Plasma lipid profiling across species for the identification of optimal animal models of human dyslipidemia[S]

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Abstract In an attempt to understand the applicability of various animal models to dyslipidemia in humans and to identify improved preclinical models for target discovery and validation for dyslipidemia, we measured comprehensive plasma lipid profiles in 24 models. These included five mouse strains, six other nonprimate species, and four nonhuman primate (NHP) species, and both healthy animals and animals with metabolic disorders. Dyslipidemic humans were assessed by the same measures. Plasma lipoprotein profiles, eight major plasma lipid fractions, and FA compositions within these lipid fractions were compared both qualitatively and quantitatively across the species. Given the importance of statins in decreasing plasma low-density lipoprotein cholesterol for treatment of dyslipidemia in humans, the responses of these measures to simvastatin treatment were also assessed for each species and compared with dyslipidemic humans. NHPs, followed by dog, were the models that demonstrated closest overall match to dyslipidemic humans. For the subset of the dyslipidemic population with high plasma triglyceride levels, the data also pointed to hamster and db/db mouse as representative models for practical use in target validation. Most traditional models, including rabbit, Zucker diabetic fatty rat, and the majority of mouse models, did not demonstrate overall similarity to dyslipidemic humans in this study.— Yin, W., E. Carballo-Jane, D. G. McLaren, V. H. Mendoza, K. Gagen, N. S. Geohagen, L. A. McNamara, J. N. Gorski, G. J. Eiermann, A. Petrov, M. Wolff, X. Tong, L. C. Wilsie, T. E. Akiyama, J. Chen, A. Thankappan, J. Xue, X. Ping, G. Andrews, L. A. Wickham, C. L. Gai, T. Trinh, A. A. Kulick, M. J. Donnelly, G. O. Voronin, R. Rosa, A-M. Cumiskey, K. Bekkari, L. J. Mitnaul, O. Puig, F. Chen, R. Raubertas, P. H. Wong, B. C. Hansen, K. S. Koblan, T. P. Roddy, B. K. Hubbard, and A. M. Strack. **Plasma lipid profiling across species for the identification of optimal animal models of human dyslipidemia.** ***J. Lipid Res.*** **2012.** 53: **51–65.**

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Cardiovascular disease (CVD) is the leading cause of morbidity and mortality worldwide (1). Dyslipidemia has been shown to be one of the most potent risk factors for coronary heart disease (CHD) (2, 3). Dyslipidemia is characterized by elevated plasma cholesterol, especially low density lipoprotein cholesterol (LDL-c) levels. Management of dyslipidemia is considered throughout the primary and secondary prevention of CHD (4). For the past 20 years, the statin (3-hydroxy-3-methylglutaryl CoA reductase inhibitors) class of cholesterol-lowering drugs has been used for the treatment of hypercholesterolemia, either alone or in combination with other classes of lipid-lowering drugs

Abbreviations: AGM, African green monkey; CHD, coronary heart disease; CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; CRP, C-reactive protein; CVD, cardiovascular disease; DAG, diacylglycerol; DIO, diet-induced obesity; DNL, de novo lipogenesis; FC, free cholesterol; FFA, free fatty acid; FPLC, fast-protein liquid chromatography; HDL-c, high-density lipoprotein cholesterol; HFD, high-fat diet; hs-CRP, high-sensitivity C-reactive protein; LDL-c, low-density lipoprotein cholesterol; LDLR, LDL receptor; LPC, lysophophatidylcholine; NHP, nonhuman primate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SFA, saturated fatty acid; TC, total cholesterol; TG, triglyceride.

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(5, 6). By inhibiting cholesterol synthesis and lowering LDL-c, statins have been proven to be effective in reducing cardiovascular risks in randomized, controlled clinical trials over 15 years involving more than 100,000 individuals (7–9). Although statins effectively lower cholesterol levels and reduce cardiovascular causes of death, a large portion of statin-treated patients still experience adverse coronary events. This has led to the vigorous search for new therapeutic agents for cardiovascular diseases, targeting the residual CVD risk that remains after statin treatment (10). However, the success rate of drug development for dyslipidemia beyond statins has lagged, and the major cause of failure in drug development has been lack of efficacy in the clinic (11). This has led us to address the need for better preclinical animal models at the target validation stage of drug discovery, in an effort to obtain better prediction of efficacy.

Traditionally, mouse models have been widely used in preclinical research and for target validation in drug discovery for dyslipidemia and atherosclerosis (12). A major difference of mouse models from humans is the absence of cholesteryl ester transport protein (CETP), a key enzyme involved in plasma cholesterol transport that transfers cholesteryl ester (CE) from HDL to apoB-containing lipoproteins such as LDL and VLDL. Rats, dogs, and pigs also have no or low plasma CETP activities, and they all display a high high-density lipoprotein cholesterol (HDL-c) and low LDL-c plasma lipoprotein distribution, similar to mice, which is associated with a low risk of CVD (13). Genetic manipulation of mice (such as $ApoE^{-/-}$ and $LDLr^{-/-}$ mice) significantly increases circulating LDL-c levels, susceptibility to atherosclerosis, and sensitivity to atherogenic diet (14–16). Expressing the human *CETP* gene in mice, such as the $CETP^{+/-}/LDLr^{-/-}$ mouse model, results in higher plasma LDL-c and lower plasma HDL-c, which is referred to as a "human-like profile," and the animal becomes atherogenic-prone (17, 18). Animal species with naturally high CETP activity, such as rabbits and nonhuman primates (NHPs) have a high LDL-c and low HDL-c profile, which is associated with increased risk of CVD. Although not inherently atherosclerotic-prone, the rabbit model develops hypercholesterolemia and aortic lesions rapidly, even with low amounts of dietary cholesterol, and has therefore been used extensively in studies of atherosclerosis (19). Historically, all the animal models mentioned above have been utilized to study disease mechanisms and for drug discovery for dyslipidemia and atherosclerosis. The question remains as to whether their dyslipidemic state is representative of human dyslipidemia, or whether these models show a similar response to anti-dyslipidemic pharmacological agents.

The classical guidelines for dyslipidemia and risk assessment for CVD focus mainly on plasma cholesterol levels (LDL-c and HDL-c) and to a lesser extent, on plasma triglyceride (TG) level (20). However, multiple abnormalities can lead to dyslipidemia. Recently, it has been examined with a variety of techniques in an attempt to more comprehensively understand the disease state and to be more reflective of the biological pathways that are relevant to specific targets (e.g., enzymes or receptors that drugs may modulate). Among these techniques, lipidomics (or lipid metabolomics) has advanced tremendously because of the ability of technology to rapidly quantify hundreds of different molecular lipid species with different structural and functional roles (21, 22).

Here we report a comprehensive and comparative lipidomic analysis of plasma lipids in 24 commonly used animal models across various animal species, including five different mouse strains, six other nonprimate species, and five primate species, including dyslipidemic humans. In addition to a comparison of classic lipid parameters (such as plasma LDL-c, HDL-c, and TG) and plasma lipoprotein profiles, we quantified the absolute and relative amounts of FAs in major circulating lipid fractions, under both basal and simvastatin-treated conditions. The aim of this study was to help us *1*) understand the differences and similarities of various animal models to dyslipidemic human; *2*) understand the applicability of different animal models to dyslipidemic humans; and *3*) identify the optimal animal model(s) for target validation and drug discovery for developing treatments for dyslipidemia.

METHODS

In vivo experiments

All animal experiments and protocols were reviewed and approved by the Merck Research Laboratories' Institutional Animals Care and Use Committee.

Unless otherwise noted, animals were housed in a temperature- and humidity-controlled environment with a 12 h light/dark cycle. In all experiments, animals received food and water ad libitum except for dogs. Dogs were on a weight maintenance diet that was available from 10 AM to 1 PM. The gender and number of animals used for each study, the exact sources of the animals, and the diets used for each animal species are all listed in supplementary Table I.

Dosing. All animals were dosed with either vehicle or simvastatin for 2 weeks. For the rodent species, simvastatin was admixed into the diet by Research Diets (New Brunswick, NJ). The dose of simvastatin was 30 mg/kg in all mouse models, 23 mg/kg in Zucker diabetic fatty (ZDF) rats, and 20 mg/kg in hamsters. For all other species, simvastatin was dosed 30 mg/kg by mouth except New Zealand White rabbit, which was dosed at 10 mg/kg. Vehicle was water in the primates, and compound was mixed into a treat (detailed in supplementary Table I), except for marmosets. Marmosets were dosed from a 3 cc syringe, with Splenda at 5% (McNeil Nutritionals) and maple syrup added as a flavor mask.

Plasma collection. Blood samples were collected with EDTA. Samples for fast-protein liquid chromatography (FPLC) analysis had lipase inhibitors added.

Human measurements. The human simvastatin study was originally reported by Chen et al. (23).

