Identification of a Novel Selective Peroxisome Proliferator-Activated Receptor α Agonist, 2-Methyl-2-(4-{3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-3-yl]propyl}phenoxy)propanoic Acid (LY518674), That Produces Marked Changes in Serum Lipids and Apolipoprotein A-1 Expression

Jai Pal Singh, Raymond Kauffman, William Bensch, Guoming Wang, Pam McClelland, James Bean, Chahrzad Montrose, Nathan Mantlo, and Asavari Wagle
Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana
Received January 5, 2005; accepted May 23, 2005

ABSTRACT

Low high-density lipoprotein-cholesterol (HDL-c) is an important risk factor of coronary artery disease (CAD). Optimum therapy for raising HDL-c is still not available. Identification of novel HDL-raising agents would produce a major impact on CAD. In this study, we have identified a potent (IC50 24 nM) and selective peroxisome proliferator-activated receptor α (PPARα) agonist, 2-methyl-2-(4-{3-[1-(4-methylbenzyl)-5-oxo-4,5-dihydro-1H-1,2,4-triazol-3-yl]propyl}phenoxy)propanoic acid (LY518674). In human apolipoprotein A-1 (apoA-1) transgenic mice, LY518674 produced a dose-dependent increase in serum HDL-c, resulting in 208 ± 15% elevation at optimum dose. A new synthesis of apoA-1 contributed to the increase in HDL-c. LY518674 increased apoA-1 mRNA levels in liver. Moreover, liver slices from animals treated with LY518674 secreted 3- to 6-fold more apoA-1 than control liver slices. In cultured hepatocytes, LY518674 produced 50% higher apoA-1 secretion, which was associated with increase in radiolabeled methionine incorporation in apoA-1. Thus, LY518674 is a potent and selective PPARα agonist that produced a much greater increase in serum HDL-c than the known fibrate drugs. The increase in HDL-c was associated with de novo synthesis of apoA-1.

Low plasma high-density lipoprotein-cholesterol (HDL-c) and its associated apolipoprotein A-1 (apoA-1) are known risk factors for coronary artery disease (CAD) (Gordon and Rifkind, 1989). Approximately 40 to 50% of the patients with established CAD have cholesterol levels that are considered in the normal range (<200 mg/dl). In a large number of these patients, low HDL-c is a common risk factor (Genest et al., 1991; Rubins et al., 1995). Moreover, low HDL-c is associated with a substantial increase in risk of CAD in diabetics. Several pharmacological strategies are currently being investigated to raise HDL-c including the inhibition of cholesterol ester transfer protein (CETP), activation of peroxisome proliferator-activated receptors (PPARs) and induction of ABCA1 (Rader, 2002; Brewer, 2004). The nuclear hormone receptor PPAR subfamily plays a major role in the regulation of lipids and glucose metabolism. PPARα, PPARγ, and PPARδ, encoded by three distinct genes, are members of the PPAR subfamily (Willson et al., 2000). PPARα is predominantly expressed in tissues that metabolize high amounts of fatty acids such as liver, kidney, heart, and muscle (Braisant et al., 1995; Auboeuf et al., 1997). High level of PPARγ is expressed in adipose tissues where its activation is associated with adipocyte differentiation and insulin sensitivity (Tontonoz et al., 1995). PPARδ is widely expressed in tissues...
(Braissant et al., 1995). Recent studies suggest that PPARδ also plays a role in lipid metabolism, presumably through mechanisms different from PPARα and PPARγ (Oliver et al., 2001). Several synthetic ligands of PPARα, including fenofibrate, gemfibrozil, bezafibrate, and clofibrate have been developed as therapy for dyslipidemia (Heller and Harvengt, 1983; Robins, 2001). However, fibrates are neither potent nor selective PPARα agonists. In rodents, fibrates lower HDL-c by reducing apoA-1 synthesis and also by inducing changes in HDL-modifying enzymes such as lecithin:cholesterol acyltransferase (Schoonjans et al., 1995, 1996). In humans, fibrates reduce triglycerides and produce a moderate elevation of HDL-c (Robins, 2001).

