Effects of peroxisome proliferator-activated receptor α/δ agonists on HDL-cholesterol in vervet monkeys

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Abstract **The objective of this study was to demonstrate the efficacy of a novel peroxisome proliferator-activated receptor (PPAR) agonist and known PPARα and PPARδ agonists to increase HDL-cholesterol (HDL-C) in the St. Kitts vervet, a nonhuman primate model of atherosclerosis. Four groups (n = 6) were studied and each group was assigned one of the following "treatments": *a*) vehicle only (vehicle); *b*) the PPARδ selective agonist GW501516 (GW); *c*) the PPARα/δ agonist T913659 (T659); and *d*) the PPARα agonist TriCor® (fenofibrate). No statistically significant changes were seen in body weight, total plasma cholesterol, plasma triglycerides, VLDL-C, LDL-C, or apolipoprotein B (apoB) concentrations. Each of the PPARα and PPARδ agonists investigated in this study increased plasma HDL-C, apoA-I, and apoA-II concentrations and increased HDL particle size in St. Kitts vervets. The maximum percentage increase in HDL-C from baseline for each group was as follows: vehicle, 5%; GW, 43%; T659, 43%; and fenofibrate, 20%. Treatment with GW and T659 resulted in an increase in medium-sized HDL particles, whereas fenofibrate showed increases in large HDL particles. These data provide additional evidence that PPARα and PPARδ agonists (both mixed and selective) have beneficial effects on HDL-C in these experimental primates.**—Wallace, J. M., M. Schwarz, P. Coward, J. Houze, J. K. Sawyer, K. L. Kelley, A. Chai, and L. L. Rudel. **Effects of peroxisome proliferator-activated receptor α/δ agonists on HDL-cholesterol in vervet monkeys. *J. Lipid Res.* 2005.** 46: **1009–1016.**

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The peroxisome proliferator-activated receptors (PPARs) are nuclear receptors that act as transcription factors to modulate specific expression of target genes. Three distinct subtypes, PPARα, PPARδ, and PPARγ, have been identified. All have tissue-specific expression patterns and form heterodimers with the retinoid X receptor to exert their effects on gene transcription (1). A number of excellent reviews describing the emerging roles of PPARs in a variety of biological processes have been published recently (1–6). Among the functions most clearly defined is the role of PPARs as dietary lipid sensors involved in fatty acid and carbohydrate metabolism.

For many years, PPARα and PPARγ agonists have been used for the treatment of dyslipidemia and type 2 diabetes, respectively. Fibrates (e.g., fenofibrate, gemfibrozil) have been shown to decrease plasma triglycerides (TGs) and increase high density lipoprotein-cholesterol (HDL-C) through the activation of PPARα, which is primarily expressed in liver, kidney, skeletal muscle, and heart. Glitazone drugs (e.g., rosiglitazone, pioglitazone) exert their antidiabetic effects through the activation of PPARγ, which is found predominantly in adipocytes and macrophages (1–3). Less is known about the function of PPARδ, which has broad tissue distribution, although emerging data also support a role in lipid homeostasis (7, 8) and fatty acid oxidation (3). Recently, the selective PPARδ agonist GW501516 (GW) was shown to decrease plasma TG levels and increase HDL-C concentrations in a nonhuman primate model of obesity and type II diabetes (7).

Currently, there are very few therapeutic drugs available to increase HDL-C concentrations in patients with levels considered to put them at risk for atherosclerotic heart disease (<40 mg/dl for men, <50 mg/dl for women) (9). Of the treatments available, PPAR agonists are among the most promising drug candidates to target this treatment gap (10). Indeed, the observation that PPARα and PPARδ agonists increase HDL-C in humans and/or animal models has enhanced interest in these two receptors as candidate targets for pharmacological intervention aimed at the treatment of dyslipidemias. The objective of this study

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was to characterize and compare a novel PPARα/δ agonist with two known PPAR agonists, GW and Tricor®, with respect to HDL-C increasing efficacy in vervet monkeys, a nonhuman primate model of atherosclerosis (11–13).

