

Safety, pharmacokinetics, and pharmacodynamics of oral apoA-I mimetic peptide D-4F in high-risk cardiovascular patients

LeAnne T. Bloedon,* Richard Dunbar,* Danielle Duffy,* Paula Pinell-Salles,* Robert Norris,[†] Bruce J. DeGroot,[§] Rajesh Movva,[†] Mohamad Navab,** Alan M. Fogelman,** and Daniel J. Rader^{1,*}

Institute for Translational Medicine and Therapeutics,* University of Pennsylvania School of Medicine, Philadelphia, PA 19104-6160; Pennsylvania Hospital,[†] Philadelphia, PA 19107; MDS Pharma Services,[§] Lincoln, NE 68502; Department of Medicine,** David Geffen School of Medicine, University of California, Los Angeles, CA 90095-1736

Abstract Patients with coronary heart disease or equivalent risk received a single dose of 30, 100, 300, or 500 mg of unformulated D-4F (n = 8, each dose) or placebo (n = 8) under fasting conditions. An additional 10 patients received 500 mg (n = 8) or placebo (n = 2) with a low-fat meal. There were no significant trends in any safety parameter. D-4F was detectable in plasma at all doses with a T_{max} of 30 min, 1 h, and 2 h for 30, 100, and ≥ 300 mg, respectively. The area under the curve_(0-t) was 27.81 ng/hr/ml and 54.71 ng/hr/ml for the 300 mg and 500 mg dose groups, respectively, and 17.96 ng/hr/ml for the 500mg dose given with food. HDL from each time point for each subject was tested for its ability to inhibit LDL-induced monocyte chemotactic activity in cultures of human aortic endothelial cells. The values obtained were normalized to 1.0 for LDL alone to obtain the HDL inflammatory index. This index significantly improved at 4 h at the 300 mg dose and at 2 h at the 500 mg dose compared with placebo (P < 0.05). There were no changes in plasma lipid or lipoprotein levels. We conclude that unformulated D-4F has low bioavailability that is improved under fasting conditions, and that a single dose of D-4F is safe and well tolerated and may improve the HDL anti-inflammatory index.—Bloedon, L. T., R. Dunbar, D. Duffy, P. Pinell-Salles, R. Norris, B. J. DeGroot, R. Movva, M. Navab, A. M. Fogelman, and D. J. Rader. **Safety, pharmacokinetics, and pharmacodynamics of oral apoA-I mimetic peptide D-4F in high-risk cardiovascular patients.** *J. Lipid Res.* 2008. 49: 1344–1352.

Supplementary key words HDL cholesterol • inflammation • atherosclerosis

Partial data were presented at the American Heart Association's 2006 Scientific Sessions. This work was supported in part by Grant M01-RR00040 (General Clinical Research Center) from the National Institutes of Health. In addition, Bruin Pharma provided financial support.

Manuscript received 16 January 2008 and in revised form 26 February 2008 and in re-revised form 6 March 2008.

Published, *JLR Papers in Press*, March 6, 2008.
DOI 10.1194/jlr.P800003-JLR200

Epidemiologically, levels of HDL cholesterol and its major protein, apolipoprotein A-I (apoA-I), are inversely related to cardiovascular risk (1). ApoA-I is widely recognized to be involved in the initial steps of macrophage reverse cholesterol transport (2). It may also be critical to the anti-inflammatory properties of HDL (3). Deficiency of apoA-I in atherosclerosis prone mice results in significantly more atherosclerosis despite preserved HDL cholesterol levels (4). Conversely, transgenic overexpression, somatic gene transfer, or intravenous infusion of apoA-I in mice and rabbits has demonstrated reduced progression and even regression of atherosclerosis (5–7). In humans, a small-scale clinical trial with intravenous recombinant apoA-I Milano complexed with phospholipids demonstrated a significant reduction in coronary atheroma volume as measured by intravascular ultrasound after 5 weeks of therapy in patients with acute coronary syndrome (8). Given such results, it is believed that directly targeting apoA-I in humans may positively influence atherosclerosis.

One approach to targeting apoA-I is to develop structurally related peptides that “mimic” apoA-I function *in vivo* (9). D-4F is an apoA-I mimetic peptide composed of 18 D-amino acids, thereby preventing degradation by gut peptidases and enhancing oral absorption (9). Like apoA-I, D-4F binds nonoxidized lipids (9) but D-4F has a structure that enhances its ability to bind and sequester fatty acid hydroperoxides and proinflammatory oxidized phospholipids (10). Oral administration of D-4F has been shown to improve HDL anti-inflammatory properties in mice and monkeys and dramatically reduce lesions in mouse models of atherosclerosis despite no change in plasma levels of HDL cholesterol (11).

Abbreviations: AE, adverse event; apoA-I, apolipoprotein A-I; AUC, area under the curve; CHD, coronary heart disease; ECG, electrocardiogram; T2DM, type 2 diabetes mellitus; TC, total cholesterol.

[†]To whom correspondence should be addressed.

e-mail: rader@mail.med.upenn.edu

We present data from the first trial of D-4F in humans. We evaluated the safety, tolerability, pharmacokinetics, and pharmacodynamics of a single oral dose of D-4F in patients with stable coronary heart disease (CHD) or a CHD equivalent state.

EXPERIMENTAL PROCEDURES

Subjects

The study included 36 men and 14 postmenopausal women (age range, 21–75 years) with stable CHD or a CHD equivalent as defined by the National Cholesterol Education Program Adult Treatment Panel III criteria (12). Eligible subjects were required to have been on a stable (>4 weeks) regimen of statin therapy. Major exclusion criteria included unstable CHD, congestive heart failure, New York Heart Association class III or IV angina pectoris, niacin >500 mg or fibrates use, uncontrolled hypertension, hemoglobin A1c >10%, serum creatine kinase above the upper limit of normal, and serum albumin <2.5 mg/dl. The protocols were approved by the General Clinical Research Center and the Institutional Review Board at the University of Pennsylvania. All subjects provided written informed consent to participate in the study.

Study design

In this double-blinded study, eligible subjects were randomized to a single oral dose of D-4F at 30, 100, 300, or 500 mg or matching placebo after a 12 h fast. The first 3 dose groups included 10 subjects (8 were randomized to D-4F, 2 received placebo). The 500 mg dose group included 20 subjects (16 received D-4F, 4 received placebo). Thus, there were a total of 10 placebo subjects in these groups that were available for comparison with the subjects receiving D-4F. The first 3 dose groups and the first 10 subjects in the 500 mg dose group (500 mg, fasted) received the single dose of test article after an overnight fast and at least 2 h prior to a standardized low-fat meal and any concomitant medications. The last 10 subjects who received 500 mg or placebo had a standardized low-fat meal provided within 10 min after dosing (500 mg, fed).