Plasma measurements

FPLC analysis. The generation of lipoprotein profiles was performed as previously reported (24). In brief, plasma lipoprotein was separated by chromatography using a Superose-6 size

exclusion column (GE LifeSciences, Inc.) on an Ultimate 3000 Series HPLC system (Dionex Corporation). Total cholesterol levels in the column effluent were continuously measured using in-line mixture with an enzymatic and colorimetric cholesterol detection reagent (Total Cholesterol E, Wako), followed by spectrophotometric detection of the reaction products at 600 nm absorbance. VLDL, LDL-c, and HDL-c were eluted from the column. The concentration for each lipoprotein fraction was calculated by multiplying the ratio of the corresponding peak area to total peak area by the total cholesterol (TC) concentration in the sample. Plasma TC level was measured with a 1:1 mixture of cholesterol E reagent and diluted plasma using a plate reader. Cholesterol standards were provided in the kit at 200 mg/dl and were serially diluted to provide a standard curve.

Biochemical analysis of circulating lipids. Plasma TG levels were measured by biochemical analysis in nonhuman primate species. In brief, TG glycerol-3-phosphate oxidase-p-aminophenazone (GPO-PAP) reagents (Roche Diagnostics; Indianapolis, IN) were used on a Roche Diagnostics Modular Analytics P Clinical Chemistry Analyzer to determine levels of TGs. Assays were carried out following all recommended procedures for instrument operation, calibration, quality control, and assay guidelines. The instrument was calibrated with Calibrator for automated systems (Roche Diagnostics) and controls were Precipath U (Roche Diagnostics) for TGs.

Measurements of FA composition in circulating major lipid fractions. The concentration and complete FA composition of plasma CE, TG, diacylglycerol (DAG), free fatty acid (FFA), phsophatidylcholine (PC), phosphatidylethanolamine (PE); and lysophophatidylcholine (LPC) were determined using TrueMass® Lipomic Panel by Lipomics Technologies (West Sacramento, CA). Lipid fractions were isolated and methylated, FAs were separated and quantified by gas chromatography, and absolute masses and percentage of each FA of the total within each lipid fraction were calculated as previously reported (25). The lipidomic study was done in all animal models except dyslipidemic African Green Monday.

Plasma CRP measurements. Plasma C-reactive protein (CRP) levels in all species were measured by Rules-Based Medicine (Austin, TX), using fully automated, bead-based multiplex sandwich immunofluorescence assays. The HumanMap antigen panel was used to determine plasma CRP levels in human, nonhuman primates, and pigs. The RodentMap antigen panel was used to determine plasma levels in dog, rabbit, and all rodent species.

CRP was also measured using the MSD 96-well multi-array human CRP assay kit obtained from Meso Scale Diagnostics, LLC (Gaithersburg, MD) for all nonhuman primate species. Plasma samples were diluted 200-fold and added 10 μl/well. Human CRP protein was used for standard curves.

Simvastatin measurements

Simvastatin levels in plasma were determined by LC-MS/MS following acetonitrile protein precipitation. Standards were prepared from 1 to 8,000 ng/ml. Internal standard L-000050672 (20 μl at 0.5 μg/ml) was spiked into samples prior to being extracted with 500 μl of acetonitrile for protein precipitation. Samples were centrifuged at 4,000 rpm for 5 min before supernatant plate transfer. A Waters Atlantis T3 (3 μm, 2.1 × 30 mm) column was used with gradient chromatography (acetonitrile, 0.1% formic acid/water, 0.1% formic acid). Negative-ion mode mass spectrometry transition monitored for simvastatin was 435.3/319.1. The levels of simvastatin are shown in supplementary Table II.

Statistical analyses

Separate studies were carried out for each animal model, and the design of the studies varied with species. The Gottingen minipig model had only a chow diet control group with no simvastatin-treated group. Rhesus diet-induced obesity (DIO), marmoset, and dog models had two groups, a vehicle-treated group and a simvastatin-treated group, with two sets of lipid measurements (pretreatment and posttreatment) per group. African green monkey (AGM), rhesus with metabolic syndrome, diabetic rhesus, and cynomolgus each had a single group of animals, with each animal measured under vehicle treatment and simvastatin treatment. Rabbit and all rodent models included a vehicle-treated group and a simvastatin-treated group, with one set of lipid measurements per group.

Basal lipid levels and percents. Means and standard deviations of basal levels of lipids and lipid composition were calculated using vehicle-treated animals and placebo-treated human subjects. The exception was pigs, for which levels in untreated animals were used.

Comparison of eight major circulating lipid fractions in various animal models to dyslipidemic humans. For each animal model, mean basal lipids for each fraction including CE, TG, DAG, FFA, PC, PE, LPC as well as free cholesterol (FC) were calculated, and the means for dyslipidemic humans were subtracted. For a given fraction, these differences were weighted (multiplied) by the square root of the proportion that fraction represented out of total lipids in humans. The weighted differences are shown in the heat map in Fig. 4. The sum (over lipid fractions) of the squared weighted differences was calculated for each model, which was the squared weighted Euclidean distance of that model from humans, and the models were sorted by distance in Fig. 3.

Estimation of simvastatin effects on lipid levels. For animals that have separate groups for different conditions, unpaired *t*-test was used to calculate the significance of simvastatin effect between groups. For models that had the same group of animals (AGM), rhesus with metabolic syndrome, diabetic rhesus, and cynomolgus) for repeat measurements, statistical analysis was done using paired *t*-test between conditions.

Estimation of simvastatin effects on FA composition. All analyses were performed after applying a $\log_{10}(x+1)$ scale transformation to measured FA values. The specific statistical analysis model varied with study, due to differences in design, but the primary result of each analysis was an estimate of the simvastatin effect on each FA endpoint for the tested species. The effect was expressed as the log of the ratio of FA values with simvastatin to values under control conditions. *P* values and confidence intervals for the effect were not multiplicity-adjusted because of the exploratory nature of the analyses.

Distance of the simvastatin effect profile of each animal species to dyslipidemic humans. The vector of simvastatin effects (log ratios) on a set of FAs for a given animal species was the effect profile for that species. To compare the effect profile of each animal species to dyslipidemic humans, three distance measures were used: *a*) euclidean distance between the vectors of log ratios; *b*) a variance-weighted Euclidean distance applied to the log ratios. (This weighted the contribution of a particular FA category to the distance calculation inversely proportional to the

estimated variance of the difference in simvastatin effects for that category. Therefore, categories with high variability were down-weighted. This metric was an approximation to the Mahalanobis distance between two effect profiles, where the off-diagonal elements of the covariance matrix were ignored.) *c*) Uncentered correlation-based distance (1-cosine distance). (This measured the distance between effect vectors by one minus the cosine of the angle between the vectors.) Unlike *a*) and *b*), *c*) assessed only the similarity of the *pattern* of simvastatin effects across FA categories, ignoring any differences between animal species based on the magnitude or scale of those effects. Each of these metrics measured a different aspect of the difference between animal models and dyslipidemic humans.

RESULTS

Basal plasma lipid levels and lipid distributions in dyslipidemic human and various animal models

The LDL-c, TC, and TG levels of the dyslipidemic humans we studied here were borderline high (154 ± 7 mg/dl, 226 ± 6 mg/dl, and 154 ± 15 mg/dl respectively) (**Table 1**) (26). This group had a fairly balanced lipid profile, with HDL-c being about 30% of LDL-c (48 ± 4 mg/dl). A representative FPLC trace of a plasma lipid profile is shown in **Fig. 1**. VLDL was not measured, but the normal range is typically ≤30 mg/dl in populations with TG levels under 150 mg/dl (26).

Based on plasma lipid parameters and lipoprotein traces, several animal models carried the majority of plasma cholesterol on non-HDL lipoproteins (i.e., VLDL and LDL) similar to dyslipidemic humans: NHPs, rabbits on cholesterol diet, $ApoE^{-/-}$ mice on chow or cholesterol diet, $LDLr^{-/-}$ mice on chow or cholesterol diet, and $CETP^{+/-}/LDLr^{-/-}$ mice on chow or cholesterol diet (Table 1 and Fig. 1). Among these animal models, the greatest similarities to humans were observed in NHPs. The AGM and cynomolgus studied here were young and healthy animals, and their plasma TC, LDL-c, HDL-c, and TG levels all fell into the normal range of healthy humans (Table 1) (26). Diseased NHP models, including dyslipidemic AGM, rhesus with metabolic syndrome, diabetic rhesus and rhesus with DIO all had characteristics of dyslipidemia in human populations, with either high TC, LDL-c, or both, or high TG levels, whereas HDL-c remained stable (Table 1). The exception among NHP models was the marmoset, which, although healthy animals, exhibited abnormally high TG with big variations (Table 1 and Fig. 1).

