Novel Formulation of a Reconstituted High-Density Lipoprotein (CSL112) Dramatically Enhances ABCA1-Dependent Cholesterol Efflux

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Objective—The ability of high-density lipoprotein (HDL) to remove cholesterol from atherosclerotic plaque is thought to underlie its inverse correlation with cardiovascular risk. Our objective was to produce and characterize a human apolipoprotein AI (apoA-I) product optimized to treat clinical atherosclerotic disease.

Approach and Results—A new formulation of full length, plasma-derived human apoA-I termed CSL112 was designed to maximize the cholesterol efflux from cells and exhibit favorable pharmacological properties. CSL112 is a disc-shaped particle that strongly elevates cholesterol esterification and shows good pharmacokinetics in rabbits. Infusion of CSL112 into rabbits caused a strong and immediate increase in the ATP binding cassette transporter A1 (ABCA1)-dependent efflux capacity of plasma, an increase in plasma unesterified cholesterol and rapid subsequent cholesterol esterification. In the presence of human plasma, CSL112 was significantly more potent than native HDL at enhancing cholesterol efflux from macrophages, and the efflux elevation was predominantly via the ABCA1 transporter. Consistent with this observation, addition of CSL112 to plasma led to generation of high levels of HDL-VS, a favorable substrate for ABCA1. The lipid profile of plasma did not affect these behaviors. In studies with whole human blood, CSL112 reduced expression of intercellular adhesion molecule 1 and cytokine secretion, and as with cholesterol efflux, these activities were substantially greater than those of native HDL assayed in parallel.

Conclusions—CSL112 has favorable pharmacological properties and strongly elevates the ability of plasma to withdraw cholesterol from cells. Preferential elevation of ABCA1-dependent efflux may target atherosclerotic plaque for cholesterol removal and this property makes CSL112 a promising candidate therapy for acute coronary syndrome. (Arterioscler Thromb Vasc Biol. 2013;33:2202-2211.)

Key Words: apolipoproteins • acute coronary syndrome • cholesterol • high-density lipoproteins, pre-β • inflammation

Cholesterol in coronary arteries builds up over decades and may eventuate in acute coronary syndrome (ACS). Current medical therapy for ACS focuses on opening of severely stenotic coronary segments with percutaneous coronary intervention and on antiplatelet drugs to reduce arterial thrombosis. Despite optimal current therapy, ACS patients experience an extremely high rate of recurrent ACS in the period immediately after an index ACS. For example, in a recent trial 12% of patients experienced recurrent ACS in the first year, with nearly half of the events occurring in the first 30 days. A potential explanation for this high rate is that neither percutaneous coronary intervention nor antiplatelet drugs act on the ultimate cause of coronary disease, plaque cholesterol.

Apolipoprotein AI (ApoA-I) is known to transport cholesterol out of cholesterol-loaded tissues, and recent studies indicate that this transport can be extremely rapid. On the basis of the in vitro rate of net cholesterol efflux from cholesterol-loaded macrophages to high-density lipoprotein (HDL), it can be calculated that a foam cell carrying 50% by weight cholesterol can efflux half of the load in 1 week. Studies in animals suggest that even faster efflux may be observed; correcting dyslipidemia in apoE−/− mice with an adenooviral vector caused loss of ≈75% of plaque cholesterol in 1 week, and transplanting atherosclerotic plaques from apoE−/− mice to wild-type recipients caused loss of ≈65% of plaque cholesterol ester in 1 week. Importantly, Feig et al showed that apoA-I is necessary for this rapid reduction, that a high apoA-I level is effective even in the presence of sustained high levels of apoB–containing lipoproteins, and that foam cell emigration from the plaque may contribute to the rapid loss. Similar results have been obtained on infusion of apoA-I in animals or in human subjects. Two infusions of apoA-I Milano into rabbits caused the loss of ≈50% of plaque cholesterol in 8 years.

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days,7 and a single infusion of reconstituted HDL caused loss of ≈60% of cholesterol ester in femoral plaque in 5 to 7 days.8 The ability of infused apoA-I to remove half of plaque cholesterol in a week suggests that it may act quickly enough to affect the high recurrent cardiovascular event rate seen in the month after ACS.

Because of its promise in treatment of ACS, infused HDL therapy has been long pursued by the pharmaceutical industry.9,10 Here, we describe a novel formulation of human apoA-I (hApoA-I) intended to treat ACS. This formulation, termed CSL112, was designed to optimize cholesterol efflux by ATP binding cassette transporter A1 (ABCA1), a transporter induced by excess cellular cholesterol and present in atherosclerotic plaque.11,12 Although CSL112 acted as a good substrate for ABCA1 in tissue culture, we found adding CSL112 to human plasma caused a more dramatic rise in ABCA1-dependent efflux capacity with activity substantially greater than that of native HDL from plasma. We propose that CSL112 is rapidly remodeled in plasma to a form that can accept cholesterol from ABCA1. We support this hypothesis with studies showing that adding CSL112 to plasma results in rapid and profound elevation of very small HDL (HDL-VS) by the nomenclature of Rosenson et al,13 also referred to as PreBeta1, the preferred substrate for ABCA1.13

Materials and Methods
Materials and Methods are available in the online-only Supplement.

Results
Infusion of CSL112 Removes Cholesterol From Tissues
Prior work has shown that infusion of therapeutic amounts of pure apoA-I in man resulted in rapid clearance with a half-life of ≈2 hours.14 However, reconstitution of the apoA-I with phosphatidylcholine (PC) resulted in a volume of distribution and clearance rate comparable with endogenous apoA-I (half-life, 3–4 days).15 The formulation chosen for CSL112 thus used reconstitution with PC. A molar ratio of 55 PC per 1 apoA-I yielded a population of particles which elute in a single symmetrical peak from size exclusion chromatography column with a slightly higher retention time than native HDL (Figure 1A). The particles had a hydrodynamic diameter of 7.5 to 8.0 nm by nondenaturing gel electrophoresis (Figure 1B) and were disc shaped by negative stain electron and cryo-electron microscopy (Figure 1C). Size heterogeneity in native gels with 2 apparent subpopulations in the 7.5 to 8.5 nm range has been seen in prior studies with reconstituted HDL and has been attributed to a subset of particles with apoA-I folds or hairpins that accommodate quantized expansion with addition of lipid.16,17 Rouleaux were clearly seen in negative stain images, and the particles in rouleaux had a diameter of 9.8±2.9 nm and height of 3.2±0.6 nm (Figure 1D). These dimensions may be biased toward larger diameters as larger discs are more often found in rouleaux. Chemical cross-linking studies demonstrated 2 apoA-I molecules per particle (not shown). The molecular size calculated on the basis of the
deduced stoichiometry (2 apoA-I and 110 PC) is 144 kDa, a value consistent with our gel-based, chromatography-based, and electron microscopy-based measures.