Creation of a human apoA-1 transgenic mouse has provided a useful model for evaluation of HDL-c–raising agents and the investigation of the mechanism of HDL-c elevation. In this model, the human apoA-1 gene expression is driven by its homologous promoter, which includes the PPAR response element (PPRE). Therefore, fibrates produce a decrease in mouse apoA-1 and an increase in human apoA-1 expression (Berthou et al., 1996, Vu-Dac et al., 1998). Using this model, we show that a highly potent and selective PPARα agonist, LY518674, produced a large increase in HDL-c. Using ex vivo studies with liver from transgenic animals dosed with LY518674 and isolated hepatocytes, we have shown that a de novo induction of apoA-1 synthesis is an important mechanism contributing to the HDL-c elevation. Thus, the highly potent and selective PPARα agonist modulating HDL-c by enhancing apoA-1 synthesis may represent important pharmacological means for the treatment of patients with dyslipidemia, particularly those with low HDL-c.

**Materials and Methods**

LY518674 and LY487, shown in Fig. 1, were synthesized at Eli Lilly and Company. Fenofibrate was obtained from Sigma (St. Louis, MO).

**Animal Dosing.** Human apoA-1 transgenic mice from Jackson ImmunoResearch Laboratories Inc. (West Grove, PA) were maintained on a chow diet. Animals were dosed orally with vehicle [1% (w/v) carboxymethylcellulose and 0.25% Tween 80], fenofibrate, or LY518674 for 1 week. Three hours after the final dose, animals were sacrificed by CO2 asphyxiation, blood was drawn by cardiac puncture, and livers were excised. Blood samples were allowed to clot at room temperature, and serum was prepared by centrifugation.

**Fast-Performance Liquid Chromatography.** Lipoproteins were separated by PFLC and quantified using an in-line detection system as described by Kief et al. (1991). Pooled serum sample (25 μl) was applied to a Superose 6HR 10 × 30 cm column (Amersham Biosciences, Piscataway, NJ) and eluted with phosphate-buffered saline, pH 7.4, containing 5 mM EDTA at 0.5 ml/min. The area under the curve corresponding to the elution of very low density lipoprotein (VLDL), LDL, and HDL was calculated using PerkinElmer Turbochrom (version 4.12F12) software (PerkinElmer Life and Analytical Sciences, Boston, MA). Serum cholesterol and triglycerides were determined by standard enzymatic methods.

**Liver Slicing.** Each liver lobe was cored and then sliced using Krebs-Henseleit buffer supplemented with 20 mM glucose and mannitol. The slices were pooled and four slices per vial were incubated in hepatocyte maintenance medium (Cambrex Bio Science Walkersville, Walkersville, MD) for 2 h in a shaking water bath maintained at 28°C under 95% O2 and 5% CO2. Aliquots of the medium were collected at the indicated time intervals.

**RNA Extraction and Northern Blot Analysis.** Total RNA was extracted with RNeasy reagent (QIAGEN, Valencia, CA), electrophoresed on a 1% agarose-formaldehyde gel, and then pressure-blotted (PosiBlot 30–30 Pressure Blotter; Stratagene, La Jolla, CA) onto BrightStar-Plus nylon membrane (Ambion, Austin, TX). Membranes were UV-cross-linked and hybridized in NorthernMax Prehyb/Hyb buffer for 18 h at 42°C with a 32P-labeled cDNA probe for apoA-1. Membranes were washed with NorthernMax High Stringency wash solution before exposure to Hyperfilm MP (Amersham Biosciences UK Ltd., Little Chalfont, Buckinghamshire, UK). Expression levels were normalized with 18S RNA.

**Western Blotting.** Protein concentration in the liver slice incubation medium collected at 30, 60, and 120 min was determined using Bradford reagent (Bio-Rad, Hercules, CA). Equal amounts of protein (253 μg) were loaded onto a 4 to 20% Tris-Gly gel (Invitrogen, Carlsbad, CA), separated by SDS-polyacrylamide gel electrophoresis (PAGE), and transferred to polyvinylidene difluoride membrane. The membranes were blocked by incubation with 5% nonfat dry milk in Tris-buffered saline with Tween 20 and then blotted with human apoA-1 monoclonal antibody (Biodesign International, Kennebunk, ME). Bands were visualized using ECL detection system (Amersham Biosciences) and quantified by scanning densitometry.

