A selective peroxisome proliferator-activated receptor δ agonist promotes reverse cholesterol transport

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The peroxisome proliferator-activated receptors (PPARs) are dietary lipid sensors that regulate fatty acid and carbohydrate metabolism. The hypolipidemic effects of the fibrate drugs and the antidiabetic effects of the glitazone drugs in humans are due to activation of the α (NR1C1) and γ (NR1C3) subtypes, respectively. By contrast, the therapeutic potential of the δ (NR1C2) subtype is unknown, due in part to the lack of selective ligands. We have used combinatorial chemistry and structure-based drug design to develop a potent and subtype-selective PPARδ agonist, GW501516. In macrophages, fibroblasts, and intestinal cells, GW501516 increases expression of the reverse cholesterol transporter ATP-binding cassette A1 and induces apolipoprotein A1-specific cholesterol efflux. When dosed to insulin-resistant middle-aged obese rhesus monkeys, GW501516 causes a dramatic dose-dependent rise in serum high density lipoprotein cholesterol while lowering the levels of small-dense low density lipoprotein, fasting triglycerides, and fasting insulin. Our results suggest that PPARδ agonists may be effective drugs to increase reverse cholesterol transport and decrease cardiovascular disease associated with the metabolic syndrome X.

The peroxisome-proliferator activated receptors (PPARs) are members of the nuclear receptor gene family that are activated by fatty acids and fatty acid metabolites (1, 2). The PPARs belong to the subset of nuclear receptors that function as heterodimers with the 9-*cis*-retinoic acid receptor (RXR) (3). Three subtypes, designated PPARα (NR1C1), PPARγ (NR1C3), and PPARδ (NR1C2), are found in species ranging from *Xenopus* to humans (1). Each receptor has a distinct tissue expression profile, with PPARα the main subtype in the liver, PPARγ the main subtype in adipose tissue, and PPARδ expressed in many tissues (4). The physiological functions of PPARα and PPARγ in lipid and carbohydrate metabolism were uncovered once it was recognized that they were the receptors for the fibrate and glitazone drugs, respectively (1). By contrast, PPARδ (NR1C2) is not reported to be a receptor for any known class of drug molecules, and its role in mammalian physiology has remained undefined (1).

In humans, lipid homestasis is a delicate balance between dietary intake, *de novo* synthesis, and catabolism. The increased incidence of cardiovascular disease in Westernized nations has been linked to dyslipidemias associated with changes in the fat content of the diet (5). Obesity, insulin resistance, and hypertension are comorbidities with these lipid disorders, which together are known as the metabolic syndrome X (6). Individuals with this condition have raised serum triglycerides and abnormally low levels of high density lipoprotein cholesterol (HDLc) (6, 7). This lipid profile is accompanied by an increase in the proportion of small-dense low density lipoprotein (LDL) particles, which are prone to accumulate in the arterial wall leading to the formation of atherosclerotic cholesterol-laden foam cells (8). HDL plays a protective role through the process of reverse cholesterol transport whereby cholesterol is removed from peripheral cells, including the macrophage-derived foam cells, and returned to the liver (9). Agents that raise the levels of HDL through reverse cholesterol transport could provide a new therapeutic option for the prevention of atherosclerotic cardiovascular disease (10).

Recently, the ATP-binding cassette A1 (ABCA1) protein has been identified as a regulator of cholesterol and phospholipid transport from cells (11). Patients with Tangier disease or familial hypoalphalipoproteinemia have been identified with loss-of-function mutations in the *ABCA1* gene (12–14). These patients have low levels of HDLc and high triglycerides and show an increased incidence of cardiovascular disease (15). Thus, therapies that increase the expression of ABCA1 could provide a new approach to treating atherogenic dyslipidemia (9). RXR heterodimers may play a role in the transcriptional regulation of ABCA1 expression, because RXR agonists have been shown to increase the expression of ABCA1 in the intestine of mice (16). In this report, we demonstrate that a selective PPARδ agonist increases ABCA1 expression and cholesterol efflux from cells and increases HDLc in primates. PPARδ agonists may provide a new approach to the treatment of cardiovascular disease by promoting reverse cholesterol transport.