Blood was obtained at baseline, 15 min, 30 min, 1 h, 2 h, 6 h, 12 h, and 24 h after study drug administration for plasma D-4F levels. Subjects returned for follow-up visits 2, 7, 14, and 30 days after study drug administration to evaluate safety laboratory measures (chemistry, hematology, coagulation, creatine kinase, and cytokine profiles), adverse events (AEs), and clinical findings. AEs were judged by a study investigator as mild, moderate or severe. Relationship of AEs to study drug was assigned by the investigator as probable, possible, unlikely or none. A single study investigator reviewed all AEs at the end of the study.

Intervention

The peptide D-4F with the primary amino acid sequence Ac-D-W-F-K-A-F-Y-D-K-V-A-E-K-F-K-E-A-F-NH₂ was synthesized by the solid phase method using an automated solid phase synthesizer (PS3 Protein Technologies, Woburn, MA) following Good Manufacturing Practice guidelines. D-amino acids were coupled to a Rink AM resin (Matrix Innovation) and were acetylated with acetic anhydride at the N-terminus. The peptides were cleaved from the solid support using 70% trifluoroacetic acid in dichloromethane and were purified via reverse-phase HPLC column. The purity of the peptides was verified via analytical reverse-phase HPLC and mass spectral analysis. Because there is no difference other than oral bioavailability between the peptide 4F when syn-

thesized from all D-amino acids (D-4F) compared with the same peptide synthesized from all L-amino acids (L-4F), the latter was used for some in vitro experiments.

D-4F is highly water-soluble, and each dose was diluted in 30 ml of 25% sucrose-water solution, yielding a test article concentration ranging from 1 mg/ml (lowest dose) to 16.67 mg/ml (highest dose). The vehicle control (25% sucrose solution) served as the placebo. The chilled diluted study agent was administered immediately after dilution and filter sterilization. Samples from each dose group were validated for purity, content uniformity, and concentration of D-4F by an independent laboratory (Alta Analytical Laboratory, Inc., El Dorado Hills, CA).

Outcome measures

Plasma levels of D-4F were determined by Alta Analytical Laboratory, Inc., using HPLC and liquid chromatography atmospheric pressure ionization tandem mass spectrometry (LC-API-MS-MS) with ¹³C, ¹⁵N-4F as the internal standard. The validated lower limit of quantification was 1.0 ng/ml. Intra-assay accuracy and precision values were within ±15%. The calibration curves were linear over a range of 1–250 ng/ml.

Pharmacokinetic calculations

Pharmacokinetic parameters including the area under the curve (AUC) from time zero to the time of the last measurable concentration [AUC_(0–t)], the AUC from time zero to infinity [AUC_(0–inf)], the terminal elimination rate (K_{el}), the apparent elimination half-life (T_{1/2}), maximum drug concentration (C_{max}), and time of the maximum drug concentration following dosing (T_{max}) were computed by MDS Pharma (Lincoln, NE) from the plasma drug concentration time data for D-4F by noncompartmental analysis, using WinNonlin version 4.0 and SAS version 8.2 (SAS Institute).

Pharmacodynamic measurements

Lipid parameters were analyzed from EDTA plasma collected after a 12 h fast in a Centers for Disease Control and Prevention standardized lipid laboratory. Plasma total cholesterol (TC), HDL cholesterol, and triglycerides (TG) were measured enzymatically on a Cobas Fara II autoanalyzer (Roche Diagnostic Systems Inc.) using Sigma reagents (Sigma Chemical Co.). LDL cholesterol and VLDL cholesterol levels were determined after ultracentrifugation at a density of 1.006 g/ml. ApoA-I was measured using Wako reagents (Wako Chemicals USA, Inc.). ApoA-I and HDL cholesterol were measured over 24 h at the same time points as the HDL inflammatory index.

Samples for analysis of the HDL inflammatory index were coded and shipped on dry ice from the University of Pennsylvania by overnight courier to the University of California-Los Angeles where the determinations were made blinded to treatments. The samples for the HDL inflammatory index were sucrose cryopreserved and the HDL inflammatory index was determined as previously described (13). Briefly, a normal control human LDL was added to human aortic endothelial cells in culture at 100 µg LDL cholesterol per ml without added HDL or together with the test HDL prepared by fast protein liquid chromatography and added at a concentration of 50 µg HDL cholesterol per ml. After 8 h the supernatants were collected and assayed in triplicate for monocyte chemotactic activity as previously described (13). The monocyte chemotactic activity produced in this assay is virtually all due to the production of monocyte chemotactic protein-1 by the endothelial cells (14). The values in the absence of HDL were normalized to 1.0. Values >1.0 after the addition of HDL indicated proinflammatory HDL; values <1.0 indicated anti-inflammatory HDL. The HDL

inflammatory index has been shown to correlate with atherosclerotic lesion area and with serum amyloid A levels in cholesterol-fed rabbits (15). Determination of LDL lipid hydroperoxide levels and paraoxonase activity were performed as previously described (16). The data from the University of California-Los Angeles and from Alta Analytical Lab (El Dorado Hills, CA) were sent to the Data Management Center at MDS Pharma (Lincoln, NE), where the samples were decoded and statistical analyses were performed.

In vitro studies

D-4F or scrambled D-4F (Ac-DWFAKDYFKKAFVEEFAK-NH₂), a peptide that does not promote α -helix formation, was added at concentrations up to 300 ng/ml to human plasma from subjects with CHD or a CHD equivalent (prior to administration of the test article). The plasma was incubated under argon for 1 h and fractionated by fast protein liquid chromatography and the HDL inflammatory index, lipoprotein lipid hydroperoxide contents and HDL-paraoxonase activity were determined as previously described (13, 16). In other in vitro studies L-4F was added to the endothelial cells at concentrations ranging from 1–1,000 ng/ml with LDL in the absence of HDL.

Statistical analyses

All individual and group mean data for pharmacokinetics pattern of D-4F were tabulated along with descriptive statistics [N, N missing, mean, SD, CV (%), SEM, median, minimum, and maximum]. Changes in pharmacodynamic measures were assessed by mean change from predose to postdose time points by dose group, dose group versus placebo, treatment versus placebo, and time. All descriptive and lipid statistical analyses were performed by MDS Pharma (Lincoln, NE) using SAS statistical software version 8.2 (SAS Institute, Cary, NC). The statistical analysis of the lipid concentration and HDL inflammatory index was performed for change from baseline values within each treatment group and compared with placebo. The analysis was conducted using ANOVA using a linear mixed model of re-

peated measurements to test the differences between D-4F dose groups and placebo at each time point. Pairwise comparisons of the LS means between D-4F dose groups and placebo at each time points of collection were examined. Analysis of data from the in vitro studies after addition of D-4F or scrambled D-4F was performed by ANOVA using GraphPad InStat version 3.05, 32 bit for Windows 95/NT (GraphPad Software, San Diego, CA). For the in vitro studies, the appropriate statistical analysis of change from baseline for HDL inflammatory index for each individual time point was based on the normality of the data within each time point and treatment. For the comparison between DF-4 and placebo at each time point, the Mann-Whitney test (non-parametric test) was used if the data were not normal. For the comparison of each postdose time point to baseline for each treatment, the Wilcoxon's signed rank test (nonparametric test) was used if the data were not normal. If the data were normal for the pairwise comparisons, ANOVA with Satterhwaite's adjustment was performed. For all statistical analyses, the α level was defined at the 0.05 statistical significance level. Because the primary objective was assessment of safety and tolerability, no formal sample size or power calculations were performed.