MATERIALS AND METHODS

Experimental animals and study design

The animals used were feral, adult male vervet monkeys (*Cercopithecus aethiops sabeus*) imported from St. Kitts Island in the Caribbean. The monkeys were part of a long-term study investigating the effect of *cis*- and *trans*-monounsaturated dietary fat on the progression of atherosclerosis. *Trans*- compared with *cis*-monounsaturated fatty acids have been found to result in lower HDL-C in this model through an unknown mechanism (40 ± 2 vs. 50 ± 3 mg/dl, respectively, for the groups in this study) (L. L. Rudel, unpublished data). The fat blends used in the diets were enriched in either *cis*-monounsaturated (18:1c; 51% of total fatty acids) or *trans*-monounsaturated (18:1t; 20% of total fatty acids) fat. Diets were made to contain fat as 35% of energy, protein as 17%, and carbohydrate as 48%, with cholesterol added to be 0.4 mg/kcal. The main ingredients were as follows (in g/100 g): fat, 16.4; flour, 35; dextrin, 9.6; sucrose, 10; casein, 9; lactalbumin, 5; alphacel, 7; Hegsted mineral mixture, 5; vitamin mix including D3 in corn oil, 2.6; and crystalline cholesterol, 0.17. At the time of this study, animals had been consuming their respective diets for ~55 months, during which time plasma lipid parameters and body weights were regularly monitored. Twelve animals from each dietary group were used in this study. Animals were housed in individual cages throughout the period of study in a facility accredited fully by the Association for the Assessment and Accreditation of Laboratory Animal Care, International. Procedures were conducted in accordance with the Federation of American Societies for Experimental Biology's Principles for the Use of Animals in Research and Education, and were approved by the Wake Forest University Institutional Animal Care and Use Committee.

Twenty-four monkeys were assigned to four equivalent treatment groups (n = 6) using pretreatment body weight, total plasma cholesterol, HDL-C, and dietary group as randomization variables. Three animals fed either type of dietary fat were included in each treatment group. Each group was then assigned one of the following four "treatments": *a*) vehicle only (vehicle); *b*) the PPARδ selective agonist GW (GW) (7); *c*) the PPARα/δ agonist T913659 (T659); and *d*) the PPARα agonist TriCor® (fenofibrate; Abbott Laboratories, Abbott Park, IL).

Oral administration of compounds was done by creating a suspension of each drug in vehicle, an aqueous mixture of Nesquik™ strawberry powder (Nestlé USA, Inc., Glendale, CA) and Carnation® nonfat dry milk (Nestlé USA). Briefly, each compound was weighed and ground with the nonfat dry milk and then mixed with Nesquik™ (91 g/l water) on a stir plate for at least 30 min. Doses were divided into aliquots with volumes (~20 ml) proportional to body weight and served immediately, just before the afternoon meal. All animals drank their doses without hesitation.

Food (70 kcal/kg/day) was weighed into morning and afternoon meals and consumption was monitored daily, with notations being made if an animal occasionally had food left over from a previous meal.

The experiment was a 19 week dose-escalating study that consisted of 1 week of baseline, 12 weeks of treatment, and 6 weeks of "washout." During baseline, monkeys in all four groups were administered vehicle once daily for 7 days per week. Test compounds other than fenofibrate were then administered in vehicle at a dose of 0.1 mg/kg once daily for 3 weeks, 1.0 mg/kg once daily for 3 weeks, and 3.0 mg/kg once daily for 6 weeks. The fenofibrate group was administered 10 mg/kg once daily for the first 3 weeks of treatment and then increased to 30 mg/kg once daily for the remaining 9 weeks of treatment. At the completion of treatment, monkeys in all groups were administered vehicle once daily and monitored for an additional 6 weeks.

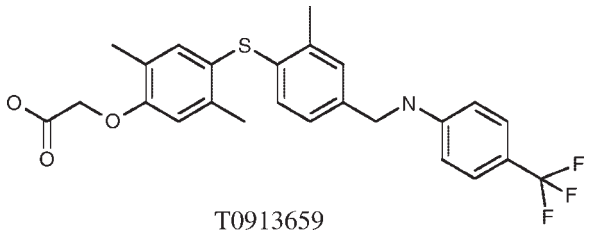
Animals were sedated with ketamine HCl (10 mg/kg) for body weight measurements and blood collection at weeks 1, 3, 4, 6, 7, 9, 10, 12, 13, 15, 16, and 19 of the study. Specifically, total plasma cholesterol, HDL-C, TG, and plasma exposure levels of each compound were measured at baseline, at least twice at each dose during the dose period (four times at the highest dose), and three times during the washout period. Cholesterol distribution among lipoprotein classes using gel filtration and ELISAs for apolipoprotein A-I (apoA-I), apoA-II, and apoB was measured at baseline and at least once at the end of each dose period. A final evaluation of lipoprotein cholesterol distribution was done at week 16, after 3 weeks of washout. To monitor the health of the animals, liver enzymes (serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, and γ-glutamyltranspeptidase) and a complete blood count with differential were done at baseline, at least once at the end of each dose period (twice during the highest dose), and twice during the washout period (liver enzymes only).

Reagents

All test material was obtained from Tularik, Inc. (South San Francisco, CA). GW and fenofibrate are well-characterized PPARδ and PPARα agonists, respectively. T659 (**Fig. 1**) emerged as a high-affinity PPARα/δ agonist from a medicinal chemistry optimization effort. Radioligand binding and cellular assays using CV-1 cells were performed as described (14). For the cellular assays, Effectene (Qiagen, Inc., Valencia, CA) was used as the transfection agent, and test compounds were added for 20 h before measuring luciferase activity.