Favorable pharmacokinetic behavior of CSL112 was confirmed by infusing 75 mg/kg into rabbits. Levels of hApoA-I in the plasma reached maximum by the end of the infusion period (0.7 hours), then steadily declined, with half of the infused hApoA-I still present in the circulation at 8 hours post infusion (Figure 2A). By comparison, lipid-free apoA-I was cleared much more quickly (Figure IIIA in the online-only Data Supplement). The infused CSL112 rapidly withdrew cholesterol from tissues as evidenced by a rise in plasma total

Figure 2. Human apolipoprotein AI (hApoA-I) pharmacokinetic profile, lecithin cholesterol acyltransferase (LCAT) activation, cholesterol efflux, and particle remodeling after the infusion of CSL112 into rabbits. CSL112 was administered to rabbits intravenously (dose 75 mg/kg, >0.7 h). Blood was collected at different time intervals post infusion as indicated. A, hApoA-I content in rabbit plasma was measured by ELISA (Mabtech). hApoA-I concentration values represent the means±SD for 8 animals. ***P<0.0001 (n=8) compared with baseline value (pre infusion). B, Total and unesterified cholesterol were measured in rabbit plasma and corresponding apoB-depleted fraction (obtained after precipitation of plasma apoB lipoproteins with polyethylene glycol [PEG]) using high-density lipoprotein (HDL) and low-density lipoprotein/very-low-density lipoprotein Cholesterol Quantification Kit (MBL International). Values are means±SD. ****P<0.0001, ***P<0.0005 (n=8) compared with baseline values (pre infusion). C, To measure LCAT activity, plasma samples of rabbits treated with CSL112 were equilibrated with [14C]-cholesterol (7.5 nmol/L per milliliter) for 4 h at 4°C. Cholesterol esterification was measured as described in Materials and Methods in the online-only Data Supplement. LCAT activity is presented as percentage change from baseline levels detected in preinfused rabbit plasma (t=0). Each value shows the means±SD of 5 animals. **P<0.002, *P<0.01, and *P<0.05 (n=5) compared with baseline values (pre infusion). D, CSL112 preferentially enhances ABCA1-dependent efflux ex vivo. Cholesterol efflux from RAW264.7 macrophages to whole and apoB–depleted plasma of rabbits dosed with CSL112. Before initiation of efflux, RAW264.7 cells were loaded with free [3H]cholesterol for 36 h and stimulated with 0.3 mmol/L 8Br-cAMP for 16 h to upregulate ABCA1. Efflux was promoted by incubating the [3H]cholesterol-labeled RAW264.7 cells with each individual rabbit plasma sample at a final concentration of 0.5%. Each fractional efflux value represents the means±SD of 6 animals in 2 groups treated with CSL112 was measured. Total efflux was measured from 8Br-cAMP–stimulated cells. The difference in efflux between stimulated and nonstimulated cells is a measure of ABCA1-mediated efflux. ****P<0.0001, ***P<0.001, and **P<0.005 (n=6) compared with baseline values (pre infusion). E, Dynamic remodeling of CSL112 after infusion into rabbits. Plasma samples of rabbits infused with CSL112 were subjected to nondenaturing gel electrophoresis on gradient 4% to 30% polyacrylamide gels followed by Western blot analysis of hApoA-I. Time (h) after infusion is indicated above the lanes. Distribution of hApoA-I among HDL subfractions before (whole plasma) and after (apoB–depleted plasma) precipitation of apoB–containing lipoproteins is shown. CSL112 used for infusion into rabbits was included in the analysis as control (CSL112). Molecular size markers (diameter in nm) and position of rabbit HDL are indicated.
cholesterol (Figure 2B). Almost all the increment in plasma cholesterol was found in the HDL fraction (Figure 2B), with little change in non-HDL (not shown). Elevation of HDL total cholesterol was driven by a rapid increase in unesterified cholesterol (0.7 hours) followed by a concomitant decline in unesterified cholesterol (2–8 hours) and a rise of cholesteryl ester (2–8 hours; Figure 2B). To confirm our hypothesis that the rise of plasma HDL cholesteryl ester is caused by elevated lecithin cholesterol acyltransferase (LCAT) activity, we measured rates of cholesterol esterification in pre- and postinfused animal plasma ex vivo. Figure 2C demonstrates that infusion of CSL112 into rabbits results in an immediate ≈1.7-fold increase of plasma LCAT activity which was sustained for 8 hours. Taken together, these data suggest that infused CSL112 augments mobilization of unesterified cholesterol from peripheral tissues to plasma HDL and elevates the rate of ongoing cholesterol esterification.

**CSL112 Elevates Capacity of Plasma to Support Cholesterol Efflux From Cells**

To characterize the net movement of cholesterol from tissue into plasma, we measured the capacity of postinfused rabbit plasma to support cholesterol efflux from RAW264.7 mouse macrophages ex vivo. Because cholesterol-loaded cells, such as those in an atherosclerotic plaque, express ABCA1,12 the contribution of this transporter to efflux was measured in RAW cells stimulated with 8Br-cAMP. CSL112 caused an immediate rise (≈3-fold) of the capacity of whole and apoB–depleted plasma to efflux cholesterol (Figure 2D). The magnitude of the rise in efflux was larger than expected given that the infusion was designed to cause less than double plasma apoA-I. More surprising was the magnitude of the elevation of ABCA1-dependent efflux which showed a >10-fold elevation (Figure 2D).