RESULTS

The baseline demographics of the subjects are presented in **Table 1**. The majority of subjects were male, Caucasian (subjects were given the option to report more than one race), and obese. Thirty-two percent of the subjects had CHD; 70% had type 2 diabetes mellitus (T2DM).

Pharmacokinetics

Fig. 1 shows the mean plasma concentrations of D-4F over time. Absorption of D-4F was rapid, less than 1%, and dose-dependent in the fasted groups. The mean maximum plasma concentrations for 30 mg, 100 mg, 300 mg,

TABLE 1. Baseline demographics by population and dose group

Trait	30 mg	100 mg	300 mg	500 mg fasted	500 mg fed	All Placebo	Overall
Male sex, n	6	7	6	4	5	8	36
Female sex, n	2	1	2	4	3	2	14
Race							
American Indian/Alaska Native	0	0	0	1	2	0	3
Asian/Pacific Islander	0	0	0	0	1	0	1
Black	3	4	2	1	0	3	13
Caucasian	5	4	6	6	5	7	33
Hispanic	0	2	1	0	0	1	4
Age (mean \pm SD), y	63.5 \pm 5.6	52.6 \pm 9.4	58.5 \pm 6.6	58.4 \pm 6.7	64.5 \pm 6.3	61.6 \pm 8.8	59.9 \pm 8.1
Weight (mean \pm SD), kg	91.5 \pm 20.0	91.3 \pm 13.6	95.0 \pm 18.1	82.9 \pm 17.3	89.3 \pm 14.3	95.9 \pm 15.5	91.2 \pm 16.3
Body mass index (mean \pm SD), kg/m ²	31.7 \pm 5.8	30.0 \pm 2.2	31.4 \pm 4.7	29.7 \pm 3.9	31.7 \pm 4.1	32.0 \pm 3.5	31.3 \pm 4.0
CHD, n	3	0	4	3	3	3	16
Receiving an angiotensin receptor blocker or angiotensin-converting enzyme inhibitor	3	7	5	1	5	5	26
T2DM, n	4	7	5	5	6	8	35
Other forms of cardiovascular disease, n	0	0	3	1	1	1	6
>20% risk if no CHD or T2DM	1	1	0	0	0	0	2
Systolic blood pressure (mean \pm SD), mm Hg	134 \pm 12.6	138 \pm 31.7	136 \pm 16.7	116 \pm 11.3	142 \pm 23.0	136 \pm 22.4	—
Diastolic blood pressure (mean \pm SD), mm Hg	76 \pm 14.5	81 \pm 19.0	79 \pm 10.5	72 \pm 6.7	76 \pm 10.0	80 \pm 12.6	—
HDL cholesterol (mean \pm SD), mg/dl	41 \pm 11.69	46 \pm 14.24	46 \pm 13.84	45 \pm 11.75	48 \pm 14.97	54 \pm 21.03	—

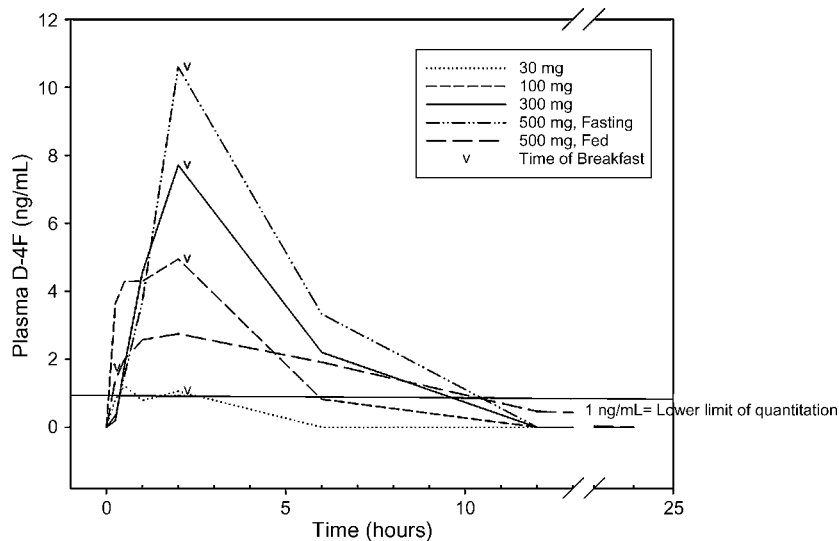


Fig. 1. Plasma D-4F concentrations increased by dose group in subjects who were administered the peptide in the fasting state and significantly decreased in subjects given a 500 mg dose of D-4F that was followed immediately by a meal.

500 mg fasted, and 500 mg fed were: 1.62 ± 1.92 ng/ml, 7.75 ± 6.43 ng/ml, 8.13 ± 5.66 ng/ml, 15.9 ± 6.53 ng/ml, and 4.49 ± 5.47 ng/ml, respectively. Five of the eight subjects (62.5%) randomized to 30 mg D-4F had at least one time point with measurable plasma levels of D-4F compared with 7 of 8 (87.5%) of the subjects in the 100 mg and 300 mg dose groups; 8 (100%) in the 500 mg (fasted) dose group and 6 (75%) in the 500 mg (fed) group. The $AUC_{(0-t)}$ values for each dose group were determined by averaging the detectable values for each dose group and were 2.17 ± 2.51 ng/h/ml (30 mg), 23.11 ± 26.19 ng/h/ml (100 mg), 27.81 ± 17.04 ng/h/ml (300 mg), 54.71 ± 22.10 ng/h/ml (500 mg fasted), and 17.96 ± 17.96 ng/h/ml (500 mg fed). The T_{max} by dose group was 0.5 ± 0 (30 mg), 0.938 ± 0.8 (100 mg), 2.45 ± 1.6 (300 mg), 2.0 ± 0 (500 mg fasted), and 1.98 ± 2.1 h (500 mg fed).

Pharmacodynamics

No formal sample size calculations were performed for pharmacodynamic readouts, because these were not the primary objective of this study. The 10 subjects in the 500 mg fed group were not analyzed for any pharmacodynamic parameter, because they were only included in the pharmacokinetic and safety studies.

