Peroxisome Proliferator-Activated Receptor Agonists Modulate Heart Function in Transgenic Mice with Lipotoxic Cardiomyopathy

Reeba K. Vikramadithyan, Kumiko Hirata, Hiroaki Yagyu, Yunying Hu, Ayanna Augustus, Shunichi Homma, and Ira J. Goldberg

Department of Medicine, Columbia University, New York, New York

Received November 6, 2004; accepted January 20, 2005

ABSTRACT

hLpLGPI transgenic mice that overexpress human lipoprotein lipase (hLpL) with a glycosylphosphatidylinositol anchor on cardiomyocytes develop lipotoxic cardiomyopathy associated with increased cardiac uptake of plasma lipids. We hypothesized that peroxisome proliferator-activated receptor (PPAR)α, PPARγ, or a PPARα/γ agonist would alter cardiac function by modulating lipid uptake by the heart. hLpLGPI mice were administered rosiglitazone (10 mg/kg/day), fenofibrate (100 mg/kg/day), or DRF2655, an alkoxy propanoic acid analog (10 mg/kg/day), for 16 days. Rosiglitazone reduced plasma triglyceride (TG) from 107.63 ± 6.98 to 77.61 ± 3.98 mg/dl, whereas fenofibrate had no effect. DRF2655 reduced TG to 33.17 ± 4.12 mg/dl. Rosiglitazone and DRF2655 decreased heart TG and total cholesterol; fenofibrate had no effect. Molecular markers for cardiac dysfunction, atrial natriuretic factor, brain natriuretic peptide, and tumor necrosis factor-α were decreased with rosiglitazone and increased with fenofibrate. Echocardiographic measurements showed reduced fractional shortening and increased left ventricular systolic dimension with fenofibrate. No changes in these parameters were observed with rosiglitazone or DRF2655 treatment. Muscle-specific carnitine palmitoyltransferase-1 and fatty acid transporter protein-1 gene expression were increased with fenofibrate and DRF2655 treatment; no change in expression of these genes was noted with rosiglitazone treatment. Rosiglitazone and DRF2655 reduced TG uptake by the heart, and fenofibrate treatment increased fatty acid uptake. Thus, in a lipotoxic cardiomyopathy mouse model, a PPARγ agonist reduced cardiac lipid and markers of cardiomyopathy, whereas an agonist of PPARα did not improve cardiac lipids and worsened heart function. These changes were paralleled by alterations in heart lipid uptake. Overall, PPAR activators exhibit differential effects in this model of lipotoxic dilated cardiomyopathy.

The three known members of the ligand-activated peroxisome proliferator-activated receptor (PPAR) family of nuclear receptors are α, β/δ, and γ (Schoonjans et al., 1997; Braissant and Wahli, 1998). PPARα is highly expressed in liver, heart, and skeletal muscle; PPARγ is predominantly present in adipose tissue; and PPARδ is ubiquitously expressed. PPARα plays a critical role in the regulation of cardiac lipid metabolism (Francis et al., 2003). How PPARγ exerts its effects on cardiac metabolism and function is not understood, mainly because drugs affecting PPARs have major metabolic actions on liver, adipose, and skeletal muscle that might secondarily alter lipid delivery to the heart (Kelly, 2003).

Lipid accumulation in the heart has been implicated as the cause of dilated cardiomyopathy in some genetic disorders of lipid oxidation, and in obesity and diabetes. Cardiomyopathy occurs in animals with altered cardiac lipid metabolism. Zucker diabetic fatty rats have reduced cardiac function associated with deposition of fat in cardiomyocytes (Zhou et al., 2000). Cardiac-specific overexpression of acyl-CoA synthetase in mouse hearts leads to increased free fatty acid (FFA) uptake and triglyceride (TG) accumulation in cardiomyocytes, apoptosis, cardiac hypertrophy, decreased systolic...
function, and premature death (Chiu et al., 2001). Overexpression of PPARα in cardiomyocytes increases myocardial lipid stores, up-regulates β-oxidation genes, and causes dilated cardiomyopathy (Finck et al., 2002).