**Apo-A-1 Production in Monkey Hepatocytes.** Cryopreserved hepatocytes from cynomologus monkeys (Cambrex Bio Science Walkersville) were cultured in hepatocyte maintenance medium for 24 h. Cells were then treated with the compounds. After 48 h, culture supernatants were collected and subjected to SDS-PAGE followed by Western blotting. For quantification, the apo-A-1 bands were normalized to transferrin band in each sample. For [35S]methionine labeling of proteins, cells were incubated in methionine-free RPMI 1640 medium for 24 h in the presence or absence of the compound. [35S]methionine (30 μCi) was then added, and the cultures were further incubated for 48 h. Apo-A1 was immunoprecipitated from the supernatants (150 μg of protein) using monoclonal antibodies coupled to protein G-Sepharose (Biodesign International). The immunoprecipitated fractions were washed and electrophoresed. The bands were quantified by scanning densitometry.

**Binding Assay.** DNA-dependent binding was performed using Scintillation Proximity Assay (Amersham Biosciences). Biotinylated oligonucleotide 5'-TAAATGTAGGTAATAGTTCAATAGGTCAAAG-GG-3' was used for binding receptor dimers to Yttrium silicate streptavidin-coated beads in the presence of the 3H-labeled corresponding ligand for each receptor. Competition binding reactions were carried out in 10 mM HEPES buffer, pH 7.8, 80 mM KCl, 0.5 mM MgCl2, 1 mM dithiothreitol, 0.5% CHAPS, and 14% glycerol using 2.5 μM each of PPARα, PPARγ, or PPARβ and retinoid X receptor α, 0.1 nM to 10 μM concentrations of compounds, and 30,000 cpm of the corresponding labeled ligand.

**Cotransfection Assay.** Cotransfection assays were performed in CV-1 cells using calcium phosphate coprecipitation method as described previously (Mukherjee et al., 1997). The reporter plasmid consisted of the acyl-CoA oxidase PPRE and thymidine kinase promoters upstream of the luciferase reporter cDNA. For PPARα, interference by endogenous PPARα was eliminated by using a GAL4 chimera in which the DNA binding domain of the transfected PPARα was replaced by that of GAL4 and the GAL4 response element was used in place of the acyl-CoA oxidase PPRE. After an overnight incubation, transfected cells were trypsinized and plated in 96-well
dishes in Dulbecco's modified Eagle's medium containing 10% charcoal-stripped fetal bovine serum. After 6 h, cells were exposed to a 0.1 nM to 10 μM concentration of test compounds. Cotransfection efficacy was determined relative to reference agonists for each receptor.

Results

LY518674 Is a Potent and Selective PPARα Agonist.

In vitro potency and selectivity for PPARs were determined by direct receptor binding, as well as cotransfection assays. Table 1 shows that, in receptor binding assays, LY518674 was >3000-fold more potent than fenofibric acid. Likewise, in a transient cotransfection assay, LY518674 was more potent than fenofibrate. In this assay, the EC50 of fenofibrate could not be determined exactly because of its low solubility in the buffer. Based on the IC50 determination, LY518674 was a 310-fold more selective agonist for PPARα compared with PPARγ. Treatment with LY518674 up to 10 μM did not reach 50% inhibition in the PPARγ and PPARδ cotransfection assays. In comparison, fenofibrate was only approximately 2-fold more selective for PPARα than PPARγ. LY518674 did not show cross-reactivity with retinoid X receptor, retinoic acid receptor, estrogen receptor, and glucocorticoid receptor when tested at 10 μM. These data show that LY518674 is a potent and selective PPARα agonist.

Effect of LY518674 on Serum Lipids in Human ApoA-1 Transgenic Mice. To study the effect of PPARα agonists on serum lipids, human apoA-1 transgenic mice were used. Animals were dosed once daily for 1 week by oral gavage. On the day of sacrifice, animals were bled by cardiac puncture. Serum lipoprotein fractions were determined using FPLC. The lipoprotein profiles of the control, fenofibrate, and LY518674-treated groups are shown in Fig. 2A. Serum from human apoA-1 transgenic mice primarily showed an HDL-c peak and small VLDL cholesterol peak (elution time of 21.7 min). Because LDL-c in human apoA-1 transgenic animals is very low (<5%), as expected, little or no LDL-c was evident in the high-pressure liquid chromatography fractions. Treatment with fenofibrate (100 mg/kg/day) increased the HDL-c peak area. A leftward shift in peak indicated an increase in particle size. Similar to fenofibrate, LY518674 produced a dose-dependent increased HDL-c peak (shown here for 0.1 mg/kg/day dose). The LY518674 dose dependence data transformed to percentage change in HDL-c are shown in Fig. 2B. In addition to increasing HDL-c, fenofibrate and LY518674 also decreased the VLDL-cholesterol peak (Fig. 2A). The overall lipoprotein profile observed here is in good agreement with PPARα agonist-induced effects in human apoA-1 transgenic mice shown by others (Berthou et al., 1996). The effect of treatments on serum triglyceride levels is shown in Fig. 2C. The dose that produced half-maximal effect on HDL-c and triglycerides (ED50), as determined by nonlinear regression analysis, is presented in Table 2. These data show that LY518674 was considerably more potent than fenofibrate. The ED50 values for HDL-c elevation and triglyceride reduction for LY518674 were 2 to 3 log lower than the corresponding values for fenofibrate. The HDL-c cholesterol achieved at the ED50 of LY518674 and fenofibrate is shown within parentheses. In addition, the maximum HDL-c level achieved by LY518674 was 208 ± 15% over control compared with 106 ± 7.6% for fenofibrate. These data demonstrate that LY518674 was highly potent and efficacious in modulating serum lipids.

