypocholesterolemic effect was related to the baseline cholesterolemia value. A *p*-value less than 0.05 has been considered as significant for all tests.[9]

Results

The main laboratory changes associated to the 2 months treatment with the tested dietary supplement and Pravastatin 20 mg are resumed in Table 2. The statistically significant differences observed in both groups regard the reduction of TC, LDL-C and TG, both in men and women. The observed cholesterolemia reduction was similar to that observed in the Pravastatin treated group ($p > 0.05$ for all parameters). In this group, however, a slight but significant increase in plasma HDL-C was also observed ($p < 0.05$, compared to the base-line value). The decrease in the TC/HDL-C risk ratio was significant in men treated with both products, but only in women treated with Pravastatin Fig. 1.

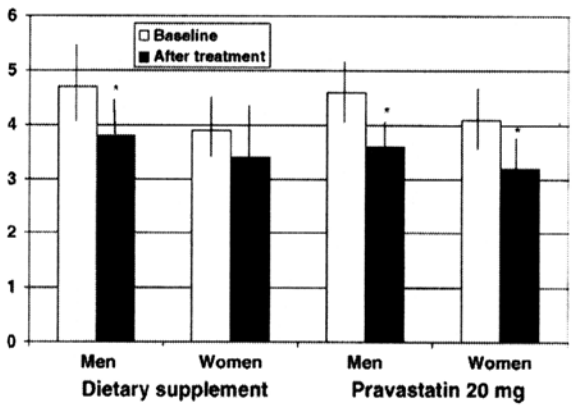

Figure 1 TC/HDL-C ratio before and after treatment with the tested dietary supplement or Pravastatin 20 mg in men and women (*$P = 0.05$).

Table 2 Effect of 2 months supplementation with the studied dietary supplement and Pravastatin 20 mg on the main laboratory parameters of our moderate hypercholesterolemic patients.

	Dietary supplement group						Pravastatin group					
	Men			Women			Men			Women		
	Mean ± S.D. change	*t*	2-tails significance	Mean ± S.D. change	*t*	2-tails significance	Mean ± S.D. change	*t*	2-tails significance	Mean ± S.D. change	*t*	2-tails significance
TC (mg/dL)	−40.1 ± 22.3	11.10	<0.001	−38.1 ± 31.9	9.78	<0.001	−38.4 ± 19.7	10.08	<0.001	−37.5 ± 25.3	9.73	<0.001
HDL-C (mg/dL)	+1.7 ± 12.6	−0.86	0.397	−1.9 ± 9.5	1.65	0.105	+3.9 ± 1.9	−0.23	0.397	+3.7 ± 2.2	1.11	0.049
LDL-C (mg/dL)	−36.8 ± 27.2	8.23	<0.001	−32.2 ± 30.3	8.48	<0.001	−37.1 ± 24.4	8.18	<0.001	−32.7 ± 19.8	8.37	<0.001
TG (mg/dL)	−24.8 ± 59.8	2.52	0.016	−22.7 ± 44.9	4.07	<0.001	−25.1 ± 53.2	2.53	0.015	−23.7 ± 41.6	4.06	<0.001
GOT (mg/dL)	−1.9 ± 9.2	1.28	0.208	+0.01 ± 5.9	−0.17	0.865	+1.4 ± 6.5	1.23	0.184	+0.09 ± 4.7	−0.15	0.669
GPT (mg/dL)	−1.3 ± 7.4	1.03	0.310	+2.5 ± 25.8	−0.77	0.444	+1.2 ± 5.9	1.01	0.455	+2.0 ± 11.7	−0.71	0.749
CPK (mU/mL)	+17.4 ± 31.9	−2.00	0.006	+1.8 ± 26.9	−0.42	0.674	+12.1 ± 24.6	−1.89	0.119	+2.5 ± 19.9	−0.35	0.591

The hypocholesterolemic effect of the dietary studied product appears to be strictly related to the baseline TC value in women ($B = 0.479$, beta = 0.114, $t = 4.203$, $p < 0.001$), but not in men ($B = 0.311$, beta = 0.168, $t = 1.852$, $p = 0.073$).

Even if CPK plasma level significantly increased in men, the CPK value never raised over the upper normality level. Two men and four women treated with the tested dietary supplement withdraw from the study because of gastrointestinal disorder (dyspepsia, colitis). The relationship with dietary supplement use was doubtful in the 50% of cases and possible and in the remaining 50%. No side effect has been observed in the Pravastatin treated group.

Discussion

Red yeast rice is the fermented product of rice on which red yeast (*M. purpureus*) has been grown. The use of red yeast rice was first documented in the Tang Diynasty in 800 ad. It has been used to make rice wine, as a food preservative to maintain the colour and taste of fish and meat,[10] and for its medicinal properties. A complete and detailed description of its manifacture is found in the ancient Chinese pharmacopoeia, Ben Cao Gang Mu-Dan Shi Bu Yi, published during the Ming Dinasty (1368–1644).[11] The hypocholesterolemic efficacy of *M. purpureus* was tested in different clinical studies,[12] also in subjects at high risk of pharmacological interactions like the HIV positive patients under antiretroviral therapy.[13] Acting through a direct inhibition of the 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase,[14] potentially it has also the same side effects of the statins.[15] Thus, to improve its safety using lower dosage of product without loosing hypocholesterolemic activity, it was combined with octacosanols. Octacosanols hypocholesterolemic efficacy was also tested in different clinical studies,[16] even on subjects at high risk for cardiovascular disease like diabetics[17] and at high risk for pharmacological interactions like elderly.[18] Octacosanols appear not to reduce liver cholesterol synthesis by a direct inhibition of the 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase, but inducing its down-regulation,[19] so acting in a synergic way with Monascus. However, both Monascus and octacosanols have a prevalent hypocholesterolemic action while their effect on plasma Triglycerides is more negligible. This is the reason why the tested product was also added in niacin that has a known positive effect on triglyceridemia and, at high dosage, on HDL-C plasma level.[20] Some preliminary tests carried out on small patients samples let

suggest that these low-dosed could have a synergistic effect on plasma lipid reduction when together assumed, and made it possible to register a brand mark (*DIF1STAT®*).

In this pilot study, the combination of *M. purpureus*, octacosanols and niacin determined a significant decrease of TC, LDL-C and TG in moderately hypercholesterolemic subjects without clinically relevant change in liver and muscular toxicity markers. The reduction of LDL-C reach the 20%, and it is similar to that obtained with a well-known effective statin like Pravastatin. The employed dose of Pravastatin was, of course, lower than that tested in large prevention trials,[21] but our aim was to test the efficacy and safety of a dietary supplement to be used in mild to moderate hypercholesterolemias of subjects with low risk for cardiovascular disease, so maybe not the best candidate for a statin treatment. A significant 16% decrease of TG plasma level and a slight not statistically significant increase in HDL-C have also been observed after 2 months of dietary supplementation with the tested products. The not impressive effect on TG and even more on HDL-C is maybe due to the choice to use a low dose of niacin in order to prevent the high doses side effects.[22]

In countries like Italy, were statin treatment is reimbursed by the government or by insurances only to subject with elevated cardiovascular risk, considering the observed effect on LDL-C, the tested product could be more cost-effective in general population, because the cost of therapy is one of the main barriers to the use of statin in cardiovascular disease prevention.[23]

Of course, we are aware that the main limitation of this study is that it is not a randomised double-blind clinical trial, and that the comparison with a reference drug was done only in an indirect way. Then, patients were not selected on the basis of a specific disease of the lipid metabolism and a relatively small range of laboratory parameters were monitored for a short time. Moreover, it is well-known that atherosclerosis and cardiovascular risk are not influenced only by the plasma lipid levels, but also by other classical risk factors (such as hypertension, diabetes, smoking habits) and emerging risk factors (such as genetic factors, prothrombotic factors, subclinical chronic inflammation, environmental factors).[24–26] So further long-term adequate studies have to be carried out before inferring about the efficacy and safety of the tested dietary supplement. The next step of our research will be in fact a double-blind three-arm clinical trial, directly comparing the long-term efficacy and safety of the tested dietary supplement, a full-dosed statin and an intensive life-style improvement program, monitoring a wider range of clinical and laboratory parameters.

A limitation shown by the tested dietary supplement is the lack of effect on HDL-C plasma level, that is of course one lipidic main determinant of cardiovascular risk.[27] However, it was specifically studied as a product with mainly antihypercholesterolemic drug and the raise in HDL-C as well as the reduction in TG are not its main indication.

Besides, the results of this pilot study are encouraging as it regards the tested dietary supplement efficacy and safety to mildly reduce cholesterolemia in subjects with low risk for cardiovascular disease.

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CLINICAL STUDY

Efficacy and safety of *Monascus purpureus* Went rice in subjects with hyperlipidemia

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Abstract

Objective: The purpose of this study was to assess the lipid-lowering effect of *Monascus purpureus* Went rice on serum lipids in patients with hyperlipidemia, and to assess its safety by reporting adverse events and clinical laboratory measurements.

Design and methods: This was a randomized, double-blind, placebo-controlled study. In all, 79 patients (aged 23–65 years) with a mean baseline low-density lipoprotein cholesterol (LDL-C) level of 5.28 mmol/l (203.9 mg/dl) received a twice daily dose of placebo or *Monascus purpureus* Went rice (600 mg) for 8 weeks.

Results: At week 8, *Monascus purpureus* Went rice therapy reduced LDL-C by 27.7%, total cholesterol by 21.5%, triglycerides by 15.8% and apolipoprotein B by 26.0%. High-density lipoprotein cholesterol and apolipoprotein A-I levels were increased by 0.9 and 3.4% respectively (not significant). No patient in the *Monascus purpureus* Went rice treatment group had an alanine aminotransferase (ALT), aspartate aminotransferase (AST) or creatine phosphokinase (CPK) measurement that was ≥ 3 times the upper limit of normal at week 4 and week 8.

Conclusion: *Monascus purpureus* Went rice significantly reduced LDL-C, total cholesterol, triglycerides and apolipoprotein B levels, and was well tolerated in patients with hyperlipidemia. However, this study only provides data from an 8-week trial and long-term safety and efficacy data are needed.

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Introduction

Monascus purpureus rice (紅麴 in Chinese), popularly called red yeast rice, is described as the fermented product of rice on which red yeast (*Monascus purpureus*) has been grown. This product has been used for centuries in China to make rice wine and to flavor foods. Traditional red yeast rice continues to be a dietary staple in many Asian countries, including China and Japan, with consumption ranging from 14 to 55 g/person per day (1). Recent studies have shown that *Monascus purpureus* rice contained 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors (statins), large quantities of unsaturated fatty acids (>125 mg/g *Monascus purpureus* rice preparation), beta-sitosterol, campesterol and stigmasterol (2–4). These components are effective in reducing serum lipid (5). The lipid-lowering effects of *Monascus purpureus* rice have been shown in several animal models of hyperlipidemia to inhibit and prevent increases in total cholesterol, low-density lipoprotein cholesterol (LDL-C) and triglycerides (6). In this study, the efficacy and safety of *Monascus purpureus* Went rice were evaluated by measuring percentage changes of lipid profiles, observing any adverse effects and completing laboratory tests at regular intervals in subjects with hyperlipidemia.

Subjects and methods

Participants

The study's clinical phase began in December 2001 and was completed in January 2003. Subjects with hyperlipidemia in this study were recruited from the outpatient clinic at the China Medical University Hospital, Taichung, Taiwan or by advertising. Study participants were men and women aged 18–65 years with a body mass index of $< 30\ kg/m^2$. Participants had to have a total cholesterol level ≥ 6.22 mmol/l (240 mg/dl), an LDL-C level ≥ 4.14 mmol/l (160 mg/dl) and a triglycerides level ≤ 4.52 mmol/l (400 mg/dL) at two qualifying visits 4 weeks apart. Women who were pregnant or breastfeeding were excluded from the study, as were patients who met any of the following conditions: hypothyroidism, nephrotic syndrome or renal dysfunction (serum creatinine >132.6 μmol/l (1.5 mg/dl)); diabetes mellitus; chronic gout; active liver disease or hepatic dysfunction (aspartate aminotransferase

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(AST) or alanine aminotransferase (ALT) > 2 times the upper limit of normal (ULN)); creatine phosphokinase (CPK) > 3 times the ULN; uncontrolled hypertension (systolic blood pressure > 160 mmHg or diastolic blood pressure > 100 mmHg); cerebrovascular disease, cardiovascular surgery, myocardial infarction, coronary angioplasty, coronary artery bypass graft, severe or unstable angina, or major operations within 6 months prior to the study period; current or recent history of alcohol abuse; significant abnormalities that the investigator believed could compromise the patient's safety in participating in the study; participation in another clinical trial within the 30-day period before consideration for entry into this study; known hypersensitivity to lipid-modifying agents; and use of any drugs known to affect lipid levels, immunosuppressive agents, drugs associated with rhabdomyolysis in combination with statins (e.g. cyclosporine and erythromycin), or mibefradil dihydrochloride. Patients taking a lipid-lowering drug could be considered for screening after a 4-week washout period, with the exception of probucol – which had to have been discontinued for at least 6 months. The study complied with the Declaration of Helsinki. The institutional ethics review boards approved the protocol, and all participants gave their informed consent.

Randomization and sample size determination

A permuted-block randomization was employed to generate the random assignment of subjects by order of entry into two different treatment groups. Each group of subjects received either *Monascus purpureus* Went rice or placebo. In this study, a sample size of 79 patients was used for characterizing and comparing the efficacy as well as the safety of *Monascus purpureus* Went rice and placebo.

The sample size for this study was based on the primary efficacy outcome, the change from baseline to 8 weeks in mean LDL-C was compared between the two groups. The standard deviation (S.D.) of the mean LDL-C level was 1 mmol/l (the S.D. of the mean for the treatment group and that of the placebo group were similar). We set the two-sided alpha (type I error) at 0.05 and the beta (type II error) at 0.10 (power of 90%). According to these assumptions, a sample size of approximately 17 subjects in each group was needed to detect a difference of 1 mmol/l in mean LDL-C.

Protocol

After a minimum of 4 weeks on an American Heart Association Step 1 diet, 79 patients were instructed to continue the diet and were randomly assigned to 8 weeks of treatment with rice powder placebo or *Monascus purpureus* Went rice (Fig. 1). All patients received dietary instruction from a registered dietitian at every research visit and were contacted by telephone every week during the study. The study was double-blind. We reviewed participants every 4 weeks and blood samples were obtained after 12-h overnight fasts. The laboratory staff responsible for analyses were blinded to treatment and received samples labeled with name codes and dates. The study protocol was approved by the Institutional Review Board of China Medical University Hospital and by the Department of Health, Taiwan.

Monascus purpureus Went rice therapy

For the treatment group, *Monascus purpureus* Went rice was pulverized and 600 mg of this milled preparation

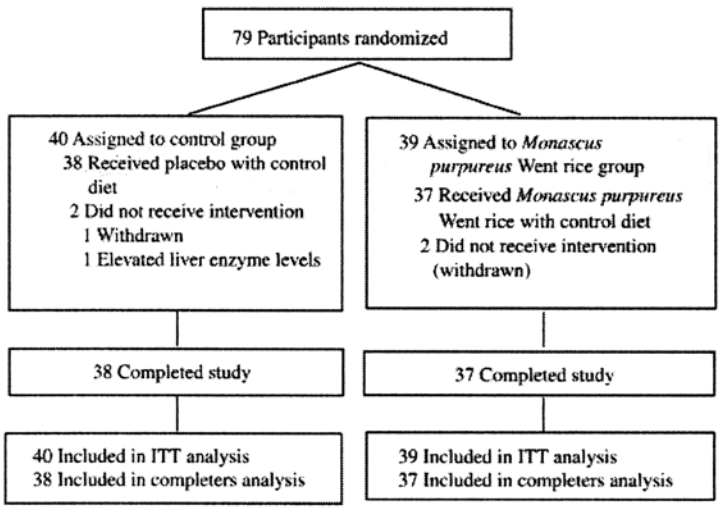

Figure 1 Flow of patients through the trial.

Table 1 Composition of *Monascus purpureus* Went rice.

	Concentration
Protein	17.00%
Starch	68.00%
Fat	4.00%
Of which:	
Linoleic acid	48.13%
Oleic acid	28.78%
Palmitic acid	18.61%
Stearic acid	4.49%
Ergosterol	0.30%
Fiber	2.00%
Water	<5.00%
HMG-CoA reductase inhibitors (statins)	1.16%
Lovastatin	0.95%
Other statins	0.21%
r-Aminobutyric acid (GABA)	2.55%
Alkaloids	
Water-soluble	0.30%
Lipid-soluble	0.05%
Glycosides	0.06%
Flavonoids	0.05%
Natural pigments	0.01%
Ethanol extracts	≥12.00%
Water extracts	≥10.00%
Citrinin	<1.5 p.p.m.[a]
Pb	<20.0 p.p.m.
Cd	<0.6 p.p.m.
Hg	<0.5 p.p.m.
As	<5.0 p.p.m.
Cu	<70.0 p.p.m.

Data are based on unpublished analyses on file with Y & B Pharmaceuticals Co., Ltd, Taipei, Taiwan. HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A. Percentage concentrations are percentage by weight.
[a] Mostly not detectable by HPLC.

encapsulated in each capsule under Good Manufacturing Practices conditions (Y & B Pharmaceuticals Co., Ltd. Taipei, Taiwan). The composition of *Monascus purpureus* Went rice is shown in Table 1. The placebo was made of grounded rice with food color mimicking the color and appearance of the active drug. Both *Monascus purpureus* Went rice and placebo capsules were dispensed by the hospital pharmacy in identical containers marked with the participant's name codes. Participants were asked to take one capsule (600 mg *Monascus purpureus* Went rice or placebo) twice daily, 30 min after breakfast and dinner, for the 56 days of the study, and to return the containers for capsule counts on each clinic visit.

Analyses

All samples from a given individual were labeled by code. Total cholesterol and triglycerides were determined by the enzymatic method using commercial kits (Beckman & Coulter, Fullerton, CA, USA). High-density lipoprotein cholesterol (HDL-C) and LDL-C were measured by the direct method (7) using commercial kits (Beckman & Coulter, LX 20 Pro, Japan). All the analyses were performed by Beckman & Coulter, Tokyo autoanalyzer. The intra-assay coefficients of variation (CV%) were 3.1, 2.3, 3.5 and 3.6% for LDL-C, total cholesterol, triglycerides and HDL-C respectively. Serum apolipoprotein A-I and apolipoprotein B were measured by nephelometry (8) (intra-assay coefficient of variation, 2.2 and 1.9 respectively).

Evaluation of efficacy

The primary analysis of efficacy endpoints was based on the intent-to-treat population and completing participants. Baseline was defined as the measurements taken at randomization. The primary measure of efficacy was the percentage change in LDL-C level from baseline to week 8. Secondary measures of efficacy were percentage changes (from baseline to week 8) in total cholesterol, HDL-C, triglycerides, apolipoprotein A-I and apolipoprotein B.

Safety analyses

Safety was evaluated in all randomized patients who had taken at least one dose of study medication and provided any follow-up information. All adverse effects that occurred during the clinical trial were recorded. Their relation to the study drug (definitely, probably, possibly, unlikely, definitely not) and their intensity (mild, moderate, severe) were assessed by the investigator. Because statins are present in *Monascus purpureus* Went rice, ALT, AST and CPK were measured. In addition, physical examinations and clinical laboratory determinations were performed at screening, randomization, week 4 and study termination.

Statistical methods

Descriptive statistics such as a number of observations, means, standard deviations and percentages were used to summarize the baseline variables. All available tests were two sided and were evaluated at the 0.05 level of significance. For subjects' demographic information, the comparability between two groups was examined using an unpaired *t*-test for continuous variables, and Fisher's exact test and Mantel–Haenszel test for categorical variables. Blood lipids, safety parameters, vital signs and laboratory examinations were analyzed based on change from baseline; they were analyzed by unpaired *t*-test for between-group variation, and by paired *t*-test for within-group variation. Fisher's exact test was used to compare the number of subjects with adverse effects between groups.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Table 2 Patient characteristics at baseline by treatment group.

	Placebo ($n = 40$)	*Monascus purpureus* Went rice ($n = 39$)	*P* value
Age (years)	46.5 ± 9.5	46.3 ± 10.1	0.922[a]
Sex, male	22 (55%)	23 (59%)	0.821[b]
BMI (kg/m^2)	23.4 ± 2.7	24.3 ± 3.3	0.161[a]
Smoking history			0.681[c]
Never smoked	34 (85%)	32 (82%)	
Current smoker	4 (10%)	6 (15%)	
Alcohol history			0.193[c]
Never	33 (83%)	36 (92%)	
Abusive	0 (0%)	0 (0%)	

Data are means ± s.d.or number with percentage in brackets. BMI, body mass index.
[a] Unpaired *t* test.
[b] Fisher's exact test.
[c] Mantel–Haenszel test.

Results

Patient characteristics at baseline

The characteristics of subjects who entered the study are summarized in Table 2. The demographics and baseline characteristics of subjects in the two groups were similar. The percentage of males and females was about equal in both groups. The age of subjects in both groups was about 46 years with a range of 23–65 years. Over 80% of subjects in both groups had never smoked. A high percentage of subjects in both groups had never had alcohol. Both groups had comparable body mass indices (BMI).

At baseline, there were no significant differences between groups for the efficacy parameters (LDL-C, total cholesterol, HDL-C, triglycerides, apolipoprotein A-I and apolipoprotein B). All these data affirmed that the distribution of subjects between the two groups was well balanced (Table 3).

Exposure

Of the 79 patients randomized to treatment, 39 received *Monascus purpureus* Went rice and 40 placebo. Four participants who were randomized did not start the study (*Monascus purpureus* Went rice, 2 patients (5.1%); placebo, 2 patients (5.0%)). Compliance with study therapy (defined as ≥80% of pills taken) was 81.6% for placebo and 89.2% for *Monascus purpureus* Went rice.

Lipid and lipoprotein response

Efficacy evaluation was carried out on completing participants, in which 75 subjects were included. LDL-C levels, the primary efficacy endpoint, were comparable between the two groups at baseline (Table 3). The *Monascus purpureus* Went rice reduced LDL-C levels by 27.7% but placebo caused only a 1.5% decrease at week 8. At baseline, the two groups showed comparable total cholesterol, triglycerides and apolipoprotein B levels ($P > 0.05$). The 8-week treatment with *Monascus purpureus* Went rice significantly reduced total cholesterol levels by 21.5%, triglycerides by 15.8% and apolipoprotein B by 26%. Worthy of special mention is that *Monascus purpureus* Went rice resulted in reductions in LDL-C level by 30.6%, total cholesterol level by 23.7%, triglycerides level by 13.4% and apolipoprotein B level by 28.9% that were observed as early as 4 weeks after its administration (Table 3). In contrast, the placebo treatment resulted in almost no reduction of total cholesterol (−0.4%) and triglycerides (+1%) at week 4 and week 8. A 3.9% reduction of apolipoprotein B levels was observed after the placebo treatment at week 8. The capacity of *Monascus purpureus* Went rice to change HDL-C and apolipoprotein A-I levels was limited. The difference in the percentage change of HDL-C and apolipoprotein A-I was comparable between the two groups ($P > 0.05$).

Safety analyses

A safety evaluation was performed based on the 'safety population', which included a total of 75 subjects ($n = 37$ in the *Monascus purpureus* Went rice group and $n = 38$ in the placebo group) who were randomized and had taken at least one dose of the study medication, with follow-up information after randomization. We recorded any complaints mentioned, however trivial. Therefore, up to 65% of the total safety population ($n = 49$) reported one or more adverse events and 8% of the total safety population ($n = 6$) had one or more drug-related adverse events. Nevertheless, the incidence of 'one or more adverse events', 'drug-related adverse events' and 'serious adverse events' between the two groups were comparable ($P > 0.05$) (Table 4). *Monascus purpureus* Went rice treatment produced a slight increase in ALT (2.3 U/l) and AST (0.8 U/l), but no patient had an ALT or AST measurement ≥3 times the ULN at week 4. At week 8, *Monascus purpureus* Went rice had the same safety profile. Baseline serum CPK was similar in both groups and was not significantly different after 8 weeks of the treatment. In the *Monascus purpureus* Went rice group, mean serum CPK at baseline was 116.4 U/l (S.D. = 66.0) and mean serum CPK at week 8 was 129.6 U/l (S.D. = 42.3). In addition, there was no patient with myopathy (defined as a CPK level ≥10 times the ULN with muscle symptoms) or CPK values ≥3 times the ULN at weeks 4 and 8. In particular, no cases of rhabdomyolysis or anaphylaxis were observed. In addition, *Monascus purpureus* Went rice did not alter other safety parameters including vital signs, results of physical examination, hematology, serum chemistry, urine analysis and electrocardiogram.