Lipoprotein lipase (LpL) is the major enzyme responsible for hydrolysis of TG contained in circulating lipoproteins (Goldberg, 1996). Of all the tissues, the most robust LpL expression is in cardiac muscle (Semenkovich et al., 1989; Preis-Sandil et al., 2002). Although fatty acid accumulation by the heart can occur via uptake of FFA associated with albumin, it is likely that LpL-generated FFAs are the primary source of heart lipids (Augustus et al., 2003; Teusink et al., 2003). Most actions of LpL on circulating lipoproteins occur in the capillaries. However, by creating mice that overexpress LpL anchored to the cardiomyocyte surface via a glycosylphosphatidylinositol (GPI) anchor (hLpLGPI), we showed that cardiomyocyte cell surface LpL can mediate cardiac lipid uptake and lead to dilated cardiomyopathy (Yagyu et al., 2003). These mice have an approximately 4-fold increase in LpL mRNA and activity compared with wild-type mice.

In the present report, we used hLpLGPI mice to assess the effects of PPAR agonists on lipid accumulation and cardiac function. hLpLGPI mice were treated with a PPARα- or PPARγ-specific agonist. In addition, a dual agonist of PPARα/γ was used. The effects of these drugs on plasma and heart lipids, cardiac function, and heart lipid uptake were determined.

Materials and Methods

Animals and Treatment. Rosiglitazone and fenofibrate were purchased from Alexis Biochemicals (San Diego, CA) and Sigma-Aldrich (St. Louis, MO). The dual PPARα/γ agonist DRF2655, an alkoxyl propanoic acid analog (Vikramadithyan et al., 2003), was a kind gift from Dr. Reddy’s Laboratories (Hyderabad, India). Protocols were approved by the Institutional Animal Care and Use Committee of Columbia University. Transgenic hLpLGPI mice have been described previously (Yagyu et al., 2003). Mice of two different age groups were used. Male hLpLGPI mice 8 weeks of age (young) were treated with PPAR agonists for 16 days. Animals were orally gavaged with rosiglitazone or DRF2655 at 10 mg/kg/day; fenofibrate was administered at 100 mg/kg/day. These doses are similar to those used by other investigators (Chaput et al., 2000; Duez et al., 2000). hLpLGPI control mice received vehicle (0.5% carboxymethylcellulose). Another set of hLpLGPI mice 6 to 9 months of age (old) were treated with the PPAR agonists for 1 month. Rosiglitazone and fenofibrate doses were the same as those used by other investigators (Chaput et al., 2000; Duez et al., 2000). TG-labeled emulsion was prepared as described by van Bennekum et al. (1999). Then, 20% Intralipid (KabiVitrum AB, Stockholm, Sweden) was diluted in sterile PBS to a final 10% concentration, labeled with 60 μCi of [3H]triolein (Amersham Biosciences Inc., Piscataway, NJ), and sonicated three times for 20 s at a power level of 40 W to incorporate the triolein. Plasma and Heart Lipids. Blood from overnight fasted mice was collected from the retro-orbital sinus for the measurement of plasma TG, total cholesterol (TC), and FFA. To measure tissue lipids, hearts were perfused with PBS and homogenized in ice-cold 1 M NaCl. Lipids were extracted by Folch method. The dried lipids were solubilized in PBS containing 1% Triton X-100. Plasmap and heart TG, TC, and FFA levels were measured enzymatically by using kits from Wako Pure Chemicals USA (Richmond, VA).

Histology. Neutral lipids were assessed in hearts from overnight fasted mice perfused with PBS and embedded in Tissue-Tek Optimal Cutting Temperature compound (Sakura Finetek Europe, Zoeterwoude, The Netherlands). Midventricular sections of myocardium (6 μm in thickness) were stained with Oil Red O and counterstained with hematoxylin.

Echocardiographic Analysis. Two-dimensional echocardiographic analysis was done using Sonos 5500 (Philips Medical Systems, Andover, MA) in conscious young and old mice after treatment with the PPAR agonists (Takuma et al., 2001). The echocardiographic images were recorded in a digital format and were analyzed off-line by a single observer blinded to the murine genotype (Kocher et al., 2001; Wang et al., 2002).

