Oral Synthetic Phospholipid (DMPC) Raises High-Density Lipoprotein Cholesterol Levels, Improves High-Density Lipoprotein Function, and Markedly Reduces Atherosclerosis in Apolipoprotein E–Null Mice

Mohamad Navab, PhD; Susan Hama, BSc; Greg Hough, MS; Alan M. Fogelman, MD

**Background**—Lecithin has been widely sold as a dietary supplement. 1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) is a phospholipid that does not exist in nature and has been used in vitro to study lipid binding. We tested DMPC in vivo in apolipoprotein (apo) E–null mice.

**Methods and Results**—DMPC or soy or egg lecithin at 1.0 mg/mL was added to the drinking water of 4-week-old apoE-null female mice. Eight weeks later, HDL cholesterol levels and apoA-I levels were markedly increased in the mice that received DMPC. HDL function was also dramatically improved in the mice receiving DMPC, and there was a significant reduction in aortic lesions \(P=0.021\) in the DMPC mice but not in those receiving lecithin. Adding 1.0 mg/mL of DMPC to the drinking water of 10-month-old apoE-null female mice for 5 weeks caused regression of aortic sinus lesions \(P=0.003\). Adding 1.0 mg/mL DMPC to the drinking water of 6-month-old apoE-null male mice for 8 weeks significantly reduced aortic sinus lesion area \(P=0.003\) and en face whole aorta lesion area \(P=0.001\), whereas adding the same concentrations of soy or egg lecithin did not significantly alter lesion area. Jejunal apoA-I synthesis and plasma apoA-I levels were increased 2- to 3-fold in mice receiving DMPC but not soy or egg lecithin.

**Conclusions**—DMPC (but not lecithin) raises HDL cholesterol and apoA-I, improves HDL function, and prevents lesions or causes their regression in apoE-null mice. *(Circulation. 2003;108:1735-1739.)*

**Key Words:** atherosclerosis • lipids • lipoproteins

There are many in vitro studies in the literature reporting on the characteristics of the association of apolipoprotein A-I (apoA-I) with 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), such as that reported by Huang et al. On searching the literature, we were unable to find any study in which DMPC was fed to animals or humans. Although DMPC has not been studied as an oral agent, lecithin has been widely sold as a dietary supplement for the prevention of a number of diseases, including atherosclerosis, for many years. The lecithin that has been fed to animals and humans has been derived primarily from soybeans or egg yolk. Studies in animals and humans have provided conflicting results. In 1982, the Food and Drug Administration asked the Federation of American Societies for Experimental Biology to review the status of development and utilization of high levels of lecithin, phosphatidylcholine, and choline as dietary supplements and the possible benefits or hazards from their consumption. The review concluded that in general, humans tolerate ≈25 g/d of commercial lecithin without side effects. It was noted that 60 to 80 g/d of phosphatidylcholine (85% purity) was given to some patients without difficulty, and none had side effects up to 40 g/d.

Another review of 24 studies on the effect of supplemental lecithin with intakes ranging from 1 to 54 g/d revealed that most studies lacked an appropriate control group, had a small sample size, or had changes in intake of foods because of increased energy intake from lecithin. The authors concluded that there was no evidence for a specific effect of lecithin on serum cholesterol independent of its linoleic acid content or secondary changes in food intake.

In vitro, phosphatidylcholine was shown to have no effect on apoB secretion but significantly increased the synthesis and basolateral secretion of apoA-I in a newborn swine intestinal epithelial cell line. In vivo, feeding soybean phosphatidylcholine to newborn swine approximately doubled the synthesis of apoA-I in the jejunum without altering apoB or apoA-IV synthesis. On searching the literature, we also found no studies of phospholipids given orally to more modern animal models of atherosclerosis, such as apoE-null mice. We report here that feeding DMPC to apoE-null mice has a profound effect on HDL cholesterol, HDL function, and atherosclerosis.
Methods

Materials
DMPC (catalogue No 850345), egg-yolk lecithin (catalogue No 830051), soy lecithin (catalogue No 830054), and 1-palmitoyl-2- arachidonoyl-sn-glycero-3-phosphocholine (PAPC) (catalogue No 850459) were purchased from Avanti Polar Lipids. 13(S)-HPODE was purchased from Biomol. Rabbit polyclonal antiumouse apoA-I antibody, affinity-purified (catalogue No K23001R), was purchased from Biodesign International. All other materials were from previously cited sources.16

Mice
Male and female apoe-null mice were purchased from The Jackson Laboratory (Bar Harbor, Maine) and were maintained on a chow diet (Ralston Purina). The lipids were weighed and added to the drinking water and mixed (vortexed for 2 minutes) to make a uniform suspension. The DMPC, obtained in the form of a fine powder, readily resulted in a uniform suspension when mixed in water. Egg lecithin and soy lecithin also formed uniform suspensions after vortexing. The suspension was added to 50-mL graduated polypropylene tubes fitted with standard rubber stoppers and stainless steel tubes of a type used routinely in mouse water bottles. The tubes were observed daily, and the lipids were found by inspection to remain in suspension. The contents of the tubes were changed every other day, and 98.2 ± 11% of the added phospholipid was determined by chemical analysis to still be in suspension at the time of the tube change. The mice drank 2 to 2.5 mL/d per mouse, and there was no significant difference in the amount of suspension consumed between the groups.