Table 3 Change from baseline in serum lipid variables, by week and treatment group (completer population).

		Placebo ($n = 38$)		*Monascus purpureus* Went rice ($n = 37$)	
Lipid parameter	**Week**	Level	Percentage change	Level	Percentage change
LDL-C (mmol/l)[a]	0	5.34+1.15		5.20+0.86	
	4	5.27+1.14	−0.5+14.1	3.61+0.83*,‡	−30.6+10.1‡
	8	5.19+1.01	−1.5+16.0	3.76+0.85*,‡	−27.7+12.2‡
TC mmol/l[a]	0	7.41+1.12		7.27+0.83	
	4	7.34+1.15	−0.5+10.9	5.54+0.76*,‡	−23.7+7.2‡
	8	7.37+1.19	−0.4+9.5	5.68+0.79*,‡	−21.5+9.4‡
HDL-C mmol/l[a]	0	1.32+0.28		1.31+0.39	
	4	1.32+0.30	0.5+11.5	1.30+0.35	0.4+8.8
	8	1.33+0.31	1.0+10.2	1.30+0.32	0.9+10.6
TG mmol/l[b]	0	1.38+0.66		1.46+0.72	
	4	1.38+0.71	5.3+32.3	1.26+0.82†§	−13.4+33.5§
	8	1.31+0.63	1.0+34.4	1.22+0.72†§	−15.8+25.1§
Apo A-I (g/l)[c]	0	1.34+0.25		1.34+0.22	
	4	1.32+0.23	0.0+16.3	1.34+0.21	1.0+12.2
	8	1.35+0.20	2.3+12.8	1.37+0.21	3.4+14.0
Apo B (g/l)[c]	0	1.57+0.30		1.54+0.27	
	4	1.51+0.30	−3.2+12.7	1.10+0.25*,‡	−28.9+8.9‡
	8	1.50+0.33	−3.9+12.2	1.14+0.27*,‡	−26.0+14.3‡

Data are means + s.d.TC, total cholestrol; TG, triglycerides; Apo A-I, apolipoprotein A-I; Apo B, apolipoprotein B.
[a] To convert total cholesterol, HDL-C and LDL-C to mg/dL, divide values by 0.0259.
[b] To convert triglyceridess to mg/dL, divide values by 0.0113.
[c] To convert Apo A-I and Apo B to mg/dL, divide values by 0.01.
* Significantly different from baseline, $P < 0.001$.
† Significantly different from baseline, $P < 0.05$.
‡ Significantly different from control group at the same week, $P < 0.001$.
§ Significantly different from control group at the same week, $P < 0.05$.

Table 4 Number and percentage of patients with adverse events (AEs).

	Placebo ($n = 38$)	*Monascus purpureus* Went rice ($n = 37$)	*P* value
All AEs	28 (74%)	21 (57%)	0.168
Drug-related AEs	3 (8%)	3 (8%)	1.000
Serious AEs	0 (0%)	1 (3%)[a]	0.494
Serious drug-related AEs	0	0	
ALT or AST ≥ 3 times ULN	0	0	
CPK ≥ 3 times ULN	0	0	
CPK ≥ 10 times ULN with muscle symptoms	0	0	

[a] Breast cancer: unrelated to the study drug.

During the trial, one serious adverse event (breast carcinoma) occurred in the *Monascus purpureus* Went rice-treated group. The 63-year-old female was diagnosed with breast cancer after a 4-week administration of *Monascus purpureus* Went rice. However, the incident was not related to *Monascus purpureus* Went rice.

Intention-to-treat analysis

This study was also analyzed on the basis of intention to treat, including the four individuals with baseline values who dropped out or were withdrawn. The differences in blood lipid levels were significantly different between the treatment groups, as was observed when these participants were not included in the analysis (Table 5). Furthermore, the mean reduction in LDL-C level across treatments was still significant at 26.3% (13.4%) ($P < 0.001$) for the *Monascus purpureus* Went rice-treated group when it was assumed that the four additional participants showed no change in response to the treatment.

Discussion

Coronary artery disease (CAD) is a leading cause of death in the developed world today. It is well established that increased total cholesterol, LDL-C and triglycerides concentrations, as well as decreased HDL-C concentrations, are strong independent predictors of CAD (9). The statins have repeatedly been shown to reduce mean serum LDL-C concentrations by 28–35% in long-term trials (10–12), with corresponding reductions in cardiovascular death of 23–32% in both primary and secondary prevention trials (11, 12). A previous study showed that red yeast rice resulted in significant reductions in LDL-C (1.00 mmol/l), total cholesterol (1.09 mmol/l) and triglycerides (0.17 mmol/l) levels (2). In this study, *Monascus purpureus* Went rice resulted in significant reductions in LDL-C (1.59 mmol/l (30.6%)), total cholesterol (1.73 mmol/l (23.7%)) and triglycerides (0.21 mmol/l (13.4%)) levels from baseline to week 4. These reductions were maintained at 8 weeks.

Table 5 Change from baseline in serum lipid variables, by week and treatment group (intent-to-treat population).

		Placebo ($n = 40$)		*Monascus purpureus* Went rice ($n = 39$)	
Lipid parameter	**Week**	Level	Percentage change	Level	Percentage change
LDL-C (mmol/l)[a]	0	5.35+1.12		5.20+0.84	
	4	5.01+1.61	−0.5+13.7	3.43+1.14*,‡	−29.1+12.0‡
	8	5.22+0.99	−1.4+15.6	3.83+0.89*,‡	−26.3+13.4‡
TC (mmol/l)[a]	0	7.40+10.9		7.28+0.84	
	4	6.97+1.97	−0.5+10.7	5.26+1.44*,‡	−22.5+8.8‡
	8	7.36+1.16	−0.4+9.3	5.78+0.89*,‡	−20.4+10.3‡
HDL-C (mmol/l)[a]	0	1.32+0.28		1.32+0.42	
	4	1.25+0.41	0.5+11.2	1.23+0.45	0.4+8.6
	8	1.33+0.30	1.0+10.0	1.32+0.36	0.9+10.4
TG (mmol/l)[b]	0	1.34+0.67		1.46+0.72	
	4	1.31+0.75	5.0+31.5	1.19+0.84†,§	−12.7+32.8§
	8	1.27+0.63	0.9+33.5	1.22+0.72†,§	−15.0+24.7§
Apo A-I (g/l)[c]	0	1.33+0.25		1.37+0.29	
	4	1.26+0.37	0.0+15.9	1.27+0.36	1.0+11.9
	8	1.34+0.20	2.2+12.4	1.40+0.28	3.2+13.6
Apo B (g/l)[c]	0	1.56+0.30		1.55+0.26	
	4	1.43+0.44	−3.1+12.4	1.05+0.35*,‡	−27.4+10.8‡
	8	1.50+0.32	−3.7+11.9	1.16+0.28*,‡	−24.7+15.1‡

Data are means + s.d. Abbreviations and symbols are as in Table 3.

The results of this study again confirm that *Monascus purpureus* Went rice has positive effects on plasma lipids.

It is noteworthy that *Monascus purpureus* Went rice reduced apolipoprotein B levels by 26.0% in this study. Each of the atherogenic particles – namely, very low density lipoprotein, intermediate-density lipoprotein, LDL and lipoprotein(a) – contain one molecule of apolipoprotein B. Thus, the serum concentration of apolipoprotein B reflects the total number of these particles. A systematic review showed the apolipoprotein B concentration to be a better estimate of the risk of vascular events than the LDL-C level (13). This is supported by the fact that apolipoprotein B has been accepted as an alternative to the cholesterol indices in the new Canadian Lipid Working Group on hypercholesterolemia and other dyslipidemias (14) and in the new Canadian Diabetes Association Guidelines (15).

A specialist commented that *Monascus purpureus* Went rice contains statins and yields similar hypolipidemic effects to statins (16), therefore the other active components may only play a minor role in the action of *Monascus purpureus* Went rice. One study (17) reported mean LDL-C reductions to be 20, 26, 19 and 24% for fluvastatin (80 mg), lovastatin (80 mg), pravastatin (40 mg) and simvastatin (20 mg) respectively; mean apolipoprotein B levels were reduced by 16, 19, 16 and 20% respectively. In this study, 1200 mg of *Monascus purpureus* Went rice contains a total of 13.9 mg statins (contains 11.4 mg lovastatin) but reduced LDL-C by 27.7% and apolipoprotein B by 26.0%. Hence, we infer that the hypolipidemic effect of *Monascus purpureus* Went rice is unlikely to be due solely to statins, but rather to result from other substances in the *Monascus purpureus* Went rice. *Monascus purpureus* Went rice also contains phytosterols, mainly beta-sitosterol with campesterol and stigmasterol. Plant sterols have been shown to decrease total cholesterol and LDL-C concentrations in several population groups (18–21).

In this study, all patients received dietary consultation but there was no record of diets of the participants. We compared BMI in the two treatment groups at baseline, at 4 weeks and at 8 weeks. There were no significant differences in BMI within or between study groups. Therefore, we infer that neither dietary fat intake or exercise had an impact on lipid profile in the study period.

Asymptomatic elevations of transaminases (>3 times the ULN) have been observed with all the statins, are relatively common (0.1–2.0%) and are dose related (22). It is interesting to note that no patient in the *Monascus purpureus* Went rice treatment group had an ALT or AST measurement ≥3 times the ULN at week 4 or week 8. Statin therapy is also known to cause increases in CPK activity, mostly during the initial stages of treatment and upward dose titration. Nevertheless, no trials have examined the effect of red yeast rice on CPK so far. In this study, no cases of CPK measurement ≥3 times the ULN or rhabdomyolysis were reported. Possible *Monascus purpureus* Went rice-related adverse events were one abnormal liver function test (ALT, 57 U/l; ULN 40 U/l), one CPK increase (151 U/l; ULN, 140 U/l), and one lactate dehydrogenase increase (208 U/l; ULN, 192 U/l). All these events were mild in severity and required no treatment. On the other hand, mild leukopenia ($n = 1$), diarrhea ($n = 1$) and nausea ($n = 1$) were found to be possibly drug-related adverse events in the placebo treated group. In general, *Monascus purpureus* Went rice was well tolerated and no

one discontinued the study due to adverse effects caused by the study drug. Extensive animal studies of red yeast rice extracts have been conducted. In an acute toxicity study in mice, there were no toxic effects noted when a single dose of the extract was administered at 533 times the typical human dose (23).

More recently, citrinin has also been isolated from *Monascus ruber* and *Monascus purpureus*, industrial species used to produce red pigments (24). Citrinin acts as a nephrotoxin in all animal species tested, but its acute toxicity varies in different species (25). The 50% lethal dose for ducks is 57 mg/kg; for chickens it is 95 mg/kg; and for rabbits it is 134 mg/kg (26). In addition, wheat, oats, rye, corn, barley and rice have all been reported to contain citrinin (27). Although citrinin is regularly associated with human foods, its significance for human health is unknown. In the present study, citrinin was not detected in *Monascus purpureus* Went rice.

Worthy of mention is the fact that *Monascus purpureus* Went is a specific strain of red yeast and a different strain of *Monascus* could result in different efficacy and safety profiles. Accordingly, red yeast rice material produced in the traditional way has yielded different amounts of active compounds compared with the *Monascus purpureus* Went rice extract. In other words, the home-processed red yeast rice may not exhibit the same hypolipidemic effect as the *Monascus purpureus* Went rice extract (16).

In summary, the significant effect of *Monascus purpureus* Went rice in reducing LDL-C, total cholesterol, triglycerides and apolipoprotein B levels was found as early as week 4 and was consistent until week 8. Moreover, no patient in the *Monascus purpureus* Went rice treatment group had an ALT, AST or CPK measurement ≥ 3 times the ULN at week 4 and week 8. However, this study only provides 8 weeks of data and further studies on the long-term safety and efficacy of *Monascus purpureus* Went rice in a larger population are needed.

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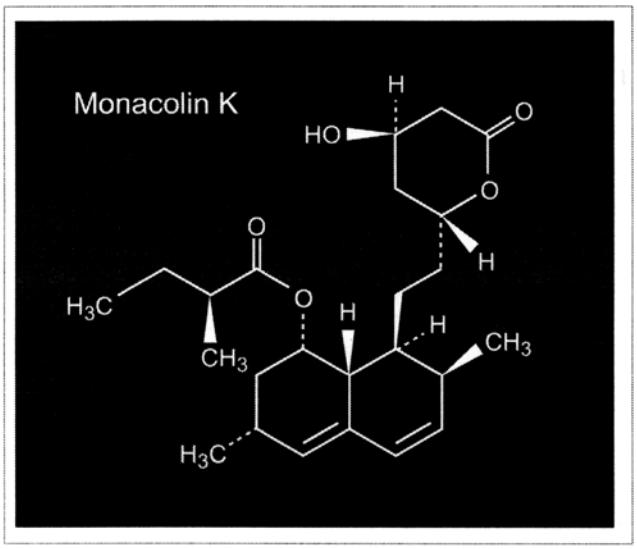

Monascus purpureus (Red Yeast Rice)

Description

Red yeast rice, a fermented product of rice on which red yeast (*Monascus purpureus*) has been grown, has been used in Chinese cuisine and as a medicinal food to promote "blood circulation" for centuries. In Asian countries, red yeast rice is a dietary staple and is used to make rice wine, as a flavoring agent, and to preserve the flavor and color of fish and meat.[1] Red yeast rice forms naturally occurring hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase) inhibitors known as monacolins. The medicinal properties of red yeast rice favorably impact lipid profiles of hypercholesterolemic patients.

Active Constituents

The HMG-CoA reductase activity of red yeast rice comes from a family of naturally occurring substances called monacolins. Monacolin K, also known as mevinolin or lovastatin, is the ingredient in red yeast rice that Merck & Co., pharmaceutical manufacturer of Mevacor® (lovastatin), asserts is a patented pharmaceutical. However, red yeast rice contains a family of nine different monacolins, all of which have the ability to inhibit HMG-CoA reductase. Other active ingredients in red yeast rice include sterols (beta-sitosterol, campesterol, stigmasterol, sapogenin), isoflavones, and monounsaturated fatty acids.[2]

Mechanisms of Action

The first documentation of the biomolecular action of red yeast rice was published in 2002. The results indicate one of the anti-hyperlipidemic actions of red yeast rice is a consequence of an inhibitory effect on cholesterol biosynthesis in hepatic cells.[3] It is unclear whether the lipid-lowering effect of red yeast rice is due solely to the monacolin K content, or if other monacolins, sterols, and isoflavones contribute to its cholesterol-lowering effect.

The monacolin K content of one proprietary preparation of red yeast rice used in a clinical trial was calculated to be 0.2 percent of the total product.[2] This trial used a daily dosage of 2.4 grams of red yeast rice (the corresponding monacolin K dosage would be 4.8 mg). The dosages used in clinical trials of lovastatin are 20-40 mg daily.[4] It is unlikely the lipid-lowering effects found in this study was a result of the monacolin K content alone.

Clinical Indications

Hyperlipidemia

The first human trial, an eight-week study conducted in China, evaluated the effect of 1.2 g/day red yeast rice on 324 hypercholesterolemic adults (total cholesterol above 230 mg/dL) who also had elevated LDL (over 130 mg/dL) and low HDL (under 40 mg/dL) versus controls.[5] Total cholesterol, LDL cholesterol, and triglycerides dropped by 23, 31, and 34 percent, respectively. Serum HDL levels increased by 20 percent. The second study included 83 hypercholesterolemic adults on 2.4 g red yeast rice daily or placebo.[2] Participants were asked to maintain a diet of 30-percent fat, 10-percent saturated fat, and a maximum of 300 mg cholesterol daily. After eight weeks the treatment group had an 18-percent lower mean total cholesterol level compared to placebo and a 17-percent drop in total cholesterol from baseline. There was also a 23-percent difference in LDL between the treatment group and the placebo group and a 23-percent drop in the treatment group, evident at eight weeks. Triglycerides also dropped 16 percent in the treatment population. The drops in total cholesterol and LDL were consistent at eight and 12 weeks. There were no changes in HDL levels.

A multicenter, self-controlled, open-labeled study used the American Heart Association's Step I diet for four weeks followed by red yeast rice 2.4 grams daily for eight weeks in 187 hypercholesterolemic patients. There were no significant differences with the diet alone, but after eight weeks of red yeast rice, total cholesterol decreased 16.4 percent, LDL by 21 percent, triglycerides by 24.5 percent, and HDL increased 14.6 percent.[6]

Clinical trials using red yeast rice with hyperlipidemic elderly patients[7] as well as HIV-related dyslipidemic patients[8] have also demonstrated ability of red yeast rice to improve lipid profiles.

Drug-Nutrient Interactions

Because HMG-CoA reductase inhibitors reduce the production of coenzyme Q10 (CoQ10),[9] supplementation of CoQ10 with long-term use of red yeast rice extract may be prudent. Theoretically, the drug-related contraindications for lovastatin are probably prudent to adhere to with red yeast rice preparations as well, including avoidance of co-administration with gemfibrozil, cyclosporin, azole anti-fungals, erythromycin, clarithromycin, nefazodone, and protease inhibitors.[10] One case report of an adverse drug-nutrient interaction between cyclosporine and a multi-ingredient herbal preparation containing red yeast rice exists in the literature.[11]

Side Effects and Toxicity

Toxicity evaluations of red yeast rice in animals for as long as four months have shown no toxicity.[1] Human trials have not shown elevations of liver enzymes or renal impairment.[2,5] Side effects have been limited to headaches and gastrointestinal discomfort.

Although larger, long-term trials will be helpful in understanding the efficacy and potential long-term effects of red yeast rice, the apparent lack of statin-like side effects in these short-term studies warrants further investigation of this hypolipidemic agent.

Dosage

No dosage standards have been established for red yeast rice. Adult dosages used in clinical studies range from 1.2-2.4 g per day[2,5] to 0.8 g/kg/day.[1] In Asian countries the average daily intake of red yeast rice is 14-55 grams.[12]

It should be noted the monacolin content of red yeast rice varies significantly according to the strain. Total monacolin content of nine different commercially available preparations evaluated by high performance liquid chromatography (HPLC) varied from zero to 0.58 percent.[13] Findings of clinical trials using red yeast rice with a standardized level and profile of monocolins may not be applicable to all commercially available red yeast rice preparations.

Warnings/Contraindications

Theoretically, the contraindications for lovastatin use are probably prudent to adhere to with monacolin K-containing red yeast rice preparations, including pregnancy, breast feeding, and hepatic or renal impairment.[10]

Individuals with known allergies to yeast or fungus should exercise caution when supplementing with red yeast rice.

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Effects of fish oil supplementation on cardiac function in chronic heart failure: a meta-analysis of randomised controlled trials

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ABSTRACT

Context The effects of fish oil on cardiac function, ventricular remodelling and functional capacity in patients with chronic heart failure (CHF) remain controversial.

Objective The aim of this meta-analysis was to evaluate effects of fish oil on cardiac function and related parameters in CHF patients.

Data Sources Medline, Embase, the Cochrane Library and references cited in related reviews and studies.

Study Selection Randomised controlled trials of fish oil supplementation on cardiac function in patients with CHF were identified.

Data Extraction Two investigators read all papers and extracted all relevant information. A fixed effect or, in the presence of heterogeneity, a random effect model, was used to estimate the combined effects.

Results 7 trials with 825 participants were included. Meta-analysis results showed that left ventricular ejection fraction was significantly increased (weighted mean difference (WMD) = 2.25%, 95% CI 0.66 to 3.83, p = 0.005) and left ventricular end-systolic volume was significantly decreased (WMD = 7.85 ml, 95% CI −15.57 to −0.12, p = 0.05) in the fish oil group compared with the placebo group, although left ventricular end-diastolic volume was not significantly affected. Meta-regression and subgroup analysis indicated that the improvement in left ventricular systolic function was more remarkable in patients with nonischaemic heart failure. Fish oil supplementation also improved the New York Heart Association functional classification and peak oxygen consumption in patients with non-ischaemic heart failure.

Conclusions Improvement in cardiac function, remodelling and functional capacity may be important mechanisms underlying the potential therapeutic role of fish oil for patients with CHF. These effects might be more remarkable in patients with non-ischaemic heart failure.

INTRODUCTION

Chronic heart failure (CHF) is a complex clinical syndrome characterised by insufficient status of cardiac function, which can be caused by various structural or functional cardiovascular disorders.[1] Despite impressive advances in medical management and device therapy in recent decades, CHF remains one of the leading causes of cardiovascular morbidity and mortality worldwide.[2 3] Thus there is an urgent need for the development of novel treatment strategies for CHF despite the standard pharmacological therapies that have been recommended by current medical guidelines.[4 5]

Numerous clinical trials have shown supplementation with fish oil, which mainly consists of two categories of marine omega 3 polyunsaturated fatty acids—eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)—may lower the risk of major cardiovascular events, such as myocardial infarction, sudden cardiac death (SCD), coronary artery disease and perhaps atrial fibrillation.[6 7] Growing evidence from experimental and epidemiological studies suggested that fish oil intake could be beneficial for patients with CHF.[8 9] Previous studies also indicated that fish oil could improve cardiac function and functional capacity in CHF patients; however, clinical trials have shown inconsistent results.[10–16] Therefore, we performed a meta-analysis to systematically evaluate the effects of fish oil on cardiac function, remodelling and functional capacity in CHF.

METHODS

The study complied with the recently reported Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement.[17]

Search strategy

We performed a literature search in Pubmed, Embase and the Cochrane Library for relevant records up to November 2011, using the term 'omega-3 fatty acids', 'fish oil', 'fish-oil', 'marine oil', 'eicosapentaenoic acid', 'EPA', 'docosahexaenoic acid', 'DHA', 'dietary therapy' paired with the following: 'heart failure', 'cardiac failure', 'cardiac dysfunction', 'ventricular dysfunction', 'ventricular insufficiency' and 'cardiomyopathy'. The search was limited to studies in humans. We also analysed reference lists of original and review articles using a manual approach.

Study selection

Studies were selected for analysis if they met the following criteria: (1) published as full length articles in any language; (2) reported as a prospective, randomised and placebo controlled trial with either a parallel or crossover design; (3) analysed adult patients (>18 years) with established CHF (regardless of the aetiology and severity of the disease) who were assigned to oral fish oil supplementation or placebo for at least 1 month, in addition to concurrent therapy; (4) reported data on at least one of the following outcomes: left ventricular ejection fraction (LVEF), left ventricular end-systolic volume (LVESV), left ventricular end-diastolic volume (LVEDV), New York Heart Association (NYHA) functional class and peak oxygen consumption (peak VO_2) in the exercise test.

Data extraction and quality assessment

Two authors (WX and WW) independently performed the literature search, data extraction and quality assessment according to the inclusion criteria. Discrepancies were resolved by consensus. Extracted data included study design characteristics, patient characteristics (number, age, sex, baseline cardiac function and concurrent medications), intervention strategies (total dose of fish oil, ratio of EPA to DHA and composition of the placebo), follow-up duration and means (SDs) for changes in the aforementioned outcomes. For studies presenting results at more than one time point from the same groups of participants, we selected a single time point at which data from the maximum number of subjects were available to avoid a unit of analysis error, as indicated in the instructions in Cochrane's handbook.[18] For studies with multiple intervention groups (eg, with different doses of fish oil), we split the shared control group into two or more groups with a smaller sample size to overcome a unit of analysis error, and included these two or more comparisons into the meta-analysis according to the instruction in Cochrane's handbook.[18]

The quality of the studies was judged by quality of randomisation, generation of random numbers, concealment of treatment allocation, blinding and reporting of withdrawals. Trials scored 1 point for each area addressed, with a possible score of between 0 and 5, where 5 represented the highest level of quality.[19]

Statistic analysis

All endpoints were estimated based on the change from baseline to follow-up, and pooled effects were presented as weighted mean difference (WMD) with 95% CI. Inter-study heterogeneity was formally tested using Cochrane's Q test, and significant heterogeneity was considered to exist if the p value was <0.10. The I^2 statistic, which describes the percentage of total variation across studies that is due to heterogeneity rather than chance,[20] was also examined, and a value of I^2 >50% indicated significant heterogeneity. If Cochrane's Q test showed significant heterogeneity, a fixed effect model was used to calculate WMD and its 95% CI. By contrast, a random effect model was applied.[21] Meta-regression and predefined subgroup analysis were performed to explore the possible source of heterogeneity. Median values of continuous variables were used as cut-off values for grouping studies. A sensitivity analysis was conducted to determine the stability of the pooled results. Furthermore, potential publication bias was assessed with the Egger regression asymmetry test[22] and funnel plots; p values were two tailed and statistical significance was set at 0.05. Meta-analysis and statistical analysis were performed with Stata software (V.12.0; Stata Corporation) and RevMan software (V.5.1; Cochrane Collaboration, Oxford, UK).