New Zealand white rabbits on chow diet had extremely low TC, VLDL-c, LDL-c, and HDL-c levels (29 ± 1 mg/dl, 4 ± 0.3 mg/dl, 8 ± 0.4 mg/dl, and 18 ± 1 mg/dl, respectively). Upon the challenge of 0.5% cholesterol in their diet, the plasma TC, VLDL-c, and LDL-c levels all dramatically increased to 811 ± 48 mg/dl, 439 ± 26 mg/dl, and 317 ± 24 mg/dl, respectively, with the majority of plasma cholesterol carried in the VLDL fraction, whereas HDL-c remained low (Table 1 and Fig. 1). $ApoE^{-/-}$ mice had high TC (412 ± 21 mg/dl), VLDL-c (226 ± 17 mg/dl), and LDL-c (178 ± 13 mg/dl), and very low HDL-c (8 ± 1 mg/dl) when on a normal chow diet; a high-fat Western diet significantly increased TC (629 mg/dl), LDL-c (402 mg/dl), and TG (154 ± 31 mg/dl), with minimal change in VLDL-c and HDL-c (205 and 22 mg/dl, respectively). The $LDLr^{-/-}$ mouse is considered a model of human familial hypercholesterolemia caused by mutations of *LDLR*. Unlike the $ApoE^{-/-}$ mouse model, $LDLr^{-/-}$ mice had only mildly elevated TC (250 ± 8 mg/dl), LDL-c (168 ± 5 mg/dl), and TG (124 ± 8 mg/dl), and they had normal VLDL-c and HDL-c (13 ± 1 and 69 ± 3 mg/dl, respectively) on chow diet. However on a low-fat Western diet, they developed extremely high TC (1,677 ± 98 mg/dl), VLDL-c (904 ± 47 mg/dl), and LDL-c (761 ± 57 mg/dl), as well as high TG (404 ± 35 mg/dl), whereas HDL-c decreased dramatically (Table 1). The $CETP^{+/-}/LDLr^{-/-}$-heterozygous mouse is a more recently developed model that was intended to mimic human lipoprotein distribution by introducing a single copy of the CETP gene along with deletion of *LDLr* (17). On normal chow, these mice had TC, VLDL-c, LDL-c, HDL-c, and TG within the lower range of normal human, and a cholesterol diet pushed their TC and LDL-c levels to borderline high (Table 1). All three mouse models above had significantly decreased HDL-c levels compared with wild-type C57BL/6 mice.

In contrast to the animals described above, the pig (Gottingen mini-pig), dog (obese beagle), Golden Syrian hamster on chow or HFD (high-fat diet), ZDF heterozygous (ZDF/+) rat, ZDF rat on an HFD, C57BL/6 and db/db mouse models all carried the majority of plasma cholesterol on HDL particles and presented athero-protective profiles (Fig. 1). The pig model had HDL-c comparable to that of healthy humans, with plasma TC, TG, and LDL-c in the low end of normal range (Table 1). The obese beagle model had a borderline high TC level (196 ± mg/dl) but the highest HDL-c level (163 ± 3 mg/dl) across all animal species, presenting the most-athero-protective lipid profile among all models studied here, because high HDL-c level is associated with reduced risk for CHD (26). The hamster on chow diet, C57BL/6, and db/db mouse models had HDL-c accounting for about 70% of TC (Table 1 and Fig. 1). An HFD induced an increase in TG levels in both hamster and ZDF rat, but to different extents: with a relatively high TG level on a chow diet, the HFD-fed hamster had a ~2.4-fold increase in TG (534 ± 43 mg/dl), whereas the ZDF rat on an HFD had a more than 17-fold increase of TG (1,482 ± 210 mg/dl).

Acute-phase-reactant CRP has been identified as a marker of inflammation; however, there are debates about whether CRP is a predictor of cardiovascular events (27, 28). The range of CRP in normal humans is 0.1–4.8 μg/ml (29). NHPs, dog, and rabbit on chow all fell at the low end of this range, whereas mouse models were at the high end. Marmoset, pig, rabbit on cholesterol diet, and hamster on both chow and HFD had very low CRP levels. Extremely high CRP levels were observed in ZDF rat models (Table 1).

FA composition analysis for three major biosynthetic plasma lipid fractions across models

Major plasma lipid fractions and the diversity of lipid molecular species in humans have been summarized

TABLE 1. Basal plasma lipid comparison across species

Species	N in each group	CETP	TG (mg/dL)	TC (mg/dL)	VLDL (mg/dL)	LDL-c (mg/dL)	HDL-c (mg/dL)	CRP (ug/ml)	
Dyslipidemic human	15	Yes	154 ± 15	226 ± 6	NA	154 ± 7	48 ± 4	0.1−8.6[29]	
Dyslipidemic African Green	6	Yes	52 ± 10	336 ± 51	33 ± 10	210 ± 36	92 ± 7	Not available	
African Green	10	Yes	54 ± 3	134 ± 5	4 ± 1	71 ± 3	59 ± 4	1.0 ± 0.5[a]	1.7 ± 0.5[b]
Cynomolgus	10	Yes	60 ± 8	139 ± 13	5 ± 2	66 ± 9	67 ± 5	0.6 ± 0.1[a]	0.3 ± 0.1[b]
Rhesus	12	Yes	42 ± 3	120 ± 5	4 ± 1	59 ± 3	58 ± 5	0.5–0.9[68]	
Rhesus, Met syn	10	Yes	118 ± 13	159 ± 12	9 ± 1	85 ± 7	65 ± 7	0.24 ± 0.03[a]	0.23 ± 0.03[b]
Rhesus, diabetic	5	Yes	192 ± 35	194 ± 18	22 ± 6	99 ± 7	62 ± 11	0.9 ± 0.1[a]	0.24 ± 0.04[b]
Rhesus DIO	12	Yes	179 ± 31	131 ± 13	12 ± 1	68 ± 9	51 ± 7	1.1 ± 0.3[a]	0.3 ± 0.1[b]
Marmoset	12	Yes	363 ± 134	161 ± 16	25 ± 11	77 ± 9	58 ± 10	Not detectable	
Pig	20	No	32 ± 2	96 ± 7	5 ± 0.4	39 ± 4	52 ± 3	0.0075 ± 0.002[a]	
Dog	10	Yes	98 ± 15	196 ± 6	7 ± 2	26 ± 3	163 ± 3	1.5 ± 0.3[c]	
Rabbit	20	Yes	47 ± 5	29 ± 1	4 ± 0.3	8 ± 0.4	18 ± 1	0.94 ± 0.07[c]	
Rabbit (Chol)	20	Yes	48 ± 4	811 ± 48	439 ± 26	317 ± 24	44 ± 4	Not detectable	
Hamster	10	Yes	226 ± 11	141 ± 7	16 ± 1	31 ± 2	94 ± 6	Not detectable	
Hamster HFD	10	Yes	534 ± 43	184 ± 4	35 ± 2	39 ± 3	110 ± 3	Not detectable	
ZDF/+ rat	6	No	85 ± 2	98 ± 4	10 ± 0.3	36 ± 3	53 ± 1	674 ± 88[c]	
ZDF rat HFD	6	No	1482 ± 210	148 ± 8	62 ± 7	20 ± 2	66 ± 2	1252 ± 44[c]	
C57BL/6	12	No	130 ± 10	127 ± 4	8 ± 1	21 ± 2	97 ± 4	10 ± 0.3[c]	
db/db	12	No	308 ± 16	170 ± 5	13±-1	30 ± 2	126 ± 4	16 ± 1[c]	
$ApoE^{-/-}$	10	No	102 ± 14	412 ± 21	226 ± 17	178 ± 13	8 ± 1	7 ± 0.3[c]	
$ApoE^{-/-}$ (Chol)	10	No	154 ± 31	629	205	402	22	9 ± 0.3[c]	
$LDLr^{-/-}$	9	No	124 ± 8	250 ± 8	13 ± 1	168 ± 5	69 ± 3	7 ± 1[c]	
$LDLr^{-/-}$ (Chol)	10	No	404 ± 35	1677 ± 98	904 ± 47	761 ± 57	12 ± 2	8 ± 0.4[c]	
$CETP^{+/-}/LDLr^{-/-}$	10	Yes	67 ± 9	89 ± 4	10 ± 2	41 ± 2	37 ± 4	6 ± 0.2[c]	
$CETP^{+/-}/LDLr^{-/-}$ (Chol)	10	Yes	59 ± 6	202 ± 4	51 ± 4	96 ± 4	54 ± 3	7 ± 0.3[C]	

Numbers in parentheses are reference citations.
[a] Measured with human kit by RBM as described in Methods.
[b] measured with BioAnalytical method as described in Methods.
[c] Measured with rodent kit by RBM as described in Methods.

previously (30). Our lipidomic study covered most of the major plasma lipid fractions, including CE, TG, DAG, FFA, PC, PE, LPC, and FC. Among these fractions, CE, TG, and PC are the three most abundant, accounting for more than 75% of total plasma lipids (30). FAs identified in plasma lipids can also be divided into three major categories: the nonessential FAs, which include saturated FAs (SFAs)and MUFAs; the linolenic acid (omega-3) pathway, which includes linolenic acid and its derivatives; and the linoleic acid (omega-6) pathway, which includes linoleic acid and its derivatives. The source of nonessential FAs can be either from de novo lipogenesis (DNL) or from diet, whereas FAs within the omega-3 or omega-6 pathways can only originate from two essential FAs obtained from diet (**Fig. 2A**).