Materials and Methods

Reagents and Assays. The human PPARδ binding assay was performed as described using [^{3}H]GW2433 as the radioligand (2, 17). Expression plasmids for the nuclear receptor-GAL4 chimeras were prepared by inserting amplified cDNAs encoding the ligand binding domains into a modified pSG5 expression vector (Stratagene) containing the GAL4 DNA-binding domain (amino acids 1–147) and the simian virus 40 large T antigen nuclear localization signal (APKKKRKVG). Transient transfection assays were performed as described using $(UAS)_5$-tk-luciferase reporter constructs (18). The synthesis of (2-methyl-4-(((4-methyl-2-(4-trifluoromethylphenyl)-1,3-thiazol-5-yl)-methyl)sulfanyl)phenoxy)acetic acid (GW501516), 2-(4-(2-(1-cyclohexanebutyl-3-cyclohexylureido)ethyl)phenylthio)-2-methylpropionic acid (GW7647), and 3-(3-(2-chloro-3-trifluoromethylbenzyl-2,2-diphenylethylamino)propoxy) phenylacetic acid (GW3965) will be described elsewhere.

Cell Culture. THP1 human monocyte cells (ATCC TID-202) were cultured in RPMI 1640 medium containing 10% FBS. Differ-

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Abbreviations: PPAR, peroxisome proliferator-activated receptor; RXR, 9-*cis*-retinoic acid receptor; HDL, high density lipoprotein; HDLc, HDL cholesterol; ABC, ATP binding cassette; LDL, low density lipoprotein; VLDL, very low density lipoprotein; apo, apolipoprotein; LXR, liver X receptor.

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entiation into macrophages was performed on 6-well plates (1×10^6 cells/well) by treatment with phorbol 12-myristate 13-acetate (100 ng/ml) for 5 days. Phorbol 12-myristate 13-acetate (100 ng/ml) was included in the medium of all subsequent experiments with these cells to maintain differentiation. 1BR3N human skin fibroblasts (European Collection of Cell Cultures 90020508) and FHS74 human intestinal cells (ATCC CCL-241) were cultured in their recommended maintenance media and plated on 6-well plates (1×10^6 cells/well) before the efflux and gene expression studies.

Cholesterol Efflux and Gene Expression Studies. Phorbol 12-myristate 13-acetate-differentiated THP1 macrophages were washed in serum-free medium and incubated in 5% FBS medium containing 1 μCi/ml of [^{3}H]cholesterol (New England Nuclear) and 1.5% fatty acid-free BSA for 24 h. The cells were washed in serum-free medium and incubated for 24 h in serum-free medium supplemented with 1.5% fatty acid-free BSA and test compound or vehicle (0.1% DMSO). The equilibration medium was removed and the cells were washed twice in serum-free medium. Serum-free RPMI 1640 medium containing test compound or vehicle ± purified human apolipoprotein (apo) AI (Athens Research & Technology, Athens, GA) was added. The final concentration of apoA1 was 10 μg/ml. The cells were maintained for 24 h before medium and cell extracts were isolated. Scintillation counting was performed to determine the percentage cellular cholesterol efflux ± apoAI. Cholesterol efflux studies with the 1BR3N fibroblasts were performed as described for the THP1 cells, except that cells were maintained for 48 h before scintillation counting was performed.

Parallel nonradioactive experiments were performed with the THP1 macrophages and 1BR3N fibroblasts to generate samples for quantitation of ABCA1, PPARδ, and liver X receptor α (LXRα) mRNA. Gene expression data with the FHS74 intestinal cells was generated after treatment with test compound or vehicle for 48 h. Total RNA was generated by using the Qiagen (Chatsworth, CA) RNeasy Mini Kit, and DNase was treated according to the manufacturer's protocol (Ambion). mRNA expression was analyzed by using real-time quantitative-PCR on an Applied Biosystems Prism 7700 sequence detection system. Primer/probe sequences used were as follows: ABCA1 forward primer, 5′-TGTCCAGTCCAGTAATGGTTCTGTGT-3′, reverse primer 5′-GCGAGATATGGTCCGGATTG-3′, probe, 5′FAM-ACACCTGGAGAGAAGCTTTCAACGAGAC-TAACC-TAMRA3′; PPARδ forward primer, 5′-CAACTGCA-GATGGGCTGTGA-3′, reverse primer, 5′-ATGCATGAA-CACCGTAGTGGAA-3′, probe, 5′FAM-CCCGGCACT-CCATGTTGAGGCT-TAMRA3′; LXRα forward primer, 5′-AGCCCTGCATGCCTACGTC-3′, reverse primer, 5′-GCATC-CGTGGGAACATCAG-3′, probe, 5′FAM-CCATCCAC-CATCCCCATGACC G-TAMRA3′. Expression data were normalized to 18S as described by the vendor.