RESULTS

Search results

A total of 1250 articles were identified, and 1210 were excluded because they did not describe randomisation or controlling, or because the objectives of these studies were irrelevant to the present meta-analysis. Of the 40 potentially relevant articles screened, seven[10–16] met the selection criteria (figure 1). Thirty-three articles were excluded because the participants in 25 trials did not have CHF; seven trials did not report any data of related outcomes and one trial included children.

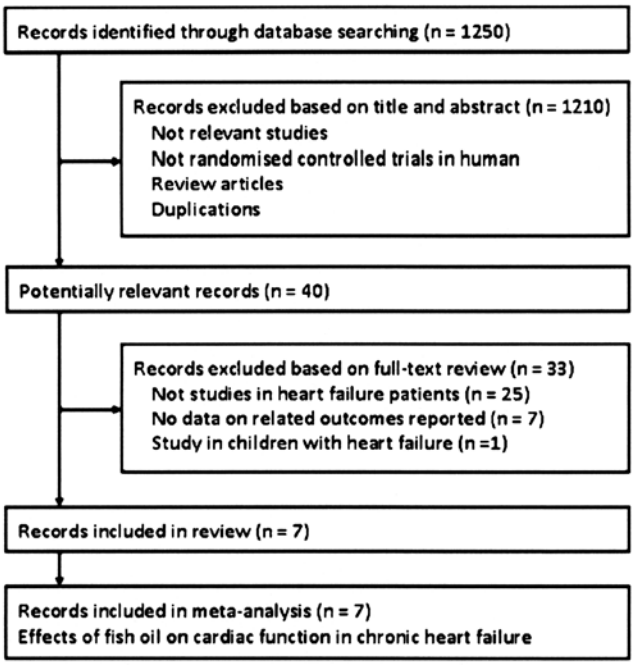

Figure 1 Flow diagram of the study selection procedure.

Study characteristics

Overall, eight study arms from seven studies[10–16] were included in the meta-analysis, which comprised a total of 825 patients with CHF, 433 in the fish oil group and 392 in the placebo group. The characteristics of the studies are presented in table 1. All studies were of parallel design. Six studies declared that the participants were clinically and haemodynamically stable CHF patients who received optimal medical therapy based on modern CHF treatment strategies, including diuretics and neurohormonal inhibitors, and the medication treatment was maintained during the follow-up interval,[11–16] while the other study did not report the use of β blockers in the included patients.[10] Mean age of the participants varied from 57 to 73 years. The aetiology of CHF was exclusively ischaemic in two studies,[10 11] non-ischaemic in three studies[12 15 16] and of both origins in two studies.[13 14] Mean baseline LVEF of the participants varied from 24% to 37%. The dose of fish oil (calculated as the total dose of EPA and DHA) ranged from 600 to 4300 mg/day, with the ratio of EPA to DHA varying from 0.60 to 1.53. Duration of the investigation varied from 3 to 12 months. None of these studies reported adverse events related to fish oil supplementation.

Data quality

The quality score of the seven studies ranged from 3 to 5. All studies were randomised and placebo controlled, with six studies conducted in a double blind fashion[10–12 14–16] and one in a single blind fashion.[13] Three studies reported methods of random sequence generation,[13–15] and three reported allocation concealment.[10 14 15] Details of withdrawals were reported in six of the included studies.[10–13 15 16]

Effects of fish oil supplementation on cardiac function and remodelling

Left ventricular ejection fraction

Six studies[11–16] with seven study arms investigated the effects of fish oil on LVEF in CHF patients. Significant heterogeneity was detected among the seven study arms (I^2=60%, p=0.02)

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Table 1 Overview and characteristics of the included studies

Study	No of patients	Mean age (years)	Male (%)	Ischaemic aetiology (%)	NYHA class	Mean LVEF (%)	ACEI/ ARB (%)	B blocker (%)	EPA+DHA dose (mg/day)	EPA/ DHA	Duration (months)	Placebo	Study design	Quality score
Skou 2001[10]	50	63	NR	100	1.70	33.0	52	NR	4300	1.53	3	Olive oil	R, DB, PC	4
Radaelli 2006[11]	25	60	96	100	2.38	35.5	92	92	1700	0.83	4	NR	R, DB, PC	3
Nodari 2009[12]	41	63	91	0	2.38	36.2	100	100	1440	0.60	6	Olive oil	R, DB, PC	3
Zhao 2009[13]	75	73	73	64	2.63	31.0	100	87	600	1.50	3	NR	R, SB, PC	3
Ghio 2010[14]	458	65	84	53	2.24	30.6	96	77	850	0.83	12	Olive oil	R, DB, PC	4
Moertl 2011 a[15]	22	57	82	0	3.11	24.3	100	100	840	1.24	3	Gelatin	R, DB, PC	5
Moertl 2011 b[15]	21	59	95	0	3.07	24.2	100	100	3360	1.24	3	Gelatin	R, DB, PC	5
Nodari 2011[16]	133	63	75	0	1.86	36.5	100	100	1950	0.60	12	Olive oil	R, DB, PC	3

The study by Moertl *et al* includes two intervention groups of different dosages of EPA+DHA (Moertl 2011a, EPA+DHA 840 mg/day and Moertl 2011b, EPA+DHA 3360 mg/day).
ACEI, ACE inhibitor; ARB, angiotensin receptor blocker; DB, double blind; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LVEF, left ventricular ejection fraction; NR, not reported; NYHA, New York Heart Association; PC, placebo controlled; R, random; SB, single blind.

and therefore the random effect model was applied. The pooled results indicated additional supplementation with fish oil significantly increased LVEF (seven study arms, 775 patients; WMD=2.25%, 95% CI 0.66 to 3.83, p=0.005) (figure 2), suggesting a beneficial effect of fish oil on cardiac systolic function.

In view of the fact that significant heterogeneity existed across the enrolled study arms, we performed predefined meta-regression analysis to explore the potential source of heterogeneity. The results indicated that the aetiology of CHF may be a potential modifier of the effect of fish oil on LVEF, and the percentage of patients with ischaemic CHF was negatively related to effect size (regression coefficient=−0.037, 95% CI −0.073 to −0.001, p=0.046), which largely explained the heterogeneity (figure 2). Other covariates, including mean age, gender of the participants, baseline LVEF, total dose of fish oil, ratio of EPA to DHA and follow-up duration were not significant modifiers.

Subsequently, we performed a subgroup analysis to specify the influence of CHF aetiology on the effects of fish oil on LVEF. We grouped the seven study arms according to the median percentage of patients with ischaemic CHF (0%). Therefore, subgroup analyses were performed in study arms which partially or wholly included patients with ischaemic CHF, and in study arms which included non-ischaemic CHF patients exclusively. For studies which partially or wholly included patients with ischaemic CHF,[11 13 14] fish oil supplementation did not significantly affect LVEF (three study arms, 558 patients; WMD=0.78%, 95% CI −0.29 to 1.86, p=0.15) (figure 2), and no significant heterogeneity among these studies was detected (I^2=0, p=0.96); however, for studies including non-ischaemic CHF patients exclusively,[12 15 16] fish oil supplementation increased LVEF significantly (four study arms, 217 patients; WMD=4.07%, 95% CI 2.38 to 5.76, p<0.00001) (figure 2), and no significant heterogeneity among these studies was found either (I^2=8%, p=0.36).

Left ventricular end-systolic volume and left ventricular end-diastolic volume

Four studies[10 12 14 16] reported the effects of fish oil supplementation on LVESV, among which no significant heterogeneity was found (I^2=0, p=0.84). The pooled results estimated with the fixed effect model indicated that fish oil reduced LVESV (four studies, 673 patients; WMD=−7.85 ml, 95% CI −15.57 to −0.12, p=0.05) (figure 3A). Only three studies[10 14 16] reported the effects of fish oil on LVEDV, and no significant heterogeneity was detected (I^2=0, p=0.92). The results indicated a decrease in LVEDV after fish oil supplementation although this was not statistically significant (three studies, 632 patients; WMD=−4.44 ml, 95% CI −14.52 to 5.65, p=0.39) (figure 3B).

Effects of fish oil supplementation on functional capacity

NYHA functional class

For the three[10 11 16] studies containing data on NYHA functional class, fish oil failed to result in a significant improvement in NYHA classification (three studies, 208 patients; WMD=−0.21, 95% CI −0.68 to 0.23, p=0.32) (figure 4A). Significant heterogeneity was detected (I^2=90%, p<0.0001). Of these studies, one study[16] included exclusively non-ischaemic CHF patients and fish oil significantly improved NYHA classification in these

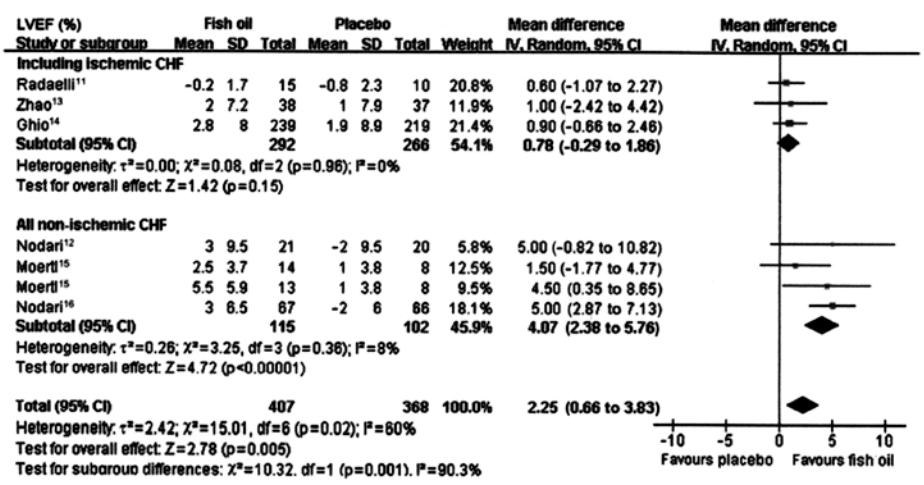

Figure 2 Forest plot from meta-analysis of weighed mean difference in left ventricular ejection fraction (LVEF) for patients with chronic heart failure (CHF), randomised to fish oil or placebo.

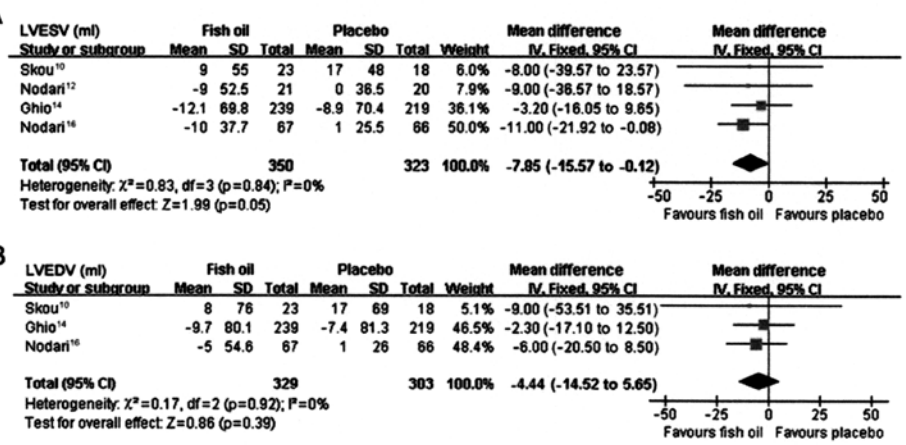

Figure 3 Forest plots from meta-analysis of left ventricular end-systolic volume (LVESV) (A) and left ventricular end-diastolic volume (LVEDV) (B) for patients with chronic heart failure, randomised to fish oil or placebo.

patients (133 patients; mean difference=−0.58, 95% CI −0.75 to −0.41, p<0.00001). The other two studies[10 11] included exclusively ischaemic CHF patients, and fish oil supplementation had no significant effect in NYHA classification in these patients (two studies, 75 patients; WMD=0.01, 95% CI −0.20 to 0.21, p=0.93) without any significant heterogeneity (I^2=0, p=0.34).

Peak oxygen consumption

Three studies[12 15 16] with four study arms, which included non-ischaemic CHF patients exclusively, investigated the effects of fish oil on peak VO_2 in the exercise test. No significant heterogeneity was detected (I^2=0, p=0.67) and the pooled results indicated fish oil significantly increased peak VO_2 in non-ischaemic CHF patients (four study arms, 199 patients; WMD=1.68 ml/kg min, 95% CI 0.52 to 2.84, p=0.005) (figure 4B).

Publication bias

Publication bias was tested based on the data for fish oil supplementation on LVEF, which included the most study arms. Funnel plot (figure 5) and results of the Egger regression test suggested no significant publication bias (Egger test, p=0.302).

DISCUSSION

In this study, we performed a meta-analysis of seven published clinical trials to investigate the effects of fish oil supplementation on cardiac function, remodelling and functional capacity in CHF. The results indicated additional fish oil supplementation caused a significant increase in LVEF and a reduction in LVESV in CHF, and the improvement in LVEF seemed to be more remarkable in patients with non-ischaemic CHF. Moreover, the benefits of fish oil were further demonstrated by improvement in NYHA classification and increase in peak VO_2, although these results were obtained from limited studies in patients with non-ischaemic CHF. These results indicated that improvement in cardiac systolic function, ventricular remodelling and functional capacity may be important mechanisms underlying the potential therapeutic role of fish oil for patients with CHF, especially those with non-ischaemic CHF.

A large number of structural and functional abnormalities may be involved in the pathogenesis of CHF,[1] and previous studies indicated that the benefits of fish oil supplementation in cardiac function and remodelling may be mediated by multiple effects on many of these processes and mechanisms.[8 9] Fish oil could preserve cardiac mitochondrial function by stimulating expression of proteins involved in cardiac lipid metabolism,[23] thereby reducing myocardial oxygen consumption and resulting in greater mechanic efficiency of the ventricle.[24] Also, by inhibition of the inflammatory nuclear transcription factor, nuclear factor κB[25] and stimulation of the anti-inflammatory hormone adiponectin,[26] fish oil may exert its anti-inflammatory effect in CHF. This is considered to be an important therapeutic mechanism because persistent inflammation has been suggested to play a pathogenic role in CHF by impairment of myocardial contractility, inducing hypertrophy, promoting apoptosis and contributing to myocardial remodelling.[27] Although other benefits have also been

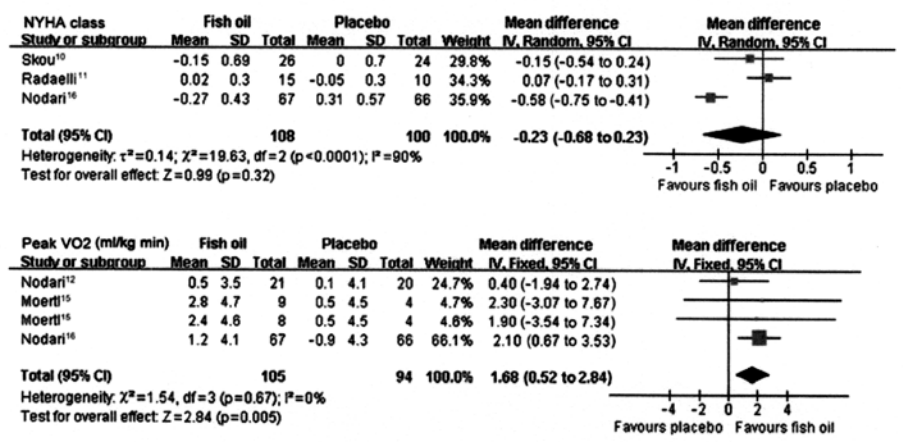

Figure 4 Forest plots from meta-analysis of New York Heart Association (NYHA) classification (A) and peak oxygen consumption (peak VO_2) (B) for patients with chronic heart failure, randomised to fish oil or placebo.

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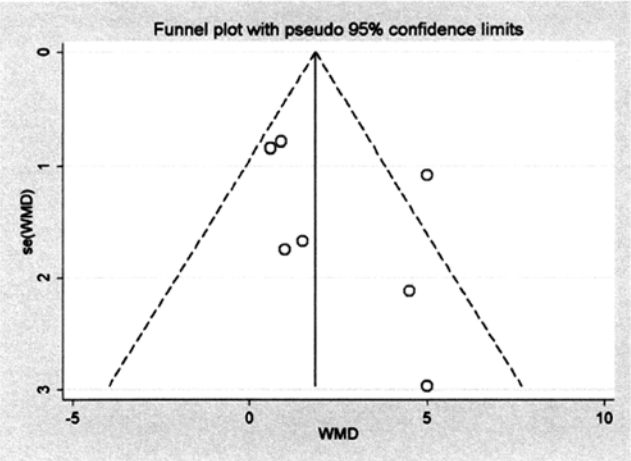

Figure 5 Funnel plot (with pseudo 95% CIs) of all individual study arms in the meta-analysis left ventricular ejection fraction for patients with chronic heart failure, randomised to fish oil or placebo. WMD, weighed mean difference.

proposed, such as improvement in endothelial function, antioxidation, inhibition of neurohormonal activation, antihypertrophy and excitation–contraction coupling of the myocardium, the exact mechanisms underlying the therapeutic effects of fish oil in CHF have yet to be determined.[8 9]

Previous evidence from epidemiological studies demonstrated that fish consumption or fish oil intake was inversely associated with the incidence of CHF in the general population.[28–30] Furthermore, the results of the GISSI-HF trial,[31] the only published large scale study to date which has evaluated the effects of fish oil on clinical endpoints in patients with CHF, showed that long term treatment with fish oil reduced mortality and hospitalisations for cardiovascular reasons in patients with CHF on evidence based therapy, which strongly indicated a therapeutic role for fish oil in CHF. However, reduction in fatal arrhythmias and SCD can only contribute up to 50% of the total benefit of fish oil on mortality, suggesting other mechanisms may be involved.[32] Results of our meta-analysis suggested that improvement in cardiac systolic function and ventricular remodelling may be important mechanisms underlying the beneficial effects of fish oil for CHF, despite its anti-SCD effects. Accumulating evidence suggested that LVEF, LVESV and LVEDV are strong predictors of mortality risk for patients with CHF,[33–35] and a recent meta-analysis also indicated that therapeutic strategies which improve cardiac function and remodelling may confer benefits on mortality in these patients.[36] In fact, according to the results of a previous study including a large contemporary CHF population, our finding of 2.25% improvement with additional fish oil supplementation would correspond to about a 6% lower risk of overall mortality.[37] Although the effect of fish oil on LVEF and mortality risk in CHF seemed to be modest, it could be important clinically in a larger population. More importantly, our results were obtained based on randomised, blinded, placebo controlled trials in patients who had already taken the current optimal medications for CHF, indicating a further beneficial effect of fish oil supplementation for CHF on the basis of treatment strategies recommended by the current guidelines.

Interestingly, the results of meta-regression and subgroup analysis indicated that the benefit of fish oil on cardiac function seemed more remarkable in patients with non-ischaemic CHF compared with those with ischaemic CHF. The exact mechanisms underlying this difference are not clear. However, a recent review suggested that significant heterogeneity exists in trials of fish oil in patients with cardiovascular disorders, and some benefits of fish oil depended on the population studied.[38] For patients with non-ischaemic CHF, our study found that fish oil supplementation significantly improved LVEF by 4.07%. Although a quantitative association between LVEF and risk of mortality in patients with non-ischaemic CHF has not been reported in previous studies, it has been suggested that increased LVEF was related to a lower risk of total mortality in these patients.[39]

Results of our meta-analysis also indicated that additional supplementation of fish oil can lead to improvement in NYHA classification and an increase in peak VO_2 although these results were obtained on the basis of studies in non-ischaemic CHF and mainly driven by one study.[16] Moreover, peak VO_2 has also been suggested to be of important prognostic value for mortality in patients with CHF, suggesting that the beneficial effects of fish oil on total mortality in CHF may be related to improvement in the functional capacity of patients.[40 41]

Several potential limitations should be addressed regarding the present meta-analysis. First, the number of studies and patients included in this meta-analysis was small, so the results of some of the estimations, such as the effects of fish oil supplementation on NYHA classification and LVEDV, should be interpreted with caution. Second, as for the meta-regression and subgroup analysis, the number of included studies and patients in each stratum was relatively small. Also, we did not have access to individual patient data. The results of the subgroup analysis might be different if larger numbers of studies were included or individual patient data were available (eg, the subgroup could be defined as patients with ischaemic CHF and non-ischaemic CHF, and not as studies partially or wholly including patients with ischaemic CHF and studies including patients with non-ischaemic CHF exclusively in the current analysis).

In conclusion, our meta-analysis suggested that improvements in cardiac function, remodelling and functional capacity may be important mechanisms underlying the benefit of fish oil on mortality in patients with CHF who are already on current optimal medications for CHF, particularly in those with non-ischaemic CHF. Additionally, large scale randomised controlled trials are needed to uncover all of the potential benefits of fish oil supplementation in patients with CHF of different aetiologies.

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Contributors All authors are in agreement with the content of the manuscript, are aware of the submission and guarantee the work. WX, WW and XL conceived and designed the experiments. WX and WW performed the experiments. WX, WW and XL analysed the data and wrote the paper.

Competing interests None.

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Heart

Effects of fish oil supplementation on cardiac function in chronic heart failure: a meta-analysis of randomised controlled trials

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Review

Omega-3 fatty acids and coronary heart disease risk: Clinical and mechanistic perspectives

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Abstract

The most common omega-3 fatty acids contain 18–22 carbons and a signature double bond at the third position from the methyl (or *n*, or omega) end of the molecule. These fatty acids must be obtained in the diet as they cannot be synthesized by vertebrates. They include the plant-derived α-linolenic acid (ALA, 18:3*n*-3), and the fish-oil-derived eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3). Normally, very little ALA is converted to EPA, and even less to DHA, and therefore direct intake of the latter two is optimal. EPA and DHA and their metabolites have important biologic functions, including effects on membranes, eicosanoid metabolism, and gene transcription. Studies indicate that the use of fish oil is associated with coronary heart disease risk reduction. A number of mechanisms may be responsible for such effects. These include prevention of arrhythmias as well as lowering heart rate and blood pressure, decreasing platelet aggregation, and lowering triglyceride levels. The latter is accomplished by decreasing the production of hepatic triglycerides and increasing the clearance of plasma triglycerides. Our focus is to review the potential mechanisms by which these fatty acids reduce cardiovascular disease risk.

Keywords: Omega-3 fatty acids; Coronary heart disease; Arrhythmia; Platelet aggregation; Triglyceride

Contents

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1. Introduction

Omega-3 fatty acids have been shown to exert cardioprotective effects in both primary and secondary coronary heart disease (CHD) prevention trials [1,2]. Proposed mechanisms to account for these findings include reduced triglyceride (TG) concentrations, antiarrhythmic effects, decreased platelet aggregation, plaque stabilization, reduced blood pressure, and/or a reduction in heart rate [2]. High TG levels have been shown to be an independent risk factor for CHD in a meta-analysis of 17 large, population-based studies ($N > 56{,}000$) [3]. After correcting for high-density lipoprotein cholesterol (HDL-C), every 88 mg/dL increase in TGs was associated with an increase in CHD risk of 14% in men and 37% in women. These findings are supported by data from families with familial hypertriglyceridemia and patients with premature familial coronary artery disease (CAD), as well as data from the Copenhagen Male Study. In this study, middle-aged men without overt CHD at baseline showed increasing incidence of ischemic heart disease over 8 years with increasing baseline TGs within each tertile of HDL-C [4]. However, while the data are strong for benefit associated with LDL cholesterol lowering, only very limited data are available to document CHD benefits from lowering of plasma TGs in randomized placebo-controlled trials. The purpose of this paper is to review the literature relating to the possible mechanisms for the TG-lowering effect of omega-3 fatty acids, as well as other potential cardioprotective mechanisms.