There was no change in HDL cholesterol or apoA-I levels over 24 h at any dose over time compared with placebo (**Fig. 2**) or when comparing all D-4F dose groups to placebo (data not shown). The change from baseline was significant for VLDL cholesterol and triglycerides at 24 h within several treatment groups (including placebo for triglycerides), but there were no differences in the change from baseline for any other plasma lipids and lipoproteins at any dose over time compared with change from baseline in placebo (**Table 2**) or when combining data from all D-4F-treated subjects to placebo (data not shown).

Nor was there a significant change in LDL lipid hydroperoxide levels or HDL paraoxonase activity (data not shown). The mean \pm SD for the baseline HDL inflammatory index values for all fasting subjects was 1.18 ± 0.32 (range, 0.59–1.97). Of the 40 subjects studied in the fasted groups, 30 subjects (75%) had an HDL inflammatory index >1.0 at baseline despite statin treatment. The number of subjects with an HDL inflammatory index >1.0 at baseline in the 30 mg, 100 mg, 300 mg, and 500 mg fasted groups was: 4 (40%), 8 (80%), 9 (90%), and 9 (90%), respectively. The change in HDL inflammatory index within each treatment group compared with placebo is presented in **Table 3**. There was a significant improvement in the HDL inflammatory index at 4 h at 300 mg and 2 h at 500 mg compared with placebo. In all treatment groups, including the placebo group, the mean HDL inflammatory index was <1.0 at 8 h post dose which returned to >1.0 at 24 h. Compared with baseline within each treatment group, the change at 8 h was significant in the 100, 300, and 500 mg dose groups, but not placebo.

In vitro studies

Adding D-4F to human plasma in vitro at 250 ng/ml was previously shown to improve HDL inflammatory index. Given the low plasma concentrations achieved in this study, concentrations of D-4F starting at 25 ng/ml up to 300 ng/ml were added to plasma from 3 patients taken at baseline and compared with the results obtained from adding the same concentration of scrambled D-4F, a peptide with the same D-amino acids as are in D-4F but arranged in a sequence that does not promote α -helix formation. The HDL inflammatory index significantly improved at the lowest dose tested and continued to improve in a dose response fashion up to 300 ng/ml (**Fig. 3A**). While the HDL inflammatory index improved significantly

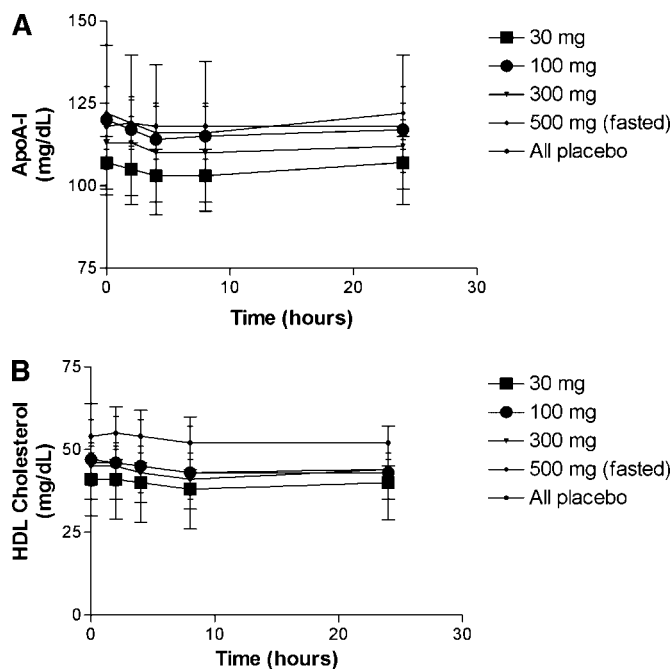


Fig. 2. There was no significant change in plasma apoA-I concentrations (A) or HDL cholesterol levels (B) after administration of D-4F.

with addition of 25 ng/ml and the mean values for LDL lipid hydroperoxide levels and paraoxonase activity improved after addition of 25 ng/ml, statistical significance for the latter two parameters was not achieved until 100 ng/ml had been added (data not shown). Thus, the HDL inflammatory index appears to be the most sensitive of the pharmacodynamic measurements made in these studies. As shown in Fig. 3B, adding as little as 10 ng/ml of 4F to cultured human aortic endothelial cells significantly ($P < 0.001$) reduced the ability of LDL to induce monocyte chemotactic activity.

Safety and tolerability

Twenty-one subjects (42%) experienced a total of 36 AEs, with none judged as serious (Table 4). The number of subjects with any reported adverse event in the 30 mg, 100 mg, 300 mg, 500 mg (fasted), or 500 mg (fed) dose groups were 1 (13%), 5 (63%), 4 (50%), 5 (63%), and 3 (30%) subjects, respectively. Three subjects (30%) in the placebo group reported at least one AE. The majority of reported AEs were rated as mild to moderate, with only 3 subjects experiencing an AE rated as severe. The AEs rated as severe were vomiting in one subject who received 300 mg, seasonal allergies in one subject who received 30 mg, and a back injury in one subject who received 100 mg. None of these events was judged related to D-4F administration, and all of these events resolved with no sequelae. The events assessed by the investigator as “possibly” related to D-4F included one report each of the following: diarrhea (30 mg), dizziness (500 mg fed), and headache (300 mg).

There were no significant trends in any of the safety laboratory parameters over the duration of this study (data

TABLE 2. Lipids over 48 hours by dose group compared with placebo

Variable (mg/dl)	30 mg (n = 8)			100 mg (n = 8)			300 mg (n = 8)			500 mg (fasted) (n = 8)			All Placebo (n = 10)		
	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h	0 h	24 h	48 h
Total cholesterol	160 (13)	166 (14) $\Delta = 6$ $P = 0.06$	168 (14) $\Delta = 8$ $P = 0.21$	174 (13)	174 (14) $\Delta = 0$ $P = 0.94$	174 (14) $\Delta = 0$ $P = 0.92$	151 (13)	151 (14) $\Delta = 0$ $P = 0.84$	153 (13)	155 (14) $\Delta = 2$ $P = 0.52$	156 (14) $\Delta = 3$ $P = 0.61$	145 (12)	149 (13) $\Delta = 3.50$ $P = 0.22$	154 (13) $\Delta = 9.20$ $P = 0.09$	
VLDL cholesterol	21 (5)	24 (5) $\Delta = 3$ $P = 0.35$	26 (5) $\Delta = 5$ $P = 0.48$	33 (5)	39 (5) $\Delta = 6$ $P = 0.18$	33 (5) $\Delta = 0$ $P = 0.29$	15 (5)	21 (5) $\Delta = 6$ $P = 0.89$	27 (5)	35 (5) $\Delta = 8$ $P = 0.74$	34 (5) $\Delta = 7$ $P = 0.92$	15 (5)	19 (5) $\Delta = 4.4$ $P = 0.09$	19 (5) $\Delta = 3.8$ $P = 0.14$	
LDL cholesterol	102 (10)	100 (10) $\Delta = 2$ $P = 0.64$	99 (10) $\Delta = 3$ $P = 0.59$	96 (10)	93 (10) $\Delta = 3$ $P = 0.25$	94 (10) $\Delta = 2$ $P = 0.70$	89 (10)	86 (10) $\Delta = 3$ $P = 0.25$	82 (10)	77 (10) $\Delta = 5$ $P = 0.07$	77 (10) $\Delta = 5$ $P = 0.42$	76 (9)	79 (9) $\Delta = 1.4$ $P = 0.56$	80 (9) $\Delta = 2.7$ $P = 0.59$	
Triglycerides	129 (19)	141 (19) $\Delta = 12$ $P = 0.47$	121 (19) $\Delta = -8$ $P = 0.38$	149 (19)	188 (19) $\Delta = 39$ $P < 0.01$	150 (19) $\Delta = 1$ $P = 0.92$	99 (19)	126 (19) $\Delta = 27$ $P = 0.03$	143 (19)	171 (19) $\Delta = 28$ $P = 0.08$	146 (19) $\Delta = 3$ $P = 0.27$	100 (17)	128 (17) $\Delta = 28.65$ $P = 0.01$	95 (17) $\Delta = -4.55$ $P = 0.68$	