Cardiac Gene Expression by Northern Blot. Total RNA from the ventricles was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA). RNA (10 μg) was separated on a 1% agarose gel containing formaldehyde and transferred to nylon filter (Hybond-N+) for Northern blotting. We used the following probes: human lipoprotein lipase (hLpL), mouse lipoprotein lipase, atrial natriuretic factor (ANF), and brain natriuretic peptide (BNP). Results are normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Quantitative Real-Time PCR for Cardiac Glucose and Lipid Metabolism and Cardiac Dysfunction Genes. Total RNA was isolated from hearts of control and treated mice using RNeasy mini kit (Qiagen, Valencia, CA). The mRNA levels for mCPT1, FATP-1, CD36, ACO, GLUT4, and TNFα were determined by SYBR Green and/or TaqMan (Applied Biosystems, Foster City, CA) real-time PCR using 10 ng of total RNA. The primer sequences are as follows: mCPT1: (S) 5′-TACAACCTGAGCATGAT-3′, (AS) 5′-GGTACAGGTTCTGAT-3′; CD36: (S) 5′-CAAGTGAGACATGCT-3′, (AS) 5′-CTCGCGGTCTCTAGTGT-3′; FATP-1: (S) 5′-GCGTTTTCCGAAAGGG-3′, (AS) 5′-CGAAGTATGAACAG-3′; GLUT4: (S) 5′-CTGGACACTTCCACTGGA-3′, (AS) 5′-GGAGAAGTGGGCGC-3′; TNFα: (S) 5′-CAAGAAAGACATCTG-3′, (AS) 5′-GCTTACAGGTCTTCA-3′; ACO: (probe) 5′-6′-FAMd (AGATGGGACCACAAGCCT CTGGC)HBQ-1-3′. (S) 5′-d(GCTTCTTGTGTCCTCCATGCT-3′), (AS) 5′-d(GTATCCCCAAGAGTATGC)-3′; and m-Actin: (probe) 5′-CAL Orange d(CACTGGCGCATCTTCTTCC CCC)-3′, (S) 5′-d(AGAGGAAATCGTGCTGCATGAC)-3′, (AS) 5′-d(CTAATTGCACTGGGCC GT)-3′. Real-time PCR standard curves were constructed by using serial dilutions of mouse total RNA isolated from heart. Cycling parameters for mCPT1, CD36, GLUT4, and TNFα were one cycle at 95°C for 10 min, 40 cycles at 95°C for 30 s, 40 cycles at 60°C for 40 s, and 40 cycles at 72°C for 30 s; ACO cycling parameters were one cycle at 50°C for 2 min, one cycle at 95°C for 10 min, 40 cycles at 95°C for 15 s, and one cycle at 60°C for 1 min. Values obtained were normalized to mouse β-actin.

Uptake of TG and Palmitate. FFA turnover used [1,4C]palmitate (PerkinElmer Life and Analytical Sciences, Boston, MA) complexed to 6% FFA free bovine serum albumin as described previously (Coburn et al., 2000). Tg-labeled emulsion was prepared as described by van Bennekum et al. (1999). Then, 20% Intralipid (KabiVitrum AB, Stockholm, Sweden) was diluted in sterile PBS to a final 10% concentration, labeled with 60 μCi of [3H]triolein (Amer sham Biosciences Inc., Piscataway, NJ), and sonicated three times for 20 s at a power level of 40 W to incorporate the triolein. Labeled FFA was injected simultaneously with Tg-labeled Intralipid particles.

Statistics. Statistical analyses were done by unpaired t test or analysis of variance. All data are expressed as mean ± S.E.M., with a statistically significant difference defined as a value of P < 0.05.

Results

Effects of PPAR Agonists on Plasma Parameters and Heart Lipids. In the hLpLGPI mice, plasma TG was significantly reduced by rosiglitazone (28%) and DRF2655 (69%) but not by fenofibrate (Fig. 1A). Plasma FFA was reduced by rosiglitazone (28%) and DRF2655 (69%) but not by fenofibrate (Fig. 1A). Plasma FFA was reduced by rosiglitazone (28%) and DRF2655 (69%) but not by fenofibrate (Fig. 1A). Plasma FFA was reduced by rosiglitazone (28%) and DRF2655 (69%) but not by fenofibrate (Fig. 1A).
none of the drugs had any significant effect on plasma glucose: control, 108.96 ± 5.52; rosiglitazone, 123.85 ± 13; fenofibrate, 107.25 ± 5.61; and DRF2655, 88.33 ± 9.10 mg/dl.