Effect of LY518674 on ApoA-1 mRNA. To determine the effect of LY518674 on steady-state levels of apoA-1 mRNA, total RNA from control and compound-treated animals was used. Animals were dosed once daily for 7 days with LY518674 (10 mg/kg/day), fenofibrate (100 mg/kg/day), or vehicle. Three hours after the last dose, blood samples were collected and serum lipoproteins were resolved by FPLC as described under Materials and Methods. A, FPLC profile of serum derived from control animals and those treated with 0.1 mg/kg/day of LY518674 or 100 mg/kg/day of fenofibrate. B and C, HDL-c and triglyceride levels at the indicated dose.

Table 1
In vitro potency and selectivity of LY518674: comparison with fenofibric acid

<table>
<thead>
<tr>
<th>Compound</th>
<th>hPPARα IC50</th>
<th>hPPARα EC50</th>
<th>mPPARα IC50</th>
<th>mPPARα EC50</th>
<th>hPPARγ IC50</th>
<th>hPPARγ EC50</th>
<th>hPPARδ IC50</th>
<th>hPPARδ EC50</th>
<th>PPARγ/PPARα</th>
</tr>
</thead>
<tbody>
<tr>
<td>LY518674</td>
<td>24.5 ± 2.8</td>
<td>45.9 ± 5.2</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>&gt;10,000</td>
<td>310</td>
</tr>
<tr>
<td>Fenofibric acid</td>
<td>68,000</td>
<td>&gt;50,000</td>
<td>962 ± 80</td>
<td>&gt;50,000</td>
<td>&gt;50,000</td>
<td>&gt;50,000</td>
<td>&gt;50,000</td>
<td>&gt;50,000</td>
<td>1.8</td>
</tr>
</tbody>
</table>
extracted and apoA-1 mRNA levels were analyzed by Northern blotting. LY518674 produced a significant increase in steady-state levels of human apoA-1 mRNA (Fig. 3A). Consistent with the previous reports, the mouse apoA-1 mRNA was reduced (Fig. 3B). In this study, we did not observe a significant change in apoA-1 mRNA in livers from the fenofibrate-treated animals. This could be the result of a lower dose used in our studies. In previous studies, the dose of fenofibrate needed to achieve increase in mRNA was 500 mg/kg/day.

**LY518674 Increased ApoA-1 Production in Liver.** To determine whether the increase in plasma HDL-c was associated with an increased synthesis of apoA-1, we conducted ex vivo studies on liver slices from animals dosed with PPARα agonists. ApoA-1 transgenic mice were treated with either 10 mg/kg LY518674 or 100 mg/kg fenofibrate. After daily dosing for 7 days, liver was excised and each liver lobe was cored. The sliced cores were incubated in hepatocyte maintenance medium. Aliquots of the medium were collected at the specified times and subjected to SDS-PAGE followed by Western blotting. Figure 4 shows that the liver slices from LY518674-treated mice produced 3- to 6-fold higher apoA-1 in the medium than the control liver slices. Consistent with the serum HDL-c levels, the level of apoA-1 secreted in LY518674-treated group was markedly higher than the fenofibrate group.