Results are reported as LS means (SE). Δ = Change compared with baseline within treatment group. δ = Net change from baseline in each dose group compared with change from baseline in placebo. Statistical comparison is to placebo.

TABLE 3. Change in HDL inflammatory index by time point and treatment group compared with placebo

Time Point	30 mg (n = 8)	100 mg (n = 8)	300 mg (n = 8)	500 mg (fasted) (n = 8)	All Placebo (n = 10)
Baseline	0.99 (0.11)	1.15 (0.11)	1.38 (0.11)	1.30 (0.11)	1.10 (0.11)
2 hours post dose	1.01 (0.08) $\Delta = 0.02$ $P = 0.87$ $\delta = 0.03$ $P = 0.80$	1.01 (0.08) $\Delta = -0.14$ $P = 0.11$ $\delta = -0.13$ $P = 0.22$	1.21 (0.08) $\Delta = -0.17$ $P = 0.07$ $\delta = -0.15$ $P = 0.14$	1.07 (0.08) $\Delta = -0.23$ $P = 0.01$ $\delta = -0.22$ $P = 0.03$	1.09 (0.08) $\Delta = -0.01$ $P = 0.90$
4 hours post dose	1.02 (0.10) $\Delta = 0.03$ $P = 0.79$ $\delta = 0.07$ $P = 0.55$	0.94 (0.10) $\Delta = -0.21$ $P = 0.02$ $\delta = -0.16$ $P = 0.17$	1.02 (0.10) $\Delta = -0.36$ $P < 0.001$ $\delta = -0.31$ $P = 0.009$	1.05 (0.12) $\Delta = -0.25$ $P = 0.01$ $\delta = -0.20$ $P = 0.09$	1.05 (0.10) $\Delta = -0.05$ $P = 0.62$
8 hours post dose	0.80 (0.12) $\Delta = -0.19$ $P = 0.06$ $\delta = -0.02$ $P = 0.92$	0.89 (0.12) $\Delta = -0.26$ $P = 0.01$ $\delta = -0.08$ $P = 0.60$	0.92 (1.12) $\Delta = -0.46$ $P < 0.0001$ $\delta = -0.28$ $P = 0.08$	0.90 (0.12) $\Delta = -0.40$ $P < 0.001$ $\delta = -0.23$ $P = 0.16$	0.92 (0.12) $\Delta = -0.17$ $P = 0.09$
24 hours post dose	1.11 (0.12) $\Delta = 0.12$ $P = 0.23$ $\delta = 0.06$ $P = 0.71$	1.12 (0.12) $\Delta = -0.03$ $P = 0.82$ $\delta = -0.08$ $P = 0.63$	1.19 (0.12) $\Delta = -0.19$ $P = 0.07$ $\delta = -0.25$ $P = 0.15$	1.17 (0.12) $\Delta = -0.13$ $P = 0.19$ $\delta = -0.19$ $P = 0.26$	1.16 (0.12) $\Delta = 0.06$ $P = 0.57$

Results are reported as LS means (SE). Δ = Change compared with baseline within treatment group. δ = Net change from baseline in each dose group compared with change from baseline in placebo.

not shown). There were no clinically significant abnormalities, including electrocardiogram (ECG) changes, in any individual in the study. There were no dose-related changes to any of the parameters measured, and no correlation of any lab with the frequency or type of AEs reported in this study. There were no clinically significant changes in the physical signs or symptoms, or changes in blood pressure, heart rate, ECG parameters, or other physical measurements (data not shown).

DISCUSSION

This first report of oral apoA-I mimetic peptide D-4F in humans demonstrated that single doses (up to 500 mg) were well tolerated, absorbed rapidly, and yielded low but dose-dependent plasma concentrations under fasting con-

ditions in high-risk cardiovascular patients. Although the study was not powered to test the effects of D-4F or dose on HDL function, the HDL inflammatory index improved at the two highest doses relative to placebo by 4 h post dose.

An unformulated version of the peptide was used, which likely accounted for the variation in absorption that was seen. The time to reach maximum plasma levels of D-4F was quick and dose dependent, with the highest dose group reaching T_{max} at 2 h. The C_{max} and AUC were greatly reduced when D-4F was administered with food. The reasons for the differences in bioavailability when D-4F is administered with or without food may include: 1) coadministration of food with D-4F decreases absorption in the stomach and upper gastrointestinal tract and results in “bolus” dosing of D-4F and/or 2) D-4F was absorbed primarily in the upper gastrointestinal tract, and absorption was limited past this area. As noted above, we

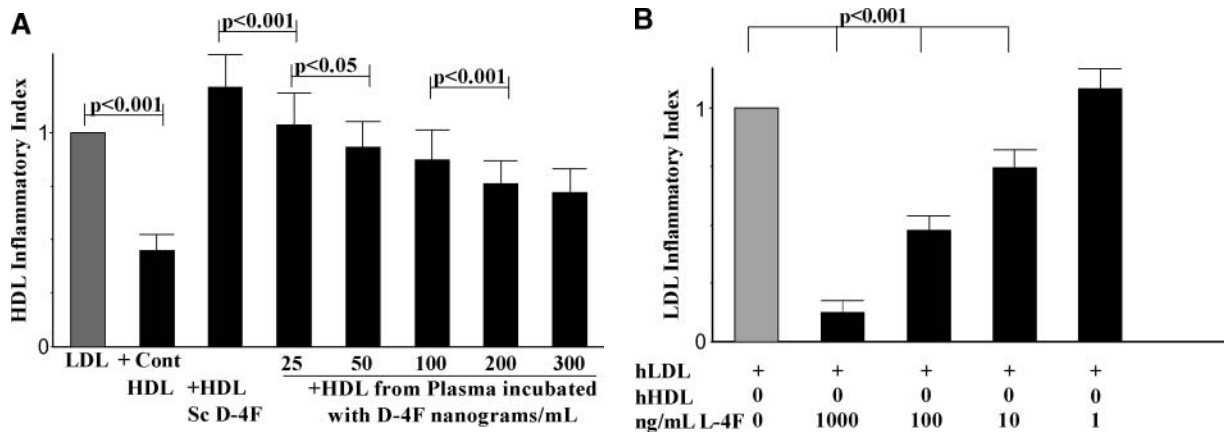


Fig. 3. A: Addition of D-4F to human plasma in vitro improves the HDL inflammatory index in a dose-dependent fashion. B: Addition of 10 ng/ml of L-4F to cultured human aortic endothelial cells significantly reduced the ability of LDL to induce monocyte chemotactic activity. Error bars indicate \pm SD.