Changes in heart lipids paralleled the alterations in plasma lipoproteins. Rosiglitazone reduced TG by 41%, but TC was unchanged. Fenofibrate did not change either lipid. DRF2655 led to a striking reduction in cardiac lipids; both lipid analysis (Fig. 1B) and histological assessment by Oil Red O showed similar results (Fig. 1C). DRF2655 reduced heart TG by 73% and cholesterol by 51%.

Effects of PPAR Agonists on Body and Tissue Weights. We next assessed how the PPAR agonists affected general metabolism. Neither rosiglitazone nor fenofibrate led to a significant change in body weight during the 16-day treatment (Fig. 2A). In contrast, DRF2655-treated mice had a 16% decrease in body weight. The body weight lowering might be due to potent PPARα (or PPARδ) activation (Vikramadithyan et al., 2003). DRF2655 treatment had no effect on food consumption. Both fenofibrate and DRF2655 reduced epididymal fat deposits (Fig. 2B); the decrease was 66% with the dual agonist and 28% with fenofibrate. Both these compounds also caused significant hepatomegaly; as has been reported previously, this is due to peroxisome proliferation (Cornwell et al., 2004). Rosiglitazone did not change fat or liver weight.

Fig. 1. Plasma and heart lipids in hLpL\textsuperscript{GPI} mice treated with the PPAR agonists. A, fasting plasma TG and TC in hLpL\textsuperscript{GPI} control, rosiglitazone-, fenofibrate-, and DRF2655-treated mice, n = 8. B, percentage of heart lipid in overnight fasted untreated and treated mice, percentage of TC and TG calculated based on heart levels of untreated mice (104 ± 7.48 μg/mg tissue for TC and 109 ± 12.7 μg/mg tissue for TG), n = 8. C, myocardial lipid accumulation in overnight fasted mice heart sections stained with Oil Red O. Values are mean ± S.E. *P < 0.05.
Cardiac Function in PPAR Agonist-Treated Mice. Young hLpLGPI mice do not have echocardiographic evidence of cardiomyopathy, although they have an increase in cardiac lipid and expression of heart failure markers (Yagyu et al., 2003). During the 2-week treatment, animals receiving fenofibrate, but not rosiglitazone, had a deterioration of heart function (Fig. 3, A and B). Fractional shortening decreased from 57 to 43%, and left ventricular systolic diameter (LVD) increased from 0.1 to 0.14 cm. In agreement with the cardiac markers, DRF2655 did not change heart function enough to be detected by echocardiography.

Expression of Genes for Heart Failure. We assessed whether PPAR agonists affected the expression of markers for heart failure in young mice. Rosiglitazone treatment reduced the expression of ventricular ANF and BNP (Fig. 4A). In contrast, fenofibrate treatment increased the levels of each marker (Fig. 4B). Despite the marked decrease in plasma and cardiac lipid, the dual agonist also increased ANF and BNP (Fig. 4C); however, these gene changes were less than those seen with fenofibrate.

Expression of TNFα, another heart failure marker was assessed in the control and treated hearts (Fig. 4D). Fenofibrate treatment increased TNFα expression about 2-fold compared with the untreated mice. Rosiglitazone treatment showed a trend to decreased expression, whereas DRF2655 treatment did not alter TNFα levels. Therefore, the changes in cardiac genes suggested that the PPARα agonist treatment was deleterious.

Expression of Cardiac Glucose and Lipid Metabolism Genes. Genes involved in cardiac glucose and lipid metabolism were assessed by quantitative real-time PCR (Fig. 5). As expected, PPARα agonistic drugs induced CPT1 mRNA, whereas rosiglitazone did not. Similarly, another PPARα target gene, FATP-1, was significantly increased with fenofibrate and DRF2655 treatment. Rosiglitazone treatment showed an increased trend; however, it was not significant. No significant changes were found in CD36, ACO, and GLUT4 mRNA levels.