Primate Study. GW501516 was evaluated in six male obese rhesus monkeys from the colony at the Obesity and Diabetes Research Center at the University of Maryland (19). The animals were housed with ad libitum access to food and water. Caloric composition of the diet was 13% fat, 18% protein, and 69% digestible carbohydrates. Each animal received repeat oral administration of α-tocopheryl polyethylene glycol succinate/polyethylene glycol 400 (vehicle) or GW501516, formulated as a solution of the sodium salt in vehicle, delivered in small food snacks twice a day. After 4 weeks of dosing with vehicle, the initial dose of GW501516 was 0.1 mg/kg for 4 weeks, rising to 0.3, 1.0, and 3.0 mg/kg during sequential 4-week periods. Food consumption was monitored daily, and body weights were recorded weekly. Decreases in food consumption have been observed in these primates with agents that lower triglycerides below 30 mg/dl (B.C.H., unpublished work). One animal, whose triglycerides were <20 mg/dl and HDLc was >130 mg/dl at the 1.0 mg/kg dose, showed a reduction in food intake. As a precaution, this animal was removed from the study before administration of the 3.0 mg/kg dose. Blood samples were drawn under light ketamine sedation on day 28 of each dose after a 16 h overnight fast and 2 h after dosing. At the completion of the study the rhesus monkeys were monitored through an 8-week washout period. Studies were approved by the Institutional Animal Care and Use committees for the University of Maryland and GlaxoSmithKline.

Serum Analysis. Serum concentrations of GW501516 were determined by HPLC/MS in the Department of BioMedical Analysis at GlaxoSmithKline. Serum total cholesterol, HDLc, triglycerides, and glucose were determined by an Instrumentation Laboratory (Lexington, MA) IL600 clinical chemistry analyzer. Serum insulin was determined by immunoassay (Linco Research Immunoassay, St. Charles, MO). Serum apoAI, apoAII, and apoCIII were determined by immunoprecipitation (Penn Medical Laboratory, Washington, DC).

Lipoprotein Subclass Analysis. Subclass analysis was performed by proton NMR spectroscopy (LipoMed, Raleigh, NC) on EDTA plasma samples as described (20). The lipoprotein subclasses were defined by NMR-determined particle size as follows: small HDL, 7.3–8.8 nm; large HDL, 8.8–13 nm; small LDL, 18.8–19.7 nm; medium LDL, 19.8–21.2 nm; large LDL, 21.3–23.0 nm; small very low density lipoprotein (VLDL), 27–35 nm; medium VLDL, 35–60 nm; and large VLDL, 60–200 nm.

Statistical Analyses. Unless otherwise stated, data are expressed as mean ± SE. The significance of differences were analyzed by using a paired Student's t test.

PHARMACOLOGY

Results

Development of a Selective PPARδ Agonist. To evaluate the therapeutic potential of PPARδ agonists, we developed a subtype-selective small molecule ligand by using combinatorial chemistry (ref. 17; D.D.S., unpublished results) and structure-based drug design (ref. 2; H.E.X. and M.H.L., unpublished results). GW501516 (Fig. 1*A*) is a high affinity ligand in a human PPARδ binding assay (2) with $K_i = 1.1 \pm 0.1$ nM (data not shown). When evaluated in a cell-based transfection assay, using the ligand binding domain of human PPARδ fused to the DNA-binding domain of the yeast transcription factor GAL4, GW501516 induced expression of a GAL4-responsive reporter gene with an $EC_{50} = 1.2 \pm 0.1$ nM. GW501516 was >1,000-fold selective for PPARδ over the other subtypes (Fig. 1*B*). At doses up to 1.0 μM, GW501516 did not promote adipocyte differentiation, bind to RXRα, or have activity on other nuclear or non-nuclear receptors (Fig. 1*C* and data not shown). GW501516 also shows $EC_{50} = 1.6 \pm 0.3$ nM on rhesus PPARδ and $EC_{50} = 24 \pm 2$ nM on mouse PPARδ with >500-fold selectivity over the other subtypes (data not shown). Other PPARδ agonists lack selectivity over either PPARα [e.g., GW2433 (1, 17)] or PPARγ [e.g., L-165041 (1, 21)] or have activity on eicosanoid receptors [e.g., carboprostacyclin (22)].

