Cardiac overexpression of hormone-sensitive lipase inhibits myocardial steatosis and fibrosis in streptozotocin diabetic mice

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—Intracellular lipid accumulation (steatosis) and resultant lipotoxicity are key features of diabetic cardiomyopathy. Since cardiac hormone-sensitive lipase (HSL) is activated in diabetic mice, we sought to explore a pathophysiological function of cardiac HSL in the development of diabetic cardiomyopathy. Transgenic (Tg) mice with heart-specific HSL overexpression were generated, and cardiac histology, function, lipid profile, and gene expressions were analyzed after induction of diabetes by streptozotocin. Electron microscopy showed numerous lipid droplets in wild-type (Wt) hearts after 3 wk of diabetes, whereas Tg mice showed no lipid droplet accumulation. Cardiac content of acylglycerides was increased ~50% with diabetes in Wt mice, whereas this was blunted in Tg hearts. Cardiac lipid peroxide content was twofold lower in Tg hearts than in Wt hearts. The mRNA expressions for peroxisome proliferator-activated receptor-α, genes for triacylglycerol synthesis, and lipoprotein lipase were increased with diabetes in Wt hearts, whereas this induction was absent in Tg hearts. Expression of genes associated with lipopapoptosis was decreased, whereas antioxidant protein metallothioneins were increased in diabetic Tg hearts. Diabetic Wt hearts showed interstitial fibrosis and increased collagen content. However, Tg hearts displayed no overt fibrosis, concomitant with decreased expression of collagens, transforming growth factor-β, and matrix metalloproteinase 2. Notably, mortality during the experimental period was approximately twofold lower in diabetic Tg mice compared with Wt mice. In conclusion, since HSL overexpression inhibits cardiac steatosis and fibrosis by apparently hydrolyzing toxic lipid metabolites, cardiac HSL could be a therapeutic target for regulating diabetic cardiomyopathy.

lipotoxicity; lipolysis; animal model; transgenic mice

CARDIOVASCULAR DISEASES, including heart failure and coronary artery disease, are the leading cause of morbidity and mortality for both type 1 and type 2 diabetic patients (4). However, a number of diabetic patients appear to develop cardiac disease in the absence of significant coronary artery disease, designated diabetic cardiomyopathy (37). Diabetic cardiomyopathy is characterized by myocyte hypertrophy, interstitial fibrosis, and increased content of triacylglycerol (TAG) and cholesterol in cardiac muscle (2, 35). These histological abnormalities are associated with functional problems, diastolic dysfunction in the early stage, and contractile dysfunction in the late stage, eventually leading to congestive heart failure.

Based on experiments using diabetic animal models, a number of mechanisms have been proposed to explain the pathogenesis of diabetic cardiomyopathy. 1) A defect of insulin signaling, as seen in type 1 diabetes, causes cardiac dysfunction with decreased fatty acid (FA) oxidation and increased immature muscle fibers (3). 2) Hyperinsulinemia, as seen in type 2 diabetes, contributes to myocyte hypertrophy via increased growth signaling. 3) Activation of the renin-angiotensin-aldosterone system leads to interstitial fibrosis by activating transforming growth factor (TGF)-β (36). 4) Chronic high glucose leads to increases in advanced glycosylation end products, polyol and hexosamine flux, and diacylglycerol (DAG) content, which stimulate collagen cross-linking, oxidative stress, impaired Ca2+ handling, and PKC, respectively (32). 5) Sympathetic dysfunction causes compromised relaxation and contraction. 6) Increased FA availability and oxidation cause a number of detrimental effects on the heart, namely, “lipotoxicity” (43).

Recent studies have been focused on the importance of lipotoxicity in the pathogenesis of diabetic cardiomyopathy. In diabetes, increased FA oxidation produces excessive reactive oxygen species (ROS), including lipid peroxide. Physiological levels of ROS can be scavenged by the action of superoxide dismutase, glutathione peroxidase, and metallothioneins (MTs) (5). However, in diabetes, excess FA oxidation overwhelms the scavenging capacity, leading to mitochondrial damage and endoplasmic reticulum stress. Increased ROS also activate TGF-β, leading to synthesis of collagens and other extracellular matrix (ECM) and, eventually, cardiac remodeling (32).