TABLE 4. Number of subjects reporting adverse events by dose group and event

Adverse Event	Dose Group						Total
	30 mg	100 mg	300 mg	500 mg (fasted)	500 mg (fed)	All Placebo	
Eye disorders	0	0	0	0	0	2 (20%)	2 (4%)
Arcus lipoides							
Gastrointestinal disorders							
Constipation	0	0	0	0	0	1 (13%)	1 (2%)
Diarrhea	1 (13%)	0	0	0	0	0	1 (2%)
Dyspepsia	0	0	1 (13%)	0	0	0	1 (2%)
Loose stools	0	0	1 (13%)	0	0	0	1 (2%)
Vomiting	0	0	1 (13%)	0	0	0	1 (2%)
General disorders							
Asthenia	0	0	0	1 (13%)	0	0	1 (2%)
Edema, peripheral	0	0	0	0	1 (13%)	0	1 (2%)
Pitting edema	0	0	0	1 (13%)	0	0	1 (2%)
Immune system disorders							
Seasonal allergies	0	2 (25%)	2 (25%)	0	0	0	4 (8%)
Infections and infestations							
Nasopharyngitis	1 (13%)	0	0	0	0	0	1 (2%)
Sinusitis	1 (13%)	0	0	0	0	0	1 (2%)
Injury, poisoning and procedural complications							
Back injury	0	1 (13%)	0	0	0	0	1 (2%)
Contusion	0	0	1 (13%)	1 (13%)	0	0	2 (4%)
Investigations (Investigators term)							
Cardiac murmur	0	1 (13%)	0	0	1 (13%)	0	2 (4%)
Carotid bruit	0	0	0	1 (13%)	0	0	1 (2%)
Heart rate irregular	0	1 (13%)	0	0	0	0	1 (2%)
Musculoskeletal and connective tissue disorders							
Arthralgia	0	1 (13%)	2 (25%)	0	0	0	3 (6%)
Back pain	0	0	1 (13%)	0	0	0	1 (2%)
Nervous system disorders							
Dizziness	0	1 (13%)	0	0	1 (13%)	0	2 (4%)
Headache	0	1 (13%)	1 (13%)	0	0	0	2 (4%)
Tremor	0	0	0	1 (13%)	0	0	1 (2%)
Respiratory, thoracic and mediastinal disorders							
Cough	1 (13%)	0	0	0	0	0	1 (2%)
Pharyngolaryngeal pain	0	0	0	1 (13%)	0	0	1 (2%)
Wheezing	0	0	0	0	0	1 (13%)	1 (2%)

used unformulated peptide for these studies. We think it is likely that the information gained from this study will lead to efforts to formulate the peptide to give significantly higher plasma levels for future studies.

Overall, the frequency and types of AEs were not unexpected, and none were thought attributable to the administration of D-4F. The most common AE reported was seasonal allergies (8%) followed by arthralgia (6%). No serious AEs occurred during the study. The frequency of AEs did not appear to be associated with the dose of D-4F. The three AEs judged as severe by the investigator were not considered related to D-4F administration. There were no dose-related changes to any of the laboratory parameters measured and no correlation of clinical chemistries, hematology, coagulation, urinalysis, or ECG measurements with frequency or type of AEs reported in the study. The majority of subjects (70%) had T2DM. The study agent was administered in 30 ml solution of 25% sucrose, and though we did not follow blood glucose concentrations immediately after D-4F administration, glucose concentrations were measured at 24 h as part of a standard safety laboratory panel and were unchanged relative to baseline within each treatment group and compared with placebo.

Although epidemiological studies consistently reveal an inverse relationship between HDL cholesterol concentra-

tions and atherosclerosis (17), recent clinical data suggest pharmacologic increase in HDL cholesterol per se is not necessarily beneficial in patients with CHD. In recent studies of the cholesteryl ester transfer protein inhibitor, torcetrapib, significant increases in HDL cholesterol in combination with significant decreases in LDL cholesterol did not affect progression of atherosclerosis (18, 19). It has been suggested that HDL function may be more important in relation to risk of atherosclerosis as opposed to plasma concentrations per se (13, 20, 21). HDL has anti-inflammatory properties that may contribute to its atheroprotective effects. Normal, anti-inflammatory HDL can inhibit LDL oxidation and reduce the migration of monocytes within the wall of the artery (22, 23). By contrast, proinflammatory HDL cannot prevent LDL oxidation or the infiltration of monocytes and may even exacerbate the inflammatory response (21, 24). HDL varies in its anti-inflammatory capacity and is influenced by systemic conditions. The acute phase response as seen after major surgery converts HDL from anti-inflammatory to proinflammatory (25). The HDL inflammatory index as used in this study is a sensitive measure of the ability of the subject's HDL to alter the inflammatory response induced by LDL when added to cultures of human aortic cells (13, 22, 23) and is correlated with levels of oxidized LDL in patients with systemic lupus erythematosus (26). This assay

has been used as one measure of HDL function in human studies in populations associated with an increased risk for atherosclerosis (13, 21, 26–30). A pro-inflammatory HDL index (i.e., HDL inflammatory index >1.0) has been associated with CHD (13) and systemic lupus erythematosus, and this index was higher in individuals with systemic lupus erythematosus and coronary artery disease (26), whereas matched healthy controls in these studies were found to have a more anti-inflammatory HDL. The HDL inflammatory index was found to separate patients with CHD from healthy control subjects better than HDL cholesterol level (21). An improvement in the HDL inflammatory index has been seen after statin administration in patients with CHD (13) and rheumatoid arthritis (27), as well as after LDL apheresis (30) and after following a low-fat, high-fiber diet in obese patients with metabolic risk factors for CHD (28). Seventy five percent of the subjects reported here had a pro-inflammatory HDL inflammatory index at baseline, despite the fact that all were regularly taking a statin. These results are consistent with the previously reported finding that the HDL inflammatory index improved significantly after statin therapy but on average remained proinflammatory (13).