PPARδ Regulates ABCA1 and Cholesterol Efflux. Recently, several nuclear receptors that form heterodimers with RXR have been evaluated for their ability to regulate expression of the cholesterol transporter ABCA1 (16). Ligands for either RXR or LXRα induce ABCA1 expression in murine peritoneal macrophages, human primary macrophages, or THP1 macrophages (16, 23, 24). To assess the potential for the PPARs to regulate this process, THP1 macrophages were dosed with ligands selective for each of the three subtypes. At 100 nM, GW7647 (see

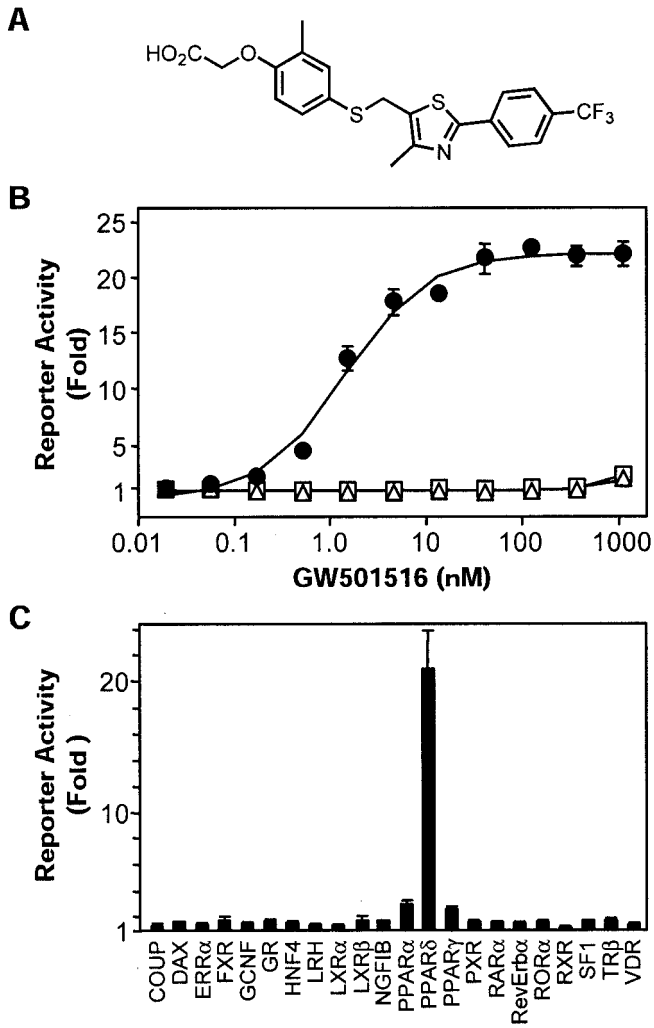

Fig. 1. GW501516 is a selective PPARδ agonist. Data are expressed as fold change compared with vehicle-treated cells and represent the mean of assays performed in triplicate ± SE. (*A*) Chemical structure of GW501516. (*B*) Activation of GAL4-PPAR ligand binding domain chimeras in transiently transfected CV-1 cells by GW501516. Dose–response curves are shown for human PPARδ (●), human PPARα (△), and human PPARγ (□). (*C*) Activation of GAL4-nuclear receptor ligand binding domain chimeras in CV-1 cells. Transfected cells were treated with 1.0 μM GW501516.

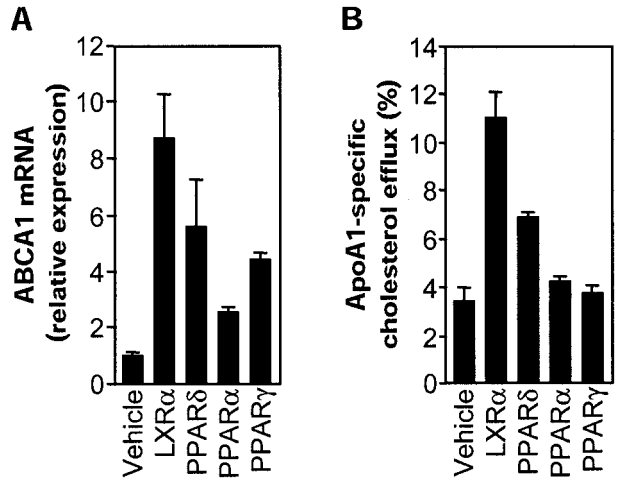

Fig. 2. Regulation of ABCA1 expression and cholesterol efflux from THP1 macrophages. Compounds were used at the following concentrations: LXRα (GW3965), 1.0 μM; PPARδ (GW501516), 100 nM; PPARα (GW7647), 100 nM; PPARγ (GW7845), 100 nM. Data are presented as the mean of assays performed in triplicate ± SD. (*A*) ABCA1 mRNA levels. (*B*) ApoA1-specific cholesterol efflux.