To explore the mechanism for the dramatic elevation in ABCA1-dependent efflux, we used nondenaturing gels to separate lipoproteins from rabbits dosed with CSL112 (Figure 2E). These studies took advantage of the fact that hApoA-I can be specifically identified on blots with an antibody that does not recognize rabbit apoA-I. We observed an identical pattern of distribution of hApoA-I among lipoproteins in rabbit whole plasma and in the HDL fraction prepared from plasma by polyethylene glycol precipitation indicating that after infusion of CSL112, hApoA-I is not associated with apoB–containing lipoproteins. However, it was clear that CSL112 underwent a prompt remodeling on infusion. By 2 hours after the start of infusion, the majority of hApoA-I was seen in particles much larger than parent CSL112. The size of these particles was ≈10 nm at 2 hours and declined slightly at 4 and 8 hours. This range is comparable with endogenous rabbit HDL (9.6–10 nm).18 Smaller species of hApoA-I were also seen during remodeling. A species slightly smaller than parent CSL112 (7.1–7.6 nm) was seen at 0.7 and 2 hours, and a second, much smaller species of a size comparable with HDL-VS (5.6 nm) peaked after 4 hours but was sustained for the duration of the study. The infusion of CSL112 into rabbits thus seemed to result in sequential formation of large forms of HDL similar to large HDL (HDL-L) and very large HDL (HDL-VL), small forms similar to HDL-VS (5.6 nm) and finally larger forms similar to the dominant HDL species of rabbit. The time course of appearance of low molecular weight particle species containing hApoA-I coincided roughly with the rise in ABCA1-dependent cholesterol efflux to plasma (Figure 2D and 2E), whereas time-dependent accumulation of larger particles (10–12 nm) was consistent with the observed increase in cholesterol esterification and reduced efflux capacity (Figure 2D and 2E). We note that drawing a correlation between structure and efflux activity must take into account remodeling of HDL that will occur during the 5-hour incubation with the cholesterol-loaded cells. Thus, peak efflux was observed in samples drawn at 0.7 hours and incubated with cells for 5 hours, whereas maximal abundance of lipid-poor apoA-I was seen in blood samples drawn at 4 hours. Because lipid-poor forms of apoA-I, such as HDL-VS,13,19 are the preferred substrates for ABCA1, the remodeling observed may explain the exaggerated elevation of ABCA1-dependent efflux on CSL112 infusion.

**Addition of CSL112 to Human Plasma Yields HDL-VS and Dramatically Potentiates ABCA1-Dependent Cholesterol Efflux**

To quantitatively explore apoA-I behavior using conditions closer to those in the clinic, we characterized functional changes on incubation of CSL112 with human plasma and serum ex vivo. CSL112 was spiked into sera derived from 5 individuals with a range of lipid phenotypes (Table I in the online-only Data Supplement), incubated at 37°C for 1 hour, and cholesterol efflux to serum was evaluated using J774 cells. Addition of CSL112 increased global efflux to serum in all samples, regardless of lipid profile (Figure 3A). As observed for rabbit samples, the increase in global efflux was mainly driven by an increase of the ABCA1-dependent efflux, whereas ABCA1-independent efflux was more modestly affected (Figure 3A). To further quantify the large increase in efflux after addition of CSL112 to serum, the contributions of endogenous and exogenous apoA-I to cholesterol efflux were normalized to levels of apoA-I (Figure 3B). Per milligram per milliliter of apoA-I, CSL112 was 3.4-fold more efficacious in promoting global cholesterol efflux from macrophages than endogenous apoA-I (Figure 3B, left). The enhanced efficacy of CSL112 versus endogenous apoA-I and CSL112 was all attributable to ABCA1-dependent efflux; per milligram per milliliter of apoA-I, CSL112 was 16-fold more efficacious than endogenous apoA-I at promoting ABCA1-dependent efflux (Figure 3B, right).

To further characterize the efflux capacity of CSL112, native HDL and apoA-I were used for direct comparison. When tested alone, CSL112 and native HDL caused comparable efflux from RAW264.7 macrophages (Figure 4A, compound alone panels). However, when CSL112 and native HDL were incubated with fresh human plasma for ≥1 hour, CSL112 was significantly more potent (2–3 fold) at enhancing ABCA1-dependent efflux to plasma than native HDL (Figure 4A, middle and right, Donors 1 and 2). The ABCA1-dependent efflux induced by CSL112 approached but was always less than that caused by apoA-I, whether assayed alone or after incubation with plasma.

In rabbits, elevated ABCA1-dependent cholesterol efflux correlated with elevated levels of HDL-VS by native gel.
Figure 3. Addition of CSL112 dramatically increases cholesterol efflux to serum regardless of lipid phenotype. A. Five sera differing in lipid profiles (normal [n=2]; low high-density lipoprotein [HDL], high triglycerides [TG; n=2], and high HDL [n=1]; (Table I in the online-only Data Supplement) were incubated with buffer control (−) or 0.8 mg/mL of CSL112 (+) for 1 h at 37°C. ApoB-containing lipoproteins were removed and sera incubated with [3H]cholesterol-labeled J774 cells for 4 h. Final concentration of CSL112 in efflux medium was 16 μg/mL; serum was at 2%. Each efflux value is the mean±SD of triplicate measurements. The far right bars labeled average represent the means±SD of all 5 donors. Statistical significance between control vs CSL112 treated was assessed by paired t test for ABCA1-dependent, ABCA1-independent, and global cholesterol efflux (**P<0.0001). B. The contributions of endogenous vs exogenous apoA-I to cholesterol efflux (global, ABCA1 independent, and ABCA1 dependent, respectively) were calculated as cholesterol efflux per milligram of apoA-I. The contribution of endogenous apoA-I to cholesterol efflux was calculated as the ratio of cholesterol efflux of control spiked serum vs the dilution corrected baseline apoA-I level. The contribution of exogenous apoA-I to cholesterol efflux was calculated as the ratio of the difference of CSL112 and control spiked serum cholesterol efflux and the spiking concentration of apoA-I (0.8 mg/mL). Shown are mean values of 5 sera±SD. Statistical significance treated was assessed by paired t test (**P=0.0042; ***P<0.0001).

To more directly examine the effects of CSL112, native HDL, and apoA-I on HDL particle size distribution in human plasma, HDL fractions prepared by polyethylene glycol precipitation were separated on nondenaturing gradient gels, and apoA-I-containing particles were detected by Western blotting with an anti-apoA-I antibody (Figure 4C, data for Donor 1 is shown). Consistent with our ELISA data, incubation of plasma with CSL112 at 37°C resulted in a strong increase in concentration of HDL-VS with no apparent changes in the distribution of medium HDL or HDL-L. Similarly, apoA-I did not alter the size distribution of larger HDL species.

CSL112 Enhances Cholesterol Esterification and Reduces Inflammatory Responses More Potently Than Native HDL

Having observed a strong difference between CSL112 and native HDL in their ability to potentiate the cholesterol capacity of plasma in vitro, we investigated whether CSL112 is also superior to native HDL in elevating human plasma LCAT activity and in modulating anti-inflammatory responses. The rates of cholesterol esterification catalyzed by LCAT after the addition of CSL112 or native HDL to pooled human plasma were compared in vitro. Plasma LCAT activity was dose dependently increased by CSL112, whereas native HDL had no effect on LCAT activity (Figure 5).