FAs within the CE, TG, or PC fractions were combined according to their synthetic pathways, and compared both quantitatively and qualitatively across animal species (Fig. 2B, C, D). In dyslipidemic humans, the mean absolute and relative amounts of FAs in plasma CE were 987 ± 50 nmol/g from the nonessential FAs (about 34% of total FAs), 58 ± 5 nmol/g from the omega-3 pathway (about 2% of total), and 1,866 ± 63 nmol/g from the omega-6 pathway (about 64% of total) (Fig. 2B). In plasma TG of dyslipidemic humans, there was 2,780 ± 197 nmol/g from the nonessential FAs (about 73% of total), 48 ± 9 nmol/g from omega-3 (about 3% of total), and 903 ± 60 nmol/g from omega-6 (about 24% of total) (Fig. 2C). In plasma PC of dyslipidemic humans, there were 2,009 ± 80 nmol/g (about 54% of total), 190 ± 28 nmol/g (about 5% of total), and 1,486 ± 52 nmol/g (about 40% of total) from the nonessential FAs, omega-3, and omega-6 pathways, respectively (Fig. 2D). Thus in plasma CE, TG, and PC of dyslipidemic humans, over 95% of FAs were from the nonessential FAs or omega-6 pathways. While the omega-6 pathway was the main source of FAs in plasma CE, the nonessential FAs provided the majority of FAs in TG and PC. Across all the animal species, the contribution of FAs from the omega-3 pathway was consistently small, with the highest percentage being 8% in CE (db/db mice), 7% in TG ($ApoE^{-/-}$ mice), and 9% in PC (rhesus with metabolic syndrome) (Fig. 2B, C, D).

The animal species most similar to dyslipidemic humans in terms of the FA composition of plasma CE were: all NHP models, pig, dog, hamster, ZDF rat, C57BL/6, db/db, $LDLr^{-/-}$, and $CETP^{+/-}/LDLr^{-/-}$ mice. Both the absolute amounts and the relative distribution of FAs in plasma CE in these animal species were similar to dyslipidemic humans, with omega-6 being the largest component (Fig. 2B). ZDF rat, C57BL/6, and db/db mice had an even greater percentage of FAs from the omega-6 pathway (over 75%). A HFD in hamster or ZDF rat did not impact either the absolute amount or the percentage of FAs from the omega-6 pathway. In $LDLr^{-/-}$ and $CETP^{+/-}/LDLr^{-/-}$ mice, however, a cholesterol-containing diet not only increased the absolute amount of FAs in CE (with a much more significant impact on $LDLr^{-/-}$ mice), it also shifted the composition of FAs from the omega-6 to the nonessential FAs

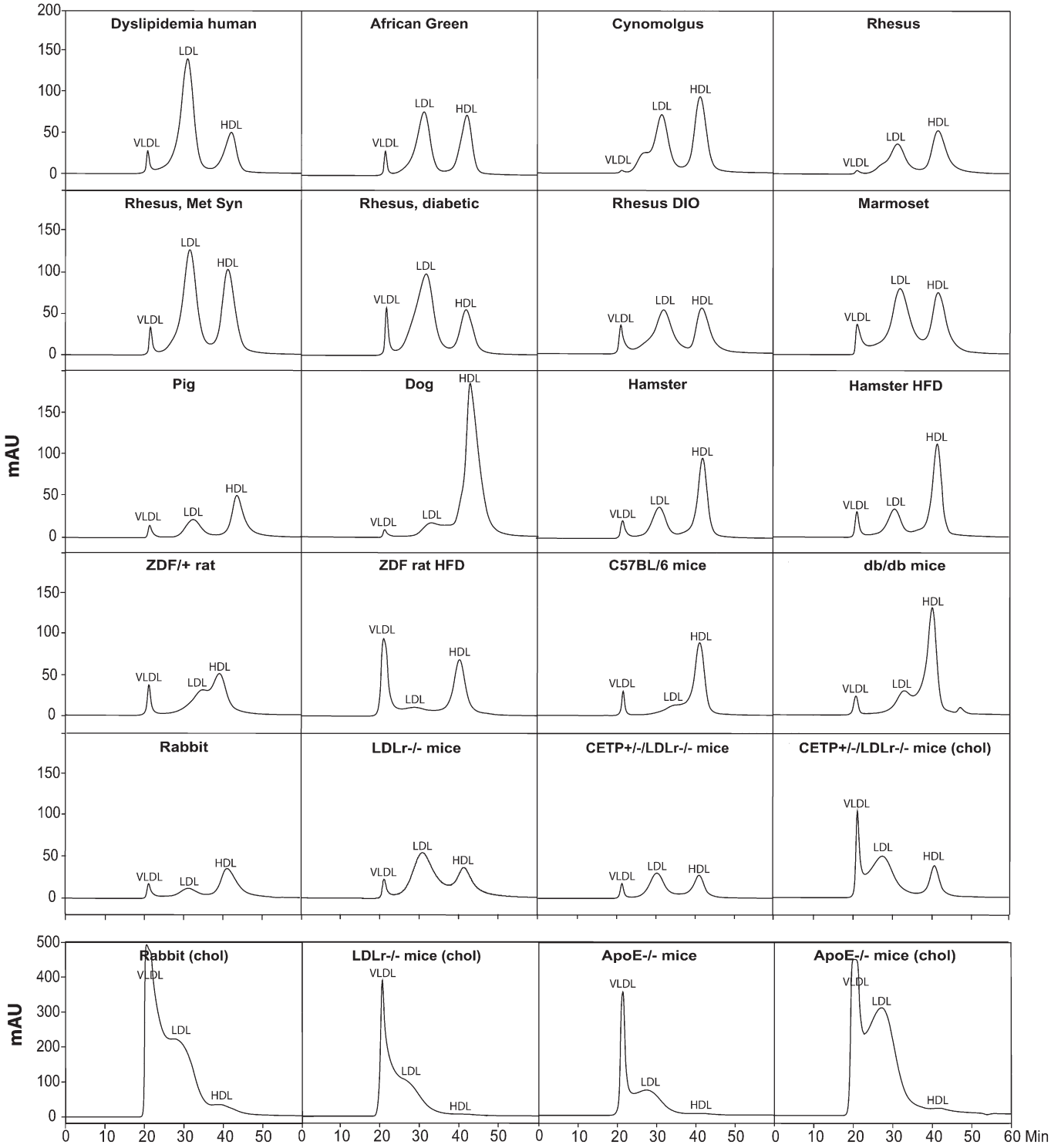

Fig. 1. Representative FPLC traces of plasma lipoprotein cholesterol levels of dyslipidemic human and various animal models. Three peaks representing VLDL, LDL, and HDL are labeled in each panel. Except in the four panels in the bottom, the Y-axis in each panel was adjusted to the same level for easier comparison.

(Fig. 2B). Rabbit and *ApoE*$^{-/-}$ mice were the animal species least comparable to dyslipidemic humans, because the nonessential FAs count for the majority of CE FAs under a chow diet condition. A cholesterol-containing diet not only dramatically increased the total amount of CE FAs, it also pushed the percentage of the nonessential FAs even higher (Fig. 2B).

The nonessential FAs were the major source for plasma TG for all animal species. However, a significant impact of diet was seen in rabbit, hamster, ZDF rat, and *ApoE*$^{-/-}$, *LDLr*$^{-/-}$, and *CETP*$^{+/-}$/*LDLr*$^{-/-}$ mice. A HFD in hamster and ZDF rat increased both the absolute amount of the nonessential FAs (with a greater increase in ZDF rat) and the percentage of the nonessential FAs in plasma TG.

A cholesterol-containing diet had little impact on total TG in rabbit, *ApoE*$^{-/-}$, and *CETP*$^{+/-}$/*LDLr*$^{-/-}$ mice, although the composition of FAs shifted toward the nonessential FAs in *ApoE*$^{-/-}$ and CETP$^{+/-}$/*LDLr*$^{-/-}$ mice (Fig. 2C). A cholesterol-containing diet in *LDLr*$^{-/-}$ mice increased both the absolute amount and the relative contribution of the nonessential FAs (Fig. 2C).

Compared with plasma CE and TG, the total amount of FAs in PC varied less, and the FA composition remained much more stable across animal species and dietary conditions About 50–60% of the total came from the nonessential FAs and 30–40% from the omega-6 pathway for all the animal species. A HFD or cholesterol-containing diet increased total FAs, but did not substantially change the FA composition of PC in rabbit, hamster, ZDF rat, *ApoE*$^{-/-}$, *LDLr*$^{-/-}$, and *CETP*$^{+/-}$/*LDLr*$^{-/-}$ mice, although slight increases in the percentage of the nonessential FAs were seen in *ApoE*$^{-/-}$, *LDLr*$^{-/-}$ and *CETP*$^{+/-}$/*LDLr*$^{-/-}$ mice (Fig. 2D).