Materials and Methods; P. J. Brown, personal communication) is a selective PPARα agonist (EC_{50} = 6, 1,070, and 6,200 nM on human PPARα, PPARγ, and PPARδ, respectively), GW7845 (1, 25) is a selective PPARγ agonist (EC_{50} = 3,500, 0.7, and >10,000 nM on human PPARα, PPARγ, and PPARδ, respectively), and GW501516 is a selective PPARδ agonist (Fig. 1*B*). Compared with a synthetic LXRα agonist GW3965 (see *Materials and Methods;* LXRα EC_{50} = 190 nM; J. L. Collins, personal communication), the PPARδ agonist GW501516 showed strong induction of ABCA1 mRNA expression (Fig. 2*A*). The PPARγ agonist GW7845 also induced ABCA1 expression, whereas the PPARα agonist GW7647 showed only a weak effect. In parallel with the measurement of ABCA1 expression, we monitored the ability of the selective PPAR agonists to effect cholesterol efflux from the THP1 macrophages (Fig. 2*B*). Although not as effective as the LXRα agonist, the PPARδ agonist GW501516 produced a 2-fold increase in cholesterol efflux to apoAI (Fig. 2*B* and Table 1). Thus, PPARδ is an RXR partner that regulates cholesterol efflux from macrophages. Both the PPARα and PPARγ agonists were inactive despite their ability to produce small increases in ABCA1 expression, which suggests that activation of pathways in addition to ABCA1 may be required to increase cholesterol efflux from these cells.

PPARδ is expressed in many tissues that contribute to cholesterol flux (4). To evaluate whether PPARδ plays a broad role in the regulation of reverse cholesterol transport, we evaluated GW501516 in human fibroblast and intestinal cell lines in addition to THP1 macrophages (Table 1). In 1BR3N human skin fibroblasts, the PPARδ agonist produced a 3.4-fold increase in ABCA1 expression and a 2-fold increase in apoA1-specific cholesterol efflux. In FHS74 human intestinal cells (26), the PPARδ agonist produced a 2.1-fold increase in ABCA1 expression. Little or no changes in the expression of LXRα (Table 1) and no changes in the expression of PPARδ (data not shown) were observed in the macrophages, fibroblasts, or intestinal cells. These results suggest that PPARδ agonists may promote cholesterol efflux from multiple tissues by increasing expression of the reverse cholesterol transporter ABCA1.

A PPARδ Agonist Raises HDLc in a Primate Model of the Metabolic Syndrome X. To assess whether the activity of GW501516 on ABCA1 expression and cholesterol efflux in cells would trans-

Table 1. Effect of GW501516 on cholesterol efflux and mRNA expression

Cell line	Cell type	Cholesterol efflux, %	ABCA1 mRNA, %	LXRα mRNA, %
THP1	Macrophage	200 ± 15	560 ± 150	190 ± 40
1BR3N	Fibroblast	210 ± 10	340 ± 25	115 ± 20
FHS74	Intestinal	N.D.	215 ± 30	120 ± 10

Data are presented as the mean of the percent change (%) relative to vehicle from assays performed in triplicate ± SD. Vehicle = 100%. N.D. = not determined.

Table 2. Effect of GW501516 on clinical chemistries in male obese rhesus monkeys

Dose, mg/kg, bid	Serum conc., ng/ml	Total chol., mg/dl	HDLc, mg/dl	Trig., mg/dl	Insulin, μU/ml	Glucose, mg/dl
0	—	125 ± 10	61 ± 9	132 ± 15	105 ± 24	69 ± 2
0.1	40 ± 91**	135 ± 6	75 ± 9	128 ± 21	113 ± 29	66 ± 2
0.3	71 ± 24**	141 ± 9	84 ± 9*	85 ± 13*	82 ± 19	66 ± 2
1.0	265 ± 100**	151 ± 12	95 ± 11*	66 ± 19*	76 ± 23	71 ± 3
3.0	706 ± 109**	159 ± 10*	109 ± 9**	58 ± 16**	55 ± 10*	68 ± 2
Nonobese	—	152	90	48	25	65

Data are presented as mean ± SE for $n = 6$ for all doses except 3.0 mg/kg where $n = 5$. *, $P \leq 0.05$; **, $P \leq 0.01$ compared to vehicle treatment. Historical data from lean rhesus monkeys in the same colony are labeled nonobese.

late to a therapeutic benefit, a primate model of human metabolic disease was selected. We previously have described a colony of rhesus monkeys that develop spontaneous adult-onset obesity on standard low-fat diets and show a high risk of developing diabetes, ultimately requiring insulin therapy to maintain glycemic control (19). In the prediabetic state these primates display many of the features of the human metabolic syndrome X, including dyslipidemia, insulin resistance, central obesity, hyperinsulinemia, and hypertension (27, 28). Importantly, the dyslipidemia observed in these primates is manifest as low HDLc and high triglycerides. Treatment of these primates with either PPARα agonists or PPARγ agonists leads to a clinical response that parallels the known effects of fibrates and glitazones in humans (19, 29).