We next assessed the anti-inflammatory properties of CSL112 and native HDL using human whole blood stimulated with phytohemagglutinin-M (PHA-M) for 20 hours as an in vitro model of inflammation. CSL112 (0.25–1 mg/mL) caused an inhibition of PHA-M stimulated human plasma LCAT activity and in modulating anti-inflammatory responses. The rates of cholesterol esterification catalyzed by LCAT after the addition of CSL112 or native HDL to pooled human plasma were compared in vitro. Plasma LCAT activity was dose dependently increased by CSL112, whereas native HDL had no effect on LCAT activity (Figure 5).

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reduced secretion of RANTES, Mip-1β, IP-10, and interleukin-6, whereas inhibitory effect of native HDL was less pronounced (IP-10, RANTES) or absent (Mip-1β). The ability of CSL112 to mediate strong anti-inflammatory effects was further supported by the finding that CSL112 caused a strong reduction of the secretion of tumor necrosis factor-α and interleukin-1β in postinfused rabbit blood stimulated with PHA-M ex vivo (Figure 6C).
Discussion

Here, we describe and characterize a formulation of hApoA-I designed to remove cholesterol from atherosclerotic plaques of coronary heart disease patients. CSL112 is a population of disc-shaped particles bearing 2 molecules of hApoA-I and ≈110 molecules of phospholipid (Figure 1). Its pharmacokinetic behavior in rabbits suggests that it avoids the rapid clearance of unformulated apoA-I (Figure 2A). Coupled with its ability to accept cholesterol from cells ex vivo, these data suggest that it may be a favorable therapeutic candidate.

In the course of this work, we discovered that on infusion of CSL112 into rabbits or on addition of CSL112 into plasma, cholesterol efflux was enhanced to a greater extent than anticipated based on apoA-I content. Direct comparison with native HDL showed that it was ≈3-fold more potent than native HDL in enhancing cholesterol efflux (Figure 4). Further work showed that the increase in efflux was preferentially mediated by ABCA1 (Figures 2D, 3, and 4A). We view this property as favorable because cellular cholesterol excess causes increased expression of ABCA1, and cholesterol withdrawal may, therefore, be targeted to cholesterol-rich tissues such as atherosclerotic plaque. Further characterization of the disproportionate rise in ABCA1-mediated efflux showed that it was seen in both normal and dyslipidemic plasma (Figure 3). Patients with low HDL and high triglyceride, such as those studied in Figure 3A, are at risk of coronary heart disease and are commonly seen in patients with ACS.

Active remodeling of CSL112 in plasma offers a mechanistic explanation for the preferential elevation of ABCA1-dependent efflux. In the hours after infusion of CSL112 into rabbits, hApoA-I was observed in particles smaller than parent CSL112 (Figure 2E), and the prevalence of these forms correlated with the rise in ABCA1-mediated cholesterol efflux. In vitro, <2% of the apoA-I in parent CSL112 reacts with an antibody against lipid-poor HDL-VS (PreBeta1-HDL; Figure 4B). However, addition of CSL112 to human plasma caused dramatic elevation of HDL-VS (Figure 4C) with nearly half of the added apoA-I reactive in a HDL-VS ELISA (Figure 4B), again consistent with active remodeling of the CSL112 particle to lipid-poor species. Lipid-poor forms of apoA-I, such as HDL-VS, are the primary acceptors of cholesterol from ABCA1, whereas HDL-L and very large HDL are poor substrates, 13,19 and thus remodeling to lipid-poor species may account for elevated ABCA1-mediated efflux, and further remodeling of apoA-I back into the main HDL fraction may account for the subsequent decline in ABCA1-mediated efflux.

Elevation of HDL-VS is thought to be beneficial in removing cholesterol from the artery wall. Waksman et al. 20 tested this hypothesis by remodeling HDL in patient plasma ex vivo with a proprietary procedure and then reinfluencing the plasma with elevated HDL-VS. Intravascular ultrasound measurements in monkeys 21 and humans 20 strongly suggested that this therapy reduced plaque size. In this setting, the total amount of apoA-I circulating in patients was not increased. These data suggest that elevation of HDL-VS by itself affects plaque. By extension, coupling HDL-VS elevation with net elevation of apoA-I should provide further benefit.

In the normal course of reverse cholesterol transport, unesterified cholesterol exits cells for incorporation into nascent HDL particles and is then promptly esterified by the action of LCAT. Esterification enables HDL to carry a much greater load of cholesterol. Moreover, by reducing the concentration of unesterified cholesterol on the surface of HDL, LCAT may prevent reuptake of unesterified cholesterol via scavenger receptor B1 or diffusion. 22,23 On infusion of CSL112 into rabbits, we observed elevation of plasma unesterified cholesterol then prompt esterification (Figure 2B). These results are consistent with the observation of dose-dependent elevation of cholesterol esterification rate on addition of CSL112 to human plasma (Figure 5). They differ, however, from results in mice where very little esterification of cholesterol was seen on infusion of a reconstituted HDL. 24 A potential explanation of the difference is the poor ability of hApoA-I to activate murine LCAT. 24

It is likely that 2 factors contribute to the degree of LCAT activity on adding CSL112 to human plasma. Human LCAT transfers the fatty acid at the sn-2 position of PC to cholesterol, with a marked preference for long chain length, unsaturated fatty acids. 25 The soy PC in CSL112 contains linoleic acid (18:2) as the dominant fatty acid, thus providing a favorable substrate. LCAT also requires allosteric activation by apoA-I and substrate particles of relatively small diameter, 26 and both of these features are present in CSL112.