Intestinal apoA-I synthesis was determined as described previously.15 Briefly, mice (4 animals per group) that had been fasted overnight were anesthetized with isoflurane by use of an anesthesia machine in accordance with the policies and with the approval of the UCLA Animal Research Committee. The abdomen was opened and the intestine exposed. The jejunum was isolated distal to the duodenum and cut laterally with a small scissors, a ball-end needle was inserted, and the jejunum/duodenum was ligated. The proximal end of the ileum was clamped. Radiolabeling was performed by instilling 0.25 mcCi (0.25 mL) of L-[4,5-3H]leucine (>120 Ci/mmol, Amersham) into the segment for 9 minutes. Body temperature was maintained by a recirculating water table. The animal was then killed by isoflurane overdose, the jejunal segment was removed and opened, and the mucosa was removed, homogenized, and subjected to immunoprecipitation. After SDS-PAGE, the newly synthesized apoA-I was determined after subtracting nonspecific counts determined from incubations with nonimmune antibody, dividing by TCA-precipitable counts, and normalizing the control to 1.0.

Lipoproteins, Cocultures, Monocyte Chemotaxis, and Lesion Scoring
Lipoproteins, cocultures, and monocytes were prepared, and monocyte chemotaxis assays were performed as described previously.16,17 Plasma samples were fractionated by a gel-permeation fast protein liquid chromatography (FPLC) system consisting of dual Pharmacia Superose 6 columns in series. Plasma (0.045 mL) was eluted with an isocratic buffer containing 154 mmol/L NaCl, 1 mmol/L EDTA, and 0.02% sodium azide, pH 8.2, at a flow rate of 0.5 mL/min, pumped by a nonmetallic Beckman high-performance liquid chromatography pump. Forty-two 1-mL fractions were collected. Fractions containing lipoproteins isolated in the absence of EDTA contained 20 μmol/L BHT. Aortic lesions were scored as described previously.18,19

Other Procedures
Plasma phospholipid levels were determined with a kit from Wako Chemicals (No. 990-54009E) and by mass spectrometry/mass spectrometry according to the protocols described previously.20,21 Paraoxonase activity was measured as described previously.22 Plasma free fatty acid concentrations were measured with a commercial kit (Wako) on the basis of a previously published method.23 Lipoprotein cholesterol concentrations were determined with a Cholesterol-20 kit (Sigma). Plasma apoA-I levels were determined by a competitive binding ELISA and by Western blot analysis. ELISA plates were obtained from Corning Costar, coated with murine apoA-I (A23100M from Biodesign International), and blocked with gelatin (G-1890 Sigma). A standard curve was constructed with murine apoA-I (A23100M from Biodesign International), and the assay included internal standards. Primary antibody was affinity-purified rabbit anti-mouse apoA-I (K23001R from Biodesign International), and the secondary antibody-conjugate was horseradish peroxidase-labeled anti-rabbit IgG F(ab)’/2 (Jackson ImmunoResearch). TMB peroxidase substrate was from Kirkegaard and Perry Laboratories. The reaction was terminated with sulfuric acid, and the absorbance was read at 450 nm. In Western analyses, murine apoA-I plasma (0.5 μL) was subjected to SDS-PAGE (4% to 20% Tris glycine from Novex) and Western transferred (semidry onto nitrocellulose from Amersham). The blots were treated sequentially with rabbit anti-mouse apoA-I (Biodesign International K23500R), horseradish peroxidase–labeled anti-rabbit IgG F(ab)’/2 (Jackson), and Amersham ECL reagent and exposed to film (Amersham). The films were scanned with a Molecular Dynamics densitometer. Murine apoA-I (0.075 μg) was included as an internal standard. Statistical significance was determined using model I ANOVA, and significance was defined as a value of P < 0.05.

Results
Twenty-four hours after DMPC (but not lecithin) had been added to the drinking water, cholesterol-containing particles with paraoxonase 1 activity appeared in the region to the right of HDL in the FPLC chromatogram (data not shown). After 24 hours, the lipids were removed from the drinking water, and the mice were bled 24 hours later, ie, 48 hours after the start of the experiment. After this second 24-hour “chase” period, the cholesterol peak containing paraoxonase 1 activity, which at 24 hours was found to the right of HDL in the FPLC chromatogram, moved into the mature HDL region, and the HDL shifted from proinflammatory to antiinflammatory in the coculture assay (data not shown).