The study was not powered to test the effects of D-4F or dose of D-4F on the HDL inflammatory index. Nonetheless, the HDL inflammatory index significantly improved by 4 h in the two highest dose groups compared with placebo, suggesting a possible dose effect. In vitro data presented here are consistent with the ability of low plasma D-4F levels to improve the HDL inflammatory index. We also found that in each dose group of 8–10 subjects (including the placebo group), the mean HDL inflammatory index dropped below 1.0 (and thus became anti-inflammatory) at 8 h and increased above 1.0 at 24 h. This may be because statins (which are known to lower the HDL inflammatory index) and perhaps other concomitant medications were administered 2 h post dose; however, we cannot rule out that a diurnal effect may also be a contributing factor. Based on the proposed mechanism of action for the HDL inflammatory index assay, it is likely that inhibition of the renin angiotensin system (e.g., by angiotensin-converting enzyme inhibitors or angiotensin receptor blockers) also would improve the HDL inflammatory index (31). Twenty-one (42%) of the subjects included in the HDL inflammatory index analysis received either an angiotensin-converting enzyme inhibitor or angiotensin receptor blocker; however, in the 500 mg dose group, in which a significant improvement was seen at 2 h compared with placebo, only one (12.5%) subject received either concomitant medication, compared with five (50%) placebo subjects. Because we did not match agents taken across treatment groups, the results of this study with regard to the improvement in the HDL inflammatory index with D-4F treatment are consistent with the known action of D-4F but cannot be considered conclusive evidence of the superiority of D-4F in improving this parameter compared with other agents or rule out a possible diurnal effect. We did not measure insulin levels or plasma levels of gut-derived peptides. Because many of the

subjects had T2DM, it is possible that gut-derived peptides may have had an effect that related to the administration of sucrose. However, the placebo group received the same amount of sucrose, and although there was a decrease in the HDL inflammatory index to below 1 in the placebo group 8 h after the dose, this change was not significant compared with baseline. There were significant changes in the HDL inflammatory index at 8 h compared with baseline in subjects receiving 100, 300, or 500 mg of D-4F.

There are no data on D-4F in humans, but preclinical data suggest that D-4F can improve HDL function in mice and monkeys (11). In apoE null mice, D-4F improved the anti-inflammatory properties of HDL (16). These properties of D-4F may account for the decrease in atherosclerotic lesions by 79% and 75% seen in LDL receptor-null and apoE-null mice, respectively, without changes in total plasma or HDL cholesterol concentrations (32). In a mouse model of influenza infection and atherosclerosis, D-4F prevented HDL from becoming pro-inflammatory (24). Data presented here provide evidence to warrant a study designed to test whether multiple doses of D-4F improve HDL function.

In summary, we have demonstrated that a single dose of unformulated D-4F appears to be safe and is absorbed at doses as low as 30 mg, and is improved when fasting and administered at higher doses. Although oral administration of D-4F to humans with high cardiovascular risk produces levels of peptide in the plasma that are in the nanogram/milliliter range, this level may be sufficient to improve the anti-inflammatory activity of HDL isolated from these individuals. These results establish proof of concept to initiate multiple dose studies using the oral administration of an apoA-I mimetic peptide to improve HDL function in humans. ■

The authors thank Rachel Mendez, Tara Santoroski, and Aoife Hughes for recruiting and following study subjects; Kim McMahon, Anna DiFlorio, and Linda Morrell for technical support; the General Clinical Research Center nurses for their help with patient care; Drs. Richard Weiss, Sue-Anne Toh, and Larry Staton for their help with patient recruitment; and, especially, the study participants. Mohamad Navab and Alan M. Fogelman are principals in Bruin Pharma, and Alan M. Fogelman is an officer in Bruin Pharma.

REFERENCES

1. Kwiterovich, P. O., Jr. 1998. The antiatherogenic role of high-density lipoprotein cholesterol. *Am. J. Cardiol.* **82**: 13Q–21Q.
2. Rader, D. J. 2006. Molecular regulation of HDL metabolism and function: implications for novel therapies. *J. Clin. Invest.* **116**: 3090–3100.
3. Barter, P. J., S. Nicholls, K. A. Rye, G. M. Anantharamaiah, M. Navab, and A. M. Fogelman. 2004. Antiinflammatory properties of HDL. *Circ. Res.* **95**: 764–772.
4. Moore, R. E., M. A. Kawashiri, K. Kitajima, A. Secreto, J. S. Millar, D. Pratico, and D. J. Rader. 2003. Apolipoprotein A-I deficiency results in markedly increased atherosclerosis in mice lacking the LDL receptor. *Arterioscler. Thromb. Vasc. Biol.* **23**: 1914–1920.
5. Badimon, J. J., L. Badimon, and V. Fuster. 1990. Regression of atherosclerotic lesions by high density lipoprotein plasma fraction in the cholesterol-fed rabbit. *J. Clin. Invest.* **85**: 1234–1243.