A large literature supports the notion that HDL can have anti-inflammatory effects on a wide range of cell types. 27 The mechanisms underlying this activity are not fully known, but in several instances, the anti-inflammatory action of HDL can be explained through its withdrawal of cholesterol from cell membranes. 28,29 For example, the action of HDL on monocytes...
can be recapitulated using cyclodextrin, a cholesterol-binding agent. Consistent with this notion, it has been observed that cells deficient in ABCA1 and ATP binding cassette transporter G1 have excess membrane cholesterol and an exaggerated inflammatory response, which can in turn be normalized with cyclodextrin or HDL. We observed that addition of CSL112 to whole human blood blunted the production of several cytokines in response to challenge with PHA (Figure 6). ACS patients have heightened inflammatory activity, and this strong anti-inflammatory activity of CSL112 may offer important benefits for these patients. Interestingly, CSL112 was significantly more potent than native HDL at inhibiting

Figure 6. Anti-inflammatory properties of CSL112 in vitro and ex vivo. A, CSL112 reduces expression of ICAM1 on primary human monocytes and neutrophils. Heparinized human whole blood was stimulated in vitro with PHA-M (1 µg/mL)±CSL112 or ±native high-density lipoprotein (HDL) at concentrations indicated. Surface expression of ICAM1 (CD54) was analyzed by flow cytometry after overnight culture. The mean fluorescent intensity (MFI) values±SD are derived from duplicate overnight cell cultures of 3 donors. **P<0.0005, ***P<0.01, and *P<0.05 (n=3) compared with PHA-stimulated cells. Cytokine expression is presented as percentage change from the levels observed in PHA-stimulated cells. ****P<0.0001, ***P<0.001, **P<0.01, and *P<0.05 (n=3) compared with PHA-stimulated cells. B and C, CSL112 inhibits the secretion of proinflammatory mediators. B, Heparinized human whole blood was stimulated as described in A. Cytokine production was measured in cell-free supernatants at 20 h after PHA stimulation. Results are derived from duplicate cell cultures of 3 donors. Cytokine expression is presented as percentage change from the levels observed in PHA-stimulated cells. Shown are mean±SD. ****P<0.0001, ***P<0.001, **P<0.01, and *P<0.05 (n=3) compared with PHA-stimulated cells. Neither CSL112 nor native HDL significantly affected expression of cytokines in PHA unstimulated cells even at the highest dose tested (1 mg/mL). C, CSL112 was administered to rabbits intravenously (dose 75 mg/kg, infusion period 0.7 h). Rabbit blood collected into NH4-heparin at different time intervals after infusion was stimulated as described above. Cytokine expression is presented as percentage change from the baseline levels detected in stimulated whole blood before CSL112 infusion. Values show the mean±SD for the 8 CSL112-treated animals. ****P<0.0001, ***P<0.001, and **P<0.005 (n=8) compared with baseline values (pre infusion).
inflammatory responses in monocytes and neutrophils in blood. A potential explanation for these differences may lie in the superior ability of CSL112 to support cholesterol efflux and HDL-VA generation (Figure 4A). The steps involved in remodeling of CSL112 to HDL-VA are not clear at this time, but prior in vitro work suggests a possible path.32,33 These studies showed that incubation of reconstituted HDL discs with LDL and LCAT caused reduction in HDL particle size after 3 hours, an effect that presumably represents the formation of small spherical HDL. These particles then underwent LCAT-mediated fusion to create larger particles in the size range of HDL-L in a process that is accompanied by the dissociation of poorly lipidated apoA-I. The sequential size changes of hApoA-I after infusion in rabbits (Figure 2E) are consistent with this sequence; hApoA-I showed an initial slight decline in particle size followed by generation of larger particles in the size range of HDL-L. The larger particles promptly diminished in size and particles of very small size, presumably lipid-poor species, appeared. In the prior studies of Liang et al,33 disc-shaped reconstituted HDL particles fused with one another to yield larger particles. In the studies described here, however, plasma supplies endogenous HDL particles, and thus fusion of CSL112 with native HDL may also occur. Additional work will be required to understand the behavior of CSL112 infused in humans. It is clear that plasma does not have the cells or cell-bound lipases that can affect HDL remodeling over and above what is caused by LCAT, cholesterol ester transport protein, and phospholipid transfer protein. Moreover, rabbits represent an imperfect model because of their deficiency in hepatic lipase.34 Nevertheless, our studies, coupled with the recent observation of a strong correlation between cholesterol efflux capacity and risk of coronary disease,35 encourage further development of interventions, such as CSL112, that can favorably affect cholesterol efflux capacity, as well as cholesterol esterification and inflammation.

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References

This is the first description of CSL112, a novel formulation of human apolipoprotein AI designed to withdraw cholesterol from atherosclerotic plaque and serve as an intravenous therapy for acute coronary syndrome. CSL112 was shown to have favorable pharmacological properties, including a very strong ability to enhance cholesterol efflux from cells ex vivo. CSL112 preferentially enhanced efflux via ATP binding cassette transporter A1, a transporter preferentially expressed in cholesterol-laden cells. CSL112 also enhanced cholesterol esterification, a key step in cholesterol transport, and reduced production of inflammatory cytokines in whole blood assays. The potency of CSL112 in enhancing cholesterol efflux, cholesterol esterification, and blocking inflammatory cytokine production was substantially greater than native human high-density lipoprotein. The high potency is explained by the observation that CSL112 is remodeled in plasma to yield the very small high-density lipoprotein, also referred to as PreBeta1-high-density lipoprotein, a form predicted to be active in removing cholesterol from plaque. These properties make CSL112 a promising candidate for further development.
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Materials and Methods

Production of CSL112

ApoA-I was purified from human pooled plasma (CSL Behring) using the cold ethanol precipitation method described by Lerch et al.\textsuperscript{1,2} to ~95% purity by SDS-PAGE. The apoA-I was combined with phosphatidylcholine (PC) using the sodium cholate dialysis method of Jonas et al.\textsuperscript{3} with modifications\textsuperscript{1}. Parenteral grade PC containing 0.2% a-tocopherol as antioxidant (Phospholipon 90) was obtained from Lipoid AG/Phospholipid GmbH and was added to achieve a 55-fold molar excess over apoA-I. The conditions of manufacture produced particles with complete apoA-I and PC incorporation (Supplemental Fig. I). After the additions, sodium cholate was removed by diafiltration using Biomax cassettes (molecular weight cut-off =10 kDa; Millipore). The principal differences between CSL112 and a previously described formulation\textsuperscript{1} (CSL111) are lower levels of PC and cholate in CSL112. After a final sterile filtration (0.22 µm), CSL112 was lyophilized, sealed under vacuum and stored at 4°C. The amount of sodium cholate remaining in CSL112 was approximately 0.03 g per g of apoA-I. This method produced disc-shaped particles with high batch-to-batch consistency. In all studies, amounts of CSL112 are given in mg of protein.