Plasma analyses

Blood samples were collected from the femoral vein via venipuncture after an overnight fast and ~5 h before daily compound administration. Blood was collected into sterile evacuated tubes containing 0.1% EDTA and 0.1% NaN_3 and placed immediately on ice. Plasma concentrations of test compounds were determined by HPLC-MS in the Department of Drug Metabolism and Pharmacokinetics at Tularik, Inc. Lipid, lipoprotein, and apolipoprotein analyses were conducted using methods described previously (11, 15). Briefly, plasma was separated by centrifugation at 2,000 *g* for 30 min at 4°C. Protease inhibitor cocktail (Sigma PZ714; Sigma-Aldrich, Inc., St. Louis, MO) was added to the isolated plasma, and total cholesterol and TGs were determined using enzymatic measurements (16, 17). Plasma lipoprotein cholesterol distributions were determined by gel filtration chromatography of whole plasma on a Superose 6 fast-protein liquid chromatography column (30 × 1 cm; Pharmacia Corp., Peapack, NJ) with on-line cholesterol determination using a modification of



T0913659

Fig. 1. Chemical structure of Tularik T913659 (T659).

the method of Kieft, Bocan, and Kraus (18). Apolipoprotein concentrations were measured using a noncompetitive sandwich ELISA with monospecific goat anti-monkey antibodies to apoA-I, apoA-II, and apoB (15).

HDL particle size determinations

To determine HDL particle size, apoA-I distribution in whole plasma was estimated using nondenaturing gradient gel electrophoresis, Western blotting, and scanning with a ChemiImager™ 5500 (Alpha Innotech Corp., San Leandro, CA). Briefly, the material from the polyacrylamide gel was blotted onto nitrocellulose and subsequently probed with goat antiserum raised against monkey apoA-I. Horseradish peroxidase-conjugated anti-goat IgG 31402 (Pierce, Rockford, IL) was used as the secondary antibody. Peroxidase signal was detected using SuperSignal® West Pico Chemiluminescent Substrate (Pierce, Rockford, IL) and captured on X-Omat film (Eastman Kodak, New Haven, CT). Images were scanned and quantified using the ChemiImager ID-Multilane densitometry analysis tool. Each lane was divided into areas representing small (two apoA-I), medium (three apoA-I), and large (four apoA-I) HDL particles using break points that corresponded to Stokes radius cutoffs of <4.2, 4.2–5.8, and >5.8 nm, respectively (19). Band intensity for each area was quantified and reported as a percentage of total apoA-I signal.

Statistical analyses

Data were tested for normality using the Kolmogorov-Smirnov test for normality and an equality of variances *F*-test. Health parameters were evaluated using repeated-measures ANOVA. Lipid and lipoprotein parameters were evaluated similarly and, when significant differences were found, subjected to subsequent analyses using paired *t*-tests to compare baseline values with values collected after 6 weeks of treatment at the highest dose administered (for fenofibrate, after 9 weeks at the highest dose).

To identify differences in HDL particle size distribution, the percentages were estimated for apoA-I in small, medium, and large HDLs after gel electrophoresis and Western blotting of whole plasma. The apoA-I concentrations in small, medium, and large particles were then calculated from whole plasma apoA-I concentration, and differences were evaluated for statistical significance using repeated-measures ANOVA.

The levels of statistical significance for identified differences were determined according to the formulae provided with the statistical software (Statview, version 5.0.1; SAS Institute, Inc., Cary, NC). Differences were considered significant at $P \leq 0.05$.

RESULTS

In vitro profile of PPAR agonists

The in vitro biological properties of T659 with respect to PPAR binding and activation are shown in **Fig. 2**. T659 bound to PPARδ, PPARα, and PPARγ with inhibition constants of 3, 90, and 420 nM, respectively. The subtype specificity of T659 was confirmed in cell-based transactivation assays, in which it activated PPARδ, PPARα, and PPARγ with EC_{50} values of 9, 510, and 1,300 nM, respectively. Thus, T659 is a very potent PPARδ agonist that also activates PPARα and, to a lesser extent, PPARγ.

T659 displayed a 1,000-fold selectivity against human forms of liver X receptor α, liver X receptor β, farnesoid X receptor, pregnane X receptor, constitutive androstane receptor, vitamin D receptor, and estrogen receptor α (data not shown). Additional criteria for selection of this compound for in vivo evaluation in the vervet nonhuman pri-