Six middle-aged obese rhesus monkeys (age = 12.9 ± 1.5 y, weight = 16.6 ± 1.7 kg, and rhesus body mass index = 53 ± 5 kg/m^2) were dosed with vehicle for 4 weeks to establish baseline characteristics (Table 2). Compared with their lean counterparts, the obese rhesus monkeys had low serum HDLc, high fasting triglycerides, and high fasting insulin levels, but were normoglycemic, which are characteristics of prediabetic insulin resistance as defined by longitudinal studies in this population (19, 30). Each animal received increasing doses of GW501516, with the dose maintained at each level for a 4-week period. A dose-dependent increase in the serum level of GW501516 was detected, reaching ≈700 ng/ml at the highest dose (Table 2). Because >99% of the circulating GW501516 is bound to plasma proteins (data not shown), we expect activation of only PPARδ *in vivo* at these serum drug concentrations (31, 32).

GW501516 had a dramatic effect on the serum lipid profile (Table 2). HDLc increased in a dose-dependent manner over the treatment period, with a 79% increase in HDLc relative to the baseline level at the 3.0 mg/kg dose. The ratio of HDLc/total cholesterol also was improved at all doses of GW501516. In parallel with the increase in HDLc, GW501516 produced a dose-dependent lowering of fasting triglycerides, with a 56% decrease at the 3.0 mg/kg dose. No changes in body weight or liver enzymes were seen (data not shown). At the end of the study the animals were monitored through a washout period, during which serum lipid parameters returned to their baseline values (data not shown).

A PPARδ Agonist Produces a Less Atherogenic Lipid Profile. Patients with low HDLc and high triglycerides often show an atherogenic lipid composition containing high levels of small-dense LDL particles (6, 7). To obtain additional characterization of the lipid particle composition in the obese rhesus monkeys after GW501516 therapy, we analyzed plasma samples by proton NMR spectroscopy (33). In humans and obese rhesus monkeys, lipoprotein subclasses distinguished by NMR correlate well with those determined by other methods (20, 33), including ultracentrifugation (D.A.W., unpublished work). At the 3.0 mg/kg dose, NMR analysis showed that GW501516 produced an 80% increase in HDLc relative to the baseline level (Fig. 3*A*), which compares well with the conventional enzymatic determination of serum HDLc levels (Table 2). In parallel, we analyzed serum to determine the effect of GW501516 on the apo composition. ApoAI and apoAII, the major lipoprotein components of HDL (34), were raised by 43% and 21%, respectively, at the 3.0 mg/kg dose of GW501516 (Fig. 4). The increases in apoAI and apoAII suggest that the rise in serum HDLc is associated with the production of more lipoprotein particles (34). Lipoprotein subclass analysis (20) (Fig. 3*B*) confirmed that there was little change in the relative proportions of the HDL subclasses. Thus, the rise in HDLc produced by GW501516 may result from an increase in the number rather than the size of HDL particles.

Consistent with the decrease in fasting triglycerides (Table 2), GW501516 produced a 50% decrease in VLDL (Fig. 3*A*). Remarkably, apoCIII, a component of VLDL and HDL that may