**PPARα Agonists Stimulate ApoA-1 Production in Primate Hepatocytes.** To demonstrate whether LY518674 stimulated apoA-1 synthesis, we used primary cultures of monkey hepatocytes. Hepatocytes from cynomologus monkeys were treated with either vehicle or LY518674 for 24 h. Culture supernatants were subjected to SDS-gel electrophoresis, Western blotted using apoA-1 antibodies, and then scanned for apoA-1 quantification. A representative blot and quantification of apoA-1 are shown in Fig. 5A. LY518674 produced a concentration-dependent increase in apoA-1 secretion in the medium. The compound-treated cultures contained approximately 50% more apoA-1 than the control cultures. To further show that the increased secretion of apoA-1 was due to de novo synthesis, [35S]methionine labeling of apoA-1 was performed. Hepatocytes were treated with LY487, a compound with potency and selectivity similar to LY518674, for 24 h in methionine-free medium, and [35S]methionine was then added. After 48 h, apoA-1 in the supernatants was immunoprecipitated using monoclonal anti-apoA-1 antibodies coupled to protein G-Sepharose. The immunoprecipitated fractions were electrophoresed. The bands were quantified by scanning densitometry. Figure 5B shows that hepatocyte cultures treated with LY487 produced approximately 2-fold higher [35S]methionine-labeled apoA-1 in the medium than the control cultures. These results confirmed that treatment with PPARα agonist stimulated synthesis and secretion of apoA-1 in hepatocytes.

**Discussion**

It is now well recognized that HDL plays an important cardioprotective role. Low HDL-c levels are associated with the development of CAD. On the other hand, the elevation in plasma HDL-c levels in humans and preclinical models of dyslipidemia has been shown to decrease atherosclerosis. Overexpression of apoA-1 increases HDL-c and protects against the development of arteriosclerosis (Rubin et al., 1991). Studies in animal models and recently in humans have shown that infusion of HDL or apoA-1 Milano reduces the development of atherosclerosis and regresses the pre-existing disease (Badimon et al., 1990; Nissen et al., 2003). Past efforts on the development of therapy for HDL-raising agents have led to agents that produce only a modest elevation of HDL-c. In various studies, an increase of only 6 to 11% in HDL-c has been achieved with fenofibrate (Robins, 2001). Likewise, only a 6% change in HDL was observed with gemfibrozil (Rubins et al., 1999). Nevertheless, treatment with

### TABLE 2
Effect of PPARα agonist on serum lipids in human apoA-1 transgenic mice

<table>
<thead>
<tr>
<th>Compound</th>
<th>HDL-c</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED₅₀</td>
<td>Max</td>
</tr>
<tr>
<td></td>
<td>mg/kg</td>
<td>% increase</td>
</tr>
<tr>
<td>LF518674</td>
<td>0.34 ± 0.11 (458 ± 52 mg/dl)</td>
<td>208 ± 15</td>
</tr>
<tr>
<td>Fenofibrate</td>
<td>106 ± 76 (300 ± 42 mg/dl)</td>
<td>122 ± 35</td>
</tr>
</tbody>
</table>
these suboptimal therapeutics has demonstrated a significant clinical benefit in patients with CAD. These studies suggest that a more potent PPARα agonist producing greater increase in HDL-c could be effective therapy for dyslipidemia and CAD. In this article, we have described a potent and selective PPARα agonist that produced major effects on serum lipids and apoA-1 synthesis. LY518674 was >2000-fold more potent and >150-fold more selective than fenofibrate. In human apoA-1 transgenic mice, LY518674 was highly potent, producing 50% reduction in triglycerides at 0.1 ± 0.04 mg/kg. More importantly, LY518674 produced a marked increase in HDL-c. The ED50 for elevating HDL-c was 0.34 ± 0.11 mg/kg for LY518674 compared with 106 ± 76 mg/kg for fenofibrate. The maximum increase in HDL-c levels was also higher with LY518674 than fenofibrate (208 ± 15 versus 122 ± 35%). Thus, LY518674 was a highly potent HDL-raising compound with greater maximum efficacy than the currently marketed fibrates.