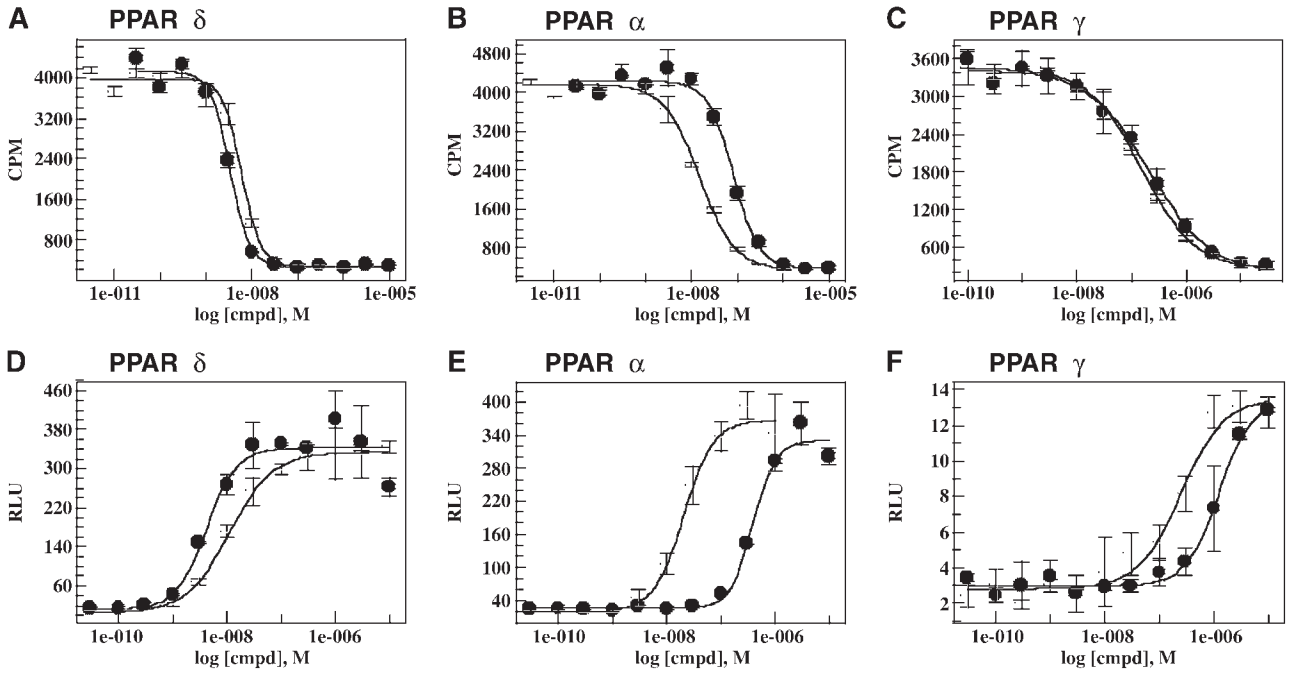


Fig. 2. In vitro properties of T659. GW501516 (GW), GW2433, and BRL49653 were used as reference compounds for peroxisome proliferator-activated receptor δ (PPARδ), PPARα, and PPARγ binding and transactivation, respectively (open circles). T659 data points are shown as closed circles. A–C: Binding affinity in radioligand displacement assays. The concentration of displaced radioligand is expressed in cpm. D–F: Cellular potency in transient transfection assays in CV-1 cells. Test compounds were added 24 h after transfection, and luciferase activity was measured in relative light units (RLU) after 20 h. Data are expressed as means of duplicate determinations in a single experiment; two additional experiments gave similar results. Error bars represent SD.

mate model were metabolic stability, low protein binding, favorable pharmacokinetic properties, lack of toxicity in rats, and HDL-C-increasing efficacy in rodent models of dyslipidemia (data not shown). In mice lacking a functional PPARα gene, 60% of HDL-C-increasing efficacy compared with wild-type controls was maintained, indicating that this effect was mediated in large part through the activation of PPARδ (data not shown).

Efficacy in vervets

All drugs were absorbed efficiently throughout the course of the study, with plasma steady-state trough concentrations increasing as a function of compound dose (data not shown). After 10 weeks of dosing, trough plasma levels of T659 were ~4-fold higher than levels of GW and ~300-fold higher than the EC_{50} value for the activation of PPARδ in the cell-based transactivation assay.

There were no statistically significant effects of treatment on body weight in any of the groups that completed the 19 week study (data not shown). The effects of the PPAR agonists on other health parameters are shown in **Table 1**. Briefly, animals in the T659 group had significant decreases in all red blood cell parameters, including red blood cell count, hemoglobin concentration, and hematocrit. All red cell values had returned to normal by the end of the 6 week washout. The parameters measured were not such that the type of anemia could be reliably classified. Significant increases were also seen in white blood cell counts of animals in the T659 group, although all values remained within the normal range for adult male vervets (International Species Information System, Apple Valley, MN). Animals in the GW group showed a statistically significant decrease in serum glutamic oxaloacetic transaminase and γ-glutamyltranspeptidase; no changes in liver enzymes were observed in any of the other groups.