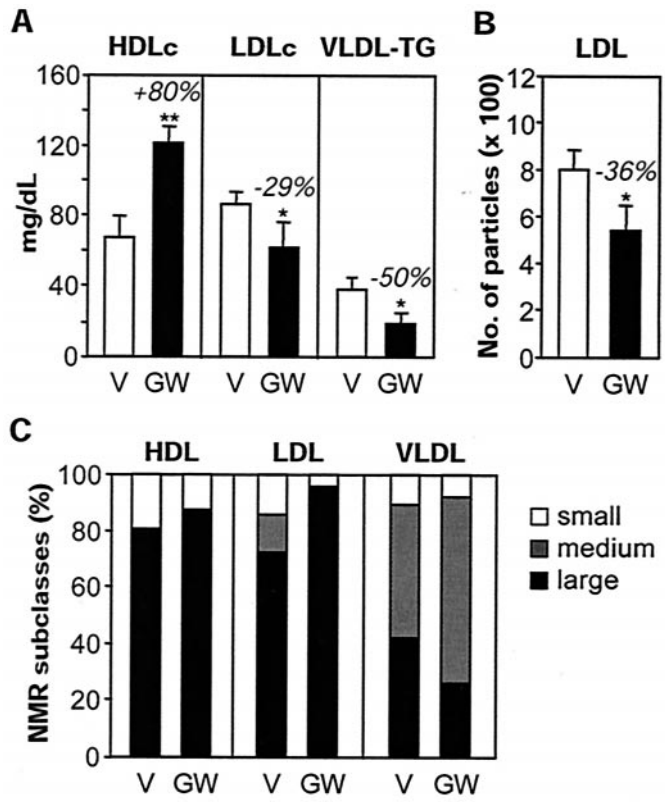

Fig. 3. NMR analysis of serum lipoproteins from primates treated with GW501516. V = vehicle, GW = 3.0 mg/kg GW501516. Data are presented as mean ± SE for $n = 5$. *, $P \leq 0.05$; **, $P \leq 0.01$. (*A*) Lipid values determined by summation of the directly measured concentrations of HDL, LDL, and VLDL subclasses after conversion to cholesterol (c) or triglyceride (TG) units. (*B*) Number of LDL particles calculated from the LDL subclasses. (*C*) Relative compositions of the HDL, LDL, and VLDL subclasses.

play a role in the regulation of peripheral lipoprotein lipase activity (35), was raised by 46% in the drug-treated primates (Fig. 4). This last result is surprising because the triglyceride-lowering activity of fibrates is accompanied by a reduction in the serum levels of apoCIII and an increase in the clearance of VLDL (36). For example, in this population of obese rhesus monkeys, fenofibrate lowered apoCIII by 29% when serum triglycerides were reduced by 55% and HDLc was raised by 35% (19). Thus, the PPARδ agonist GW501516 and the PPARα agonist fenofibrate produce opposite effects on serum apoCIII levels, which is an indication that they regulate lipid metabolism by different biochemical mechanisms.

NMR analysis revealed a 29% decrease in LDL cholesterol and a 36% decrease in the number of LDL particles in the drug-treated primates (Fig. 3 *A* and *B*). Although these changes are not as dramatic as those in the HDL fraction, further analysis (Fig. 3*C*) showed that GW501516 produced a reduction in the proportion of small and medium LDL subclasses, while increasing the proportion of large LDL and medium VLDL subclasses. Therefore, the reduction in LDL is due largely to a decrease in the number of small-dense particles (6). Overall, our analysis by three independent methods indicates that GW501516 changes the serum lipoprotein composition by increasing the number of HDL particles while producing fewer, yet larger, LDL and VLDL particles. Together, these effects should result in a less atherogenic lipid composition.

A PPARδ Agonist Corrects Hyperinsulinemia in Primates. The rhesus monkeys in this study were hyperinsulinemic but maintained glucose concentrations in the normal range. Although not a primary endpoint of the *in vivo* study, we noted that GW501516 produced a dose-dependent decrease in the serum insulin levels (Table 2). At the 3.0 mg/kg dose, a 48% decrease in serum insulin was seen relative to the baseline level, whereas no changes in fasting glucose levels were detected throughout the study. Thus, GW501516 partially corrects the hyperinsulinemia in these primates without adverse effects on glycemic control. The mechanism by which PPARδ lowers insulin in these primates remains to be determined.

Discussion

During the past decade the role of the orphan nuclear receptors PPARα and PPARγ in the regulation of lipid metabolism has been clearly established through their association with the fibrate and glitazone drugs, respectively (1). There have been several tantalizing clues that PPARδ also may modulate aspects of lipid homeostasis: PPARδ binds to many of the same fatty acids as the other subtypes (2), signifying that it also may be a dietary lipid sensor; L-165041, a weak nonselective PPARδ agonist (1), raised total cholesterol levels in *db/db* mice (37); and PPARδ null mice displayed a reduction in the size of adipose tissue depots (38), although no lipid phenotype was reported. However, in the absence of potent and selective ligands, the physiological role of PPARδ has remained an enigma. In this report, we have described a truly selective PPARδ agonist, GW501516. Using GW510516 as a chemical tool, we have provided evidence that PPARδ increases cholesterol efflux from cells, in part, through an increase in the expression of the ABCA1 reverse cholesterol transporter. These data suggest that PPARδ is an important regulator of cholesterol metabolism with unique pharmacology that distinguishes it from the other PPAR subtypes.