We assessed the effects of treatment on expression of human LpL in the heart. Not surprisingly, human LpL expression driven by the MHC promoter was not altered by treatment with any of the three agonists (data not shown). In contrast, DRF2655, but not the other drugs, induced mouse LpL expression (Fig. 5B).

Effects of PPAR Agonists on Cardiac Lipid Uptake. At this point, we had two unresolved issues. 1) Two treatments, fenofibrate and DRF2655, increased CPT1 equiva-
lently, but only fenofibrate led to a deterioration in heart function. 2) Two treatments, rosiglitazone and DRF2655, reduced heart lipid content, but only rosiglitazone reduced expression of ANF and BNP. We therefore questioned whether expression of genes involved in lipid uptake and uptake of lipids themselves were both required to increase toxicity. Therefore, we next assessed whether these three drugs altered heart uptake of circulating plasma TG or FFA by simultaneously injecting labeled TG-Intralipid and palmitate. The uptake of each tracer was calculated using the plasma concentrations. Rosiglitazone treated mice had significantly less TG-Intralipid tracer taken up by heart (Fig. 6A). Animals treated with DRF2655 showed a similar trend in the TG uptake as that of rosiglitazone. Muscle and liver also had reduced TG uptake; however, adipose uptake of TG was not altered. Neither rosiglitazone nor DRF2655-treated mice had a significant change in FFA uptake by the heart (Fig. 6B). However, FFA uptake by adipose was increased.

In contrast, mice treated with fenofibrate had greater uptake of lipids into the heart than did control mice, or mice treated with the other two compounds. Cardiac TG-Intralipid uptake was not significantly increased; however, FFA uptake increased 62% (Fig. 6). There were no significant differences in lipid uptake by skeletal muscle and adipose. Liver FFA and TG uptake were slightly reduced. Thus, only fenofibrate led to greater lipid delivery to the heart.

Effects of PPAR Agonists on Older Mice. We next treated 9-month-old hLpLGPI mice with an established cardiomyopathy with DRF2655 for 1 month. A lower dose (3 mg/kg/day) of DRF2655 was used for the long-term study as the compound is known to be a highly potent activator of PPARγ and PPARα (Vikramadithyan et al., 2003). At this dose, there was a 51% reduction in plasma TG (from 129.75 ± 5.76 to 63.00 ± 7.43 mg/dl) and 22% reduction in FFA (from 0.39 ± 0.02 to 0.31 ± 0.05 mM).

Compared with similarly aged wild-type mice, hLpLGPI
mice had 54% reduction in cardiac fractional shortening (52.32 ± 2.37, wild type versus 23.85 ± 5.77%, hLpL<sup>GPI</sup>) that continued to deteriorate (48.59 ± 2.04, wild type versus 12.88 ± 4.36%, hLpL<sup>GPI</sup>) during the 1-month study. DRF2655 totally arrested the deterioration of fractional shortening (24.98 ± 2.42%, day 0 versus 32.74 ± 3.85%, day 30; Fig. 7A). In addition, the increase in LVDs (0.125 ± 0.009 cm, wild type versus 0.293 ± 0.034 cm, hLpL<sup>GPI</sup>) in the hLpL<sup>GPI</sup> mice was prevented by the dual agonist (0.262 ± 0.018 cm, day 0 versus 0.240 ± 0.02 cm, day 30; Fig. 7B). No significant change in ANF or BNP was found with this treatment (data not shown).

We also treated older mice with rosiglitazone or fenofibrate. As was found in younger animals, rosiglitazone reduced expression of heart failure markers. However, no echocardiographic effects of either treatment were evident (data not shown). The reason for less response to both rosiglitazone and fenofibrate in these older mice could be because these mice already had a deteriorated cardiac function that could not have been improved or deteriorated any further, unless a very potent PPAR transactivation agent such as DRF2655 was used.