2. Treatment of hypertriglyceridemia with omega-3 fatty acids

The National Cholesterol Education Program Third Adult Treatment Panel (NCEP ATP III) recommends that patients with borderline (150–200 mg/dL) and high (>200 mg/dL) TG levels be treated with lifestyle modifications [5]. The NCEP ATP III also indicates that patients with high TG levels (200–499 mg/dL) may need pharmacologic therapy that targets non-high-density lipoprotein cholesterol (non-HDL-C): statins, fibrates, and nicotinic acid. It is not clear whether TG reduction, especially when combined with low-density lipoprotein cholesterol (LDL-C) reduction, contributes more to cardiovascular event rate reduction than that attained through LDL-C lowering alone.

The TG-lowering effect of omega-3 fatty acids in humans is well established [6]. A meta-analysis of 36 crossover and 29 parallel-design studies demonstrated that omega-3 fatty acids lowered serum TG levels in a dose-dependent manner, with the TG lowering being generally proportional to baseline levels [7]. In trials of subjects with TG levels >150 mg/dL (>1.69 mmol/L) taking the omega-3 fatty acids eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA) in dosages of 3.4–4 g/day, TG levels decreased by an average of 29% (range 16–45%).

In addition to fibrates and nicotinic acid, a highly concentrated, prescription omega-3 (P-OM3) preparation (Lovaza™,[1] Reliant Pharmaceuticals, Inc., Liberty Corner, NJ) is now available for the treatment of hypertriglyceridemia as an adjunct to diet. P-OM3 contains over 90% long-chain omega-3 fatty acid ethyl esters, primarily EPA (465 mg/g) and DHA (375 mg/g) [8]. Multiple randomized, controlled trials confirm the efficacy of P-OM3 as a TG-lowering therapy, including the combined results from two trials (Fig. 1). In these trials a 45% increase in LDL-C produced a final mean LDL-C of 129 mg/dL [6,9]. While these increases were clearly not inconsequential and could theoretically diminish the overall cardioprotection afforded by omega-3 fatty acids, the actual clinical relevance of this finding is uncertain in view of the favorable effects on TG, HDL-C, and associated enrichment of tissue omega-3 fatty acid levels. Regulation of LDL-C levels in subjects with hypertriglyceridemia is complex. In apoB-100 kinetic studies, P-OM3 increased the percent conversion of very low-density lipoprotein (VLDL) to LDL without increasing LDL apolipoprotein B-100 (apoB) levels [10]. Interestingly, weight loss in overweight subjects with hypertriglyceridemia can also raise LDL-C, and it appears to do so by reducing the fractional catabolic rate of LDL [11]. Studies in non-human primates suggest that omega-3 fatty acid-enriched LDL particles have physical, chemical, and biological properties that may render them less atherogenic than control LDL particles [12–14]. Specifically, cultured human THP-1 macrophage cells incubated with acetylated LDL from monkeys fed an omega-3 fatty acid-enriched diet accumulated significantly less cholesteryl ester than cells incubated with similar LDL obtained from animals fed diets enriched with other fats [12]. It must be

[1] Lovaza™ was formerly known as Omacor®. Reliant Pharmaceuticals, Inc. has changed the name of Omacor to Lovaza™ (omega-3-acid ethyl esters). Reliant took this step at the request of the FDA and in response to a limited number of reports of prescribing and dispensing errors [Data on File. Reliant Pharmaceuticals, Inc.] due to similarity in name between the company's Omacor capsules and Xanodyne Pharmaceuticals' Amicar® (aminocaproic acid) [Amicar® is a registered trademark of Xanodyne Pharmaceuticals, Inc.]. The name change is intended to minimize the potential for such errors in the future.

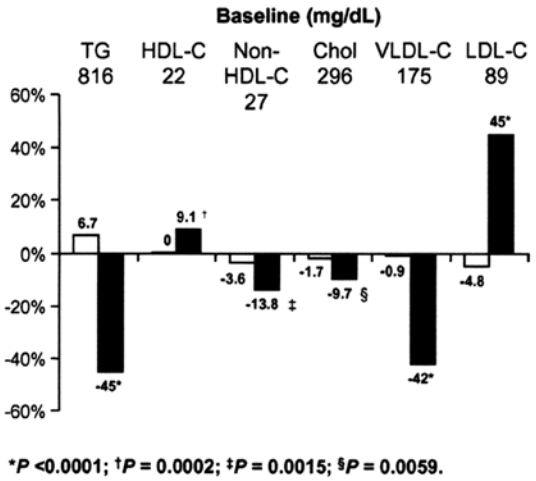


Fig. 1. Prescription omega-3 acid ethyl esters (P-OM3) and lipid levels in subjects with hypertriglyceridemia (≥500 mg/dL). Compared with placebo, P-OM3 (4 g/day for 6 weeks [9] or 4 months [6]) significantly reduced triglyceride (TG) levels. Non-high-density lipoprotein cholesterol decreased because the decrease in very-low-density-lipoprotein cholesterol was greater (in absolute values) than the increase in low-density lipoprotein cholesterol (LDL-C). In this patient population, the normal conversion of very-low-density-lipoprotein to low-density lipoprotein is inhibited (which contributes to the severe elevation in TG). In these studies the baseline low-density lipoprotein cholesterol (LDL-C) was 89 mg/dL. This 45% increase in LDL-C produced a final mean LDL-C of 129 mg/dL. Data from studies by Harris et al. [6] ($N=42$) and Pownall et al. [9] ($N=40$) were pooled.

appreciated, however, that these monkeys were given doses of omega-3 fatty acids that were about five-fold higher than are used in humans to lower TGs; thus the clinical relevance is unclear. Nevertheless, evidence reviewed below indicates that the rise in LDL in patients with hypertriglyceridemia may be offset by concurrent treatment with statins.

3. Biochemistry of omega-3 fatty acids

Fatty acids are straight chains of carbon atoms (usually 12–24) with an alpha (or carboxylic acid) end and an omega (or methyl or *n*) end. Fatty acid nomenclature begins with the number of carbons, then after a colon, the number of double bonds, followed by the position of the first double bond counting from the omega (or *n*th) carbon. The major saturated fatty acids in plasma are palmitic acid (16:0) and stearic acid (18:0); they are called 'saturated' because all carbon–carbon bonds are saturated with hydrogens, meaning there are no double bonds. The major monounsaturated fatty acid, oleic acid (18:1*n*9), contains one double bond.

Omega-3 fatty acids are polyunsaturated fats in which the first double bond counting from the omega carbon is at position 3, hence the name omega-3 (or *n*-3). Major omega-3 fatty acids include α-linolenic acid (ALA, 18:3*n*-3), EPA (20:5*n*-3), and DHA (22:6*n*-3), and comprise one of the two classes of essential fatty acids. Preformed EPA and DHA are best obtained from fatty fish or fish-oil supplements. ALA may be obtained from certain seed oils, but only a small percentage of ALA is converted to EPA in mammals, and further transformation to DHA is very low (Fig. 2). The other class of essential fatty acids is the omega-6 fatty acids, comprised mainly of linoleic acid (LA, 18:2*n*-6) and arachidonic acid (AA, 20:4*n*-6).

Essential fatty acids play a key role in many metabolic processes, and cannot be synthesized by mammals because the necessary enzymes to place a double bond at the omega-3 or -6 positions are absent [15–17]. Omega-6 fatty acids and their derivatives play a role in the immune response and in thrombosis, whereas omega-3 fatty acids and their derivatives are less active in these processes. After absorption, fatty acids are incorporated into triglycerides (3 fatty acids on a glycerol backbone), phospholipids (2 fatty acids attached to phosphatidic acid backbone), and cholesteryl esters (1 fatty acid attached to free cholesterol). About 70% of the cholesterol in plasma is in the form of cholesteryl ester. Phospholipids are critical for the formation of every cell membrane in the body. The phospholipid bilayer of the membrane is oriented so that the polar head groups interface with the aqueous environment inside and outside of the cell while the fatty acid chains are oriented towards the interior of the membrane, providing a water-impermeable barrier. In this membrane are embedded cholesterol and a large variety of proteins (e.g., receptors, ion channels, signaling complexes). The fluidity of the membrane may be very important for receptor function and recycling as well as the efficiency of signaling pathways. The fluidity of the membrane is determined in part by the fatty acid content of the membrane phospholipids. Fatty acids with multiple double bonds confer increased fluidity to cell membranes, which may partially account for their benefits in preventing cardiac arrhythmias, as well as in the maintenance of neurologic function. While only about 4% of the fatty acids in the bloodstream are DHA, almost 30% of the fatty acids in phospholipids in the brain and retina are DHA. This observation suggests an important role for DHA in neurologic and visual function.

The final step in the conversion of ALA to DHA is a beta oxidation step converting 24:6*n*3 to 22:6*n*3, and this step occurs in liver peroxisomes. Rare patients lacking peroxisomes (Zellweger's disease) or having peroxisomal dysfunction (neonatal adrenal leukodystrophy) have marked plasma DHA deficiency, develop severe neurologic dysfunction, and die at an early age. Therefore, DHA appears to be important for central nervous system function [18,19]. Cross-sectional studies have linked low DHA levels with dementia, while prospective studies have linked both all-cause dementia and Alzheimer's disease with decreased fish intake and low plasma phospholipid DHA levels [20–34]. In the Framingham Heart Study subjects who were in the highest quartile of plasma phospholipid DHA levels consumed on average at least 180 mg of DHA per day, and had a 50% reduction in the risk of all-cause dementia and Alzheimer's disease [28]. Recent data have also linked less rapid progression of coro-

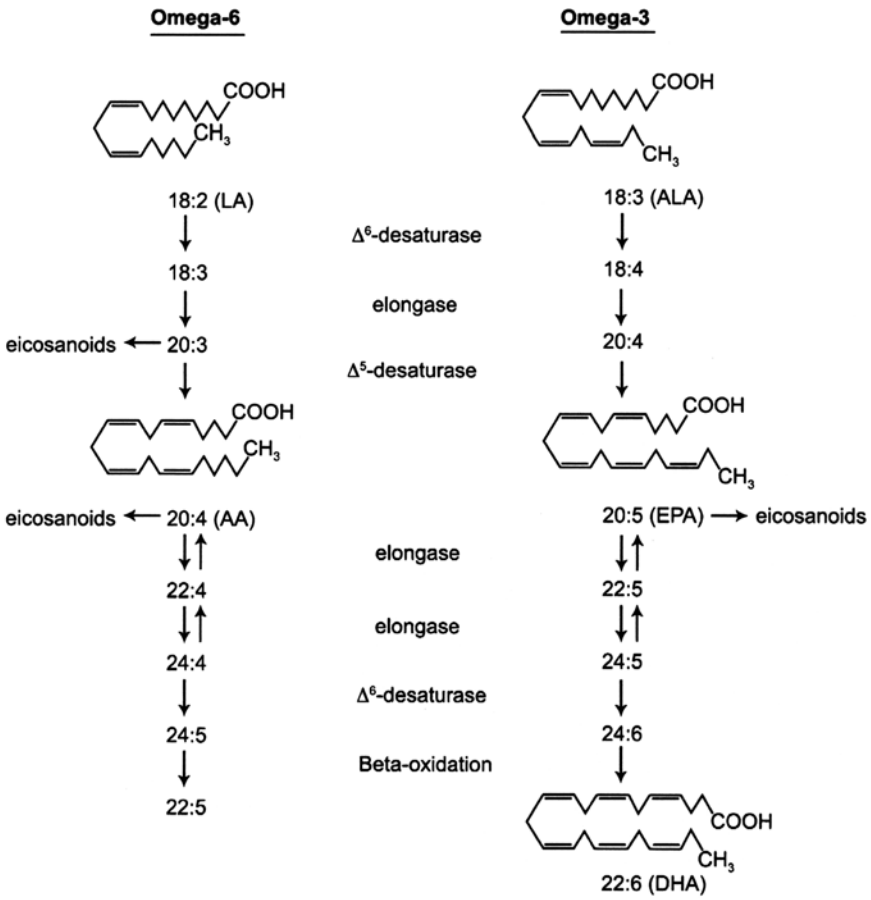


Fig. 2. Synthesis of omega-6 and omega-3 fatty acids in mammals. The primary dietary omega-6 fatty acid is linoleic acid (LA) which has 18 carbons and 2 double bonds (18:2*n*-6). α-linolenic acid (ALA) is a short-chain omega-3 fatty acid (18:3*n*-3) found in plant products such as flaxseed and soybean oils. Essential fatty acids cannot be synthesized by mammals because the necessary enzymes to place a double bond at the omega 3 or 6 positions are absent. The final step in the conversion of ALA to docosahexaenoic acid (DHA) is a β-oxidation step converting 24:6*n*-3 to 22:6*n*-3. In adult men, about 1–5% of ALA is converted to eicosapentaenoic acid (EPA), and conversion to DHA is very low (<0.1%). In women, fractional conversion to DHA appears to be somewhat greater. The initial introduction of a double bond into ALA by Δ^6-desaturase is the rate-limiting reaction of the pathway. Although the affinity of Δ^6-desaturase is higher for ALA than for LA, the typically higher cellular concentrations of LA result in greater net conversion of long-chain omega-6 fatty acids. Diets high in omega-6 fatty acids can reduce the conversion of ALA to EPA and DHA. Adapted from Jump [43] and Calder [2].

nary atherosclerosis in patients with higher levels of plasma DHA [35].

4. Omega-3 fatty acids: TG-lowering mechanisms

Elevated TG levels may result from genetic or metabolic abnormalities that lead to increased plasma residence time of potentially atherogenic chylomicron and/or VLDL remnants. Hypertriglyceridemia associated with elevations in VLDL can be due to overproduction of VLDL particles by the liver, reduced intravascular lipolysis of VLDL-TG, and/or delayed clearance of small (remnant) VLDL particles from the plasma. VLDL particles are formed in the liver from apo B, cholesterol, cholesteryl ester, phospholipids, and TG, the latter originating from long-chain free fatty acids extracted from the plasma, recycled fatty acids, and/or de novo synthesis from acetyl co-enzyme A (CoA).

The omega-3 fatty acids found in fish oil lower fasting and postprandial plasma TG concentrations without clinically significant effects on fat absorption [36]. In general, clinical studies indicate that both EPA and DHA have similar TG-lowering effects [37]. Treatment with 3.4 g/day of EPA and DHA for 4 months increased EPA and DHA proportions in phospholipids two- to threefold from baseline levels [6].

The molecular mechanisms by which EPA and DHA reduce serum TGs are not completely understood, but several potential mechanisms derived from preclinical studies are illustrated in Fig. 3. These studies provide compelling evidence that these fatty acids can both reduce hepatic VLDL-TG synthesis and secretion and enhance TG clearance from chylomicrons and VLDL particles. It is also known that EPA (and DHA) are preferentially shunted into phospholipid synthesis pathways, compared to other fatty acids (i.e., oleate) which are preferentially incorporated into triacylglycerol [38].

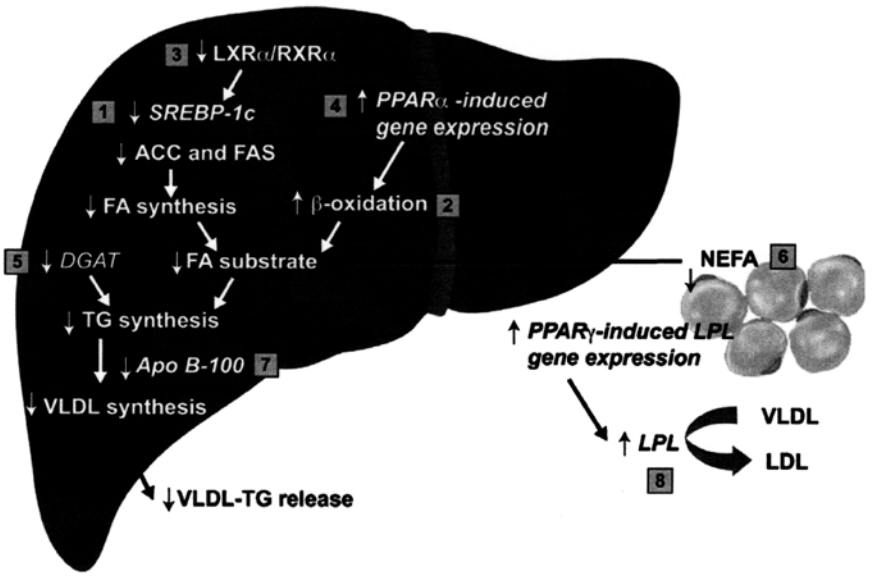


Fig. 3. Potential triglyceride (TG)-lowering mechanisms of the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid. Reduced very-low-density-lipoprotein cholesterol triglyceride (VLDL-TG) secretion may be due to decreased expression of sterol regulatory element-binding proteins (SREBP)-1c (1) or increased rates of mitochondrial and/or peroxisomal β-oxidation (2), leading to reduced substrate for TG synthesis. (3) Decreased SREBP-1c expression may be mediated by inhibition of liver X receptor (LXR) ligand binding to LXR/retinoid X receptor. Increased rates of peroxisomal β-oxidation may be a consequence of peroxisome proliferator-activated receptor (PPAR)-α-induced increase in acyl-coenzyme A oxidase gene expression (4). Decreased activity of TG-synthesizing enzymes (5), decreased non-esterified fatty acid delivery from adipose tissue (6), and decreased availability of apo B (7) are potential mechanisms for reduced VLDL-TG release. In the periphery, increased lipoprotein lipase activity (8) may lead to increased VLDL-TG clearance, possibly due to increased PPAR-γ and/or PPAR-α gene expression. Adapted from Harris and Bulchandani [39] and Davidson [49].

5. Reduced VLDL-TG synthesis

As noted above, omega-3 fatty acids reduce serum TG concentration in humans partly via inhibition of hepatic VLDL-TG secretion rates secondary to decreased synthesis of TG. Thus, reductions in hepatic TG synthesis will lead to reduced production and secretion of VLDL [39]. Studies using perfused monkey liver system show that EPA and DHA decrease hepatic TG secretion through relatively poor utilization of EPA as a substrate for VLDL-TG [40], resulting in a lipid-poor hepatic VLDL [41]. Omega-3 fatty acid-induced decreases in VLDL-TG synthesis appear to be associated with decreases in transcription factors that control the expression of the enzymes responsible for TG assembly within hepatocytes and for fatty-acid oxidation. EPA and/or DHA can also increase intracellular degradation of apo B in primary rat hepatocytes, resulting in decreased VLDL production [42]; however, the importance of this pathway in humans is not clear. Studies in African green monkeys show that fish oil feeding does not significantly affect hepatic apo B secretion [40]. Omega-3 fatty acids can also lower circulating non-esterified fatty acid (NEFA) concentrations (discussed below). Although all of these mechanisms may play a role in the reduction of VLDL-TG synthesis by omega-3 fatty acids, a systematic review of preclinical studies in rats concluded that EPA and/or DHA is most consistently associated with decreased hepatic lipogenesis [39].

Dietary fat has been shown to affect gene transcription via ligand-activated nuclear transcription factors. All peroxisome proliferator-activated receptor subtypes (PPAR-α, -β, and -γ) bind EPA [43] and it has been postulated that omega-3 fatty acids may modulate fatty acid β-oxidation by interacting with PPAR-α (see below). However, effects of omega-3 fatty acids on lipogenic gene expression were observed in PPAR-α null mice, ruling out an absolute requirement for PPAR-α in omega-3 fatty acid-induced suppression of lipogenic gene expression [44]. Thus, details of the mechanism(s) of action by which PPARs might be involved in the TG-lowering effect of EPA and DHA are still lacking.

Sterol regulatory element-binding proteins (SREBPs) are transcription factors that regulate cholesterol-, fatty-acid-, and TG-synthesizing enzymes. One of the main molecular pathways for hepatic lipogenesis involves activation of the transcription factor SREBP-1c, which in turn stimulates the synthesis of acetyl-CoA carboxylase-1 (ACC1) and fatty-acid synthase (FAS), critical lipogenic enzymes (Fig. 3) [45]. The liver X receptor alpha/retinoid X receptor alpha (LXRα/RXRα) heterodimer regulates expression of the SREBP-1c gene via two LXR-responsive elements (LXREs) in the SREBP-1c promoter. Fish-oil feeding in mice is associated with a significant decrease in plasma TG levels and a marked decrease in the level of hepatic SREBP-1c mRNA [46], an effect that may be due to EPA- and DHA-induced inhibition of binding of the LXR/RXR heterodimer to the LXREs in the SREBP-1c promoter, thereby

suppressing SREBP-1c gene expression [47]. In addition, suppression of SREBP-1c mRNA and the SREBP-1 protein by EPA was associated with decreased TG synthesis in HepG2 human hepatoma cells [48]. However, studies in rats suggest that EPA-induced suppression of SREBP-1c and its targeted lipogenic genes is independent of LXRα (reviewed in Davidson) [49]. Thus, inhibition of LXRα binding to LXREs is likely not the only important cause of EPA- and DHA-induced suppression of SREBP-1c mRNA.

Regulation of SREBP-1c expression may not be specific to long-chain omega-3 fatty acids since levels of SREBP-1c mRNA were decreased in HepG2 cells cultured in medium containing not only EPA and DHA but also ALA and omega-6 fatty acids [46]. Neither of the latter two lowers serum TGs in humans. Another study found no change in the levels of SREBP-1 in HepG2 cells cultured in medium containing EPA and DHA [50]. These discrepant findings may be due to differences in experimental models, and thus additional studies are needed to determine the role of hepatic SREBP-1c in the TG-lowering effects of EPA and DHA.

The farnesoid X receptor (FXR) is a nuclear receptor for bile acids that also plays a central role in lipid homeostasis [51]. Studies in HepG2 cells demonstrate that FXR suppresses hepatic lipase and apo CIII gene expression and induces apo CII and VLDL-receptor gene expression [52–55], all of which may contribute to the TG-lowering action of FXR agonists [49]. Notably, mice lacking a functional FXR protein had a proatherogenic serum lipoprotein profile, including elevated TGs [56]. Since DHA is a ligand for FXR [57], a mechanism for the TG-lowering effects of DHA may involve FXR-induced changes in gene expression [49].

Phosphatidic acid phosphohydrolase (PAP) and acyl-CoA:diacylglycerol acyltransferase (DGAT) are key enzymes in TG biosynthesis, catalyzing the conversion of phosphatidate to diacylglycerol and diacylglycerol to TG, respectively. Although a number of studies have shown that EPA and EPA plus DHA can inhibit the activity of DGAT and PAP in rat liver microsomes, other studies have shown no effect of EPA and DHA on DGAT and PAP activity. Importantly, most of these studies used EPA or EPA and DHA at supraphysiological doses and employed different experimental conditions [39]. Thus, the extent to which the TG-lowering effects of EPA and DHA depend on the inhibition of DGAT and/or PAP remains speculative.

NEFAs, which appear to enter cells via fatty-acid transport proteins [58], are rapidly converted by acyl-CoA synthetases into fatty acyl-CoA thioesters that are potential substrates for TG synthesis [43]. Reduced serum NEFAs could potentially reduce hepatic TG synthesis. However, reduced plasma TG levels may themselves lead to decreased circulating NEFA concentrations, in which case the reduced NEFA levels may be an effect of omega-3 fatty acid-induced TG lowering, not a cause [39]. Alternatively, individual fatty acids may be differentially processed. For example, a study by Parks et al. [40] showed that in livers from monkeys fed fish oil (vs. lard), there is preferential incorporation of EPA into hepatic phospholipids and a lower percentage incorporated into secreted TG [41]. Although human data are lacking, studies using non-human primates suggest that, compared with other fatty acids, differences in the intrahepatic processing of free EPA and DHA may contribute to their TG-lowering effects.

Slower formation of TG-rich VLDL in rodents that were fed fish oil or EPA has been linked to a faster rate of hepatic fatty-acid oxidation. Evidence suggestive of this effect has also been seen in healthy human subjects receiving dietary supplementation with 9 g of EPA + DHA per day [59]. Of the rat studies that show an EPA- and/or DHA-induced increase in β-oxidation, about half report an increase in peroxisomal oxidation and the other half, mitochondrial (reviewed in Harris and Bulchandani) [39]. In vitro and ex vivo studies have shown that EPA and DHA can induce acyl-CoA oxidase gene expression in rat hepatocytes in a PPAR-α-dependent manner. However, other studies in rats [39] and monkeys [40] have found that EPA and/or DHA had no significant effect on β-oxidation. Hence, the extent to which increased β-oxidation plays a role in reducing the production of VLDL-TG in humans taking 3–4 g of EPA and DHA remains unknown.