Amounts of eight major circulating lipid fractions in various animal models compared with dyslipidemic humans

To get a more comprehensive understanding of the similarities and differences of different animal models compared with dyslipidemic humans, the amounts of eight major circulating lipid fractions (CE, TG, DAG, FFA, PC, PE, LPC, and FC) in all the animal models were measured and compared with dyslipidemic humans. For any given lipid fraction, first the mean for that lipid fraction was calculated in each animal species, then the difference was compared with the mean of the same lipid fraction in dyslipidemic humans. The differences were weighted based on the proportion of that lipid fraction over total lipid in dyslipidemic humans so that abundant lipid fractions (such as CE, TG, and PC) contributed more than did less-abundant lipid fractions in the comparison. The animal models were then sorted by distance (**Fig. 3**). Most NHP models were relatively close to dyslipidemic humans, with the rhesus metabolic syndrome model having the greatest similarity. Although dietary manipulation is most commonly used in generating dyslipidemic or atherosclerotic models, in most non-NHP models, such as *LDLr*$^{-/-}$ mice, *ApoE*$^{-/-}$ mice, ZDF rat, rabbit, and hamster, the similarity between these animal species and dyslipidemic humans with respect to the distribution of the eight major lipid fractions actually decreased with a cholesterol-containing diet or HFD, with most significant changes seen in *LDLr*$^{-/-}$ mice, hamster, and ZDF rat (Fig. 3). *CETP*$^{+/-}$/*LDLr*$^{-/-}$ mice on a cholesterol-containing diet is the only model that showed closer similarity to dyslipidemic human than the mice on chow diet. It also showed the closest similarity to dyslipidemic human among all NHP models (Fig. 3). The mean values of each lipid fraction in each animal model are also shown in supplementary Table III.

Impact of simvastatin treatment on plasma lipid profiles

To further understand the utility of different animal models for identification of new targets in drug discovery, the response of plasma lipid profiles in various animal models to simvastatin treatment was determined. Percent changes in plasma lipids (TC, VLDL-c, LDL-c, HDL-c, and TG) upon simvastatin treatment and their statistical significance are shown in **Table 2**. Consistent with the literature, statin treatment caused a significant decrease in plasma TC and LDL-c level in the dyslipidemic humans studied here (16% and 24% decrease, respectively, upon 40 mg/day simvastatin treatment for 2 weeks) (31). Statin treatment also has been shown to decrease plasma VLDL-c and TG and increase HDL-c in a subset of patients (31, 32). Here, simvastatin treatment significantly decreased plasma TG (−18%), but the effect on HDL-c was minimal (+1%).

Similar to dyslipidemic humans, a greater than 15% decrease in TC and 20% decrease in LDL-c were seen in all the NHP models, with dyslipidemic AGM being the most-responsive model to simvastatin treatment (Table 2). Decreases of VLDL-c were seen in most NHP models except rhesus with metabolic syndrome. Among the nonprimates, dog was the only species that showed responses of plasma TC, VLDL-c, and LDL-c (−34%, −43%, and −80% respectively). Significant decreases in plasma TC and VLDL-c were seen in rabbit on cholesterol, hamster on a HFD, and ZDF rat on a HFD; however, no decrease was seen in LDL-c. In ZDF rat on a HFD, statin treatment increased LDL-c more than 2-fold (+112% increase). All mouse models had no change or increases in plasma TC, VLDL-c, and LDL-c upon simvastatin treatment.

Changes in plasma TG levels varied among NHP models, with AGM and cynomolgus having the most-significant decrease, similar to dyslipidemic humans, and no decreases in plasma TG in dyslipidemic AGM, rhesus with metabolic syndrome, and marmoset. In nonprimate species, dog, hamster on a HFD, ZDF rat on a HFD, and db/db mice showed significant decreases in plasma TG upon simvastatin treatment. Similar to our observation in dyslipidemic humans, no significant increase in HDL-c was seen in any animal species, including NHP models (Table 2).

Changes in the FA composition of plasma lipids (CE, TG, and PC) upon statin treatment

To obtain more-detailed insight into the similarities and differences of various animal models to dyslipidemic humans in statin responsiveness, statin-induced changes in SFAs, MUFAs, omega-3 pathway-derived FAs, and omega-6 pathway-derived FAs in plasma CE, TG, and PC upon simvastatin treatment were calculated (**Fig. 4**). In dyslipidemic humans, significant decreases were seen in CE and TG FAs across all four categories (except MUFAs in CE), along with a consistent but not statistically significant decrease in PC FAs. AGM, cynomolgus, and hamster on HFD were the animal models closest to dyslipidemic humans in overall responsiveness on CE, TG, and PC (Fig. 4). Three diseased rhesus models (rhesus with metabolic syndrome, diabetic rhesus, and rhesus with DIO) showed responses in plasma CE and PC to simvastatin treatment that were similar to dyslipidemic humans; however, changes in plasma TG were not significant, due to the large biological variation.

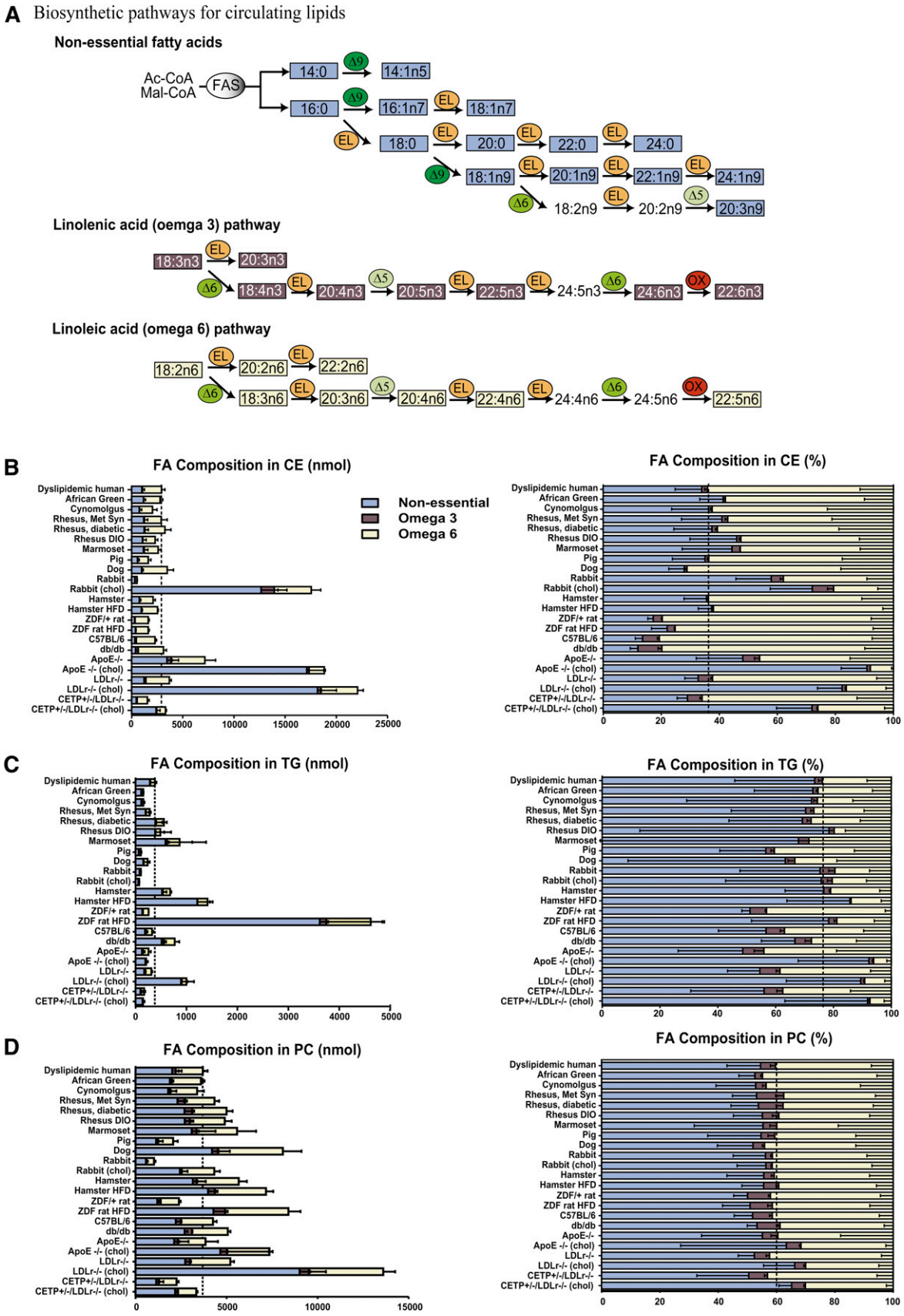

Fig. 2. FA composition in plasma CEs, TGs, and PCs across animal species. A: Schematic showing FAs within three categories: nonessential FAs (light blue), omega-3 (maroon) pathway FAs, and omega-6 (light yellow) pathway FAs. Highlighted FAs were measured in this study. B: Measured FAs were summed for each pathway. Left panel shows the absolute amounts (nmol) of FAs in each pathway and right panel shows the relative amounts (%) for plasma CEs, (C) TGs, and (D) PCs.

Marmosets did not show any statistically significant changes across CE, TG, and PC in any FA categories, despite significantly decreased plasma TC upon simvastatin treatment (Fig. 4 and Table 2).

Hamster on HFD, dog, and rabbit were the only nonprimate species that showed decreased FAs in all four categories of CE upon simvastatin treatment. $CETP^{+/-}/LDLr^{-/-}$ mice on a cholesterol-containing diet showed decreased omega-3 and omega-6 pathway related FAs, but increased SFAs. ZDF rat on HFD and all the other mouse models either had no change or had increases in the majority of FA categories in plasma CE (Fig. 4). For plasma TG, ZDF rat on HFD, db/db, and $ApoE^{-/-}$ mice showed similar response in FA composition compared with dyslipidemic humans. Three atherogenic-diet animal models, rabbit on cholesterol-containing diet, $ApoE^{-/-}$ mice on cholesterol-containing diet, and $CETP^{+/-}/LDLr^{-/-}$ mice on cholesterol-containing diet, showed increases in each component of TG upon simvastatin treatment. A trend toward a small decrease, either significant or nonsignificant, was seen in most of the animal models, except dog, rabbit on cholesterol-containing diet, and ZDF rat on a HFD.