**Discussion**

PPAR activation has a number of metabolic effects on cardiac muscle, and activation of these transcription factors is associated with changes in cardiac function. In addition insulin sensitivity of these drugs could have other actions such as changing blood flow through myocardial capillaries. Using hLpL<sup>GPI</sup> mice that develop cardiomyopathy and lipid accumulation, we assessed whether and how these drugs affect the heart in a model of lipotoxic dilated cardiomyopathy. Our data show the following: 1) PPAR<sub>γ</sub> activation reduced plasma and cardiac lipids. 2) Along with reduced heart lipid, rosiglitazone reduced heart failure markers. In contrast, fenofibrate increased ANF, BNP, and TNFα and led to reduced ejection fraction. DRF2655 had an intermediate effect. 3) A lower dose of DRF2655 reduced plasma TG in older mice and prevented deterioration of cardiac function. 4) Rosiglitazone and DRF2655 reduced cardiac uptake of TG; fenofibrate increased cardiac uptake of FFA.

PPARs have tissue-specific effects on gene expression and also secondary effects due to global changes in metabolism. Fenofibrate lowers plasma TG in hypercholesterolemic and hypertriglyceridemic animals, but it had no effect in mice with normal TG levels (Olivier et al., 1988). Since hLpL<sup>GPI</sup> mice have normal TG levels, as expected, fenofibrate did not
alter fasting plasma lipids in our study. Other more potent agents do reduce TG in mice; however, their use would have complicated our ability to dissect the cardiac and systemic actions of PPARγ activation (Ye et al., 2001). Moreover, fenofibrate is a commonly used clinical drug, and unlike some other agents, it does not have significant PPARγ actions (Lehmann et al., 1997).

PPARγ agonists produce small reductions in plasma TG and increase HDL in humans (Diamant and Heine, 2003). Rosiglitazone also reduces plasma TG in mice, as was found in our study. In comparison, DRF2655 is a more potent agent, and it led to greater plasma TG reductions in rodents (Vikramadithyan et al., 2003). These effects on plasma lipoproteins could be due to either stronger PPARγ or γ actions, both of which have been shown using a luciferase reporter assay and in vivo experiments (Vikramadithyan et al., 2003).

Cardiac lipids were reduced only with agents having PPARγ activity. Both rosiglitazone and DRF2655 reduced heart lipids in young mice. Although the drugs could have a direct effect on the myocardium, we suspected that the changes reflected reduced accumulation of plasma lipids. By performing kinetic studies, we showed that both of these drugs decreased cardiac TG and fatty acid uptake. In part, the reduced heart uptake may have resulted from uptake by other tissues, especially adipose. It has been reported by other investigators as well that rosiglitazone increases fatty acid uptake into adipose tissue while lowering the uptake in skeletal muscle and liver (Ye et al., 2004). One of the main reasons for this could be that rosiglitazone activates PPARγ, the transcription factor predominantly expressed in adipose tissue, whereas the expression level in skeletal muscle and liver is relatively very little. Although muscle acts as a lipid sink, the reduced uptake of lipids in skeletal muscle could be an indirect effect of rosiglitazone in this tissue, which has also been demonstrated in muscle-specific PPARγ knockout mice (Hevener et al., 2003; Norris et al., 2003).

In our model, PPARα activation by fenofibrate led to increased heart failure markers and decreased cardiac function. PPARα activation could lead to increased reactive oxygen species production. Our genetic and lipid uptake data showing increased expression of cardiac fatty acid oxidation genes, increased fatty acid uptake, and no change in cardiac lipids are consistent with greater oxidation in the fenofibrate-treated mouse hearts. In many ways, this observation confirms those found in cardiac PPARα transgenic mice, and as hypothesized by others, may be due to the generation of toxic oxidation products due to excess FFA metabolism in the peroxisome (Finch et al., 2003). In a rat model of pressure-overloaded cardiac hypertrophy, PPARα agonists prevented substrate switching to glucose and resulted in severe depression of cardiac power and efficiency in the hypertrophied heart (Young et al., 2001). A very recent report by Sharma et al. (2004) showed that intramyocardial lipid accumulation and up-regulation of PPARα-regulated transcripts in failing human heart resembles the lipotoxic rat heart. The pathway responsible for increased FFA uptake in the hearts of fenofibrate-treated mice is unclear. CD36, a known cardiac FFA transporter, did not change, but another potential transporter, FATP-1, was induced.