Overall, EPA and DHA have demonstrated effects in reducing hepatic VLDL-TG synthesis. While the molecular mechanisms for this noted reduction are not fully understood, they are likely due to the modulation of transcription factors involved in hepatic fatty-acid uptake, synthesis, and oxidation, as well as those involved in TG synthesis and VLDL assembly.

6. Enhanced TG clearance

Chylomicrons and VLDL are competitive substrates for lipoprotein lipase (LPL), a TG hydrolase present on the capillary endothelium of various tissues. EPA and DHA, when given individually (4 g/day), both significantly increased the rate of chylomicron clearance (Fig. 4A), an effect associated with shorter chylomicron TG half-life [37]. The accelerated chylomicron TG clearance was associated with increased pre-heparin LPL activity (Fig. 4B). All of these effects were statistically significant only in the fed, not the fasted state suggesting that insulin may play a role in this phenomenon. Additional studies are needed to determine if omega-3 fatty acids amplify insulin-induced LPL activity and/or enhance blood flow to adipose tissue and muscle, thereby exposing postprandial chylomicrons to tissues enriched with endothelial LPL. Khan et al. demonstrated that the TG-lowering effects of EPA and DHA in subjects with an atherogenic lipoprotein profile were associated with increased LPL gene expression in adipose tissue (Fig. 5) and significantly increased post-heparin plasma LPL activity [60]. EPA was shown to increase PPAR-γ mRNA in isolated adipocytes [61], and PPAR-γ mRNA levels in adipose tissue have been positively correlated with plasma EPA concentra-

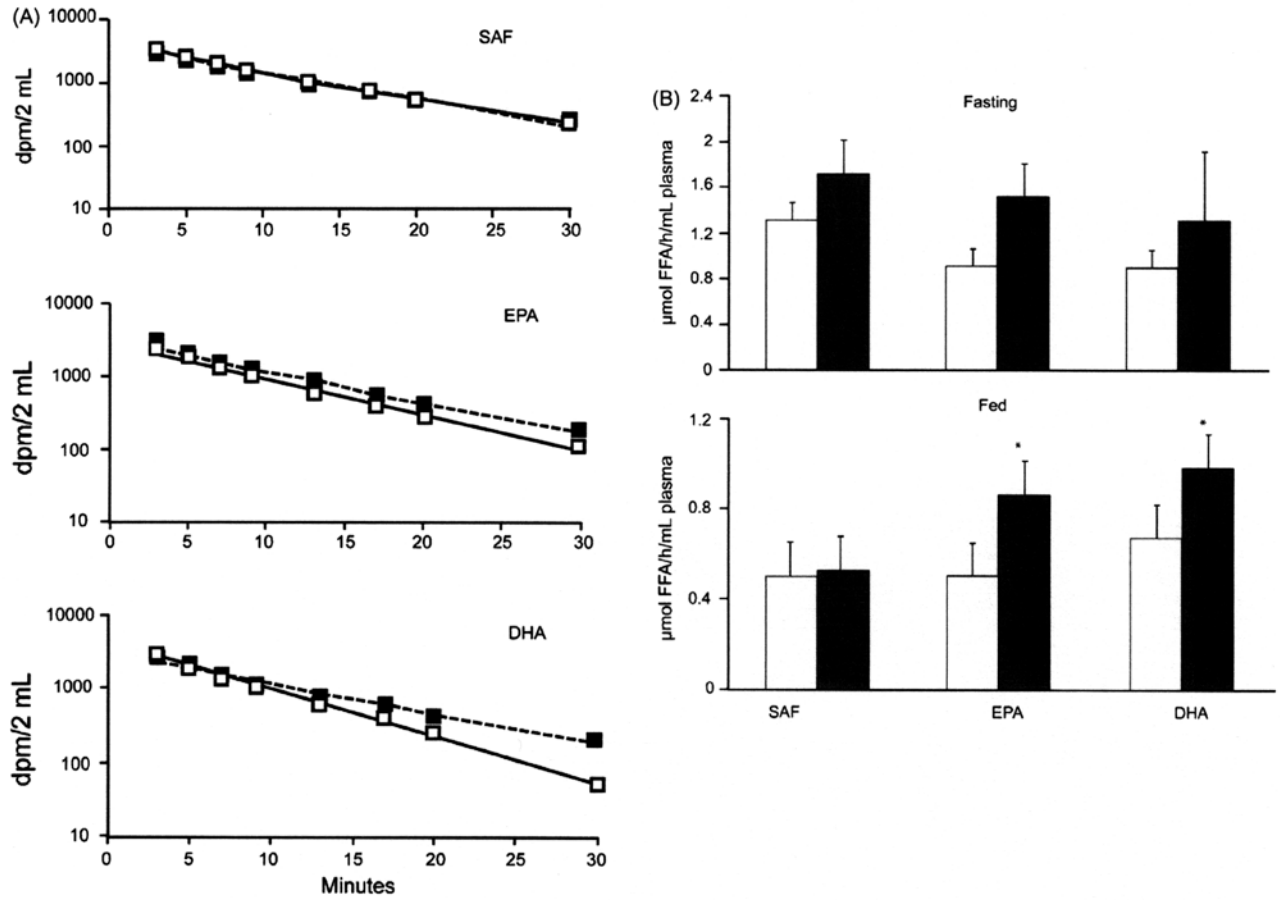


Fig. 4. Effect of omega-3 fatty acids [4 g/day of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), or safflower oil (SAF)] on chylomicron clearance and lipolytic activity in normolipidemic humans. (A) Clearance of [^{3}H]triolein-labeled lipid emulsion from chylomicron fraction measured during the fed state during placebo (olive oil) treatment (*black boxes*) and EPA, DHA, or SAF treatment (*white boxes*). (B) Pre-heparin lipolytic activities during the fed state increased from placebo (*white bars*) to active treatment (*black bars*), with increased activity being observed with both DHA (+47%) and EPA (+73%) treatments compared with SAF. Post-heparin lipoprotein lipase activities were not affected by any treatment (data not shown). *$P < 0.05$ vs. change in SAF group. From Park and Harris [37].

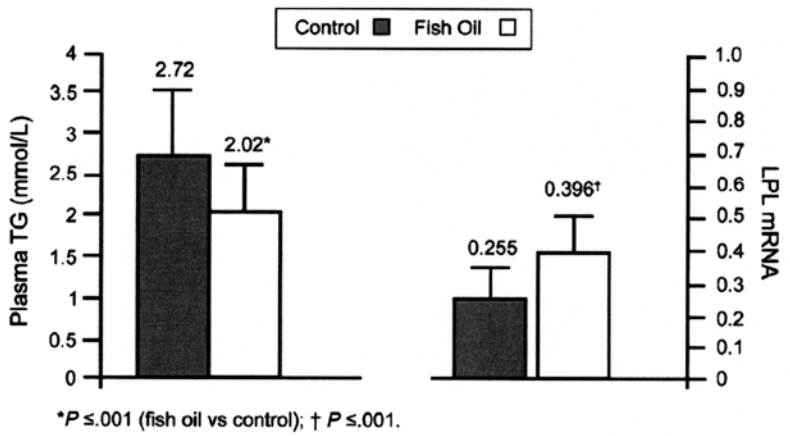


Fig. 5. Triglyceride-lowering effects of eicosapentaenoic acid plus docosahexaenoic acid in subjects with an atherogenic lipoprotein profile are associated with increased lipoprotein lipase (LPL) gene expression in adipose tissue. Not shown: post-heparin plasma LPL activity was significantly increased at 5 min post-injection (+31%, $P < 0.036$). From Khan et al. [60].

tions in obese subjects [61]. Since LPL activity in adipose tissue from obese ob/ob mice was shown to be increased by a PPAR-γ agonist [62], increased LPL activity associated with EPA plus DHA treatment may be a consequence of PPAR-γ induction.

The majority of clinical studies have not demonstrated a significant change in the fractional catabolic rate of apo B particles and chylomicron remnants, which suggests that whole particle clearance rates per se are not accelerated [39]. This does not, however, contradict the data supporting an increased rate of TG removal from VLDL and chylomicron particles in the circulation, which appears to be enhanced via activation of LPL by omega-3 fatty acids.

7. The combined effects of omega-3 fatty acids and statins

Statins (inhibitors of 3-hydroxy-3-methylglutaryl-CoA reductase) decrease LDL levels primarily by raising the number of LDL receptors, and thus, enhance the removal of LDL from plasma. Studies suggest that co-administration of P-OM3 with a statin improves the lipid profile in patients with hypertriglyceridemia to a greater extent than statin treatment alone [10,63,64]. Chan et al. showed that in insulin-resistant obese men, P-OM3 lowered TG to a similar extent with or without statin therapy [10]. P-OM3 decreased the rate of VLDL secretion and increased the conversion of VLDL to intermediate-density lipoprotein (IDL) and LDL (Fig. 6). Combined treatment with atorvastatin and P-OM3 also increased conversion of VLDL to IDL or LDL, but the pool sizes of IDL and LDL decreased by 35% and 40%, respectively, because of the statin-induced activation of LDL receptors. The results of this study indicate that there are differential mechanisms by which atorvastatin and P-OM3 reduce plasma TGs, and thus there is a role for combined therapy in insulin-resistant obese subjects with dyslipidemia.

8. Other cardioprotective effects of omega-3 fatty acids

An extensive body of data supports a cardioprotective effect of omega-3 fatty acids [1,65–69]. Diets enriched with omega-3 fatty acids protect against coronary artery atherosclerosis in non-human primates, an effect that appears to be independent of plasma lipoproteins [70]. Indeed, in some European countries, P-OM3 is approved for use in post-myocardial infarction (MI) patients to prevent CHD events [71]. The American Heart Association (AHA) advises ~1 g/day of EPA plus DHA for cardiovascular protection in patients with documented CHD, and in those without documented CHD, the consumption of a variety of fatty fish at least twice per week. The AHA recommends that treatment of elevated TGs with omega-3 fatty acids at higher doses

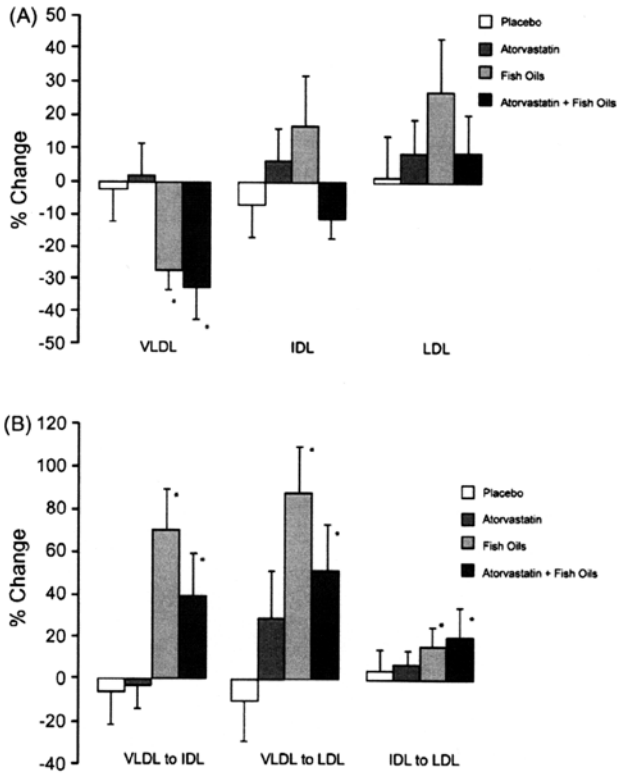


Fig. 6. Effects of omega-3 acid ethyl esters (P-OM3, 4 g/day) and atorvastatin (singly and in combination) on apolipoprotein B-100 (apo B) kinetics. (A) Percentage change in the secretion rate of apo B-containing lipoproteins into the plasma. (B) Percentage change in the interconversion of apo B–containing lipoproteins. *$P<0.01$ compared with placebo group. From Chan et al. [10].

(2–4 g/day) be undertaken under a physician's supervision [68].

Meta-analyses of primary and secondary CHD prevention trials have shown that omega-3 fatty acids can significantly decrease the risk of all-cause mortality, CHD death, and sudden death [1]. The largest single study to test the efficacy of omega-3 fatty acid for secondary prevention of CHD was the GISSI-Prevenzione Study [67]. Patients who had survived a heart attack ($n=11,324$) were randomized to either 300 mg of vitamin E, 850 mg of omega-3 fatty acid ethyl esters, both, or usual care alone. After 3.5 years, the group given the omega-3 fatty acid alone experienced a 20% reduction in all-cause mortality ($P=0.01$), and a 45% reduction in sudden death ($P<0.05$) compared to the usual care group. Vitamin E provided no additional benefit. This trial, although very large and carried out in a relatively "real-life" setting, did not include a placebo arm and drop out rates were high (>25%) in both the omega-3 and the vitamin E groups. Thus, there remains a need for further research to determine the efficacy, the optimal dose and mechanism of action of omega-3 fatty acids for the prevention of CHD death. Further evidence in secondary prevention was observed in a high-fish-consuming population in Japan. The Japan EPA Lipid Intervention Study (JELIS) [69]

Table 1
Factors involved in CHD that may be affected by EPA and/or DHA

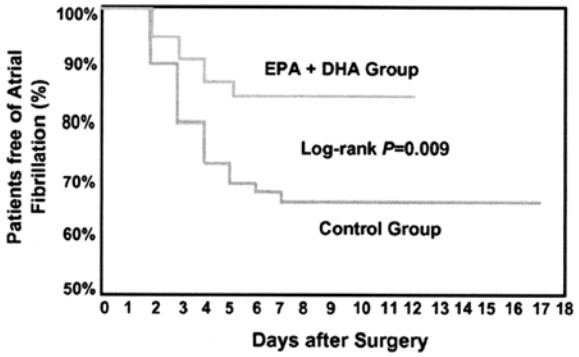
Factor	Effect
Serum TG	↓
Production of chemoattractants	↓
Production of growth factors	↓
Cell surface expression of adhesion molecules	↓
Production of inflammatory eicosanoids	↓
Blood pressure	↓
Endothelial relaxation	↑
Thrombosis	↓
Cardiac arrhythmias	↓
Heart rate variability	↑
Atherosclerotic plaque stability	↑

Adapted from Calder [2]; ↑ = increase; ↓ = decrease.

included 18,645 patients (14,981 patients with no history of coronary artery disease and 3664 patients with a history), all on statin treatment, who were randomized to 1.8 g/day EPA (no DHA) or to usual care and followed for 4.6 years for major coronary events. Compared with the statin-only group, the EPA-plus-statin group demonstrated a 19% reduction in major coronary events ($P = 0.011$). The effect was virtually the same in both the primary and secondary subgroups, but reached statistical significance only in the secondary group ($P = 0.048$). The fact that effect sizes were the same strongly suggests that EPA was equally effective in both settings, and it was the low number of events in the primary prevention group that prevented the results from reaching statistical significance. The beneficial effects of EPA on CHD events was not associated with changes in the levels of total cholesterol, TG, HDL-C, or LDL-C, indicating that non-lipid factors played a major role in a cardioprotective effect of EPA. Among the proposed factors that may account for the cardioprotective effects of omega-3 fatty acids are antiarrhythmic effects, decreased platelet aggregation, stabilization of atherosclerotic plaques, and blood-pressure lowering (Table 1) [2,68].

9. Cardiac arrhythmia suppression

The cardioprotective effects of fish oil have been attributed to antiarrhythmic effects of EPA plus DHA (reviewed in Reiffel and McDonald) [72]. Preclinical data indicate that several mechanisms may account for the antiarrhythmic action of omega-3 fatty acids. Omega-3 fatty acids are incorporated into myocardial cell membranes [73], potentially altering both eicosanoid production and ion-channel function [65]. Atrial fibrillation is the most common cardiac arrhythmia observed clinically and is a cause of particularly costly cardiovascular morbidity (stroke and heart failure). Caló et al. demonstrated that administration of P-OM3 at 2 g/day in patients undergoing coronary artery bypass graft surgery substantially reduced the incidence of postoperative AF (Fig. 7) [74]. Omega-3 fatty acids may produce an antiarrhythmic action by preventing cytosolic free calcium levels from reaching toxic levels in cardiac myocytes. Dhein et al. showed



	Control (n=81)	EPA + DHA (n=79)	P
Post CABG AF	33%	15%	0.013
Hours of AF	24	16	0.12
Hospital Length of Stay After Surgery	8.2 ± 2.6 days	7.3 ± 2.1 days	0.017

Fig. 7. Omega-3-acid ethyl esters reduce the incidence of atrial fibrillation (AF) post-coronary artery bypass graft surgery (CABG). Patients ($N = 160$) awaiting CABG were randomized to usual care or prescription omega-3-acid ethyl esters (2 g/day) for at least 5 days pre-surgery through hospitalization. AF, detected by electrocardiography during hospitalization, was defined as an electrocardiography-confirmed episode of AF > 5 min or requiring intervention. From Caló et al. [74].

that infusion of EPA, DHA, or α-linolenic acid in spontaneously beating isolated rabbit heart (Langendorff technique) produced negative inotropic and chronotropic effects [75]. Although omega-3 fatty acids have been shown to suppress both L-type calcium channels and sodium channels in rat cardiomyocytes, omega-6 fatty acids have similar effects [76].

Several recent clinical trials have examined whether omega-3 fatty-acid supplementation suppresses arrhythmias in patients with implantable cardioverter defibrillators (ICD). In the Fatty Acid Antiarrhythmia Trial (FAAT), Leaf et al. randomized 402 patients with ICDs to 2.6 g/day EPA plus DHA vs. placebo and found significant reductions in time to first ICD discharge, with the most benefit observed in patients with preexisting CHD [66]. In contrast, Raitt et al. observed no benefit of EPA plus DHA (1.3 g/day), although the study did exclude patients with a recent MI [77]. The most recent clinical trial was the Study on Omega-3 Fatty Acids and Ventricular Arrhythmia (SOFA), which examined 546 patients with ICDs who were randomized to either 0.8 g/day of EPA and DHA or placebo, to assess appropriate ICD discharges for ventricular tachycardia/ventricular fibrillation [78]. While no difference in the primary endpoint was identified, there was a trend ($P = 0.13$) towards longer event-free survival in the EPA and DHA group among the prespecified subgroup with prior MI ($n = 342$). The prevention of triggered arrhythmic

afterpotential discharges that accompany ischemia has been proposed as an important mechanism underlying omega-3 fatty acid supplementation [65]. Therefore, these data support the use of omega-3 fatty acids in post-MI patients with or without ICD placement. However, in non-ischemic patients with ICDs, there is little support for the use of fish oils in arrhythmia suppression.

10. Decreased platelet aggregation

The antithrombotic potential of omega-3 fatty acids was one of the first effects reported in Greenlandic Eskimos, who consume large amounts of whale and seal meat. Omega-6 fatty acids and certain of their derivatives can enhance thrombosis, while omega-3 fatty acids and their derivatives have an opposing effect [2]. AA is the precursor for the 2-series eicosanoids, which have a wide range of effects on metabolic pathways relevant to atherosclerosis. Thromboxane A2 stimulates platelet aggregation and produces vasoconstriction, and 5-lipoxygenase metabolites (e.g., leukotrienes) have been linked to inflammation and atherogenesis. On the other hand, the AA-derived prostacyclin is a potent vasodilator and opposes platelet aggregation. These essential metabolic functions of AA metabolites, if internally imbalanced and unopposed by sufficient omega-3 fatty acids, may contribute to a proatherogenic state. Consumption of EPA and DHA can lower tissue levels of AA by inhibiting its synthesis and by taking its place in membrane phospholipids [2,6,73]. EPA-derived 3-series eicosanoids are typically less vasoconstrictive and produce less platelet aggregation than those made from AA [2]. The net result of higher tissue omega-3 fatty acid levels is thus antithrombotic. Although EPA plus DHA have been associated with modest increases in bleeding times, no published studies have reported clinically significant bleeding episodes among patients treated with antiplatelet drugs and relatively high doses (3–7 g/day) of EPA plus DHA [79].

11. Atherosclerotic plaque stabilization

Thies et al. demonstrated that atherosclerotic plaques from patients treated with fish oil were less heavily infiltrated with macrophages than those in the placebo group [80]. Moreover, plaques from patients treated with fish oil were more likely to be fibrous-cap atheromas (type IV plaque; considered more resistant to rupture), and less likely to be thin, inflamed-cap atheromas (type V plaque) compared to plaques from patients given placebo (Fig. 8).

12. Blood pressure and heart-rate reduction

A meta analysis of 36 randomized trials found that fish-oil intake (median dose 3.7 g/day EPA plus DHA) reduced systolic blood pressure by 2.1 mm Hg ($P<0.01$) and diastolic blood pressure by 1.6 mm Hg ($P<0.01$) [81]. At least two mechanisms could account for this effect. First, incorporation of EPA and DHA into membrane phospholipids could increase systemic arterial compliance [82]. Second, EPA and DHA could improve endothelial function [83]. This is consistent with the observation that the antihypertensive effect of fish oil may be greater in populations with arterial stiffness and/or microvascular dysfunction, i.e., populations with hypertension and older populations [81]. In addition, a meta-analysis of 30 randomized trials found that fish-oil intake (median dose 3.5 g/day EPA plus DHA) reduced heart rate by 1.6 bpm compared with placebo ($P=0.002$) [84].

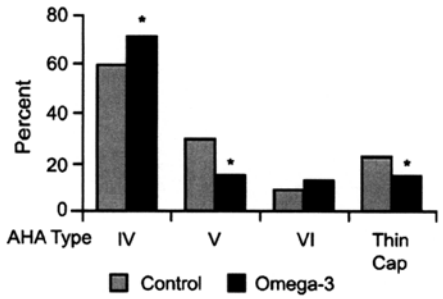


Fig. 8. Effect of omega-3 fatty acids on carotid plaque composition. Patients awaiting carotid endocardectomy were randomized to control (vegetable oil [$n=57$]) or omega-3 fatty acids ($n=53$) for a median of 42 days pre-procedure. AHA, American Heart Association. *$P<0.05$. From Thies et al. [80].

13. Conclusion

Multiple factors that affect CHD risk may be affected by omega-3 fatty acids (Table 1). The TG-lowering effects of omega-3 fatty acids appear to be due to a combination of decreased hepatic TG secretion combined with enhanced clearance of TG from the plasma. Gaps in our understanding of the mechanisms that link omega-3 fatty acids and CHD risk are due, in part, to variability in study designs and animal models, and to the use of supraphysiological doses of fish oil in some animal studies. Very limited mechanistic data in humans are available. Nonetheless, clinical studies with omega-3 fatty acids have demonstrated multiple cardioprotective benefits. Omega-3 fatty acids reduce

Table 2
Effects of omega-3 fatty acids on plasma lipids in patients with mixed dyslipidemia

Lipid parameter	Effect
TGs	↓ (20–50%)
LDL-C	↑/neutral
Total cholesterol	↑/neutral
HDL-C	↑/neutral

TGs, triglycerides; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; ↑ = increase; ↓ = decrease. Adapted from Harper and Jacobson [85].

the level of plasma TGs (Table 2), which is an independent risk factor for CHD. In addition, they exert antiarrhythmic effects in ischemic, post-MI patients; decrease platelet aggregation; increase plaque stabilization; reduce blood pressure; and reduce heart rate. Additional studies are needed to define more clearly the cellular and molecular basis for the cardioprotective effects of omega-3 fatty acids in humans.

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Fish and Long-Chain ω-3 Fatty Acid Intake and Risk of Coronary Heart Disease and Total Mortality in Diabetic Women

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Fish and Long-Chain ω-3 Fatty Acid Intake and Risk of Coronary Heart Disease and Total Mortality in Diabetic Women

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Background—Although several prospective cohort studies have found an inverse association between fish consumption and risk of coronary heart disease (CHD) or sudden cardiac death in the general population, limited data are available among diabetic patients.