The effects of the PPAR agonists on plasma lipids and lipoproteins can be characterized by the values in the week 13 sample taken at the end of the drug treatment period, and these values are contrasted with the baseline values in **Table 2**. No significant differences in total plasma cholesterol or TG, which are already naturally low in male monkeys fed atherogenic diets, were seen in any of the groups. Similarly, there were no effects of any of the treatments on serum VLDL-C, LDL-C, or apoB concentrations. By contrast, significant increases in HDL-C concentration were seen in all treatment groups. Although dose-dependent trends for HDL-C increases were observed at the lower doses, Table 2 illustrates the statistically significant effects seen after 6 weeks of treatment at the highest dose administered (after 9 weeks in the fenofibrate group), which is at the end of 12 total weeks of drug treatment. The percentage increase in HDL-C from baseline for each group was as follows: vehicle, 5%; GW, 43%; T659, 43%; and fenofibrate, 20%. The increase in HDL-C concentration seen in treatment groups was accompanied by significant increases in plasma apoA-I (fenofibrate: $P = 0.054$) and apoA-II concentrations. Animals in the vehicle group showed no significant differences in HDL-C, apoA-I, or apoA-II throughout the study.

TABLE 1. Effects of mixed and selective PPARα/δ agonists on health parameters

Group	Baseline	Treatment	Washout	P^a
		mm^2		
White Blood Cell Count				
Vehicle	6.0 ± 0.9	5.9 ± 1.0	6.7 ± 1.4	NS
GW	7.7 ± 1.6	7.1 ± 1.2	7.4 ± 1.9	NS
T659	5.1 ± 0.8	7.3 ± 0.8	7.8 ± 0.5	0.02
Fenofibrate	5.7 ± 0.6	5.0 ± 0.6	4.7 ± 0.7	NS
		10^6 mm		
Red Blood Cell Count				
Vehicle	7.1 ± 0.3	7.2 ± 0.5	7.3 ± 0.6	NS
GW	6.9 ± 0.3	6.6 ± 0.2	7.2 ± 0.4	NS
T659	6.6 ± 0.1	5.5 ± 0.3	7.0 ± 0.2	<0.001
Fenofibrate	6.4 ± 0.2	7.0 ± 0.2	7.2 ± 0.2	0.003
		g/dl		
Hemoglobin				
Vehicle	17.1 ± 1.3	17.0 ± 1.4	16.3 ± 1.7	NS
GW	15.0 ± 0.6	15.3 ± 0.6	15.7 ± 0.7	NS
T659	16.5 ± 0.5	13.5 ± 0.8	16.4 ± 0.3	0.008
Fenofibrate	16.2 ± 0.5	16.0 ± 0.4	16.4 ± 0.4	NS
		%		
Hematocrit				
Vehicle	48 ± 3	49 ± 3	46 ± 4	NS
GW	42 ± 1	44 ± 1	45 ± 2	NS
T659	45 ± 1	39 ± 2	45 ± 1	0.03
Fenofibrate	45 ± 2	46 ± 1	45 ± 1	NS
		IU/l		
Serum Glutamic Oxaloacetic Transaminase				
Vehicle	68 ± 11	58 ± 5	53 ± 7	NS
GW	55 ± 3	39 ± 4	34 ± 6	0.02
T659	67 ± 9	44 ± 6	90 ± 28	NS
Fenofibrate	63 ± 11	36 ± 4	61 ± 13	NS
		IU/l		
γ-Glutamyltranspeptidase				
Vehicle	46 ± 3	46 ± 7	44 ± 5	NS
GW	37 ± 3	22 ± 3	30 ± 5	0.02
T659	53 ± 12	33 ± 8	71 ± 32	NS
Fenofibrate	76 ± 35	29 ± 2	59 ± 7	NS

PPAR, peroxisome proliferator-activated receptor. Values shown are means ± SEM.

[a] Repeated-measures ANOVA comparing baseline with treatment for each group with significant *P* values as shown.

The distribution of apoA-I on HDL particles in plasma of each of the animals throughout the study was evaluated using nondenaturing gradient gel electrophoresis, and the data are shown in **Fig. 3**. Statistical significance, as indicated by asterisks in this figure, is an indication of a significant trend for any one HDL particle population, as obtained by repeated-measures ANOVA. Large HDLs are equivalent to particles with four apoA-I molecules per particle, medium HDLs are particles with three apoA-I molecules, and small HDLs are particles with two apoA-I molecules. In the vehicle group, no statistically significant differences in the concentration of apoA-I in any of the size fractions occurred at any time during the study. Essentially, apoA-I concentrations were nearly equivalent in each of the size fractions. Overall, the data in the treatment groups show that the PPARδ agonists in this study caused increases in apoA-I concentrations in the medium-

TABLE 2. Effects of mixed and selective PPARα/δ agonists on plasma lipids and lipoproteins