In other experiments, apoE-null mice were given drinking water alone, drinking water containing DMPC, or soy lecithin or egg lecithin, all at 1.0 mg/mL. After 8 weeks, there were no significant differences in plasma triglyceride levels for the mice in the different treatment groups (water, 569 ± 74 mg/dL; egg lecithin, 138 ± 18 g/dL; soy lecithin, 161 ± 8.5 mg/dL; and DMPC, 158.4 ± 6.3 mg/dL). Total cholesterol was also not different (water, 142 ± 16 mg/dL; egg lecithin, 138 ± 18 g/dL; soy lecithin, 161.6 ± 8.5 mg/dL; and DMPC, 158.4 ± 6.3 mg/dL). HDL phospholipid levels were 85.2 ± 7.4 mg/dL, or 0.02% of the total plasma phospholipid concentration in the group that received DMPC in their drinking water, cholesterol-containing particles with paraoxonase 1 activity appeared in the region to the right of HDL in the FPLC chromatogram (data not shown). After 24 hours, the lipids were removed from the drinking water, and the mice were bled 24 hours later, ie, 48 hours after the start of the experiment. After this second 24-hour “chase” period, the cholesterol peak containing paraoxonase 1 activity, which at 24 hours was found to the right of HDL in the FPLC chromatogram, moved into the mature HDL region, and the HDL shifted from proinflammatory to antiinflammatory in the coculture assay (data not shown).

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were 267 042 /11006 the lesion scores of this third group that had received DMPC drinking water at 1.0 mg/mL. After the additional 5 weeks, a third group of the 10-month-old mice was continued on the chow diet for another 5 weeks, but DMPC was added to their drinking water at 1.0 mg/mL. After 8 weeks, mice were bled, and plasma apoA-I levels were determined by a specific ELISA. *P<0.05, statistically significant difference.

Figure 1. DMPC increases plasma apoA-I levels. Four-week-old female apoE-null mice received drinking water alone (Water) (n=12), egg lecithin (n=8), soy lecithin (n=8), or DMPC (n=8), all at 1.0 mg/mL. After 8 weeks, mice were bled, and plasma was used to determine apoA-I levels. Plasma apoA-I levels were determined by specific ELISA. *P<0.05, statistically significant difference.

Figure 2. DMPC improves HDL function. Four-week-old female apoE-null mice received drinking water alone (Water) (n=12), egg lecithin (n=8), soy lecithin (n=8), or DMPC (n=8), all at 1.0 mg/mL. After 8 weeks, mice were bled, and HDL was isolated by FPLC. PAPC 20 μg and HPODE 1.0 μg were added or not added (No Addition) to human artery wall cell cocultures with normal human HDL at 350 μg cholesterol (Control h HDL) or murine HDL at 50 μg cholesterol from mice that had received drinking water without additions (Water) or drinking water with egg lecithin, soy lecithin, or DMPC at 1.0 mg/mL. After 8 hours, supernatants were collected and assayed for monocyte chemoattractant activity. Data are mean±SD of number of migrated monocytes in 9 fields for triplicate samples from each of 2 separate experiments. *P<0.05, statistically significant difference.

As shown in Figure 2, when DMPC or soy or egg-yolk lecithin at 1.0 mg/mL was added to the drinking water of 4-week-old apoE-null mice for 8 weeks, HDL function was dramatically improved in the mice receiving DMPC but not in those receiving lecithin.

Figure 3 demonstrates that there was a significant reduction in aortic sinus lesions in the mice receiving DMPC but not in the mice receiving either egg-yolk or soy lecithin.

In other experiments, 1 group of 10-month-old female apoE-null mice maintained on a chow diet were killed and found to have lesion scores of 405 198 ± 77 231 μm² (Figure 4A). A second group of these mice was continued on the chow diet for an additional 5 weeks, with no additions to their drinking water. After the additional 5 weeks, the lesion scores of this second group were 471 233 ± 64 521 μm² (Figure 4A). A third group of the 10-month-old mice was continued on the chow diet for another 5 weeks, but DMPC was added to their drinking water at 1.0 mg/mL. After the additional 5 weeks, the lesion scores of this third group that had received DMPC were 267 042 ± 42 105 μm² (P=0.044 compared with the first group and P=0.003 compared with the second group) (Figure 4A).