There are significant differences in the regulation of lipid metabolism in rodents compared with humans (39). This is highlighted by the fact that there is no single rodent model of dyslipidemia in which both PPARα and PPARγ agonists are active (40). We chose to study the pharmacology of a PPARδ ligand in obese rhesus monkeys where the lipid profile more

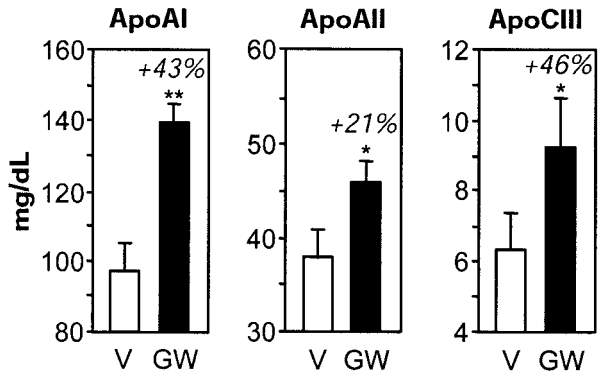

Fig. 4. Serum apo concentrations determined by immunoprecipitation. V = vehicle, GW = 3.0 mg/kg GW501516. Data are presented as mean ± SE for $n = 5$. *, $P \leq 0.05$; **, $P \leq 0.01$.

closely matches that seen in humans (19). In these primates, GW501516 has beneficial effects on multiple cardiovascular risk factors, including lipoprotein size and composition, resulting in a potentially less atherogenic lipid profile (6). These changes, which include a marked increase in HDLc, are consistent with an increased flux of cholesterol from peripheral tissues to nascent HDL particles. Patients with Tangier disease and familial hypoalphalipoproteinia have low circulating levels of HDLc and high triglycerides due to mutations in the *ABCA1* gene (11). Fibroblasts from these patients show a reduced capacity for cholesterol efflux, which correlates with the decrease in HDLc and an increased risk of cardiovascular disease (15). Because, GW501516 increases ABCA1 expression, promotes cholesterol efflux from peripheral cell types, and raises HDLc in primates, it appears that activation of PPARδ provides a novel mechanism for promoting reverse cholesterol transport (9). Additional studies will be required, however, to determine the global effect of GW501516 on cholesterol flux from peripheral tissues to the liver.

Fibrates are a class of drugs that have been used for decades for their beneficial effects on serum lipids. Although fibrates are predominantly triglyceride-lowering drugs that only modestly raise HDLc (19, 36), clinical trials have shown that they lower the incidence of atherosclerosis and coronary artery disease in patients with normal levels of LDLc (36, 41). Most fibrate drugs are only weakly active on human PPARα and show low selectivity over human PPARδ and PPARγ (1). It was recently reported that the experimental fibrate drug Wy14,643 induces ABCA1 expression and cholesterol efflux from macrophages (42). However, at the concentrations used in the study (50 μM), Wy14,643 has significant PPARδ activity [EC_{50} = 35 μM for human PPARδ (1)]. Using compounds that are selective for each of the three PPAR subtypes, we have now shown that their relative ability to induce ABCA1 expression is PPARδ > PPARγ > PPARα. These data argue that the reported effects (42) of high doses of fibrates on cholesterol efflux are mediated primarily through PPARδ. Glitazone PPARγ agonists also were reported to increase ABCA1 expression through the induction of LXRα expression (42, 43). Interestingly, we observed no consistent increase in LXRα expression with GW501516 (Table 1) suggesting that alternate mechanisms may contribute to the regulation of ABCA1 expression by PPARδ agonists.

The therapeutic effects of fibrates are due, in part, to their effects on hepatic gene expression mediated by PPARα (36). In particular, decreases in the expression of apoCIII have been associated with the triglyceride-lowering activity of PPARα agonists (44–46). We were surprised to find that GW501516 raised serum apoCIII levels in the obese rhesus monkeys.

Although both PPARα and PPARδ agonists lower triglycerides in primates, GW501516 has a greater effect on HDLc compared to a fibrate. It is possible that the observed increase in apoCIII after activation of PPARδ is due to an increase in the number of these apos associated with the HDL particles. In addition, GW501516 does not affect apoA1 or apoCIII mRNA levels in human hepatocytes (data not shown), providing further evidence that changes in hepatic gene expression are unlikely to explain the changes in serum HDLc or triglycerides.

Hyperinsulinemia and the lipid triad of low HDLc, small LDL particles, and elevated serum triglycerides are characteristics of dyslipidemia associated with the metabolic syndrome X (6, 7). Individuals with this atherogenic lipoprotein phenotype have a higher incidence of premature coronary artery disease (6). Our results demonstrate that PPARδ agonists are likely to have beneficial effects on the lipid triad and the atherogenic particle composition through a mechanism that increases cholesterol flux from peripheral tissues. These activities, combined with the benefit of lowering serum insulin levels, suggest that PPARδ agonists may be powerful drugs for decreasing the incidence of cardiovascular disease associated with the metabolic syndrome X.

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