To statistically compare the responsiveness to statin treatment between each animal model to dyslipidemic human and obtain an overall ranking of the similarity, we calculated the distance between the set of 12 log ratios in Fig. 4 for dyslipidemic humans and each of the animal models. Three distance measures were used, each reflecting a different aspect of the difference between sets of log ratios. Euclidean method measures the "ordinary" distance which reflects the magnitude of statin responsiveness in each animal model. Variance-based Euclidean method also considers the variation of each FA within the same category, so categories with high variability were downweighted. Uncentered correlation-based (1-Cosine distance) method assesses only the pattern of statin responsiveness across FA categories (**Fig. 5**; see *Methods* for more detailed description). Rhesus DIO, cynomolgus, AGM, hamster on HFD, and db/db mouse models were closest to dyslipidemic humans in their responses to simvastatin treatment, followed by rhesus with metabolic syndrome, dog, marmoset, and diabetic rhesus. Those models showed close distances to dyslipidemic human in all three measures. $LDLr^{-/-}$ mice on cholesterol-containing diet, $CETP^{+/-}/LDLr^{-/-}$ mice on chow or cholesterol-containing diet, and $ApoE^{-/-}$ mice on chow or cholesterol-containing diet had large cosine-based distances from dyslipidemic humans, indicating that the patterns of their response in FA composition of plasma

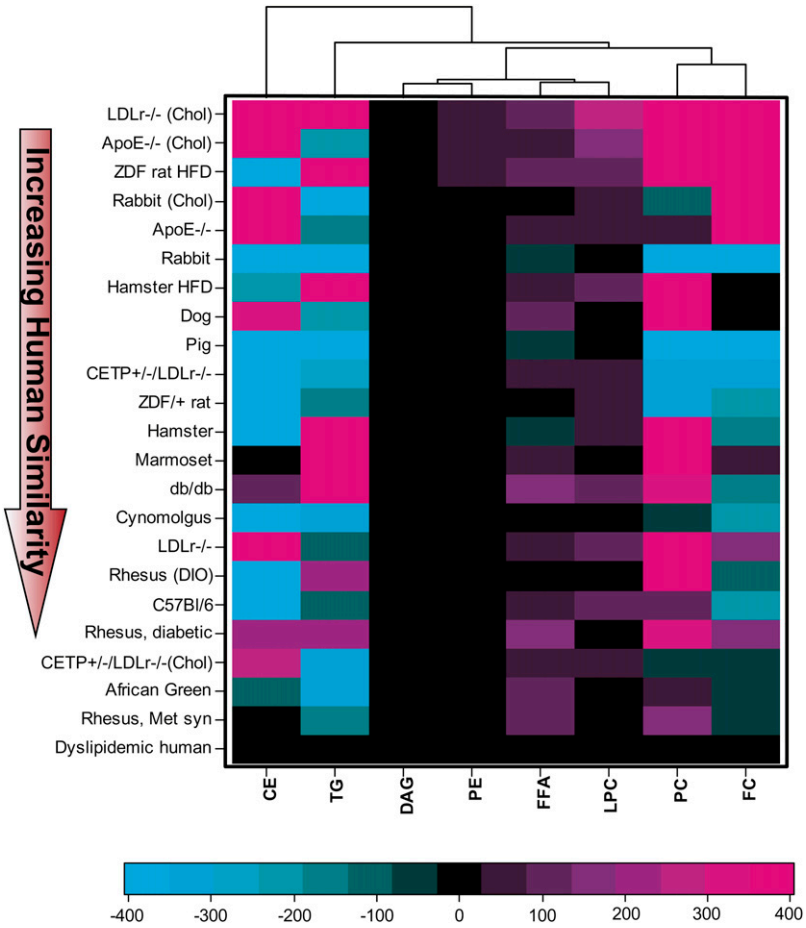

Fig. 3. Dendrogram comparison of baseline plasma lipid similarity based on eight major circulating lipid fractions (CE, TG, DAG, PE, FFA, LPC, PC, and FC) across species. The difference of any given lipid fraction between the means of each animal model and dyslipidemic human was calculated and weighted according to the proportion the same lipid fraction over total lipid in humans. The overall weighted distance of each animal model from dyslipidemic humans was calculated, and the models were sorted by distance (see METHODS for a more-detailed description).

CE, TG, and PC were very different from dyslipidemic humans (Fig. 5). In ZDF rat on a HFD the magnitudes of the simvastatin effects were very different from dyslipidemic humans, shown by the large values of the Euclidean and variance-weighted distances. Simvastatin effects in rabbit on a cholesterol-containing diet were unlike dyslipidemic humans by all three distance measures (Fig. 5).

In summary, considering basal levels and composition of plasma lipids and their response to simvastatin treatment, the NHP models, including two AGM models, cynomolgus, and three rhesus models, were the species most similar to dyslipidemic humans. They were thus the best models among those tested for both studying disease mechanism and validation of potential drug targets for dyslipidemia. The exception among the NHPs was the marmoset. Marmosets were the only new world monkeys in our study, and they are much smaller in size than the other NHPs. In addition, in our study, marmosets exhibited extremely elevated cortisol levels (data not shown), which may account for the high variability seen across numerous endpoints (33). The dog displayed significant similarities to dyslipidemic humans, despite being an HDL-dominated animal species, especially in response to statin treatment, supporting the use of the dog model in studies of many aspects of dyslipidemia, in particular LDL lowering. Data from the hamster on a HFD and db/db mouse models suggested that they can be used as models for the study of hypertriglyceridemia. Most of the commonly used dyslipidemic or atherosclerotic animal models, such as $ApoE^{-/-}$ mice on a chow or cholesterol-containing diet, $LDLr^{-/-}$ mice on a cholesterol-containing diet, rabbit on a cholesterol-containing diet, and ZDF rat on a HFD, however, fell out of the range of dyslipidemic humans and demonstrated the least similarity of all models, raising concern about relating experimental data from those models to dyslipidemic humans.

DISCUSSION

Lipid metabolism is a dynamic process that involves multiple pathways, with both intracellular and extracellular regulation and modulation. Although the cross-talk and interactions among these pathways are essential to maintaining lipid homeostasis, disregulation of each pathway can contribute substantially to the development of dyslipidemia. Management of dyslipidemia (with plasma LDL-c over 130 mg/dl and TC over 200 mg/dl) with first-line medications such as statins has been shown to decrease risk for CHD by epidemiological studies. Hypertriglyceridemia (with plasma TG over 200 mg/dl) is also linked to CHD risk, and management of non-HDL-c levels in those patients has been proposed as secondary prevention (34). Considering those risk factors as well as responsiveness of lipid profiles to treatment with statins, we compared plasma lipid profiles under basal and simvastatin-treated conditions for various animal models to dyslipidemic humans. Our goal was to identify the optimal model(s) for validating new targets for treatment of dyslipidemia in humans.

Suitable preclinical models for dyslipidemia with elevated plasma LDL-c

At baseline, the dyslipidemic human subjects studied here had borderline high plasma TC and LDL-c, and a balanced LDL-c-to-HDL-c ratio, which are representative of the majority of dyslipidemic patients with elevated CVD risk. Looking across the 24 nondiseased or diseased animal models in this study, the NHP models, especially the diseased NHP models such as dyslipidemic AGM and three diseased rhesus models, were the most similar to humans with respect to basal plasma TC, ratio of LDL-c to HDL-c, and lipoprotein traces. Also similar to humans, in NHP models, the majority of FAs within plasma CE were linoleic acid and its derivatives (the omega-6 pathway), and the

TABLE 2. Comparison of changes on plasma lipids upon simvastatin treatment across the species

		Change of lipids upon statin treatment				
Species	Statin respsonsiveness	TC	VLDL-c	LDL-c	HDL-c	TG
Dyslipidemic human	Yes	−16%[b]	NA	−24%[b]	1% NS	−18%[a]
Dyslipidemia African green	Yes	−67%[b]	−57%[b]	−69%[b]	−74%[b]	23% NS
African Green	Yes	−36%[c]	−36% NS	−51%[b]	−18%[b]	−14%[b]
Cynomolgus	Yes	−25%[b]	−55%[a]	−22%[a]	−26%[b]	−19%[a]
Rhesus, Met syn	Yes	−16%NS	3% NS	−22%[c]	−11% NS	17% NS
Rhesus, diabetic	Yes	−24%[b]	−33% NS	−23% NS	−7% NS	−15% NS
Rhesus DIO	Yes	−15%NS	−24% NS	−26% NS	2% NS	−17% NS
Marmoset	Yes	−29%[a]	−66% NS	−25% NS	−19% NS	1% NS
Dog	Yes	−34%[b]	−43% NS	−80%[b]	−26%[b]	−39%[a]
Rabbit (Chol)	No	−46%[b]	−76%[b]	−7% NS	6% NS	15% NS
Hamster HFD	No	−8%[a]	−33%[b]	7% NS	−5% NS	−25%[a]
ZDF rat HFD	No	−28%[b]	−51%[b]	112%[b]	−50%[b]	−76%[b]
db/db	No	−4% NS	−4% NS	19% NS	−10%[a]	−30%[b]
$ApoE^{-/-}$	No	59%[b]	89%[b]	25% NS	−19% NS	−31% NS
$LDLr^{-/-}$ (Chol)	No	14% NS	19% NS	7% NS	−32% NS	−7% NS
$CETP^{+/-}/LDLr^{-/-}$	No	13% NS	25% NS	53%[b]	−34%[b]	−8% NS
$CETP^{+/-}/LDLr^{-/-}$(Chol)	No	7% NS	20% NS	12% NS	−13% NS	12% NS

NA, not available; NS, not significant.
[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$ by paired or unpaired *t*-test as indicated in Methods.