Size Exclusion Chromatography and Electron Microscopy

Size exclusion chromatography (HPSEC) was performed on a Superose 6 column (GE Healthcare) using phosphate buffered saline (PBS), pH 7.4 as an eluent. The column was calibrated with molecular size standards from Bio-Rad (vitamin B12, 1.35 kDa; equine myoglobin, 17 kDa; chicken ovalbumin, 44 kDa; bovine γ-globulin, 158 kDa; thyroglobulin, 670 kDa).

For cryo-electron tomography, reconstituted CSL112 was vitrified in liquid ethane prior to transfer to a JEM-1400 120 kV transmission electron microscope. Tomographic tilt-series were recorded using low-dose acquisition techniques over an angular range of at least ±60 degrees in 2 degree increments using the ‘TEMography Recorder’ software (System in Frontier, Inc.) and an Ultrascan 1000 2k x 2k CCD camera (Gatan) with a pixel size of 0.69 nm or 0.81 nm. The total dose was kept below 70 e⁻/Å². Aligned tilt series were reconstructed using weighted back projection or iterative algorithms.

For negative stain electron microscopy (NS-EM), 3 µg of reconstituted CSL112 was applied to a Formvar coated grid using 2% (w/v) sodium phosphotungstate (pH 7.0) as a negative stain and allowed to dry. Micrographs were recorded with a Philips CM10 TEM and recorded at 44,000 fold magnification on 35 mm film. Calibration was done at the same magnification with a carbon grating replica (ProSciTech S003; 2,160 lines/mm). A well-known artifact of NS-EM imaging of lipoprotein disks is the stacking of lipoprotein in rouleaux.\textsuperscript{4} The disk shapes and sizes were similar in cryo- and negative stain EM. For statistical analysis, disk diameters of CSL112 were measured in five different negative stain micrographs. Only particles in rouleaux were assessed.

In Vivo Studies

To characterize the biological effects of CSL112, a conscious rabbit model was used. Female CHB rabbits (Bauer, Neuental, Germany) were 3–4 months old weighing 2.5–3.5 kg (four
animals per group, two repeats). CSL112 was administered via a single intravenous infusion lasting 0.7 hours into the marginal ear vein at a dose of 75 mg/kg. Blood was taken from the auricular artery immediately before (time 0) and 0.7, 2, 4 and 8 hours after the start of the infusion and collected into NH₄-heparin, EDTA and streptokinase (SK 150 U/mL). Blood samples were processed to plasma which was stored in aliquots at -70°C until analysis. The animals tolerated CSL112 very well, and no elevations of hepatic transaminases were seen at any time point (not shown).

**Concentrations of Lipids and Apolipoproteins**

Measurements of total and unesterified cholesterol in whole and apoB-depleted rabbit plasma were performed using commercial HDL and LDL/VLDL Cholesterol Quantification Kits (MBL). Separation of rabbit HDL from LDL/VLDL was performed by polyethylene glycol (PEG)-precipitation using 2x LDL/VLDL precipitation buffer included in the kit. Human apoA-I (hApoA-I) content in rabbit plasma was determined by ELISA (Mabtech). HDL-VS was measured in human EDTA-plasma using a PreBeta1-HDL ELISA (Sekisui/American Diagnostica GmbH). Assays were performed according to the manufacturer's recommendations.

**Non-Denaturing Pore Gradient Gel Electrophoresis (PGGE) and Western Blotting**

For the analysis of HDL particle subclasses, plasma samples were subjected to non-denaturing gel electrophoresis on 4–30% polyacrylamide gradient gels (LPE System, CBS Scientific Company Inc.). Molecular size markers were from GE Healthcare (Amersham High Molecular Weight Calibration Kit for Electrophoresis, range: 669/17 thyroglobulin, 440/12.2 ferritin, 232/9.5 catalase, 140/8.4 lactate dehydrogenase, 66/7.1 BSA; kDa/nm).

Proteins were then transferred on to polyvinylidene difluoride membranes using the wet blotting procedure (Invitrogen). Membranes were blocked with 5% non-fat milk (Bio-Rad) in Tris-buffered saline (TBS: 25 mM Tris, 150 mM NaCl, 2 mM KCl, pH 7.4) containing 0.05% Tween 20, and probed with goat anti-human apoA-I polyclonal antibody (Rockland, 1:3,000 dilution) in TBS/0.05% Tween 20 containing 2.5% BSA overnight at 4°C. After extensive washing with TBS/0.05% Tween 20 for 30 minutes, membranes were incubated with the secondary rabbit anti-goat IgG coupled to horseradish peroxidase (DAKO, 1:3,000 dilution in TBS/0.05% Tween 20 containing 2.5% BSA) for 1 h at room temperature. Immunoreactive bands were visualized using enhanced chemiluminescence detection system (ECL-Kit Dura PIECE) and Luminescent Image Analyzer (LAS4000, GE Healthcare).

**Isolation of HDL**

Native HDL was isolated from pooled human plasma (CSL Behring) by sequential ultracentrifugation at 4°C in a Kontron TFT 70.38 rotor, Centrikon T2080 ultracentrifuge. Briefly, plasma density was adjusted to 1.13 g/mL with solid potassium bromide (KBr) and centrifugation was carried out for 18 hours at 55,000 rpm (330,000 g). The bottom fraction was collected, density was adjusted to 1.21 g/mL with KBr and centrifugation was repeated as above. The top fraction was collected, density was measured by densitometer and adjusted to 1.21 g/mL with KBr, and third spin was continued for 24 hours. The resulting fraction corresponds to HDL-M and HDL-S by the nomenclature of Rosenson et al. or to HDL₃ in older nomenclature and was dialyzed against endotoxin-free PBS (pH 7.4).
**Cholesterol Efflux Assay**