To study the action of the phospholipids in male mice, groups of 6-month-old male apoE-null mice (n=8 per group) were provided with water alone, egg lecithin, soy lecithin, or DMPC, all added to the drinking water at 1.0 mg/mL. After 8 weeks on this treatment, the lesion scores for the aortic root serial sections were not different for mice given water, egg lecithin, or soy lecithin (Figure 4B). However, the mice given DMPC had significantly smaller lesion scores (Figure 4B; P=0.0031 for the DMPC group compared with the water control group). A representative aortic sinus section is shown for the water control group (Figure 4C) and for mice receiving DMPC (Figure 4D). Aortic sections from the mice that received egg lecithin or soy lecithin were similar to the
The correlation coefficient for aortic sinus lesion scores versus plasma HDL cholesterol levels was 0.894, and the correlation coefficient for aortic sinus lesion scores and plasma apoA-I levels was 0.945. In addition, en face lesion area for the entire aortic surface was determined in these male mice and was found to be significantly less only in the mice that received DMPC (Figure 4E; \( P = 0.001 \) for the DMPC group compared with the water control group). A representative aorta en face is shown for the water control group (Figure 4F) and for mice receiving DMPC (Figure 4G). Aortas from the mice that received egg lecithin or soy lecithin were similar to the water control group (data not shown). The correlation coefficients for en face lesion area for the entire aortic surface versus plasma HDL cholesterol levels or plasma apoA-I levels were 0.991 and 0.967, respectively.

As shown in Figure 5, adding DMPC at 1.0 mg/mL to the drinking water of apoE-null mice significantly increased apoA-I synthesis in the jejunum of male mice compared with water alone, and adding soy or egg lecithin to the drinking water at the same concentration did not significantly alter jejunal apoA-I synthesis. Similar results were found in female mice (data not shown).

**Discussion**

When the apoE-null mice were allowed access to DMPC for 24 hours, particles containing cholesterol and paraoxonase 1 activity were found in the region to the right of HDL in the FPLC chromatogram, suggesting that DMPC induced the formation of pre-\( \beta \)-HDL–like particles, which in the absence of a significant difference for DMPC group vs group maintained on water alone. C and D, Representative aortic sinus section for water control group (C) and for mice receiving DMPC (D). E, Entire aortas of mice described in B were removed, fixed, and stained, and en face lesion scores were determined as described in Methods. *\( P = 0.001 \), statistically significant difference between values for DMPC-treated mice vs water alone. F and G, Representative aorta en face for water control group (F) and for mice receiving DMPC (G).
of continued access to DMPC appeared to move into the mature HDL fractions over the next 24 hours.

The increase in paraoxonase activity together with increased intestinal synthesis of apoA-I (Figure 5) and the increase in plasma apoA-I (Figure 1) may have contributed to the decreased atherosclerosis (Figures 3 and 4) seen in the mice receiving DMPC. The very strong negative correlation coefficients between lesion area and plasma HDL cholesterol levels and plasma apoA-I levels indicate an inverse correlation between plasma HDL cholesterol and apoA-I levels and lesions in this model of mouse atherosclerosis and are consistent with this hypothesis. We previously reported that infusion of apoA-I into both mice and humans decreased LDL oxidation in our human artery wall coculture model.20,21 We also recently reported that an apoA-I mimetic peptide synthesized from all d-amino acids when given orally improved HDL function and dramatically reduced atherosclerosis in both LDL receptor–null and apoE-null mice.16

DMPC prevented lesion formation (Figure 3) and appeared to cause lesion regression (Figure 4). The increase in apo A-I synthesis after DMPC (Figure 5) is the presumed reason for the marked increases seen in HDL cholesterol levels. This and the dramatic improvement in the ability of HDL to prevent the induction of monocyte chemotactic activity in response to oxidized phospholipids in the artery wall model system (Figure 2) are probably the main causes of the marked reduction in lesions, because total plasma cholesterol, triglycerides, phospholipids, and fatty acids were not significantly altered by any of the treatments. In addition, the antiatherogenic response to DMPC would not be predicted from the known effects of myristic acid, which has proatherogenic properties.24 Because the mice drank 2.5 mL/d per mouse, we can assume that the mice received 2.5 mg of DMPC per mouse in the experiments described in Figures 1 to 4. The equivalent dose for a 70-kg human would be 8.75 g/d of DMPC. The usual dose of lecithin recommended as a food supplement is on the order of 10 g/d. Most humans seem to tolerate up to 25 g/d of lecithin without serious side effects.12 The molecular mechanisms by which DMPC (but not soy or egg lecithin) increases HDL cholesterol, improves HDL function, increases apoA-I synthesis in the intestine, raises plasma apoA-I, and prevents atherosclerosis or causes its regression in apoE-null mice remain to be elucidated. This report, together with our previous report,16 suggests that oral HDL–based therapies may hold considerable therapeutic promise.

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Circulation. 2003;108:1735-1739; originally published online September 22, 2003;
doi: 10.1161/01.CIR.0000089375.60050.35

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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