Methods and Results—We examined prospectively the association between intake of fish and ω-3 fatty acids and risk of CHD and total mortality among 5103 female nurses with diagnosed type 2 diabetes but free of cardiovascular disease or cancer at baseline. Between 1980 and 1996 (45 845 person-years of follow-up), we documented 362 incident cases of CHD (141 CHD deaths and 221 nonfatal myocardial infarctions) and 468 deaths from all causes. Compared with women who seldom consumed fish (<1 serving/mo), the relative risks (RRs) (95% CI) of CHD adjusted for age, smoking, and other established coronary risk factors were 0.70 (0.48 to 1.03) for fish consumption 1 to 3 times per month, 0.60 (0.42 to 0.85) for once per week, 0.64 (0.42 to 0.99) for 2 to 4 times per week, and 0.36 (0.20 to 0.66) for 5 or more times per week (*P* for trend=0.002). Higher consumption of fish was also associated with a significantly lower total mortality (multivariate RR=0.48 [0.29 to 0.80] for ≥5 times per week [*P* for trend=0.005]). Higher consumption of long-chain ω-3 fatty acids was associated with a trend toward lower incidence of CHD (RR=0.69 [95% CI 0.47 to 1.03], *P* for trend=0.10) and total mortality (RR=0.63 [95% CI, 0.45 to 0.88], *P* for trend=0.02).

Conclusions—A higher consumption of fish and long-chain ω-3 fatty acids was associated with a lower CHD incidence and total mortality among diabetic women. ***(Circulation.* 2003;107:1852-1857.)**

Key Words: coronary disease ■ nutrition ■ fish ■ fatty acids ■ women

The role of long-chain ω-3 fatty acids in the management and treatment of diabetes has received much attention in the literature. Fish oil supplementation substantially lowers triglyceride levels in diabetic individuals.[1] Because hypertriglyceridemia is a hallmark of diabetic dyslipidemia and an important risk factor for cardiovascular disease among diabetic patients,[2] fish oil may have an important role in treating hypertriglyceridemia in diabetics. In addition, fish oil has been shown to decrease endothelial cell activation and improve endothelial dysfunction among diabetics.[3] Other potential benefits of long-chain ω-3 fatty acids for diabetes include reduction of platelet aggregability[4] and antiarrhythmic effects.[5] Furthermore, higher fish intake has been associated with lower risk of microalbuminuria in type 1 diabetic patients.[6] A potential concern is that fish oil may worsen glycemic control among diabetic patients,[7,8] but this adverse effect was not substantiated in 2 recent meta-analyses of metabolic studies.[9,10]

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Although several prospective cohort studies have found an inverse association between fish consumption and risk of coronary heart disease (CHD) or sudden cardiac death in the general population,[11–15] limited data are available among diabetic patients. We therefore examined prospectively the association between fish and long-chain ω-3 fatty acid intake and incidence of CHD and total mortality among diabetic women in the Nurses' Health Study cohort.

Methods

Study Population

The Nurses' Health Study cohort was established in 1976 when 121 700 female registered nurses, 30 to 55 years old and residing in 11 large US states, completed a mailed questionnaire about their medical history and lifestyle. Every 2 years, follow-up questionnaires have been sent to update information on potential risk factors and to identify newly diagnosed cases of CHD and other illness. The

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present study included the 5103 women who reported physician-diagnosed type 2 diabetes mellitus on any questionnaire from 1976 to 1994 (1694 prevalent diabetic women in 1980 and 3409 incident diabetic women during the follow-up). Women with a history of CHD (including myocardial infarction, angina, and/or coronary revascularization), stroke, or cancer reported on the 1980 questionnaire (when diet was first assessed), or before, were excluded at baseline.

Confirmation of Diabetes Mellitus

A supplementary questionnaire regarding symptoms, diagnostic tests, and hypoglycemic therapy was mailed to women who indicated on any biennial questionnaire that they had been diagnosed with diabetes. A case of diabetes was considered confirmed if at least 1 of the following was reported on the supplementary questionnaire: (1) classic symptoms plus fasting plasma glucose of ≥140 mg/dL (7.8 mmol/L) or random plasma glucose ≥200 mg/dL (11.1 mmol/L), (2) ≥2 elevated plasma glucose concentrations on different occasions (fasting plasma glucose ≥140 mg/dL [7.8 mmol/L] or random plasma glucose ≥200 mg/dL [11.1 mmol/L] and/or concentration ≥200 mg/dL after ≥2 hours on oral glucose tolerance testing) in the absence of symptoms, or (3) treatment with hypoglycemic medication (insulin or oral hypoglycemic agent). The validity of this questionnaire has been verified in a subsample of this study population.[16] Among a random sample of 84 women classified by the questionnaire as having type 2 diabetes mellitus, 71 gave permission for their medical records to be reviewed, and records were available for 62. An endocrinologist blinded to the information reported on the supplementary questionnaire reviewed the records according to National Diabetes Data Group criteria.[17] The diagnosis of type 2 diabetes mellitus was confirmed in 61 of 62 of the women (98%). We used the National Diabetes Data Group diagnostic criteria because the analytic cohort preceded the American Diabetes Association guideline published in 1997.[18] In our primary analyses, we used self-reported diabetes to define the analytic cohort. Secondary analyses including only diabetic women confirmed by the supplementary questionnaire yielded similar results (consisting of ≈80% of cases).

Ascertainment of Diet

The semiquantitative food frequency questionnaire used in 1980 included 61 foods, including a single question assessing fish intake.[19] A common unit or portion size for each food (eg, 6 to 8 oz for fish) was specified, and each woman was asked how often on average during the previous year she had consumed that amount. Nine responses were possible for each food item, ranging from "almost never" to "6 or more times per day." In 1984, 1986, 1990, and 1994, the dietary questionnaire was expanded to include 4 fish and seafood items: (1) dark-meat fish such as mackerel, salmon, sardines, bluefish, or swordfish (3 to 5 oz); (2) canned tuna (3 to 4 oz); (3) other fish (3 to 5 oz); and (4) shrimp, lobster, or scallops as main dish (3 to 5 oz). The average daily intake of nutrients was calculated by multiplying the frequency of consumption of each item by its nutrient content per serving and totaling the nutrient intake for all food items. We first collected information on fish oil supplements in 1990. However, we were not able to study the effects of fish oil supplements because the use was very low in our cohort (≈1.6% in 1990).

The calculation of long-chain ω-3 fatty acid intake was described in detail elsewhere.[14] Briefly, to calculate intake of ω-3 fatty acids (eicosapentaenoic acid and docosahexanoic acid), we assigned grams per serving as follows: 1.51 for dark-meat fish, 0.42 for canned tuna fish, 0.48 for other fish, and 0.32 for shrimp, lobster or scallops. These ω-3 fatty acid values were derived by weighting the mean values of ω-3 fatty acids for the most common types of fish on the basis of US landing data in 1984 (US Department of Commerce). For the 1980 questionnaire, we assigned 1.16 g of long-chain ω-3 fatty acids per portion (6 to 8 oz) of fish. This number was calculated as a weighted average of the relative composition of ω-3 fatty acid composition from dark-meat fish, canned tuna, and other fish on the 1984 dietary questionnaire. In a validation study, the energy-adjusted intake of eicosapentaenoic acid from fish estimated by the food frequency questionnaire was significantly correlated with percentage of eicosapentaenoic acid in adipose tissue (Spearman correlation coefficient, 0.49; $P<0.001$).[20]

End Point Ascertainment

The end point for this study was incidence of CHD (including CHD deaths and nonfatal myocardial infarction) and all-cause mortality occurring after return of the 1980 questionnaire but before June 1, 1996. We sought to review medical records for all self-reported myocardial infarctions. Records were reviewed by physicians with no knowledge of the self-reported risk factor status. Myocardial infarction was confirmed according to World Health Organization criteria: symptoms plus either diagnostic ECG changes or elevated cardiac enzymes.[21] Infarctions that required hospital admission and for which confirmatory information was obtained by interview or letter but for which no medical records were available were designated as probable (17%). Excluding probable cases from the analyses did not materially alter the results.

Deaths were identified from state vital records and the National Death Index or reported by next of kin and the postal system. Follow-up for the deaths was >98% complete.[22] We obtained copies of death certificates and medical records and determined causes of death (classified according to the categories of the International Classification of Diseases, Ninth Revision [ICD-9]). Fatal coronary disease was defined as fatal myocardial infarction if this was confirmed by hospital records or autopsy or if coronary disease was listed as the cause of death on the certificate and this was the underlying and most plausible cause and evidence of previous coronary disease was available. We designated as presumed coronary disease (15% of fatal cases) those in which coronary disease was the underlying cause on the death certificate but no records were available.

Statistical Analyses

For women who reported diabetes on the 1980 or earlier questionnaires, person-months of follow-up were calculated from the date of return of the 1980 questionnaire to the first end point, death, or June 1, 1996, whichever came first. For women who developed diabetes during the follow-up, person-months were calculated from the date of return of the questionnaire on which diabetes diagnosis was reported. Women who reported having cardiovascular disease on previous questionnaires were excluded from subsequent follow-up.

Because of the long follow-up period, dietary variables were updated to better represent long-term dietary patterns, using the information from 1980, 1984, 1986, 1990, and 1994 dietary questionnaires. We calculated intakes of fish and ω-3 fatty acids as a cumulative average of intake from all available dietary questionnaires up to the start of each 2-year follow-up interval in which events were reported.[23] We stopped updating diet when a person developed hypertension or hypercholesterolemia during the follow-up, because development of these conditions may lead to changes in diet.[24] The other nutrient variables (fiber, trans fat, and the ratio of polyunsaturated to saturated fats) and intake of fruits and vegetables and red meat (beef, pork, or lamb as main dish or mixed dish) were also calculated as cumulative averages of intake.

We divided women into 5 categories according to frequency of fish consumption (<1/mo, 1 to 3/mo, 1/wk, 2 to 4/wk, and 5+/wk) or quintiles of ω-3 fatty acids (as percentage of total energy) and calculated incidence rates by dividing the number of events by person-time of follow-up in each category. The relative risk (RR) was computed as the rate in a specific category of fish or ω-3 fatty acid consumption divided by that in the lowest category, with adjustment for age in 5-year categories. In multivariate analyses using pooled logistic regression (asymptotically equivalent to Cox regression with time-varying covariate),[25] we simultaneously included total energy intake, cigarette smoking, body mass index, menopausal status and postmenopausal hormone use, alcohol use, history of hypertension and high cholesterol, multivitamin use, vitamin E supplement use, family history of myocardial infarction, physical activity (number of hours spent on moderate to vigorous exercise per week), and aspirin use. We also adjusted for duration of diabetes and use of insulin or other hypoglycemic therapy reported

TABLE 1. Age-Adjusted Baseline Characteristics According to the Intake of Fish and ω-3 Fatty Acids Among Diabetic Women in 1980

	Fish Consumption, Servings			ω-3 Fat Intake		
	<1/mo (n=159)	1/wk (n=676)	5+/wk (n=82)	Lowest (n=361)	Intermediate (n=405)	Highest (n=331)
Mean value						
Age, y	47.8	48.8	50.2	47.2	48.7	49.5
Body mass index, kg/m^2	27.8	28.1	28.7	27.5	27.9	29.0
Physical activity, h/wk	3.1	3.2	4.0	3.1	3.1	4.0
Alcohol, g/d	2.7	3.9	1.9	4.2	3.9	3.0
Saturated fat, g/d	31.4	28.7	21.7	30.6	29.0	25.0
Polyunsaturated fat, g/d	9.5	9.6	8.3	9.5	9.7	8.8
Trans fat, g/d	4.2	4.1	2.7	4.3	4.2	3.3
Fiber, g/d	13.7	14.9	17.4	13.2	14.6	16.6
Glycemic index	53.9	53.2	50.9	53.7	53.5	51.5
Glycemic load	98.6	96.4	93.3	97.4	97.1	94.4
α-Linolenic acid, g/d	1.1	1.1	1.1	1.1	1.1	1.1
Fruits, servings/d	2.1	2.6	3.3	2.2	2.5	2.9
Vegetables, servings/d	2.0	2.1	2.9	1.7	2.0	2.7
Red and processed meats, servings/d	1.7	1.6	1.1	1.7	1.7	1.3
Duration of diabetes, y	7.0	8.1	8.0	8.4	8.0	7.9
Percent of women						
Parental history of MI before age 60 y	16.2	20.2	15.7	18.0	19.7	21.2
Current smoking	37.2	26.7	23.8	33.2	27.3	27.7
History of hypertension	44.6	46.4	63.7	40.1	44.8	51.1
History of hypercholesterolemia	15.9	15.0	20.2	13.7	17.8	22.4
Multivitamin use	39.6	34.7	52.2	36.2	30.5	42.5
Vitamin E supplement use	9.6	12.6	27.3	12.2	12.3	23.9
Current estrogen therapy among postmenopausal women	19.6	20.3	14.9	19.2	27.9	26.7
Aspirin use ≥1×/wk	40.9	37.9	40.6	42.6	42.1	38.4
Insulin use	37.5	45.3	41.7	43.7	47.3	44.9
Oral hypoglycemic medication only	25.6	27.0	24.0	25.9	27.4	29.7

on the supplementary diabetes questionnaire or the 1988 main questionnaire.

Results

Between 1980 and 1996 (45 845 person-years of follow-up), we documented 362 incident cases of CHD (141 CHD deaths and 221 nonfatal myocardial infarctions) and 468 deaths from all causes (161 from CHD or stroke, 172 from cancer, and 135 from other causes). Compared with diabetic women who seldom ate fish, those with a higher fish consumption were older and slightly heavier, had a lower prevalence of current smoking, and had a higher prevalence of hypertension, high blood cholesterol, and multivitamin and vitamin E supplement use (Table 1). Fish consumption was positively associated with intake of fruits and vegetables and inversely associated with intake of red and processed meats.

Table 2 presents RRs of CHD and total mortality according to fish intake. We observed significant inverse associations between fish intake and incidence of CHD after adjustment for age (P for trend=0.003). After further adjustment for other cardiovascular risk factors, RRs (95% CI) were 0.70 (0.48 to 1.02) for fish consumption 1 to 3 times per month, 0.60 (0.42 to 0.85) for once per week, 0.65 (0.43 to 0.99) for 2 to 4 times per week, and 0.38 (0.21 to 0.68) for ≥5 times per week (P for trend=0.002). Further adjustment for other dietary factors did not appreciably alter the results. A higher fish consumption was associated with a significantly lower risk of both fatal CHD (multivariate RR for 5+/wk, 0.41; 95% CI, 0.18 to 0.94) and nonfatal myocardial infarction (multivariate RR for 5+/wk, 0.28; 95% CI, 0.11 to 0.71). Additional adjustment for fruits, vegetables, and red meat did not materially affect the RRs. Among the 4 different types of fish on which we first collected information in 1984, only dark-meat fish and shrimp intakes were inversely associated with risk of CHD (multivariate RRs comparing ≥2 times/wk with <1/mo, 0.38 [0.05 to 2.75] for dark-meat fish and 0.43 [0.06 to 3.14] for shrimp). The wide 95% CIs reflect a small number of cases.

For total mortality, the age-adjusted RRs across categories of fish intake were 1.0, 0.64, 0.61, 0.56, and 0.54 (P for

TABLE 2. RR (95% CI) of CHD in a Cohort of 5103 Diabetic Women Followed Up From 1980 to 1996 According to the Average Frequency of Fish Intake

	Average Frequency of Fish Intake					
	<1/mo	1–3/mo	1/wk	2–4/wk	5+/wk	*P* for Trend
CHD incidence						
No. of cases	41	92	161	52	16	...
Person-years	3170	11 685	21 705	6495	2790	...
Age adjusted*	1.0	0.63 (0.43–0.91)	0.55 (0.39–0.78)	0.59 (0.39–0.99)	0.40 (0.22–0.71)	0.003
Multivariate I†	1.0	0.70 (0.48–1.02)	0.60 (0.42–0.85)	0.65 (0.43–0.99)	0.38 (0.21–0.68)	0.002
Multivariate II‡	1.0	0.70 (0.48–1.03)	0.60 (0.42–0.85)	0.64 (0.42–0.99)	0.36 (0.20–0.66)	0.002
Total mortality						
No. of deaths	48	114	219	60	27	...
Person-years	3209	11 784	21 837	6554	2808	...
Age adjusted*	1.0	0.64 (0.46–0.91)	0.61 (0.44–0.83)	0.56 (0.38–0.83)	0.54 (0.34–0.88)	0.01
Multivariate I†	1.0	0.75 (0.53–1.07)	0.67 (0.49–0.93)	0.69 (0.47–1.02)	0.49 (0.30–0.81)	0.006
Multivariate II‡	1.0	0.75 (0.53–1.07)	0.66 (0.48–0.92)	0.67 (0.45–1.01)	0.48 (0.29–0.80)	0.005

*Adjusted for age (5-year categories) and time intervals.

†Adjusted for factors cited above and for smoking status (never, past, current 1–14.9, 15–24, ≥25 cigarettes/d), body mass index (<22, 22–22.9, 23–24.9, 25–28.9, ≥29 kg/m^2), alcohol intake (0, 0.1–4.9, 5–14, ≥15 g/d), parental history of myocardial infarction, menopausal status and postmenopausal hormone use, moderate to vigorous activities (<1, 1–1.9, 2–3.9, 4–6.9, ≥7 h/wk), usual aspirin use (<1/wk, 1–2/wk, 3–6/wk, 7–14/wk, and 15+/wk), multivitamin supplement use (yes vs no), vitamin E supplement use (yes vs no), history of hypertension (yes vs no), hypercholesterolemia (yes vs no), duration of diabetes(<5, 5–10, 11–15, >15 y), and hypoglycemic medication (none, oral medicine only, insulin use).

‡Adjusted for factors cited above and *trans* fat, the ratio of polyunsaturated fat to saturated fat, and dietary fiber (all in quintiles).

trend=0.01). After adjustment for lifestyle and dietary factors, the RRs were 1.0, 0.75, 0.66, 0.67, and 0.48 (*P* for trend=0.005). The inverse association was observed for both cardiovascular disease mortality (multivariate RR comparing extreme categories of fish intake was 0.47; 95% CI, 0.21 to 1.03) and noncardiovascular mortality (corresponding RR, 0.50; 95% CI, 0.26 to 0.93).

Intake of long-chain ω-3 fatty acids was associated with a significantly lower risk of CHD in age-adjusted analysis (RR comparing extreme quintiles, 0.67; 95% CI, 0.46 to 0.98; *P* for trend=0.03) (Table 3). The RRs were slightly attenuated in multivariate analyses (RR comparing extreme quintiles, 0.69; 95% CI, 0.47 to 1.03; *P* for trend=0.10). We considered the possibility that the weaker association with ω-3 fatty acids than with fish might be because of a more extreme contrast with the latter and perhaps greater measurement error in calculating ω-3 fatty acids. Also, the event rate for CHD in the reference group for ω-3 fatty acid analysis was somewhat lower than that in the reference group for fish analysis (7.5/1000 versus 12.9/1000).

TABLE 3. RR* (95% CI) of CHD in a Cohort of 5103 Diabetic Women Followed Up From 1980 to 1996 According to Quintiles of ω-3 Fatty Acid Intake

	Quintiles of Average ω-3 Fatty Acids (Median Intake, g/d)					
	1 (0.04)	2 (0.06)	3 (0.09)	4 (0.15)	5 (0.25)	*P* for Trend
CHD incidence						
No. of cases	56	113	77	67	49	...
Person-years	7421	11 822	10 334	8462	7806	...
Age adjusted	1.0	1.04 (0.77–1.41)	0.88 (0.62–1.23)	0.92 (0.66–1.28)	0.67 (0.46–0.98)	0.03
Multivariate I	1.0	0.97 (0.71–1.32)	0.86 (0.61–1.21)	0.94 (0.67–1.32)	0.71 (0.48–1.04)	0.11
Multivariate II	1.0	0.96 (0.71–1.31)	0.85 (0.60–1.20)	0.92 (0.66–1.30)	0.69 (0.47–1.03)	0.10
Total mortality						
No. of deaths	77	131	101	87	72	...
Person-years	7475	11 924	10 420	8515	7857	...
Age adjusted	1.0	0.83 (0.64–1.08)	0.80 (0.60–1.07)	0.75 (0.56–1.01)	0.63 (0.46–0.86)	0.004
Multivariate I	1.0	0.78 (0.60–1.02)	0.77 (0.58–1.04)	0.79 (0.59–1.06)	0.65 (0.47–0.90)	0.02
Multivariate II	1.0	0.77 (0.58–1.00)	0.76 (0.56–1.02)	0.77 (0.57–1.05)	0.63 (0.45–0.88)	0.02

*The covariates in the models are the same as those listed in Table 2.

Because aspirin also has antiplatelet activity, analyses were stratified by regular aspirin use. The inverse association between ω-3 fatty acids and CHD appeared to be stronger for non–aspirin users (multivariate RR comparing extreme quintile, 0.56; 95% CI, 0.29 to 1.06) than for regular aspirin users (corresponding RR, 0.82; 95% CI, 0.49 to 1.36). However, the test for interaction between regular aspirin use and ω-3 fatty acid intake was not statistically significant (P for interaction=0.30).

For total mortality, the age-adjusted RRs according to quintiles of ω-3 fatty acid intake were 1.0, 0.83, 0.80, 0.75, and 0.63; P for trend=0.004. Adjustment for lifestyle and dietary factors did not appreciably change the RRs for total mortality (RR comparing extreme quintiles of ω-3 fatty acids, 0.63; 95% CI, 0.45 to 0.88; P for trend=0.02).

The statistical test for interaction between diabetes status and fish consumption on CHD risk was not statistically significant (P for interaction=0.21), suggesting that the associations did not differ significantly between women with and without diabetes. The multivariate RRs of CHD for nondiabetic women (1257 CHD cases) across categories of fish consumption were 1.0, 0.88, 0.80, 0.75, and 0.78 (P for trend=0.028).

Discussion

In this prospective cohort study of diabetic women, higher consumption of fish and ω-3 fatty acids was associated with a lower incidence of both CHD and total mortality, even after adjustment for established cardiovascular risk factors. The inverse association was not explained by dietary predictors of CHD, including fiber, trans fatty acids, the ratio of polyunsaturated to saturated fats, and intake of fruits, vegetables, and red meat.

Type 2 diabetes is characterized by lipid and lipoprotein metabolism abnormalities, increased platelet aggregation and clotting, endothelial dysfunction, and increased cardiac arrhythmia risk, all of which are associated with accelerated cardiovascular incidence and mortality.[26] ω-3 fatty acids may reduce CHD incidence and mortality among diabetics through multiple mechanisms, including reduction of blood triglycerides,[27] inhibition of platelet aggregability,[4] and antiarrhythmic effects.[5] In addition, fish oil may improve endothelial dysfunction, an early marker of atherosclerosis, especially among diabetic patients.[28,29] The antiarrhythmic effects of ω-3 fatty acids are well established.[5] Because diabetics are more prone to ventricular arrhythmia and sudden cardiac death,[30,31] an adequate intake of long-chain ω-3 fatty acids may be particularly important for diabetic patients. In the present study, we were not able to study sudden cardiac death as an end point because of a relatively small sample size.

In addition to cardiovascular benefits, higher fish consumption may reduce the risk of microvascular complications. In a nested case-control study of 1150 patients with type 1 diabetes,[6] higher fish intake was associated with a significantly lower risk of microalbuminuria, after adjustment for HbA1c, age, sex, and other potential confounding variables. Although we were not able to study the association between fish consumption and microvascular complications, our analyses suggest that a higher consumption of fish intake was associated with a lower mortality from noncardiovascular causes.

Concerns have been raised that fish oil may worsen glycemic control by diverting substrates from lipogenesis to gluconeogenesis in the process of inhibiting hepatic triglyceride synthesis.[7,8,32] Two recent meta-analyses, however, found no significant adverse effects of fish oil supplementation on glycemic control, despite fish oil's lowering triglyceride levels by ≈30%.[9,10] There is some evidence that fish oil supplements cause a slight increase in LDL cholesterol among diabetic patients.[9,10] Thus, the combined effects of fish oil and statins on diabetic dyslipidemia need to be investigated. In a recent study, fish oil supplementation was shown to potentiate the beneficial effects of statins on lipid profile for coronary patients with combined hyperlipemia by reducing postprandial hyperlipemia and small dense LDL.[33]

One limitation of our study is that we do not have direct measures of glycemic control and severity of diabetes. However, we adjusted for duration of diabetes and use of insulin and hypoglycemic medications and obtained similar results, suggesting that our findings are unlikely to be explained by confounding because of severity of the disease. Because diabetes is self-reported, there is a potential for misclassification. Nonetheless, self-report of this diagnosis has previously been shown to be valid in this cohort, and analyses restricted to confirmed cases yielded similar results.