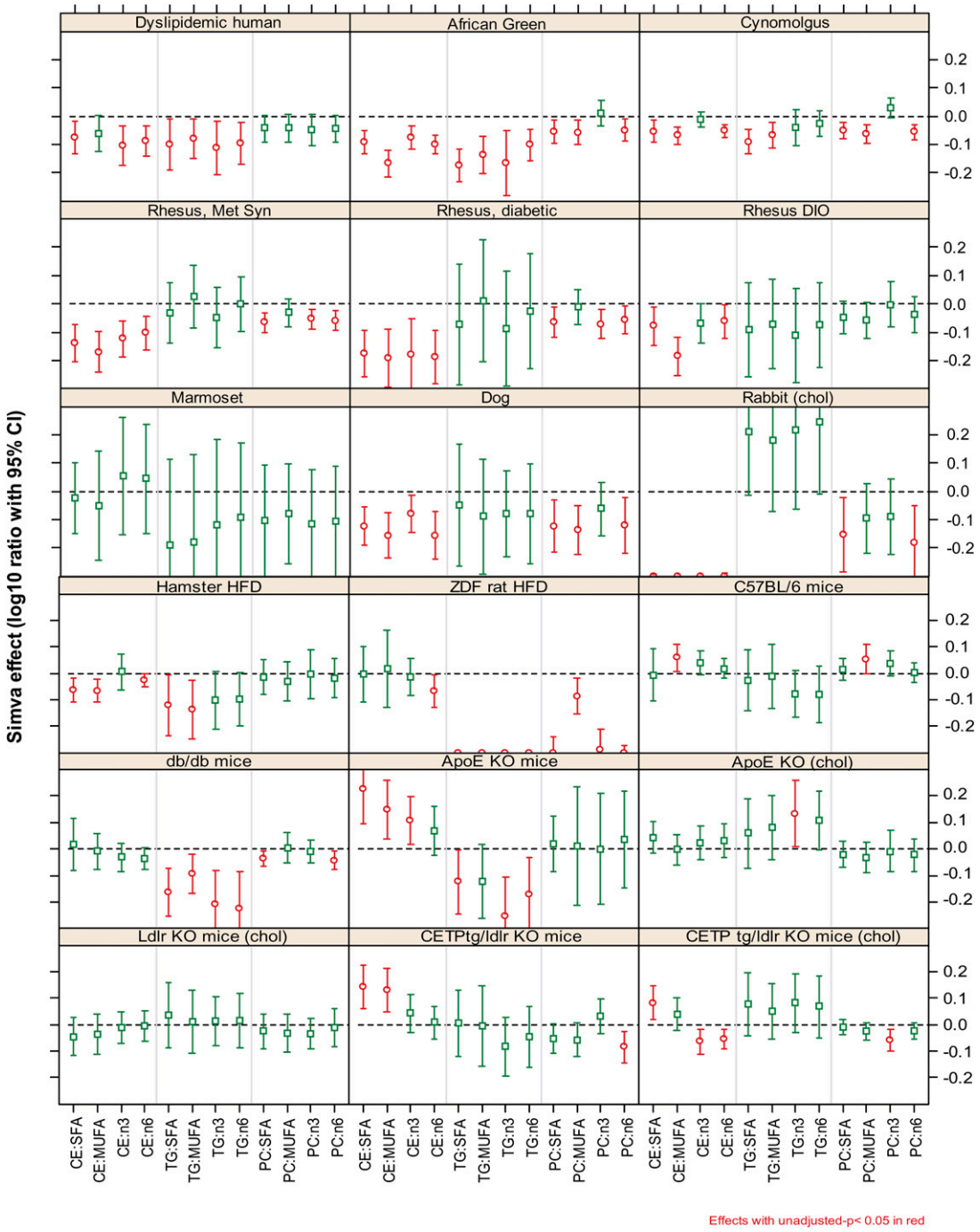

Fig. 4. Simvastatin effect on FA composition in plasma CEs, TGs, and PCs in dyslipidemic humans and various animal models. FAs were summed into four categories: SFA, MUFA, omega-3, and omega-6. The effect of simvastatin treatment on each category was estimated, and is shown as a $\log_{10}$ ratio with 95% confidence interval. Statistically significant effects ($P < 0.05$) are highlighted in red.

vast majority of FAs in TG were SFAs and MUFAs. Statin treatment lowered plasma TC and LDL-c levels in NHP models to a similar extent as in dyslipidemic humans, as well as decreasing FAs from all three categories (the non-essential FAs from DNL or diet, FAs from omega-3 pathway, and FAs from omega-6 pathway). By these parameters, NHP models are the best preclinical models for dyslipidemia for both mechanistic studies and target validation. This is not surprising, because, phylogenetically, NHPs are the closest mammals to humans. They also have similar omnivorous dietary preferences, as well as similar activity and substrate specificity for key plasma lipid enzymes, such as CETP and LCAT (13, 35). Although CETP directly determines cholesterol content distribution within plasma lipoproteins by transferring CE from HDL to LDL, LCAT and ACATs are responsible for the conversion of FC to CE. LCAT takes FAs from the *sn*-2 position of PC (which contains mostly PUFAs) and converts FC to CE, whereas PC is converted to LPC in plasma during HDL biogenesis (36). ACATs add FFAs (MUFAs from the DNL pathway) from

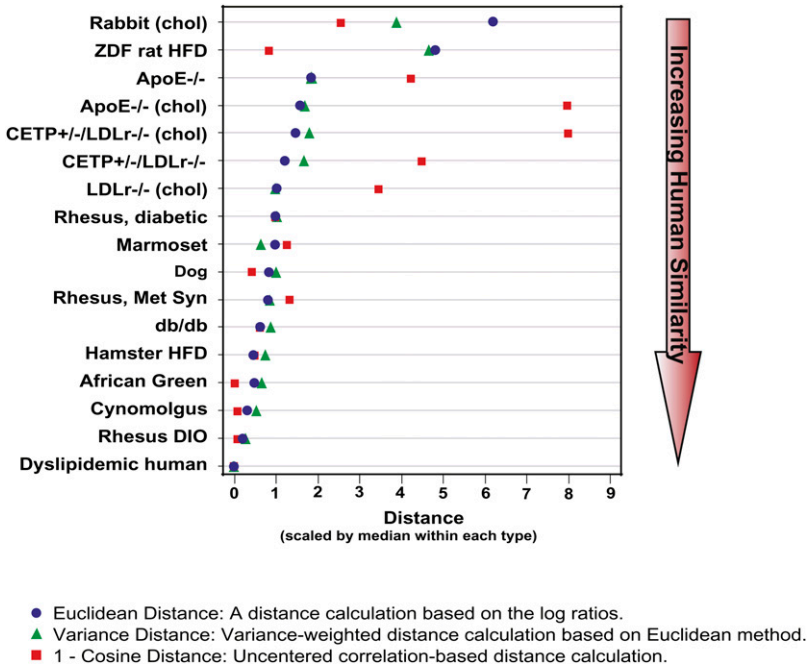

Fig. 5. Distance comparison from various animal models to dyslipidemic humans with respect to the responsiveness to simvastatin treatment on plasma CE, PC, and TG. Three distance measures, each representing a different aspect of the difference on the comparison, are shown: Euclidean (purple solid circle), a distance calculation based on the log ratios from Fig. 4, which represents the similarity based on the magnitude of responsiveness; variance-weighted Euclidean (green solid triangle), which represents also the Euclidean comparison, but with the contribution of the variability of the responses weighted, so the FA categories with high variability were down-weighted; uncentered-correlation-based distance (1-cosine distance, red solid square), which represents the similarity based on pattern of statin responsiveness.

either exogenous dietary sources or endogenous lipogenesis to FC (37). Although activation of LCAT increases athero-protective HDL-c levels, ACAT-derived CE is the predominant atherogenic lipid in blood (38). The fact that the majority of FAs in plasma CE in humans and NHPs are from the omega-6 pathway suggests that LCAT could be the major enzyme for CE biosynthesis in these species.