Plasma HDL-c levels are modulated by a number of different mechanisms. ApoA-1 synthesis in the liver and intestine are the major sources of newly appearing HDL-c in plasma. Decrease in catabolism of apoA-1–containing particles involving CETP, lecithin:cholesterol acyltransferase, endothelial and hepatic lipase activities, and scavenger receptor B type 1 or an increased efflux of cholesterol from periphery as a result of induction of ABCA1 expression could also contribute to plasma HDL-c (Rader, 2002; Cuchel and Rader, 2003). It is not clear whether plasma HDL-c elevation achieved by these different mechanisms produces similar effects on the development of atherosclerosis. For example, CETP deficiency increases HDL-c, which may be associated with increased risk of coronary heart disease (Hirano, et al., 2000). Patients deficient in hepatic lipase exhibit increased HDL-c levels (Connelly and Hegele, 1998). Deficiency of hepatic lipase is also associated with dyslipidemia and thus may be a risk of coronary artery disease. Deficiency of scavenger receptor B type 1 in mice produces elevated HDL-c (Rigotti et al., 1997) but was associated with increased atherosclerosis (Trigatti, et al., 1999; Huszar, et al., 2000). An elevation of HDL-c achieved by increasing apoA-1 synthesis is considered a favorable mechanism. Some evidence suggests that the weak PPARα agonist and fibrates increase HDL-c by increasing apoA-1 synthesis (Berthou, et al., 1996). However, because fibrates exhibit relatively low potency and selectivity for PPARα and the effects on apoA-1 occur at very high concentrations, questions remain whether the pharmacological effects observed with fenofibrate are mostly attributed to their direct effect on PPARα. For example, in a recent study in hyperlipidemic primates, fenofibrate increased HDL-c but an increase in plasma apoA-1 was not observed (Winegar, et al., 2001). Fenofibrate increases apoA-1 production in human hepatocytes. However, these effects are observed at very high concentrations (500 μM), which potentially could produce off-target effects, because fibrates are known to interact with other nuclear hormone receptors (Wurch, et al., 2002).

We have shown, using several experimental approaches, that the increase in HDL-c by LY518674 was associated with an increase in apoA-1 synthesis. In LY518674-treated animals, liver apoA-1 mRNA levels were significantly elevated. Ex vivo determination of apoA-1 showed that liver slices from animals treated with LY518674 produced a 3- to 6-fold higher apoA-1. Finally, the treatment of cultures of monkey hepatocytes with LY518674 resulted in 50% more apoA-1 secretion in the culture medium than the control cultures.

**Fig. 4.** LY518674 increases apoA-1 production in liver. Human apoA-1 transgenic mice were dosed with 10 mg/kg/day LY518674 or 100 mg/kg fenofibrate for 7 days. Animals were sacrificed, and livers were excised. Liver slices were prepared and incubated in hepatocyte maintenance medium. Aliquots of the incubation medium were removed at 0.5, 1, or 2 h, and equal amounts of protein (235 μg) were immunoprecipitated with apoA-1 antibodies. The immunoprecipitates were resuspended in SDS-gel buffer and subjected to SDS-gel electrophoresis followed by Western blotting. The lanes in the inset from left to right shown here and in the bar graph at each time point are control ( ), fenofibrate ( ), and LY518674 ( ), respectively. The bands from three different animals were scanned and plotted on a bar graph. The data show the mean of three experiments ± S.D.

**Fig. 5.** LY518674 induces de novo synthesis of apoA-1. A, primary monkey hepatocytes were cultured in hepatocyte maintenance medium for 2 days. LY518674 or vehicle (1% dimethyl sulfoxide) was added and further incubated for 72 h. Culture supernatants were collected and subjected to SDS-gel electrophoresis followed by Western blotting. The Western blot bands at each concentration are shown at the bottom of the bar graph. The bar graph (A) shows the quantification after densitometric scanning of bands and normalizing with transferrin. To demonstrate de novo synthesis of apoA-1, hepatocytes were incubated in methionine-free medium. After 24 h, 10 nM LY487 was added to the culture and further incubated for 48 h in the presence of [35S]methionine. Supernatants were collected, and apoA-1 was immunoprecipitated using human apoA-1 antibodies. Precipitates were solubilized in gel buffer. Equal amount of protein from each sample was loaded onto SDS-polyacrylamide gel electrophoresis. Autoradiograms were scanned and quantified.
The increase in apoA-1 by LY518674 was associated with increased $^{35}$Smethionine incorporation in apoA-1 protein. These data support that LY518674 induces de novo synthesis of apoA-1.

We conclude that LY518674 is a novel potent and selective PPARα agonist that produced a profound increase in HDL-c in human apoA-1 transgenic mice. The increase in HDL was at least partly contributed by a de novo synthesis of apoA-1. Based on its effect of serum lipids and HDL, LY518674 represents a unique therapeutic agent for the treatment of dyslipidemia and atherosclerosis.

References


Downloaded from aspetjournals.org at AT&T Journals on July 21, 2017