The observed increase in cardiac CPT1 expression in both fenofibrate- and DRF2655-treated mice could have allowed greater mitochondrial oxidation of the acquired FFA. However, only fenofibrate led to worsening of cardiac function. This led us to conclude that increased expression of FFA oxidation pathway genes only affects heart function if it is coupled with greater lipid uptake. Whether the toxicity is then due to oxidation products or accumulation of lipid intermediates is unknown. Mice overexpressing LpL in the heart only develop cardiomyopathy when bred with PPARα knockout mice, animals that have reduced FFA oxidation (Nohammer et al., 2003). In this model, toxicity is associated with reduced FFA oxidation.

Rosiglitazone reduced ANF and BNP in the hLpL<sup>GPl</sup> model. PPARγ agonists are beneficial in leptin-deficient lipotoxicity (Zhou et al., 2000). Either the drugs have a direct effect on the heart or their actions are secondary to more global metabolic effects. When new models are created with specific overexpression or deletion of PPARγ in the heart, this issue can be addressed in genetic experiments. PPARγ expression is low in heart relative to adipose tissue but similar to expression in skeletal muscle (Desvergne and Wahli, 1999). PPARγ agonists induce insulin-mediated glucose uptake into skeletal muscle and heart; increased glucose uptake could have improved heart function in the hLpL<sup>GPl</sup> mice (Hevener et al., 2003; Muurling et al., 2003). However, the cardiomyopathy in the hLpL<sup>GPl</sup> mice is clearly associated with excess uptake of plasma lipid and not an intrinsic metabolic defect of cardiomyocytes. For that reason, it is logical that rosiglitazone reduction of cardiac lipid uptake should lower the cardiac levels of toxic metabolites and ameliorate the cardiomyopathy. In this context, Listenberger et al. (2003) have shown that palmitate, but not oleic acid, is toxic to cells and have suggested that accumulation of nonesterified lipids causes lipotoxicity.

Cardiac lipid uptake was a better marker for the beneficial effects of PPAR agents than was cardiac lipid content. Although DRF2655 reduced cardiac lipid uptake in young mice, it did not do this as well as rosiglitazone. Beneficial effects of DRF2655 might have been counterbalanced by negative PPARα effects. In older mice, when we reduced the amount of drug used such that the beneficial actions were not overwhelmed by the toxic effects of PPARα activation, cardiomyopathy was improved.

In contrast to the reported effects of PPAR agonists in young mice with developing disease, the same doses of fenofibrate and rosiglitazone did not affect cardiac function and did not alter gene expression in older mice (data not shown). Therefore, once disease is established, it seems that the response to PPAR agonist therapy differs.

In summary, our study illustrates a pharmacological approach toward understanding lipotoxicity. Because cardiac FFA oxidation is already induced by the presence of the hLpL<sup>GPl</sup> transgene, further PPARα activation may lead to greater cardiac dysfunction due to accumulation of additional lipids. PPARγ agonists have beneficial effects, perhaps because they reduce plasma TG and route more TG and FFA to adipose, and less to the heart. Although models may differ, our data suggest that PPAR agents have great potential both for good and harm in this form of cardiomyopathy. Most importantly, our data suggest that their effects on heart function may reflect changes in overall body metabolism and plasma lipid levels and not direct actions on the myocardium. It should be noted that the actions of PPAR agonists in
humans differ from those in mice; this is most evident in the observed effects on lipoprotein profiles. Moreover, the pathogenesis of cardiomyopathy in humans with obesity or diabetes may differ from that in mouse models of cardiotoxicity. Thus, the effects of these treatments in humans might be different. Nonetheless, these types of animal studies provide a basis for exploring drug actions and dissecting their effects on disease.

References

discussion.


Address correspondence to: Dr. Ira J. Goldberg, Department of Medicine, Columbia University College of Physicians and Surgeons, 630 West 168th St., New York, NY 10032. E-mail: igig@columbia.edu