Because of the observational nature of our study, we cannot completely exclude the possibility that the observed association is because of unmeasured or residual confounding, although we have carefully controlled for important dietary and lifestyle confounding variables. Previous clinical trials[34–36] have demonstrated protective effects of increased fish consumption or fish oil supplementation against coronary mortality and sudden cardiac death among patients with coronary disease, but controlled trials of the effects of fish oil supplementation on prevention of CHD and mortality among diabetic patients have not been conducted and would be useful. A recent study suggested that a higher mercury intake might attenuate the benefits of long-chain ω-3 fatty acids.[37] We were not able to test this hypothesis because our study did not assess mercury exposure.

In conclusion, this prospective study provides evidence for an inverse association between fish and long-chain ω-3 fatty acid consumption and risk of CHD and total mortality among diabetic women. These findings suggest that regular fish consumption should be considered as part of a healthy diet for diabetic management.

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BLOOD LEVELS OF LONG-CHAIN n−3 FATTY ACIDS AND THE RISK OF SUDDEN DEATH

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ABSTRACT

Background Experimental data suggest that long-chain n−3 polyunsaturated fatty acids found in fish have antiarrhythmic properties, and a randomized trial suggested that dietary supplements of n−3 fatty acids may reduce the risk of sudden death among survivors of myocardial infarction. Whether long-chain n−3 fatty acids are also associated with the risk of sudden death in those without a history of cardiovascular disease is unknown.

Methods We conducted a prospective, nested case–control analysis among apparently healthy men who were followed for up to 17 years in the Physicians' Health Study. The fatty-acid composition of previously collected blood was analyzed by gas–liquid chromatography for 94 men in whom sudden death occurred as the first manifestation of cardiovascular disease and for 184 controls matched with them for age and smoking status.

Results Base-line blood levels of long-chain n−3 fatty acids were inversely related to the risk of sudden death both before adjustment for potential confounders (P for trend = 0.004) and after such adjustment (P for trend = 0.007). As compared with men whose blood levels of long-chain n−3 fatty acids were in the lowest quartile, the relative risk of sudden death was significantly lower among men with levels in the third quartile (adjusted relative risk, 0.28; 95 percent confidence interval, 0.09 to 0.87) and the fourth quartile (adjusted relative risk, 0.19; 95 percent confidence interval, 0.05 to 0.71).

Conclusions The n−3 fatty acids found in fish are strongly associated with a reduced risk of sudden death among men without evidence of prior cardiovascular disease. (N Engl J Med 2002;346:1113-8.)

WE previously reported that fish consumption was associated with a reduced risk of sudden death from cardiac causes, but not a reduced risk of myocardial infarction, in the Physicians' Health Study.[1] It is hypothesized that the long-chain n−3 polyunsaturated fatty acids found in fish, primarily eicosapentaenoic acid and docosahexaenoic acid, may be responsible for this association. Experimental data from studies in animals and at the cellular level suggest that these n−3 fatty acids have antiarrhythmic properties,[2,3] and a recent randomized trial testing supplements of these n−3 fatty acids in survivors of myocardial infarction found a statistically significant 45 percent reduction in the risk of sudden death, with no effect on nonfatal myocardial infarction.[4] However, prospective data on blood levels of long-chain n−3 fatty acids and sudden death from cardiac causes are sparse, and there have been no randomized trials of the effects of long-chain n−3 fatty acids in the diet or as supplements among persons without a history of cardiovascular disease, who represent over half of all cases of sudden death from cardiac causes.[5]

To address the hypothesis that the long-chain n−3 fatty acids found in fish are associated with a reduced risk of sudden death from cardiac causes in those without known cardiovascular disease, we performed a prospective, nested case–control analysis of the fatty-acid composition of whole blood in men without a confirmed history of cardiovascular disease who were participants in the Physicians' Health Study.

METHODS

Study Population and Collection of Whole-Blood Samples

The methods of the Physicians' Health Study have been described in detail elsewhere.[6,7] Briefly, 22,071 male physicians, who were 40 to 84 years old in 1982 and had no history of myocardial infarction, stroke, transient ischemic attacks, or cancer, were assigned at random according to a two-by-two factorial design to receive aspirin, beta carotene, both active drugs, or both placebos. Informed consent was obtained from all subjects, and the research protocol was approved by the institutional review board at Brigham and Women's Hospital in Boston. At base line, the physicians completed questions on their health status and risk factors for cardiovascular disease. Dietary intake of fish was ascertained at 12 months with an abbreviated, semiquantitative food-frequency questionnaire,[8] as described previously.[1] Information on cardiovascular events was updated every six months for the first year and annually thereafter with follow-up questionnaires.

Before randomization, which occurred between August 1982 and December 1984, potential participants were asked to provide base-

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line blood samples, which were collected in EDTA and processed for long-term storage at −80°C. Of the randomized study participants, 14,916 (68 percent) provided base-line blood samples. More than 70 percent of these specimens were received between September and November 1982.

Confirmation of End Points and Selection of Controls

The end point of sudden death from cardiac causes was ascertained by a two-step process. First, deaths from any cause were generally reported by postal authorities or next of kin, and an end-point review committee confirmed deaths from cardiovascular causes by examining medical records obtained from hospitals and attending physicians. A participant's next of kin was interviewed about the circumstances of the death if they were not adequately documented in the medical record.

Second, to ascertain the specific end point of sudden death from cardiac causes, two cardiologists who were unaware of the subject's exposure status reviewed medical records and reports from the next of kin of all deaths from cardiovascular causes (excluding strokes). In this second review, sudden death from cardiac causes was defined as death within one hour after the onset of symptoms or a witnessed cardiac arrest or abrupt collapse that occurred within one hour after the onset of symptoms and that resulted in death. For all these deaths, no probable noncardiac cause was suggested by the history or autopsy. To increase specificity for death from cardiac arrhythmia, we excluded any death for which there was evidence of collapse of the circulation (hypotension, exacerbation of congestive heart failure, or altered mental status) before the disappearance of the pulse.[9] Unwitnessed deaths with no information on timing but with an autopsy report consistent with death from cardiac arrhythmia (i.e., acute coronary thrombosis or severe coronary artery disease without myocardial necrosis or other pathological findings to explain death) were considered possible sudden deaths from cardiac causes. Analyses were performed both including and excluding these deaths, with similar results.

Over the 17 years of study follow-up, 201 sudden deaths from cardiac causes were documented; for 119 of these, an adequate base-line blood sample was available for analysis. Ninety-four of these subjects had been free of confirmed cardiovascular disease before death. Each of these subjects was matched with two control subjects, who had also provided an adequate base-line blood sample and who were alive and remained free of confirmed cardiovascular disease at the time of case ascertainment. A diagnosis of confirmed cardiovascular disease required a report of angina with a positive stress-test result or coronary angiography documenting clinically significant coronary artery disease, myocardial infarction confirmed according to World Health Organization criteria, or stroke confirmed by a typical neurologic deficit lasting longer than 24 hours. Using a risk-set sampling method, we randomly selected controls from among study participants who met the matching criteria for age (within one year), smoking status (formerly, currently, or never), and length of time since randomization (in six-month intervals). For four case subjects, only one adequate control could be found.

Laboratory Analysis

Whole blood collected and stored at base line was thawed. Although there are no data on their long-term storage in blood, long-chain n−3 fatty acids have been documented to have high reliability coefficients (0.64 to 0.66) and minimal oxidation in serum samples stored at −80°C for up to 12 years.[10] The fatty acids from whole blood were extracted[11,12] and quantitated by gas–liquid chromatography on a fused silica capillary cis–trans column (SP2560, Supelco, Bellefonte, Pa.). We identified the peak retention times from 47 peaks and the percentage of total fatty-acid peak area representing 95.2 percent of the total peak area by injecting known standards (NuCheck Prep, Elysian, Minn.), with the use of ChemStation A.08.03 software for analysis (Agilent Technologies). Blood specimens were analyzed in groups of three blinded samples, with the position of the case subject's specimen varied at random within the groups to reduce the possibility of systemic bias and to decrease variability between assays. Coefficients of variation for all fatty-acid peaks were measured by analyzing quality-control samples (indistinguishable from other study samples) randomly distributed throughout the study samples. The major peaks of long-chain n−3 fatty acids included docosahexaenoic acid ($C_{22:6n-3}$), eicosapentaenoic acid ($C_{20:5n-3}$), and docosapentaenoic acid ($C_{22:5n-3}$). The coefficients of variation for these major long-chain n−3 fatty acids were 7.7, 13.7, and 9.2, respectively. These three peaks were summed for each participant to arrive at the total long-chain n−3 fatty acid level.

Statistical Analysis

For base-line risk factors, means or proportions were calculated for men who died suddenly (case subjects) and controls. The significance of associations was tested with the chi-square statistic for categorical variables and with Student's t-test for continuous variables. Associations between base-line risk factors and levels of long-chain n−3 fatty acids, expressed as percentages of total fatty acids, were tested with Student's t-test. The Spearman rank-correlation coefficient was used to test the association between base-line levels of long-chain n−3 fatty acids and fish consumption, measured at 12 months.

Means (expressed as percentage of total fatty acids) for each fatty-acid peak were calculated for case and control subjects, and the significance of differences was tested by Student's t-test for those that were normally distributed and the Wilcoxon rank-sum test for those that were not normally distributed. To estimate the relative risk of sudden death according to the blood level of long-chain n−3 fatty acids, we first categorized each subject according to quartiles determined by the distribution of fatty acid levels in the controls. We then performed logistic-regression analysis, conditioned on the matching variables of age and smoking. Adjusted estimates of risk were obtained with multivariate models that also controlled for body-mass index; presence or absence of a history of diabetes, hypertension, or hyperlipidemia; presence or absence of a parental history of premature myocardial infarction; alcohol intake; frequency of physical activity; and random assignment to aspirin and beta carotene or placebo. After the quartile analysis suggested a linear relation, tests for trend were performed by entering a continuous variable in the conditional-regression model.

In secondary analyses, to assess for confounding by other fatty acids, each fatty-acid group (saturated, monounsaturated, n−6 polyunsaturated, and trans unsaturated fatty acids) was entered separately according to quartile into the multivariate model, and the change in the parameter estimate for the continuous value of long-chain n−3 fatty acid was observed. If a change of more than 15 percent was noted, the fatty acid remained in this multivariate model. All P values were two-tailed, and all confidence levels were computed at the 95 percent level.

RESULTS

Table 1 shows the base-line characteristics of the 94 subjects in whom sudden death was the first manifestation of cardiovascular disease and the 184 matched controls. The mean time from study enrollment to sudden death was 8.7 years (range, 0.7 to 16.9). In this sample, the men who died suddenly were significantly more likely to have a history of hypertension, significantly more likely to have a parental history of early coronary artery disease (i.e., before 60 years of age), and significantly less likely to have been randomly assigned to receive aspirin. The relation with alcohol intake was □-shaped: men who died suddenly were more

TABLE 1. BASE-LINE CHARACTERISTICS OF THE STUDY PARTICIPANTS.

CHARACTERISTIC	GROUP WITH SUDDEN DEATH FROM CARDIAC CAUSES (N=94)	CONTROL GROUP* (N=184)	P VALUE
Age — yr	58.5±9.2	58.3±9.1	Matching factor
Body-mass index†	25.4±2.9	25.1±3.3	0.33
Vigorous exercise less than once weekly — no. (%)‡	23 (24.7)	55 (30.1)	0.19
Smoking status — no. (%)			Matching factor
Current smoker	13 (13.8)	25 (13.6)	
Former smoker	42 (44.7)	83 (45.1)	
Never smoked	39 (41.5)	76 (41.3)	
Medical history — no. (%)			
Diabetes	5 (5.3)	6 (3.3)	0.41
High cholesterol level§	8 (9.2)	24 (15.5)	0.73
Hypertension¶	44 (48.4)	54 (30.0)	0.003
Parental history of myocardial infarction before 60 yr — no. (%)	14 (15.6)	8 (4.4)	0.002
Alcohol intake — no. (%)			
Less than weekly	34 (36.6)	42 (23.1)	0.004
1–6 Drinks weekly	29 (31.2)	96 (52.8)	
Daily	30 (32.3)	44 (24.2)	
Aspirin assignment — no. (%)	40 (42.6)	105 (57.1)	0.02

*Plus–minus values are means ±SD. Percentages are based on the numbers of subjects for whom data were available.

†The body-mass index is the weight in kilograms divided by the square of the height in meters.

‡Vigorous exercise was defined as "exercise vigorous enough to work up a sweat."

§The self-reported total cholesterol level was at least 240 mg per deciliter (6.2 mmol per liter), or the subject was taking cholesterol-lowering medication.

¶The self-reported blood pressure was at least 140/90 mm Hg, or the subject was taking antihypertensive medication.

likely to drink less than once a week or daily, and less likely to drink one to six drinks per week. Of the risk factors listed in Table 1, significantly lower levels of long-chain n−3 fatty acids were found among current smokers than among former smokers or those who had never smoked (mean [±SD], 4.47±1.31 vs. 5.20±1.30 percent of total fatty acids; P=0.002). In addition, the base-line blood level of long-chain n−3 fatty acids was significantly correlated with fish intake at 12 months (R^2=0.24, P=0.001) (data not shown).

The base-line blood levels of each major fatty acid among men who died suddenly and controls are shown in Table 2. The mean level of total long-chain n−3 fatty acids was significantly lower among the men who died suddenly than among the controls (4.82±1.31 vs. 5.24±1.32 percent of total fatty acids, P=0.01). In contrast, the levels of the other fatty acids, including the short-chain n−3 polyunsaturated fatty acid (α-linolenic acid), saturated fatty acids, monounsaturated fatty acids, n−6 polyunsaturated fatty acids, and trans unsaturated fatty acids, did not differ significantly between men who died suddenly and control subjects.

Table 3 shows the relation of base-line blood levels of long-chain n−3 fatty acids to the risk of sudden death. The level of long-chain n−3 fatty acids was significantly inversely related to the risk of sudden death in both the analysis adjusted for age and smoking status (P for trend = 0.004) and the multivariate analysis (P for trend = 0.007). As compared with the men with levels of long-chain n−3 fatty acids in the lowest quartile (mean, 3.58 percent of total fatty acids), those with levels in the highest quartile (mean, 6.87 percent) had a relative risk of sudden death of 0.19 (95 percent confidence interval, 0.05 to 0.71) after known confounders were controlled for (multivariate model 1). These results were not materially affected when 15 possible sudden deaths were excluded from the analysis (relative risk for highest vs. lowest quartile, 0.14; 95 percent confidence interval, 0.03 to 0.75; P for trend = 0.01).

Since the level of long-chain n−3 fatty acids was significantly associated with levels of other fatty acids (inversely with saturated, trans unsaturated, and monounsaturated fatty acids and directly with n−6 polyunsaturated fatty acids), each fatty-acid group was entered in the multivariate model to evaluate the independent association of long-chain n−3 fatty acids with the risk of sudden death. Adjustment for saturated and n−6

TABLE 2. BASE-LINE BLOOD FATTY-ACID LEVELS OF STUDY PARTICIPANTS WHO DIED SUDDENLY FROM CARDIAC CAUSES WITHOUT EVIDENCE OF CARDIOVASCULAR DISEASE AND CONTROLS MATCHED FOR AGE AND SMOKING STATUS.*

FATTY ACID	GROUP WITH SUDDEN DEATH FROM CARDIAC CAUSES (N=94)	CONTROL GROUP (N=184)	P VALUE
	percentage of total fatty acids		
Total saturated	31.6±1.88	31.3±1.80	0.21
Palmitic	19.2±2.16	18.8±2.00	0.16
Stearic	10.6±1.02	10.6±0.91	0.75
Total monounsaturated	19.8±3.25	19.5±2.69	0.72
Oleic	17.2±2.69	17.0±2.28	0.89
Total n−6 polyunsaturated	38.1±3.81	38.3±3.49	0.65
Linoleic	24.0±3.31	24.2±3.61	0.56
Arachidonic	10.6±1.88	10.6±1.75	0.93
Total long-chain n−3 polyunsaturated	4.82±1.31	5.24±1.32	0.01
Eicosapentaenoic	1.72±0.59	1.84±0.53	0.06
Docosahexaenoic	2.12±0.65	2.38±0.78	0.005
Docosapentaenoic	0.98±0.23	1.01±0.21	0.25
Short-chain n−3 polyunsaturated			
α-Linolenic	0.39±0.16	0.37±0.15	0.28
Total trans unsaturated	1.77±0.63	1.79±0.55	0.55
18:1 trans isomers	1.17±0.48	1.18±0.46	0.67
18:2 trans isomers	0.41±0.16	0.41±0.11	0.17

*Plus–minus values are means ±SD.

TABLE 3. RELATIVE RISK OF SUDDEN DEATH FROM CARDIAC CAUSES ACCORDING TO BASE-LINE BLOOD LEVEL OF LONG-CHAIN n−3 POLYUNSATURATED FATTY ACIDS.*

VARIABLE	QUARTILE OF n−3 POLYUNSATURATED FATTY ACID				P VALUE FOR TREND
	1	2	3	4	
Fatty acid level (%)†					
Mean	3.58	4.76	5.63	6.87	
Range	2.12–4.32	4.33–5.19	5.20–6.07	6.08–10.2	
Model adjusted for age and smoking status					
Relative risk	1.0	0.47	0.37	0.31	0.004
95% confidence interval		0.22–1.00	0.17–0.83	0.13–0.75	
Multivariate model 1‡					
Relative risk	1.0	0.55	0.28	0.19	0.007
95% confidence interval		0.18–1.70	0.09–0.87	0.05–0.71	
Multivariate model 2§					
Relative risk	1.0	0.52	0.19	0.10	0.001
95% confidence interval		0.16–1.72	0.05–0.69	0.02–0.48	

*Long-chain n−3 polyunsaturated fatty acids found in fish include docosahexaenoic acid, eicosapentaenoic acid, and docosapentaenoic acid.

†Levels are expressed as a percentage of total fatty acids.

‡Multivariate model 1 controlled for assignment to aspirin and beta carotene treatment or placebo, body-mass index (subjects were classified as having values of less than 25, 25 to 30, or more than 30), history of diabetes, history of hypertension, history of hypercholesterolemia, alcohol consumption (monthly or less, one to six drinks per week, or daily), frequency of vigorous exercise (less than weekly, or at least once weekly), and parental history of myocardial infarction before the age of 60 years.

§Multivariate model 2 included the variables in multivariate model 1 and the quartile of trans unsaturated fatty acid and monounsaturated fatty acid levels.

polyunsaturated fatty acids did not appreciably alter the association between long-chain n−3 polyunsaturated fatty acids and the risk of sudden death. Adjustment for monounsaturated and trans unsaturated fatty acids resulted in a further strengthening of the relation (Table 3, multivariate model 2). With this adjustment, the relative risk of sudden death among men with levels of long-chain n−3 fatty acids in the highest quartile as compared with the lowest quartile was 0.10 (95 percent confidence interval, 0.02 to 0.48; P for trend = 0.001).

DISCUSSION

In this prospective, nested case–control study of healthy male physicians without evidence of cardiovascular disease at enrollment, the base-line blood level of long-chain n−3 fatty acids was inversely associated with the subsequent risk of sudden death, even after known confounders had been controlled for. The association was linear, with a statistically significant inverse trend across quartiles of levels of long-chain n−3 fatty acids. As compared with men with levels of long-chain n−3 fatty acids in the lowest quartile, those with levels in the highest quartile had an 81 percent lower risk of sudden death. This relation persisted when blood levels of other fatty-acid groups were controlled for in the model. Therefore, the association did not appear to depend on compensatory changes in the levels of other fatty acids.

These prospective findings are remarkably similar to those reported in a population-based case–control study involving 82 cases of primary cardiac arrest.[13] That study found a strong inverse association between red-cell n−3 fatty-acid composition at the time of the arrest and the risk of primary cardiac arrest among subjects with no history of clinically recognized cardiac disease. Taken together, these data support the hypothesis that long-chain n−3 fatty acids are responsible for the observed inverse association between fish consumption and sudden death.[1,13] There was no evidence of a threshold effect for blood levels of long-chain n−3 fatty acids, although a threshold effect was previously reported for fish intake in this cohort.[1]

Because the present study did not examine other cardiovascular end points or death from other causes, we cannot present direct data on the selectivity of the association between long-chain n−3 fatty acids and sudden death. However, previous studies of this cohort found no association between plasma levels of long-chain n−3 fatty acids[14] or fish intake[1] and the risk of myocardial infarction. In addition, a selective beneficial effect on the risk of sudden death was found in a randomized trial.[4] In that trial, men with a history of myocardial infarction who were assigned to a fish-oil supplement had a statistically significant 45 percent reduction in the risk of sudden death, which translated into an overall significant reduction in total mortality. However, there was no reduction in the risk of nonfatal cardiovascular events or in other causes of mortality.

The apparent beneficial effect on the risk of sudden death from cardiac causes in observational studies and randomized trials could be due in part to the antiarrhythmic effects of n−3 fatty acids, as reported from experimental models.[3,15,16] Plausible mechanisms for these antiarrhythmic effects include modulation of sodium, potassium, and L-type calcium channels[2,17,18]; inhibition of thromboxane production[19,20]; and beneficial effects on heart-rate variability.[21,22] Other indirect effects of long-chain n−3 fatty acids include lowering of the nonesterified fatty-acid concentration in plasma and cell membranes. Nonesterified fatty acids have multiple proarrhythmic properties and have recently been associated with an increased risk of sudden death, but not of fatal myocardial infarction, among men enrolled in the Paris Prospective Study I.[23]

The limitations of these data merit consideration. First, our analyses are based on a single base-line measurement and therefore may not accurately reflect levels of long-chain n−3 fatty acids over long periods. Furthermore, although the coefficients of variation were low, misclassification due to laboratory error cannot be ruled out. It is important to note, however, that neither of these sources of variability can account for the strong inverse association we observed between long-chain n−3 fatty acid levels and sudden death, since any random misclassification would bias results toward the null hypothesis. The use of a single base-line measurement does, however, limit our ability to assess accurately the relation between the shorter-chain n−3 fatty acid, α-linolenic acid, and sudden death, since this fatty acid is largely metabolized,[24] and if it is stored, it is elongated to docosahexaenoic acid. Therefore, blood levels of α-linolenic acid would be more dependent on what foods were eaten recently and less likely to reflect average dietary intake.

The use of whole blood is a possible limitation, because it combines two different pools of long-chain n−3 fatty acids, the plasma and the stored red-cell pools. Alternatively, this composite measure may be viewed as a strength, since these pools have different half-lives and provide complementary information. Finally, as with any observational study, the inverse association between blood levels of long-chain n−3 fatty acids and sudden death could be due, at least in part, to residual confounding by other dietary and lifestyle factors. However, control for major known confounders and other fatty-acid groups had little effect on the estimates of relative risk.

In summary, taken together with previous data from observational studies and randomized trials, these prospective data suggest that the long-chain n−3 fatty ac-

ids found in fish may reduce the risk of sudden death from cardiac causes, even among men without a history of cardiovascular disease. Because more than 50 percent of all sudden deaths from cardiac causes occur in people with no history of cardiac disease,[5] preventive efforts must address this segment of the population to have a substantial effect on the overall incidence of sudden death from cardiac causes. If the observed association is causal, increasing the intake of n−3 fatty acids by eating more fish or by taking supplements is an intervention that could be applied to this segment of the population at low cost and little risk.

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Overview of the use of CoQ_{10} in cardiovascular disease

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Abstract. The clinical experience in cardiology with CoQ_{10} includes studies on congestive heart failure, ischemic heart disease, hypertensive heart disease, diastolic dysfunction of the left ventricle, and reperfusion injury as it relates to coronary artery bypass graft surgery. The CoQ_{10}-lowering effect of HMG-CoA reductase inhibitors and the potential adverse consequences are of growing concern. Supplemental CoQ_{10} alters the natural history of cardiovascular illnesses and has the potential for prevention of cardiovascular disease through the inhibition of LDL cholesterol oxidation and by the maintenance of optimal cellular and mitochondrial function throughout the ravages of time and internal and external stresses. The attainment of higher blood levels of CoQ_{10} (>3.5 μg/ml) with the use of higher doses of CoQ_{10} appears to enhance both the magnitude and rate of clinical improvement. In this communication, 34 controlled trials and several open-label and long-term studies on the clinical effects of CoQ_{10} in cardiovascular diseases are reviewed.