	Total Plasma Cholesterol		Triglyceride		LDL-C		ApoB		HDL-Cholesterol		ApoA-I		ApoA-II	
Group	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment	Baseline	Treatment
	mg/dl													
Vehicle	382 ± 70	359 ± 55	13 ± 1	14 ± 1	343 ± 67	306 ± 57	159 ± 27	168 ± 28	43 ± 7	45 ± 7	165 ± 30	201 ± 21	13.1 ± 2.2	14.3 ± 2.0
GW	374 ± 43	348 ± 50	15 ± 3	16 ± 2	313 ± 45	278 ± 50	135 ± 17	150 ± 15	42 ± 7	60 ± 7[a]	177 ± 21	253 ± 27[a]	12.1 ± 1.7	19.4 ± 2.9[a]
T659	380 ± 32	381 ± 32	20 ± 7	19 ± 6	334 ± 32	297 ± 39	160 ± 21	153 ± 13	47 ± 7	67 ± 6[a]	185 ± 27	261 ± 30[a]	16.1 ± 2.4	21.0 ± 3.0[a]
Fenofibrate	360 ± 35	326 ± 34	16 ± 6	12 ± 2	318 ± 44	267 ± 41	152 ± 22	177 ± 45	41 ± 3	49 ± 5[a]	174 ± 20	232 ± 14	12.6 ± 1.3	19.1 ± 1.7[a]

ApoB, apolipoprotein B.
[a] Paired *t*-test comparing baseline with treatment for each group ($P < 0.05$).

sized HDL particles. For the PPARα agonist fenofibrate, the trend for an increase in apoA-I in medium-sized particles was not statistically significant. However, a significant trend for apoA-I to increase in large HDL particles was present. At no time in any of the groups was an increase in the concentration of small HDLs observed.

DISCUSSION

Current pharmacologic treatment for atherosclerotic heart disease consists mainly of the administration of HMG-CoA reductase inhibitors or "statin" drugs to reduce LDL-C concentrations. Although this drug class is effective at decreasing LDL-C and reducing coronary events, it is of limited use in patients with metabolic syndrome, who, in addition to increased LDL-C levels, have low HDL-C concentrations, hypertriglyceridemia, and increased insulin resistance (10, 20). For these patients, PPAR agonists are believed to have greater therapeutic potential (10). Indeed, PPARα and PPARγ agonists have been used for a number of years to treat metabolic syndrome and type 2 diabetes, respectively. However, these drugs, like statins, are limited in their ability to increase HDL-C (10, 20). Although fibrates are known to cause slight increases in HDL-C concentration, glitazone drugs have little or no effect on HDL-C (10, 20). The emerging role of PPARδ in lipid metabolism (7, 21) and the demonstrated ability of PPARδ agonists to increase HDL-C in animal models (7, 22) have enhanced interest in this receptor as a target for the therapeutic intervention of dyslipidemias. More potent PPAR agonists and/or agonists with mixed receptor selectivity are thought to have significant potential as novel antiatherosclerotic agents (19, 20).

In this study, selective PPARα and PPARδ compounds, and a compound with mixed PPARα/δ selectivity, increased plasma HDL-C, apoA-I, and apoA-II concentrations in St. Kitts vervets. The percentage increase in HDL-C from baseline for treatment groups ranged from 20% to 43%, with the greatest increases seen in groups treated with agonists with mixed PPARα/δ receptor selectivity. GW and T659 showed similar biochemical and cellular potency for PPARδ binding and activation and caused a similar increase of HDL-C in vervet monkeys, but the plasma trough concentration of T659 was on average 4-fold higher than that of GW at each time point measured. The lack of an effect on fasting insulin and glucose levels (data not shown) suggests that the in vitro potency of T659 for PPARγ activation did not translate into a metabolic effect in this model.

These findings confirm and expand previous reports of increased plasma HDL-C in obese prediabetic primates treated with the PPARα agonist fenofibrate (23) or the same selective PPARδ agonist (GW) used in this study (7). Unlike in previous reports, the St. Kitts vervets in this study were fed fat-enriched atherogenic diets and had naturally low plasma TG concentrations that were unaffected by treatment with the selective and mixed PPARα/δ agonists evaluated here. This is not unexpected, because the previous studies showing a decrease in TG were done in a rhesus monkey model of obesity and type 2 diabetes in which the diet was a low-fat chow. Affected rhesus monkeys are atypical in that they have aged to the point that they become hyperinsulinemic and hypertriglyceridemic, even in a prediabetic state (23). In contrast, most monkeys fed fat-enriched diets have very low plasma TG concentrations, presumably as a result of the induction of lipoprotein lipase by the dietary fat (24). The tight inverse relationship observed between plasma concentrations of HDL-C and TG makes the HDL-C increases in this study even more striking, because they occurred in the face of normal plasma TG concentrations that were unaffected by treatment.