Cholesterol efflux from murine macrophage cell lines J774 and RAW 264.7 is highly responsive to cAMP stimulation, which leads to the up-regulation of ABCA1. RAW264.7 cells were obtained from the American Type Culture Collection. Cells were cultured in DMEM (Dulbecco’s modified Eagle’s medium, Gibco) supplemented with 10% (v/v) fetal calf serum (FCS, Gibco), 2 mM glutamine, 100 units/mL penicillin and 100 µg/mL streptomycin in a humidified CO₂ incubator at 37°C. For efflux experiments, cells were seeded into 24-well plates at a density of 0.35 x 10⁶ cells per well. The following day, cells were labeled with [1,2-³H]cholesterol (1 µCi/mL, GE) in DMEM supplemented with 5% (v/v) FCS. After a labeling period of 36 hours, cells were washed with PBS and then incubated in DMEM containing 0.2% fatty-acid-free bovine serum albumin (BSA) in the absence or presence of 0.3 mM 8-bromoadenosine 3’,5’-cyclic monophosphate sodium salt-cAMP (8Br-cAMP) for 16 h to up-regulate ABCA1. Following two washes with PBS, cells were incubated with different cholesterol acceptors in DMEM/0.2% fatty-acid-free BSA medium. After 5–6 hours of incubation, plates were centrifuged at 500 g for 10 minutes to remove any floating cells and cellular debris. Radioactivity in cell supernatants was measured by liquid scintillation counting. Total cell-associated [³H]cholesterol was determined after extraction of cells in control wells for at least 30 minutes with 0.1 M Triton X-100. Cholesterol efflux was expressed as the percentage of the radioactivity released from cells into the medium relative to the total radioactivity in cells and medium. The difference in efflux between control and 8Br-cAMP-stimulated cells was taken as a measure of ABCA1-dependent efflux. Efflux was linearly dependent on dose of both CSL112 and native HDL (Supplemental Fig. II).

In some studies efflux was assayed using cAMP-stimulated J774 cells loaded with [³H]cholesterol. These studies were performed at Vascular Strategies LLC, Wynnewood, PA, USA, as previously described by de la Llera-Moya et al. Efflux was carried out for 4 hours.

**Addition of CSL112 to Plasma of Single Donors**

All human plasma and serum samples were obtained from donors with informed consent. Five subjects were selected to encompass a range of lipid profiles (normal [n=2]; low HDL and high triglycerides [n=2] and high HDL [n=1]; Table I, Suppemental). Serum samples obtained from these subjects were spiked with CSL112 at a concentration of 0.8 mg protein/mL or control and incubated for 1 hour at 37°C to allow remodeling. After removal of apoB-containing lipoproteins by precipitation of samples with 0.4 volumes of 13% (w/v) PEG 6000, HDL-containing fractions were used for the efflux assay from J774 cells. Final concentration of CSL112 in efflux medium was 16 µg/mL of protein; serum was at 2%.

For the comparison of CSL112 and native HDL, CSL112 and native HDL were added to fresh human EDTA-plasma at a concentration of 1 mg total protein/mL such that the final concentration of plasma was 90%. PBS was added to the control plasma sample. Following incubation at 37°C for 0, 1, 4 and 18 hours, samples were snap frozen in liquid nitrogen and stored at -70°C until analyzed for cholesterol efflux and HDL-VS content. To obtain the HDL fraction, apoB lipoproteins were precipitated from plasma as described by Asztalos et al. Briefly, plasma was mixed with 0.4 volumes of 20% PEG 8000 MW (Sigma P-2139) in 200 mM glycine pH 7.4. Following the incubation for 15 minutes, the solution was centrifuged at 10,000 rpm for 30 minutes at 4°C. Supernatant containing HDL fraction was collected and used for electrophoretic analysis and for the measurements of cholesterol efflux from
RAW264.7 cells. Final concentrations of CSL112 and native HDL in efflux medium was 5 µg/mL; serum was at 0.45%. All concentrations of CSL112 and native HDL$_3$ in this paper are given as mg of protein.

**Lecithin-cholesterol acyltransferase (LCAT) Activity Determination**

To determine LCAT activity in human plasma, esterification of $[^{14}\text{C}]$cholesterol in human plasma was assayed as described by Stokke and Norum. Briefly, $[^{14}\text{C}]$cholesterol (Perkin Elmer) was evaporated under a nitrogen stream, re-dissolved in a small amount of ethanol and vigorously mixed with human albumin solution (20 mg/mL HSA (CSL Behring) in PBS, pH 7.4). CSL112 or native HDL$_3$ were incubated with pooled human plasma (CSL Behring) after the addition of $[^{14}\text{C}]$cholesterol/albumin solution for 1.5 hours at 4°C to allow both the $[^{14}\text{C}]$cholesterol and LCAT to equilibrate between the bulk solution and the surface of the lipoprotein particles. The final concentration of $[^{14}\text{C}]$cholesterol in reaction mixture was 7.5 nM/mL; 0.37 µCi/mL. To initiate the esterification of cholesterol, the reaction mixture was placed at 37°C for 30 minutes. Following extraction with n-hexane, cholesteryl ester (CE) was separated from unesterified cholesterol using a solid phase extraction column (SampliQ Amino, Agilent) and quantified by scintillation counting. LCAT activity is expressed as nmol CE/mL/h.

Measurements of LCAT activity in rabbit plasma were performed as follows. $[^{14}\text{C}]$cholesterol was evaporated under nitrogen, re-dissolved in a small amount of ethanol and added to an albumin solution (20 mg/mL BSA in 20 mM Tris-HCl pH 7.4). The cholesterol-albumin solution was vigorously mixed and added to pre-cooled plasma samples. Equilibration was carried out for 4 hours at 4°C. LCAT activity in kinetic experiments is expressed in percent of the activity measured in the pre-infusion plasma sample (=100%).

**Stimulation of Whole Blood, Cytokine Measurements and Flow Cytometry**

Rabbit blood was collected in NH$_4$-heparin at different time periods after the initiation of CSL112 infusion. Shortly after collection, heparinized rabbit whole blood was stimulated with 0.8 µg/mL phytohemagglutinin-M (PHA-M). Following incubation for 20 hours at 37°C in a CO$_2$ incubator, blood cells were pelleted by centrifugation (1,000 g for 15 minutes). Cell-free supernatants were snap frozen in liquid nitrogen and stored at -70°C until required for analysis of cytokines. Rabbit IL-1β was assessed using Human IL-1β Flex Set (Bead B4 from BD Biosciences). TNF-α was measured by a sandwich ELISA using antibodies from BD Biosciences (purified goat anti-rabbit TNF and biotin mouse anti-rabbit TNF).
Human blood collected in NH₄-heparin was stimulated with 1 µg/mL PHA-M₁₁,₁₂ in the presence or absence of CSL112 or native HDL and incubated without mixing for 16–20 hours at 37°C in a CO₂ incubator. IL-1β, IL-6, and TNF-α were measured in cell-free supernatants using Cytometric Bead Array for human inflammatory cytokines (BD Biosciences). Mip-1β, RANTES and IP-10 were measured using Human Cytokine Magnetic 25-Plex Panel from Invitrogen. Pre-incubation of PHA with polymyxin B before stimulation of blood did not affect cellular responses ruling out potential effects of endotoxin contamination (not shown). To document that CSL112 does not bind PHA, biotinylated PHA was prepared with the EZ-link sulfo-NHS-LC-biotinylation kit from Pierce Chemical (Thermo Scientific). CSL112 did not bind to biotinylated PHA immobilized on streptavidin sepharose beads (GE Healthcare) as detected by Western blot (not shown).