6. Benoit, P., F. Emmanuel, J. M. Caillaud, L. Bassinet, G. Castro, P. Gallix, J. C. Fruchart, D. Branellec, P. Deneffe, and N. Duverger. 1999. Somatic gene transfer of human apoA-I inhibits atherosclerosis progression in mouse models. *Circulation*. **99**: 105–110.
7. Plump, A., C. Scott, and J. Breslow. 1994. Human apolipoprotein A-I gene expression increases high density lipoprotein and suppresses atherosclerosis in the apolipoprotein E-deficient mouse. *Proc. Natl. Acad. Sci. U.S.A.* **91**: 9607–9611.
8. Nissen, S. E., T. Tsunoda, E. M. Tuzcu, P. Schoenhagen, C. J. Cooper, M. Yasin, G. M. Eaton, M. A. Lauer, W. S. Sheldon, and C. L. Grines, et al. 2003. Effect of recombinant ApoA-I Milano on coronary atherosclerosis in patients with acute coronary syndromes: a randomized controlled trial. *JAMA*. **290**: 2292–2300.
9. Navab, M., G. M. Anantharamaiah, S. T. Reddy, S. Hama, G. Hough, V. R. Grijalva, N. Yu, B. J. Ansell, G. Datta, and D. W. Garber, et al. 2005. Apolipoprotein A-I mimetic peptides. *Arterioscler. Thromb. Vasc. Biol.* **25**: 1325–1331.
10. Anantharamaiah, G. M., V. K. Mishra, D. W. Garber, G. Datta, S. P. Handattu, M. N. Palgunachari, M. Chaddha, M. Navab, S. T. Reddy, and J. P. Segrest, et al. 2007. Structural requirements for anti-oxidative and anti-inflammatory properties of apolipoprotein A-I mimetic peptides. *J. Lipid Res.* **48**: 1915–1923.
11. Navab, M., G. M. Anantharamaiah, S. T. Reddy, and A. M. Fogelman. 2006. Apolipoprotein A-I mimetic peptides and their role in atherosclerosis prevention. *Nat. Clin. Pract. Cardiovasc. Med.* **3**: 540–547.
12. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. 2001. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*. **285**: 2486–2497.
13. Ansell, B. J., M. Navab, S. Hama, N. Kamranpour, G. Fonarow, G. Hough, S. Rahmani, R. Mottahedeh, R. Dave, and S. T. Reddy, et al. 2003. Inflammatory/antiinflammatory properties of high-density lipoprotein distinguish patients from control subjects better than high-density lipoprotein cholesterol levels and are favorably affected by simvastatin treatment. *Circulation*. **108**: 2751–2756.
14. Navab, M., S. S. Imes, S. Y. Hama, G. P. Hough, L. A. Ross, R. W. Bork, A. J. Valente, J. A. Berliner, D. C. Drinkwater, and H. Laks, et al. 1991. Monocyte transmigration induced by modification of low density lipoprotein in cocultures of human aortic wall cells is due to induction of monocyte chemotactic protein 1 synthesis and is abolished by high density lipoprotein. *J. Clin. Invest.* **88**: 2039–2046.
15. Van Lenten, B. J., A. C. Wagner, M. Navab, G. M. Anantharamaiah, S. Hama, S. T. Reddy, and A. M. Fogelman. 2007. Lipoprotein inflammatory properties and serum amyloid A levels but not cholesterol levels predict lesion area in cholesterol-fed rabbits. *J. Lipid Res.* **48**: 2344–2353.
16. Navab, M., G. M. Anantharamaiah, S. T. Reddy, S. Hama, G. Hough, V. R. Grijalva, A. C. Wagner, J. S. Frank, G. Datta, and D. Garber, et al. 2004. Oral D-4F causes formation of pre-beta high-density lipoprotein and improves high-density lipoprotein-mediated cholesterol efflux and reverse cholesterol transport from macrophages in apolipoprotein E-null mice. *Circulation*. **109**: 3215–3220.
17. Gordon, D. J., and B. M. Rifkind. 1989. High-density lipoproteins—the clinical implications of recent studies. *N. Engl. J. Med.* **321**: 1311–1316.
18. Kastelein, J. J., S. I. van Leuven, L. Burgess, G. W. Evans, J. A. Kuivenhoven, P. J. Barter, J. H. Revkin, D. E. Grobbee, W. A. Riley, and C. L. Shear, et al. 2007. Effect of torcetrapib on carotid atherosclerosis in familial hypercholesterolemia. *N. Engl. J. Med.* **356**: 1620–1630.
19. Nissen, S. E., J. C. Tardif, S. J. Nicholls, J. H. Revkin, C. L. Shear, W. T. Duggan, W. Ruzyllo, W. B. Bachinsky, G. P. Lasala, and E. M. Tuzcu. 2007. Effect of torcetrapib on the progenesis of coronary atherosclerosis. *N. Engl. J. Med.* **356**: 1304–1316.
20. Navab, M., G. M. Anantharamaiah, S. T. Reddy, B. J. Van Lenten, B. J. Ansell, G. C. Fonarow, K. Vahabzadeh, S. Hama, G. Hough, N. Kamranpour, et al. 2004. The oxidation hypothesis of atherogenesis: the role of oxidized phospholipids and HDL. *J. Lipid Res.* **45**: 993–1007.
21. Navab, M., S. Y. Hama, G. P. Hough, G. Subbanagounder, S. T. Reddy, and A. M. Fogelman. 2001. A cell-free assay for detecting HDL that is dysfunctional in preventing the formation of or inactivating oxidized phospholipids. *J. Lipid Res.* **42**: 1308–1317.
22. Navab, M., S. Y. Hama, C. J. Cooke, G. M. Anantharamaiah, M. Chaddha, L. Jin, G. Subbanagounder, K. F. Faull, S. T. Reddy, and N. E. Miller, et al. 2000. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: step 1. *J. Lipid Res.* **41**: 1481–1494.
23. Navab, M., S. Y. Hama, G. M. Anantharamaiah, K. Hassan, G. P. Hough, A. D. Watson, S. T. Reddy, A. Sevanian, G. C. Fonarow, and A. M. Fogelman. 2000. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. *J. Lipid Res.* **41**: 1495–1508.
24. Van Lenten, B. J., A. C. Wagner, G. M. Anantharamaiah, D. W. Garber, M. C. Fishbein, L. Adhikary, D. P. Nayak, S. Hama, M. Navab, and A. M. Fogelman. 2002. Influenza infection promotes macrophage traffic into arteries of mice that is prevented by D-4F, an apolipoprotein A-I mimetic peptide. *Circulation*. **106**: 1127–1132.
25. Van Lenten, B. J. 1995. Antiinflammatory HDL becomes proinflammatory during the acute phase response. Loss of protective effect of HDL against LDL oxidation in aortic wall cell cocultures. *J. Clin. Invest.* **96**: 2758–2767.
26. McMahon, M., J. Grossman, J. FitzGerald, E. Dahlin-Lee, D. J. Wallace, B. Y. Thong, H. Badsha, K. Kalunian, C. Charles, and M. Navab, et al. 2006. Proinflammatory high-density lipoprotein as a biomarker for atherosclerosis in patients with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum.* **54**: 2541–2549.
27. Charles-Schoeman, C., D. Khanna, D. E. Furst, M. McMahon, S. T. Reddy, A. M. Fogelman, H. E. Paulus, G. S. Park, T. Gong, and B. J. Ansell. 2007. Effects of high-dose atorvastatin on antiinflammatory properties of high density lipoprotein in patients with rheumatoid arthritis: a pilot study. *J. Rheumatol.* **34**: 1459–1464.
28. Roberts, C. K., C. Ng, S. Hama, A. J. Eliseo, and R. J. Barnard. 2006. Effect of a short-term diet and exercise intervention on inflammatory/anti-inflammatory properties of HDL in overweight/obese men with cardiovascular risk factors. *J. Appl. Physiol.* **101**: 1727–1732.
29. Kalantar-Zadeh, K., J. D. Kopple, N. Kamranpour, A. M. Fogelman, and M. Navab. 2007. HDL-inflammatory index correlates with poor outcome in hemodialysis patients. *Kidney Int.* **72**: 1149–1156.
30. Opole, I. O., J. M. Belmont, A. Kumar, and P. M. Moriarty. 2007. Effect of low-density lipoprotein apheresis on inflammatory and noninflammatory high-density lipoprotein cholesterol. *Am. J. Cardiol.* **100**: 1416–1418.
31. Fogelman, A. M. 2005. When pouring water on the fire makes it burn brighter. *Cell Metab.* **2**: 6–8.
32. Navab, M., G. M. Anantharamaiah, S. Hama, D. W. Garber, M. Chaddha, G. Hough, R. Lallone, and A. M. Fogelman. 2002. Oral administration of an apo A-I mimetic peptide synthesized from D-amino acids dramatically reduces atherosclerosis in mice independent of plasma cholesterol. *Circulation*. **105**: 290–292.