The measures of HDL particle size found in vehicle and treated vervets suggest that PPAR agonists, both α and δ, favor the production of larger HDL particles in primates. Previous experiments in our laboratory have shown that small, medium, and large HDL particles typically contain two, three, and four apoA-I molecules per particle, respectively, and there is a unidirectional maturation in the plasma from small to medium and small to large HDL particles (19). The larger HDL particles are cleared from plasma preferentially by the liver (25). The shift toward increased amounts of medium and large particles as seen in animals treated with GW, T659, and fenofibrate suggests that PPARα/δ agonists may be involved in the process by which HDL particles acquire cholesterol, apoA-I, and apoA-II and are matured in plasma. In humans, small HDL particles are associated with coronary artery disease progression and larger HDL particles are thought to be protective (26).

The mechanism for the observed increases in plasma HDL-C concentration and larger apoA-I HDL particles

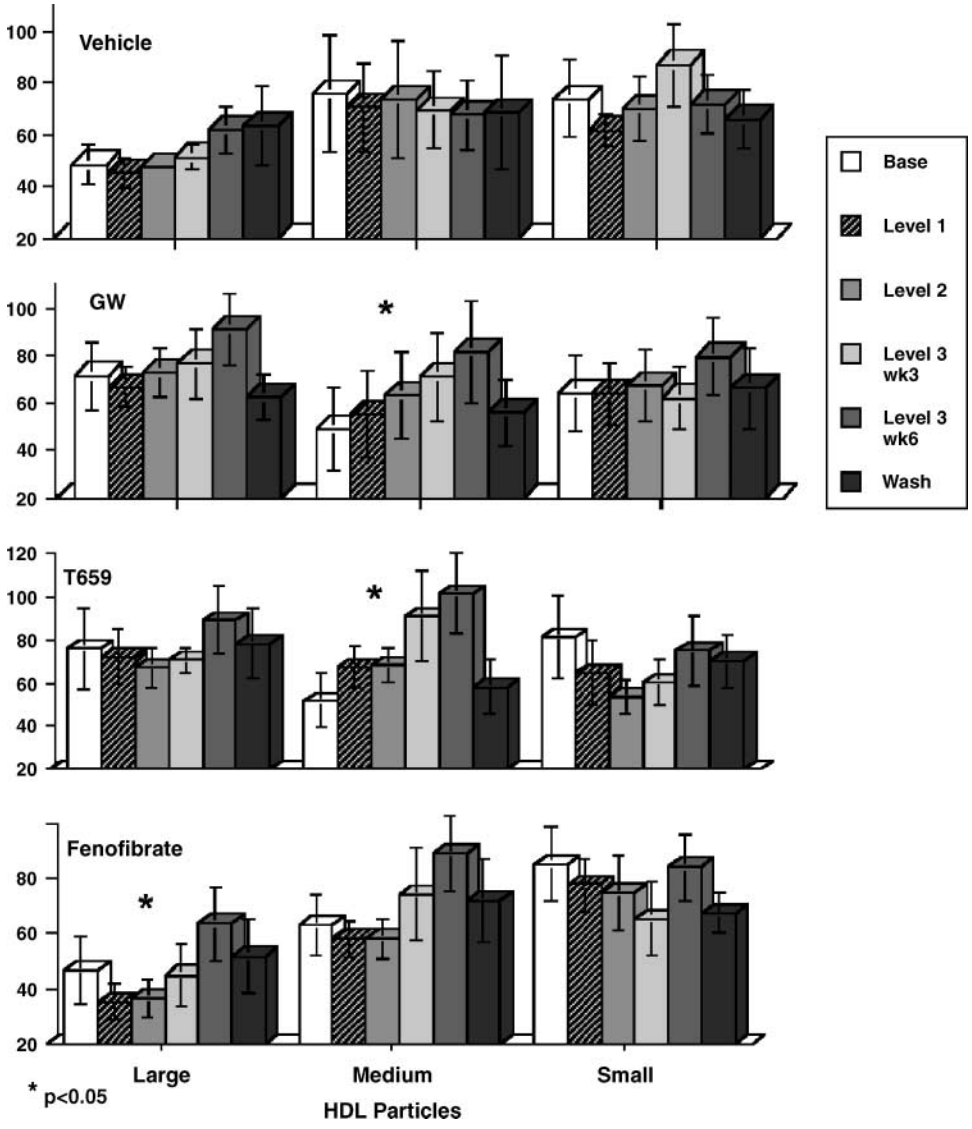


Fig. 3. Effects of mixed and selective PPARα/δ agonists on apolipoprotein A-I (apoA-I) distribution in HDL particles of different size. Data represent concentrations of apoA-I in small, medium, and large HDLs as determined after nondenaturing gradient gel electrophoresis and Western blotting of aliquots of whole plasma from each animal. Means were obtained for six animals within each group, and differences were evaluated for significance using repeated-measures ANOVA. Error bars represent SEM.