The Gottingen mini-pig and obese beagle models in this study showed omega-6 pathway-enriched FA composition in plasma CE, and nonessential FAs enriched FA composition in plasma TG, similar to humans. However, their lipoprotein profiles were distinctly different. In pigs and dogs, CETP activity is either very low or not detectable (39, 40). It has been shown that CETP expression can be upregulated by an atherogenic diet in humans and rabbits; whether the same might be true in pigs or dogs, or to what extent the increase contributes to the susceptibility to CVDs, remains to be studied (41–43). Previous studies showed that under an atherogenic diet, a different pig strain, the Yucatan mini-pig, can develop atherosclerotic plaques, which are similar to human athero-lesions (44). Statin treatment was not examined in the pig model in our study, but has very little effect on plasma TC and LDL-c levels in Yutacan mini-pigs (45). Whether all mini-pigs, or a specific mini-pig model, are suitable for the study of dyslipidemia in humans requires further validation. Although the basal plasma lipid profile is not similar to dyslipidemic human, the dog model showed significant TC and LDL-c lowering upon statin treatment in our study, which was also seen in a previous report (46). Looking at FA composition of CE in the dog model, statin treatment decreased FAs synthesized from all three categories (nonessential FAs, omega-3, and omega-6) with magnitudes similar to those seen in dyslipidemic humans (Fig. 4). Dog also demonstrated close similarity to dyslipidemic human in all three distance analyses looking at simvastatin effects on CE, PC, and TG (Fig. 5). These data suggested that the dog is another useful model for examining the effect of LDL-c-lowering agents for the treatment of hypercholesterolemia.

Rabbits on a normal diet had very low plasma TC and LDL-c levels (~30 mg/dl and 8 mg/dl, respectively), which is due to the very low amount of cholesterol in the normal rabbit diet and the high metabolic clearance rate (47). Upon cholesterol diet (0.5% cholesterol) challenge, there was a 27-fold increase in TC and a 100-fold LDL-c increase, due to a lowered metabolic clearance rate (47). This was one of the most dramatic changes among all the models we analyzed. Unlike dyslipidemic humans, in cholesterol-fed rabbits, over 75% of the FAs were SFAs and MUFAs, suggesting that CE biosynthesis might be via ACATs instead of LCAT. Another major difference of rabbits from humans is the absence of HL, an enzyme that has both TG lipase and phospholipid lipase activity (48). Under a cholesterol-fed condition, absence of HL in rabbit causes massive cholesterol accumulation in chylomicron remnant and β-VLDL lipoprotein particles (49).

Dramatic changes in plasma TC and LDL-c were also seen in cholesterol-fed $ApoE^{-/-}$, $LDLr^{-/-}$, and $CETP^{+/-}/LDLr^{-/-}$

mice. Unlike the same models on chow diets, which have the majority of FAs in plasma CE from the omega-6 pathway, the majority of FAs in CE in these cholesterol-fed models were from the nonessential FAs, consistent with the previous observation that expression of ACATs increases on a cholesterol-containing diet (50).

Statin treatment did not decrease plasma LDL-c levels in any of the rabbit or rodent models. Although hamster on chow and on a HFD had similar FA compositions in plasma CE and TG as human, statin had no effect on LDL-c levels. The data suggest that these models are not suitable for target validation or drug discovery for dyslipidemia with elevated LDL-c.

Representative preclinical models for dyslipidemic populations with other risk factors such as elevated non-HDL cholesterol and TG

Recently, non-HDL cholesterol (including LDL-c and remnant lipoproteins such as VLDL-c and IDL-c) has been proposed to be a better estimate of total atherogenic burden than LDL-c, especially in patients with elevated plasma TGs between 200 and 500 mg/dl (26, 34). Elevated plasma TG levels are also often associated with low HDL-c levels. Although highly variable due to dietary status and often associated with other complications such as type 2 diabetes and metabolic syndrome, elevated TG has been considered another independent risk factor for CHD by a number of studies (34). Among the animal models in our study that had similar omega-6 pathway-enriched FA compositions as humans in plasma CE, many showed elevated VLDL-c and/or TG levels, including several NHP models (dyslipidemic AGM, rhesus with metabolic syndrome, diabetic rhesus, and DIO rhesus), dog, hamster on HFD, and db/db mouse (Table 1) (51, 52). Statin treatment lowered VLDL-c and/or TG levels in all these models (Table 2), suggesting that they are also useful as models for studying plasma remnant lipoprotein and TG metabolism and secondary risk prevention for dyslipidemic humans. Marmosets, as the exception, showed no decrease in TG upon statin treatment and are a suboptimal model due to their sensitivity to stress (data not shown). ZDF rats on a HFD also had elevated TG and demonstrated VLDL-c and TG-lowering upon statin treatment. However their plasma baseline TG level was abnormally high (1,482 ± 210 mg/dl), and the majority of cholesterol resided in less-atherogenic larger VLDL and chylomicron particles. Thus, the lowering of non-HDL levels is less reliable as an indicator of lowered CHD risk (26).

Other than statins, which, in several clinical trials, have confirmed the benefit in patients with atherogenic dyslipidemia (26), fibrates also demonstrated beneficial effects. For example, fenofibric acid alone or in combination with statins has been shown to improve plasma TG, HDL-c, and LDL-c in dyslipidemic humans (53). In other species, fibrates were shown to be effective in lowering plasma TG and cholesterol levels in pig and dog, as well as in species for which statins are not effective and are poorly tolerated, such as hamster, rat, and mouse models (54–56). Other agents for treatment of atherogenic dyslipidemia include nicotinic acid (niacin) alone or in combination with statins, CETP inhibitors, and long-chain omega-3 PUFAs (57, 58). In future studies, it might be useful to evaluate the effects of one or more of these agents, alone or in combination with statins, to provide more information for better assessment of the suitability of different models for target validation in human dyslipidemia.

In the study of atherosclerosis and plaque progression, the most widely used preclinical models are rabbit, *ApoE*$^{-/-}$ mice, and *LDLr*$^{-/-}$ mice on a cholesterol–containing diet, due to their ability to quickly form plaques. Although not studied here, it has been shown that the histology of lesion development in mouse models is similar to that in humans; however, the major limitation of mouse models is that the most-common complications of plaque rupture and superimposed thrombosis (and subsequent acute myocardial infarction and ischemic stroke) rarely occur in mouse models (59). Recently, NHPs, pigs, dogs, and hamsters on atherogenic diets have been used as models of human atherosclerosis (60–62). The hamster model is questioned because no consistent lesion development has been observed in several hamster strains (63). Larger animals, such as dog, pig, and NHPs are more attractive due to the ability to apply interventional procedures and imaging on the larger vessels, making them more suitable as models for clinical practice (59). Future analysis of lipid composition in plaques before and after statin treatment will be helpful to further understand the applicability of those animal models to dyslipidemic humans.

Given the role of phospholipids as precursors of eicosanoid synthesis, their FA composition is of clear interest to model selection for cardiovascular disease research. Figure 2D shows the relative abundance and FA composition of plasma PCs. There is a marked degree of similarity in the latter between the animal species. The ratio of saturated to unsaturated FA in this particular lipid fraction is carefully balanced by de novo synthesis and strongly influenced by remodeling via hydrolysis and reacylation (the Lands cycle). The result is a relatively well-controlled composition in which a saturated FA occupies the *sn*1 position and an unsaturated FA occupies the *sn*2 position (64, 65). The acyl chain specificities for some of the enzymes involved in remodeling of phospholipids have been investigated in several animal species, including rat and pig, and in many cases, were found to have similar preferences for n6 and n3 FAs (66). Given this, the similarity in PC composition between animal species reported here is not surprising. Further complexities involving dietary PCs and their bacterial breakdown products, and correlation with cardiovascular disease risk in humans and in mice, suggest that more-detailed studies of dietary PC and the plasma sequelae in models of interest are warranted.

Inflammation is involved in all phases of atherosclerosis. As a sensitive marker for inflammation, CRP has been proposed as a predictor of cardiovascular risk. The recently completed JUPITER trial found that rosuvastatin reduces vascular events in low-risk older subjects with normal LDL-c levels but elevated high-sensitivity C-reactive protein (hs-CRP), supporting the inclusion of hs-CRP levels among

cardiovascular risk factors (67). However, due to methodology limitations on measuring CRP levels in nonhuman, nonrodent species, the level of CRP failed to improve our understanding of applicability of animal species. The evaluation of CRP as well as other inflammatory markers, and the effect of statins, will be discussed in detail in subsequent manuscripts focusing on specific animal models.

Taken together, our findings indicated that among the animal models we considered, NHPs are in general the best for both mechanistic studies and for target validation of primary and secondary pharmacological treatments for dyslipidemia in humans. The dog model can also be considered for developing pharmacological agents to lower LDL-c. For validation of targets related to secondary CVD risks such as high non-HDL cholesterol and high TG, dog, hamster on HFD, and db/db mice can also be considered. Further profiling of the lipid composition of lipoprotein fractions, plaque, and activated macrophages, as well as responsiveness to additional pharmacological agents targeting dyslipidemia, such as fibrates, niacin, CETP inhibitors, and fish oil, will provide more insights for understanding the applicability of various animal models. Clearly, choice of an animal model requires careful consideration of the specific target or pathway of interest, inasmuch as each model can have common characteristics with humans and can be uniquely useful for addressing questions in dyslipidemia research. The information gathered as more mechanisms and animal models are evaluated out will allow the circular translation from the bench to the clinic and back.

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