Keywords: Coenzyme Q_{10}, ubiquinone, cardiovascular disease, heart failure, hypertension, ischemia, diastolic dysfunction, angina, cardiac surgery, HMG-CoA reductase inhibitors

1. Introduction

Since the discovery of the vitamin-like nutrient coenzyme Q_{10} (ubiquinone, CoQ_{10}) by Frederick Crane et al. in 1957 [10] and by other investigators [57], and since the first patients with heart failure were treated with coenzyme Q by Yuichi Yamamura [87–89], there has been a slow but steady accumulation of worldwide clinical experience with CoQ_{10} in heart disease over the ensuing 30 years.

CoQ_{10} is a coenzyme for the inner mitochondrial enzyme complexes involved in oxidative phosphorylation [44,45,49]. This bioenergetic effect of CoQ_{10} is believed to be of fundamental importance in its clinical application, particularly as relates to cells with exceedingly high metabolic demands such as cardiac myocytes. The second fundamental property of CoQ_{10} involves its antioxidant (free radical scavenging) functions [5,15,62,82]. CoQ_{10} is the only known naturally occurring lipid soluble antioxidant for which the body has enzyme systems capable of regenerating the active reduced ubiquinol form [15]. CoQ_{10} is known to be closely linked to vitamin E and serves to regenerate the reduced (active) α-tocopherol form of vitamin E [9]. Other aspects of CoQ_{10} function include its involvement in extramitochondrial electron transfer, e.g., plasma membrane oxidoreductase activity [41,82], involvement in cytosolic glycolysis [41,46,48], and potential activity in both Golgi apparatus and lysosomes [11,12]. CoQ_{10} also plays a role in improvement in membrane fluidity [42,43,62] as evidenced by a decrease in blood viscosity with CoQ_{10} supplementation [29].

The rationale behind the use of CoQ_{10} in heart failure has focused primarily on the correction of a measurable deficiency of CoQ_{10} in both blood and myocardial tissue with the degree of CoQ_{10} deficiency correlating directly with the degree of impairment in left ventricular function [55]. CoQ_{10} supplementation corrects measurable deficiencies of CoQ_{10} in blood and tissue [16,17,30,31,34,47,55]. Exogenous

CoQ_{10} is taken up by CoQ_{10}-deficient cells and can be demonstrated to be incorporated into the mitochondria [59]. The role of free radicals in cell injury and in cell death in settings of ischemia and reperfusion is becoming increasingly well established. CoQ_{10}'s antioxidant properties and its location within the mitochondria (the center of free radical production) make it an obvious candidate for a potential therapeutic agent in these situations [92].

2. Congestive heart failure controlled trials

Since the Japanese pioneering studies in the late 1960's, there have been at least 15 randomized controlled trials involving a total of 1,366 patients with both primary and secondary forms of myocardial failure. The first randomized controlled trial by Hashiba et al. in 1972 involving 197 patients [21] documented significant improvement using 30 mg CoQ_{10} per day. Similar observations were made in another controlled trial by Iwabuchi et al., again using 30 mg of oral CoQ_{10} in 38 patients with heart failure [24].

The first controlled trial in idiopathic dilated cardiomyopathy in the United States was published by Per Langsjoen in 1985 using 100 mg of CoQ_{10} per day in 19 patients with double-blind crossover design and three month treatment periods [34]. Significant improvements were noted in ejection fraction as well as functional status. Three controlled trials in 1986 by VanFraechem et al., Judy et al., and Schneeberger et al. confirmed these findings, again using 100 mg of CoQ_{10} per day [25,71,81]. In 1990 Oda documented normalization of load-induced cardiac dysfunction in 40 patients with mitral valve prolapse using a double blind placebo controlled design [60]. In 1991, Rossi et al. showed significant improvement in ischemic cardiomyopathy in 20 patients using 200 mg of CoQ_{10} per day [68]. Poggesi et al. documented significant improvement in myocardial function in 20 patients with either ischemic or idiopathic dilated cardiomyopathies using 100 mg of CoQ_{10} per day [65]. Judy et al. randomized 180 patients to receive either 100 mg per day of CoQ_{10} versus placebo and noted significant improvement in long-term survival with patients followed up to eight years [26].

The only controlled study to show no benefit in heart failure was published by Permanetter et al. in 1992 [64]. This was a well designed study looking specifically at idiopathic dilated cardiomyopathy in 25 patients with documented normal coronary anatomy by cardiac catheterization. After a two month stabilization period, patients were treated with either placebo or CoQ_{10} for a duration of four months in a double-blind crossover design. No significant improvement in exercise tolerance or measurements of myocardial function could be demonstrated. Possible reasons for the lack of therapeutic efficacy of CoQ_{10} in this trial merit discussion. Although the 100 mg per day dose of CoQ_{10} used in this trial was shown to give a three-fold increase plasma CoQ_{10} levels in healthy volunteers, the plasma levels in the patients during the trial were not measured and it is conceivable that many of the cardiomyopathy patients may have had poor absorption of the CoQ_{10} and, therefore, may have had only marginal increases in their plasma Q levels. Another point is that all prior trials in heart failure involved patients with a mixture of etiologies, which frequently included patients with ischemic heart disease. It has become clear in recent years that the ischemic cardiomyopathy patients with viable but weak myocytes, often show the most dramatic improvements with supplemental CoQ_{10} (author's observations) perhaps related to the large free radical burden in ischemic tissue. Furthermore, the duration of idiopathic dilated cardiomyopathy prior to the institution of CoQ_{10} supplementation was not specified in this study and is of considerable importance in so much as those patients treated shortly after the diagnosis of dilated cardiomyopathy show the greatest degree of improvement as opposed to patients with long-standing dilated cardiomyopathy who frequently show minimal changes, presumably related to the gradual loss of myocytes in this disease with an increasingly thin and fibrotic myocardium.

In 1993, Rengo et al. documented clinical and echocardiographic improvement in 60 patients treated with 100 mg of CoQ_{10} for seven months [66]. The largest controlled trial to date was published in 1993 by Morisco et al., in which 641 patients were randomly assigned to receive either placebo or CoQ_{10} at 2 mg/kg per day in a one year double-blind trial [51]. 118 patients in the controlled group required hospitalization for heart failure in the one year follow-up compared to 73 in the CoQ_{10} treated group ($P < 0.001$). In addition to the obvious improvement in quality of life for these patients, the reduction in hospitalization rate has strong implications in the growing problem of health care cost containment. A year later in 1994, Morisco et al. documented significant improvements in ejection fraction, stroke volume and cardiac output as measured by radio nuclide scanning in six patients treated with 150 mg of CoQ_{10} per day in a double-blind crossover design [52]. Lastly, in 1995, Swedberg et al. published a study on 79 patients with severe chronic congestive heart failure whose mean ejection fraction at rest was 22 $\pm$ 10% [75]. There was a slight but significant improvement in volume load ejection fraction measurement and a significant improvement in quality of life assessment. A meta analysis of controlled studies in heart failure by Soja et al. demonstrated significant improvement in measurements of cardiac function [73].

3. Open label and long-term congestive heart failure trials

Dr Yuichi Yamamura published an excellent review of all early Japanese trials prior to 1984 [91]. By mid 1980's it became apparent that CoQ_{10} was safe and effective in the short-term treatment of patients with heart failure. Several long-term trials were undertaken to determine if this effect would be sustained and to determine long-term safety.

In 1985, Mortensen et al. observed sustained benefit and safety in idiopathic dilated cardiomyopathy on 100 mg per day of CoQ_{10} [53]. In 1990, we published our observations on 126 patients with dilated cardiomyopathy followed for six years, again noting sustained benefit with remarkable long-term safety and lack of side effects [35]. In 1994, Baggio et al. published the largest open trial in heart failure involving 2,664 patients treated with up to 150 mg of CoQ_{10} per day, again noting significant benefit and lack of toxicity [3]. Also, in 1994, we published observations on 424 patients with a broader spectrum of myocardial disease including ischemic cardiomyopathy, dilated cardiomyopathy, primary diastolic dysfunction, hypertensive heart disease, and valvular heart disease [37]. Patients were treated with an average of 240 mg of CoQ_{10} per day and followed for up to eight years with mean follow-up of 18 months. We observed significant improvement in NYHA functional classification, improvement in measurements of myocardial function, an average of 50% reduction in the requirement for concomitant cardiovascular drug therapy, and a complete lack of toxicity. Myocardial function became measurably improved within one month with maximal improvement usually obtained by six months and this improvement appears to be sustained in the majority of patients. The withdrawal of CoQ_{10} therapy resulted in a measurable decline in myocardial function within one month and a return to pretreatment measurements within three to six months. This return to baseline myocardial function after withdrawal of CoQ_{10} therapy was also observed by Mortensen et al. [54].

4. Diastolic dysfunction

After initial favorable observations in advanced congestive heart failure with predominant systolic dysfunction, our group and others began to look at earlier stages of myocardial dysfunction, specifically, diastolic dysfunction. This filling phase of the cardiac cycle involves the ATP-dependent clearance of

calcium which in turn is required for the breaking of the actin–myosin binding. Diastolic dysfunction often precedes more advanced stages of congestive heart failure and is commonly seen in a wide variety of clinical syndromes, including symptomatic hypertensive heart disease with left ventricular hypertrophy, symptomatic mitral valve prolapse, hypertrophic cardiomyopathy, the aging heart, and is often seen in fatigue states such as chronic fatigue syndrome.

In 1993, we published observations on 115 patients with isolated diastolic dysfunction: 60 with hypertensive heart disease, 27 with mitral valve prolapse syndrome, and 28 with chronic fatigue syndrome [36]. The administration of CoQ_{10} resulted in improvement in diastolic function, a decrease in myocardial thickness, and an improvement in functional classification. In 1994, Oda published results on 30 patients with load-induced diastolic dysfunction and documented normalization of diastolic function in all patients after CoQ_{10} supplementation [63]. In 1994, we published data on 109 patients with hypertensive heart disease and again noted not only improvement in NYHA functional classification and left ventricular hypertrophy, but we also observed significant improvement in diastolic function as measured by Doppler echocardiography [38]. We noted improvement in blood pressure and a lessening in the requirement for antihypertensive drug therapy which occurred in tempo with the improvement in diastolic function. In 1997, we published data on seven patients with hypertrophic cardiomyopathy and again noted significant improvement in diastolic function as well as a lessening in hypertrophy and an improvement in functional status [39]. Also in 1997, we published data on 16 otherwise healthy elderly patients over the age of 80, all of whom had significant diastolic dysfunction prior to treatment and all of whom had normalization of diastolic function within three months of CoQ_{10} supplementation [40].

In summary, there appears to be an improvement in diastolic function in all categories of cardiac disease and this improvement occurs earlier and is more consistent than improvements in systolic function. This is understandable given the frequent occurrence of permanent myocardial fibrosis in advanced idiopathic dilated cardiomyopathy and the permanent myocardial scarring seen in advanced ischemic heart disease. Diastolic dysfunction is easily identified by non-invasive techniques and appears to be readily reversible with supplemental CoQ_{10} with gratifying clinical improvement.

5. Ischemic heart disease controlled trials

Controlled trials in angina did not begin until the mid 1980's with the first publication by Hiasa in 1984 in which 18 patients were randomized to receive either intravenous CoQ_{10} or placebo [22]. The treated patients showed an increase in exercise tolerance of one stage or greater in a modified Bruce protocol as compared to no increase in exercise tolerance in the placebo group, showed less ST-segment depression with exercise and experienced less angina with no alteration in heart rate or blood pressure. A year later in 1985, Kamikawa et al. studied 12 patients with chronic stable angina in a double-blind placebo controlled randomized crossover protocol using 150 mg a day of oral CoQ_{10} [28]. Exercise time increased significantly from 345 to 406 seconds with CoQ_{10} treatment and time until 1 mm of ST depression increased significantly from 196 to 284 seconds ($P < 0.01$). Again, no significant alteration in heart rate or blood pressure was observed. In 1986, Schardt et al. studied 15 patients with exercise-induced angina treated with 600 mg per day of CoQ_{10} with a placebo controlled double-blind crossover design [70]. Again, a significant decrease in ischemic ST-segment depression was noted with CoQ_{10} treatment. Since the CoQ_{10} treatment caused no significant alteration in heart rate or blood pressure, it was concluded that the mechanism of action was related to a direct effect on myocardial metabolism.

In 1991, Wilson et al. studied 58 patients with up to 300 mg per day of CoQ_{10} compared to placebo and again noted significant improvement in exercise duration to the onset of angina without a change in peak rate pressure product, suggesting an improvement in myocardial efficiency [84]. Also, in 1991, Serra et al. showed significant improvement in 20 patients with chronic ischemic heart disease using 60 mg of CoQ_{10} per day for 4 weeks, documenting improvements in myocardial function measurements, improved exercise capacity, and a significant reduction in the number of anginal episodes and nitrate consumption [72]. In 1994, Kuklinski et al. studied 61 patients with acute myocardial infarction, randomized to obtain either placebo or 100 mg of CoQ_{10} with 100 μg of selenium for a period of one year [32]. The treatment group showed no prolongation of the QT-interval whereas, in the placebo group, 40% showed prolongation of the corrected QT-interval of greater than 440 milliseconds ($P < 0.001$). Although there were no significant differences in the acute hospitalization, the one year follow-up revealed six patients (20%) in the control group died from re-infarction, whereas one patient in the treatment group suffered a noncardiac death. The prevention of QT-interval prolongation can be explained by an enhancement in myocardial bioenergetics with an improvement in sodium potassium ATPase function, thereby optimizing membrane repolarization.

6. LDL cholesterol oxidation

The antioxidant properties of CoQ_{10} and the fact that 60% of CoQ_{10} is carried in the plasma with LDL cholesterol [2], has led to investigations as to whether or not CoQ_{10} has any clinically relevant antioxidant function in terms of decreasing the oxidation of cholesterol [23,79]. It is generally believed that the oxidation of LDL cholesterol is of primary importance in the development of atherosclerosis. In 1996 in Australia, Stocker's group showed *in vitro* that supplemental CoQ_{10} prevented the pro-oxidant effect of alpha-tocopherol [78]. Supplementation with vitamin E alone resulted in an LDL which was more prone to oxidation as compared to the combination of CoQ_{10} and vitamin E which increased the resistance to oxidation. Alleva et al. showed that supplemental CoQ_{10} increased the amount of CoQ_{10} in LDL (especially LDL3) and lowered the peroxidizability of the LDL. Aejmelaeus et al. documented a doubling of CoQ_{10} content in LDL particles after CoQ_{10} supplementation at 100 mg/day [1].

7. Statins and CoQ_{10}

Harry Rudney was among the first to recognize the importance of HMG-CoA reductase in the biosynthesis of CoQ_{10}. In January 1981 at the 3rd International Symposium on the Biomedical and Clinical Aspects of Coenzyme Q held in Austin, Texas, USA, he stated "... a major regulatory step in CoQ_{10} synthesis is at the level of HMG-CoA reductase" [69]. In 1990 Willis et al. [83] studied 40 rats and demonstrated significant tissue CoQ_{10} deficiency in heart and liver in the lovastatin treated rats which could easily be prevented by co-administration of CoQ_{10}. Later in the same year, Langsjoen et al. noted not only a decline of CoQ_{10} blood levels, but also a significant clinical decompensation with a reduction in ejection fraction in 5 heart failure patients after the addition of lovastatin to their standard medical therapy plus 100 mg CoQ_{10} per day [18]. This decompensation was reversed by a doubling of their CoQ_{10} dose from 100 to 200 mg/day.

In 1992 Ghirlanda et al. showed in a double blind controlled trial in 40 hypercholesterolemic patients a 40% drop in blood CoQ_{10} level after treatment with either pravastatin or simvastatin [19]. In 1994 Bargossi et al. randomized 30 patients to receive either 20 mg simvastatin or 20 mg simvastatin plus

100 mg CoQ_{10} and followed them for 90 days [4]. The lowering of cholesterol was significant and similar in both groups and the simultaneous CoQ_{10} therapy prevented both the plasma and platelet CoQ_{10} depletion induced by simvastatin administration. In 1997 Mortensen et al. observed similar reductions in serum CoQ_{10} levels in a placebo controlled double blind trial [56]. The authors concluded that "although HMG-CoA reductase inhibitors are safe and effective within a limited time horizon, continued vigilance of possible adverse consequences from CoQ_{10} lowering seems important during long term therapy". Also in 1997, Palomaki et al. documented a decrease in the resistance of LDL cholesterol to oxidative stress after 6 weeks of lovastatin therapy in a double blind, placebo controlled, cross over trial on 27 hypercholesterolemic men [63]. This enhanced oxidizability of LDL cholesterol is believed to be related to a decrease in the number of molecules of CoQ_{10} per each LDL cholesterol particle and may lessen the benefit of LDL cholesterol reduction.

The CoQ_{10} lowering effect of statins is now well established with a significant depletion in plasma and platelets in humans and with a significant depletion in blood, liver and heart in rats. Human skeletal muscle CoQ_{10} may actually increase with statin therapy as documented by a Finnish study [33] but human heart muscle tissue CoQ_{10} data are presently lacking and, when available, should help clarify the mechanism of clinical deterioration noted in some cardiomyopathy patients treated with statins. The concern over the long term consequences of statin-induced CoQ_{10} deficiency is heightened by the rapidly increasing number of patients treated and the increasing dosages and potencies of the statin drugs. As the "target" or "ideal" cholesterol level is steadily lowered, the CoQ_{10}-lowering effect will be more pronounced and the potential for long term adverse health effects enhanced. Before the results of this vast human experiment become obvious over the next decade, it is incumbent upon the medical profession to more closely evaluate the clinical significance of this drug-induced CoQ_{10} depletion. The combined use of CoQ_{10} and statins not only prevents the depletion of CoQ_{10}, but may also enhance the benefits of the cholesterol lowering by lessening the oxidation of LDL cholesterol.

8. Hypertension

A tendency to decrease blood pressure in patients with established hypertension has been noted as far back as 1976 by Nagano, who studied 45 patients on 30–60 mg of CoQ_{10} per day [58]. A year later, Yamagami published data on 29 patients using 1–2 mg of CoQ_{10} per kg body weight per day [86]. From 1980 through 1984, three smaller studies again showed favorable improvement in hypertension with CoQ_{10} supplementation [20,67,80] and in 1986, Yamagami evaluated 20 patients in randomized controlled fashion using 100 mg of CoQ_{10} per day and again observed a favorable effect [86]. Further uncontrolled open studies [14,38,50] all uniformly found a favorable influence on hypertension when CoQ_{10} supplementation was added to standard antihypertensive drug therapy. We postulate that the blood pressure lowering effect of CoQ_{10} may in part be an indirect effect, whereby improved diastolic function leads to a lessening in the adaptive high catecholamine state of hypertensive disease. In addition, effects on vascular endothelium may be involved. It is also possible that the blood viscosity lowering effect of CoQ_{10} may favorably influence hypertension [29].

9. Controlled trials in cardiovascular surgery

The first controlled study evaluating the effectiveness of CoQ_{10}, administered preoperatively, was published by Tanaka et al. in 1982 [77]. Fifty patients undergoing heart valve replacement were randomized

to receive either placebo or CoQ_{10} at a dose of 30–60 mg per day for six days before surgery. The treatment group showed a significantly lower incidence of low cardiac output state during the postoperative recovery period. In 1991, Sunamori et al. studied 78 patients undergoing coronary artery bypass graft surgery [74]. Sixty of these patients were given 5 mg per kg of CoQ_{10} intravenously two hours prior to cardiopulmonary bypass. Postoperatively, there was a significant benefit to left ventricular stroke work index in the CoQ_{10} treated group as compared to controls and a significant decrease in postoperative CPK MB measurements in the treated group. In 1993, Judy et al. studied 20 patients undergoing either coronary artery bypass surgery (16 patients) or combined bypass surgery with valve replacement (4 patients) [27]. Patients were randomized to receive either placebo or administration of oral 100 mg per day of CoQ_{10} for 14 days prior to surgery and continued for 30 days postoperatively. The treatment group showed significant elevations not only in blood CoQ_{10} level but also in myocardial tissue CoQ_{10} content as measured in atrial appendage. Significant improvement in postoperative cardiac index and left ventricular ejection fraction were noted in the treatment group, and a significant shortening of the postoperative recovery time was observed. In 1994, Chello et al. randomized 40 patients to receive either placebo or 150 mg per day of oral CoQ_{10} one week prior to coronary artery bypass graft surgery [6]. A significant decrease in postoperative markers of oxidative damage was observed in the treatment group with lower concentrations of coronary sinus thiobarbituric acid reactive substances, conjugated dienes and cardiac isoenzymes of creatine kinase. The treatment group also showed a significantly lower incidence of ventricular arrhythmias in the recovery period and the mean dose of dopamine required to maintain stable hemodynamics was significantly lower in the CoQ_{10} treated group. In 1994, Chen et al. randomized 22 patients to receive either CoQ_{10} or placebo prior to coronary artery bypass surgery and observed improvement in left atrial pressure and an improvement in the incidence of low cardiac output state in the postoperative period [8]. Right and left ventricular myocardial ultrastructure was better preserved in the CoQ_{10} treated group as compared to placebo. In 1996, Chello randomized 30 patients to receive either placebo or 150 mg oral CoQ_{10} for 7 days before abdominal aortic surgery and documented a significant decrease in markers of peroxidative damage in the CoQ_{10} treated patients [7]. In 1996 Taggart et al. randomized 20 patients undergoing coronary revascularization surgery to receive either placebo or 600 mg of oral CoQ_{10} 12 hours prior to operation with no significant effects observed, confirming the lack of acute pharmacologic or clinical changes with CoQ_{10} [76]. Typically, oral CoQ_{10} supplementation rarely causes measurable effect before one week and is not maximal for several months.

10. Conclusions

In summary, coenzyme Q_{10} is a deceptively simple molecule which lies at the center of mitochondrial ATP production and appears to have clinically relevant antioxidant properties manifested by tissue protection in settings of ischemia and reperfusion. Congestive heart failure has served as a model for measurable deficiency of CoQ_{10} in blood and tissue, which when corrected, results in improved myocardial function. Ischemic heart disease, anginal syndromes, and most recently the ischemia reperfusion injury of coronary revascularization has provided clear evidence of clinically relevant antioxidant cell protective effects of CoQ_{10}. Newer ^{31}P NMR spectroscopy studies such as those conducted by Whitman's group in Philadelphia have documented enhanced cellular high energy phosphate concentrations with CoQ_{10} supplementation in models of ischemia and reperfusion [13]. Sophisticated biochemical markers of oxidative injury are now demonstrating *in vivo* the antioxidant cell protective effects of CoQ_{10}.

Upon review of the 30 years of clinical publications on CoQ_{10} and the author's own clinical experience, it is clear that there are several consistent and unique characteristics of the clinical effects of

CoQ_{10} supplementation which are worthy of discussion and may for simplicity be termed the "Q effect". The benefits of CoQ_{10} supplementation are likely not due solely to a correction of deficiency in so far as clinical improvements are frequently seen in patients with "normal" pre-treatment CoQ_{10} blood levels and optimum clinical benefit requires above normal CoQ_{10} blood levels (2 to 4 times higher). High blood levels may be required to attain an elevation of tissue CoQ_{10} levels or to rescue defective mitochondrial function perhaps by driving cytosolic glycolysis or the plasma membrane oxidoreductase or by directly enhancing the function of defective mitochondria. There is almost always a delay in the onset of clinical change of one to four weeks and a further delay in maximal clinical benefit of several months. Possible reasons for this delay include time to attain adequate tissue levels of CoQ_{10} or time to synthesize CoQ_{10}-dependent apoenzymes. Supplemental CoQ_{10} appears to affect much more than just cardiac myocytes and many aspects of patients' health tend to improve which cannot be explained by the observed improvement in heart function. CoQ_{10} does not lend itself to traditional organ-specific or disease-specific strategy and requires a reassessment and a rethinking of medical theory and practice.

The combination of the ready availability of pure crystalline CoQ_{10} in quantity from the Japanese pharmaceutical industry and increasingly sophisticated and standardized methodology to directly measure CoQ_{10} in both blood and tissue, brings us to a point where we can more readily and accurately expand upon the preceding 30 years of pioneering clinical work on this extraordinary molecule.

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