seen here in nonhuman primates treated with PPARα/δ agonists has yet to be determined, but presumably it involves an enhanced capacity for reverse cholesterol transport by HDL in treated animals. In the presence of PPARα and PPARδ agonists, the ability of apoA-I to be assembled into larger HDLs for cholesterol transport may be promoted, presumably indicating that the availability of apoA-I for HDL particle assembly is higher (27). PPAR agonist effects on the transcription of key proteins involved in reverse cholesterol transport are of interest given the relative paucity of pharmacological agents known to promote HDL-C concentrations. PPARα has been shown previously to mediate fibrate action on HDL-C levels via transcriptional induction of the synthesis of the major apolipoproteins of HDL as well as LPL (28, 29). Fibrates have been reported to upregulate ABCA1 (30) and scavenger receptor class B type I (SR-BI) (31) expression in human monocytes and macrophages and to decrease hepatic apoC-III transcription (2). More recently, fibrate treatment was found to suppress hepatic SR-BI protein expression in mice without changing SR-BI mRNA levels (32). The reduced SR-BI protein expression in fibrate-treated mice correlated with enlarged HDL particle size. When hepatic SR-BI levels were normalized by recombinant adenoviral gene transfer, the fibrate-induced HDL enlargement was abolished, suggesting a role of SR-BI in the formation of large HDL particles (32).

Less is known about the potential role of PPARδ in reverse cholesterol transport, because there are fewer receptor-specific agonists available for study. In vitro cholesterol-loading studies have generated conflicting results. In one study, the PPARδ agonist GW was found to promote

cholesterol efflux in multiple tissues by increasing expression of the reverse cholesterol transporter ABCA1 (7). In another study involving a different selective PPARδ agonist, total macrophage lipid accumulation was increased, despite an increase in ABCA1 expression, apparently as a result of a relative upregulation of class A and B scavenger receptors and adipophilin and repression of other key genes involved in lipid efflux (21). The extent to which the different agonists used may explain the observed differences cannot be determined. More recently, comparison of PPARδ$^{-/-}$ and wild-type bone marrow revealed no differences in cellular cholesterol balance, leading the authors to conclude that PPARδ does not appreciably modulate either cholesterol uptake or efflux (33). Studies in genetically obese mice (Lepr$^{db/db}$) and transgenic mice with selective expression of an active form of PPARδ in adipose tissue reveal interesting parallels between PPARδ and PPARα, and coupled with in vitro data, they suggest that PPARδ may also be an important promoter of fatty acid oxidation and energy uncoupling (34). The key enzyme involved in the hydrolysis of TG, LPL, has been implicated in the generation of endogenous PPARα and PPARδ ligands (29) and is known to correlate positively with HDL-C levels (35). Whether PPARα- and PPARδ-mediated lipolysis drives reverse cholesterol transport by increasing the availability of HDL-associated apoA and apoC in plasma is unknown.

Clearly, additional studies are needed to determine the complex mechanism by which PPARα and PPARδ agonists influence lipid metabolism in a manner that results in beneficial effects on HDL-C concentration and particle size. A caveat that must be considered, however, is that many of the published studies have been done in mice, and for unknown reasons the responsiveness of rodent models to PPAR agonists is not typical of that in primates. One explanation may be found in the observation that PPARs induce human apoA-I gene expression in vivo via a positive PPAR response element (PPRE) located in the apoA-I promoter A site (36). A three nucleotide difference renders the positive PPRE nonfunctional in rodents. The beneficial effect of PPARs observed in apoE-deficient mice overexpressing human apoA-I (37), but not in other mouse models (37, 38), lends support to the premise that a difference in rodent apoA-I PPRE is responsible for the observed variability in rodent responsiveness to PPAR agonists. Using sequence alignment software, we have determined that the PPRE sequence in African green (vervet) and cynomolgus monkey apoA-I is identical to that of human apoA-I. Therefore, it is reasonable to assume that individual PPARs (α, δ, and γ) activate vervet monkey apoA-I in a manner analogous to that of human apoA-I. Whether this mechanism is responsible for the improved HDL profiles seen in vervets in this study is unknown. However, the similarity between human, cynomolgus, and vervet apoA-I PPRE suggests that primates are good models for future investigations of the effects of PPARs on human HDL metabolism and atherosclerosis.

In summary, each of the PPARα and PPARδ agonists investigated in this study appeared to increase plasma HDL-C, apoA-I, and apoA-II concentrations and increase HDL particle size in St. Kitts vervets. Whether these changes were the result of increased production or decreased clearance of HDL-C is only a matter of speculation at this time and will require further work. However, these data provide additional evidence that PPARα and PPARδ agonists (both mixed and selective) have beneficial effects on HDL-C in primates. With respect to increasing HDL-C, GW and fenofibrate were as efficacious in the vervet monkey as reported for obese rhesus monkeys (7, 23). Thus, the vervet can be considered a valid primate model in which to conduct future investigations of the therapeutic potential of PPARα and PPARδ agonists for the treatment of dyslipidemias and the mechanism(s) behind the observed effects on plasma HDL-C concentrations.

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