Blood cells were resuspended in 0.1 mL PBS and stained with the antibodies specific for CD54, CD14 and CD45. All antibodies were obtained from BD Biosciences (CD54-PE, CD14-FITC, CD45-PerCP). Following incubation of cells with indicated antibodies for 30 minutes on ice, cells were fixed and permeabilized with OptiLyseB 250T lysis buffer (Beckman Coulter International, SA) for 10–15 minutes. Remaining erythrocytes were lysed by addition of 1 mL of ddH₂O. Cells were pelleted by centrifugation (1,500 rpm, 10 minutes, 4°C), resuspended in 0.2 mL of PBS and analyzed by flow cytometry. Leukocytes were gated using pan-leukocyte surface marker CD45. Further subdivisions were made by granularity and CD14 expression allowing for the distinction between monocytes, neutrophils and lymphocytes.

Statistics

Values are presented as mean + standard deviation (SD) or percentage of control + SD. All results were analyzed for statistical significance using one-way ANOVA followed by Dunnet post-hoc test. Statistical significance was set at P<0.05.
References

Supplemental Information

Supplemental Figure I. Phospholipid and protein are completely incorporated into reconstituted HDL particles. One minute fractions of a SE-HPLC run of CSL112 were collected and analysed for protein and phospholipid content. Protein was determined using a modified Lowry test kit (Thermo Scientific) whereas phospholipids were assayed using an enzymatic test kit based on the modified Trinder method (MTI diagnostics).

![SE-HPLC graph]

Supplemental Figure II. Efflux to CSL112 and native HDL shows linear dose-dependence. RAW267.4 cells were incubated for 5 hours with increasing concentrations (2.5, 5, 10 and 20 µg/mL) of CSL112 or native HDL. Before initiation of the efflux, RAW cells were loaded with free [3H]cholesterol for 36 hours, and then stimulated with 0.3 mM 8-Br-cAMP to up-regulate ABCA1. ABCA1-independent efflux represents efflux from non-stimulated cells. Total cholesterol efflux represents efflux from 8-Br-cAMP-stimulated cells. The difference in efflux between stimulated and non-stimulated cells is taken as a measure of ABCA1-mediated efflux. Each fractional cholesterol efflux value is the mean ± standard deviation of triplicate measurements.
**Supplemental Figure III.** Unformulated human apoA-I is rapidly cleared upon infusion in rabbits. Human apoA-I was administered to rabbits intravenously (infusion dose 75 mg/kg, infusion time 0.7 hours, four animals per group), and blood was collected at time intervals post-infusion as indicated. For comparison, comparable data using CSL112 is also shown. (A) Human apoA-I content in rabbit plasma was measured by ELISA (Mabtech). hApoA-I concentration values represent the mean ± standard deviation for the group of four animals. (B) Plasma samples after precipitation of apoB-containing lipoproteins were subjected to non-denaturing gel electrophoresis on gradient 4-30% polyacrylamide gels followed by western blot analysis of human apoA-I. Time post infusion (hours) is indicated above the lines, and the distribution of human apoA-I among HDL subfractions is shown. ApoA-I used for the infusion into rabbits was included in the analysis as control (C). (C) Cholesterol efflux from RAW macrophages to plasma of rabbits dosed with human apoA-I or CSL112. Before initiation of the efflux, RAW cells were loaded with free [³H]cholesterol for 36 hours, and then stimulated with 0.3 mM 8Br-cAMP for 18 hours to up-regulate ABCA1. Efflux was promoted by incubating the [³H]Cholesterol-labelled RAW cells with each individual rabbit plasma sample at a final concentration of 0.5%. Each fractional efflux value represents the mean ± standard deviation for animals infused with apoA-I (n=4) or CSL112 (n=8). Time 0 represents pre-infusion plasma. Total cholesterol efflux represents efflux from 8Br-cAMP-stimulated cells. The difference in efflux between stimulated and non-stimulated cells is taken as a measure of ABCA1-mediated efflux. (D) Rabbit blood collected into NH₄-heparin at different time intervals post-infusion was stimulated with PHA-M (0.8 µg/mL) for 18 hours. TNF-α was measured in cell-free supernatants by a sandwich ELISA using antibodies from BD Biosciences (purified goat anti-rabbit TNF and biotin mouse anti-rabbit TNF). Cytokine expression is presented as a percentage change from the baseline levels detected in stimulated whole blood before infusion with apoA-I or CSL112. Each value shows the mean ± standard deviation for animals treated with apoA-I (n=4) or CSL112 (n=8).

We note that formulation with phospholipid conferred a much greater effect on plasma apoA-I levels in man than in rabbit. In man, unformulated apoA-I is cleared with a half-life more than 30-fold faster than formulated apoA-I, leading to undetectable levels at 8 hours (refs 25 and 26 in manuscript). In contrast, unformulated apoA-I in rabbit appears to clear rapidly in the first two hours but clearance then slows to resemble that of formulated apoA-I, and significant blood levels are still present after 8 hours (panel A). A potential explanation for this interspecies difference is the very slow incorporation of apoA-I into human HDL, (Figure 4) and the more rapid incorporation of apoA-I into rabbit HDL (panel B).
Table I. Donor lipid profiles at baseline. Five donors were selected to encompass a range of lipid phenotypes including normal (Donors 1 and 2), dyslipidemic with high triglycerides (TG) and low HDL (Donors 3 and 4) and high HDL (Donor 5). Phospholipids, triglycerides and total cholesterol were determined on Roche P-Module using reagents from Roche (Triglyceride (GB) Kit) and Wako (Wako Cholesterol E, Wako Phospholipids C). Triglycerides were corrected for free glycerol. LDL cholesterol was calculated by the Friedewald equation. ApoA-I was assessed by an immunoturbidimetric method run on the Roche Modular P. Spiking recovery of CSL112 was determined by measuring apoA-I concentration at baseline and after spiking and was acceptable (105 ± 1% of targeted spiking concentration).

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<th>Donor lipid profiles at baseline (mg/dL)</th>
<th>Donor 1 (normal)</th>
<th>Donor 2 (normal)</th>
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<th>Donor 4 (high TG, low HDL)</th>
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