In diabetic Zucker rats, it appears that excessive FA flux causes myocardial TAG accumulation, and excess FA released from TAG droplets stimulates ceramide synthesis. Ceramide then increases inducible nitric oxide synthase (iNOS) expression via NF-κB, and the resultant lipid peroxide leads to myocyte apoptosis (50). In addition, there is an increase in cardiac DAG and PKC activation in streptozotocin (STZ)-induced diabetic mice (18). The PKCβ and PKCδ activated by DAG stimulate TGF-β, leading to ECM synthesis and fibrosis (7). Thus intracellular accumulation of acylglycerides appears to play an important role in the pathogenesis of diabetic cardiomyopathy.

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Intracellular lipolysis in cardiomyocytes is catalyzed by two major cytosolic lipases: hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) (16, 51). The classic enzyme, HSL, is expressed in many organs and cells, including adipose tissues, skeletal and cardiac muscles, and pancreatic β-cells (17). HSL activity is regulated by hormones, such as catecholamines, which stimulate its activity via phosphorylation of the protein by cAMP-dependent protein kinase. Phosphorylated HSL translocates from the cytosol to the surface of lipid droplets at the onset of lipolysis. Insulin inhibits HSL activity via dephosphorylation of HSL by phosphodiesterase 3B (23). ATGL is expressed primarily in adipose tissues and at a low level in cardiac muscle, although ATGL null mice have been shown to develop steatotic cardiomyopathy (14). HSL hydrolyzes TAG, DAG, and cholesterol ester (CE), with the highest hydrolytic activity against DAG, as evidenced by the fact that HSL null mice accumulate DAG in many tissues, including heart (13). In contrast to HSL, ATGL specifically removes the first FA from TAG, generating FA and DAG. Thus HSL and ATGL are thought to catalyze intracellular lipolysis cooperatively in the tissues expressing these lipases.

In diabetes, impaired insulin action stimulates adipose lipolysis, and we previously reported that mRNA and protein expression of HSL were also increased in adipose tissues of STZ-diabetic rats (42). Since heart expresses HSL, cardiac HSL might be expected to be activated in diabetes; however, there are a limited number of studies investigating the function and regulation of cardiac HSL in diabetes. In a previous study we generated transgenic (Tg) mice with tetracycline-inducible HSL overexpression in heart and reported that Tg mice lacked fasting-induced TAG accumulation in cardiomyocytes (40). In the current study we have sought to clarify the pathophysiological functions of cardiac HSL in the development of diabetic cardiomyopathy by using Tg mice with constitutive overexpression of HSL in the heart. The results show that HSL activation is beneficial in preventing cardiac steatosis and fibrosis by decreasing toxic lipid metabolites, with a resultant decrease in overall mortality.

EXPERIMENTAL PROCEDURES

Generation of heart-specific HSL-overexpressing mice. Rat HSL cDNA was inserted under the myosin heavy chain (MHC)-α promoter, kindly provided by Dr. J. Robbins (University of Cincinnati Medical Center, Cincinnati, Ohio) (39) (see Fig. 2A). The transgene was injected into embryos derived from C57BL/6 × DBA2 F1 background in the Division of Laboratory Animal Resources at the University of Fukui. Out of 30 survivors, 6 founders were identified by PCR screening followed by Southern blot analysis. The founders were backcrossed to C57BL/6 mice for six to seven generations. All animals were fed a chow diet ad libitum and were maintained on a 12:12-h light-dark cycle. All procedures were performed in accordance with National Institute of Health guidelines for the care and use of animals and were approved by the guidelines for animal experiments of the University of Fukui.

Induction of diabetes. Twelve- to sixteen-week-old mice were injected intraperitoneally with 150 mg/kg of STZ every 3–4 days until plasma glucose concentrations were higher than 350 mg/dl. Urine glucose and ketone bodies were also tested. Vehicle-treated mice served as nondiabetic controls. Three weeks after diabetes was documented, mice were killed at 10:00–11:00 AM and analyzed. Each experimental group consisted of equal numbers of male and female mice.

Blood chemistry. Blood was drawn by puncturing the left ventricle or descending aorta. Plasma concentrations of nonesterified FA (NEFA), TAG, total cholesterol (T-Ch), and insulin were measured using commercially available kits (Wako, Osaka, Japan). Plasma glucose concentration was measured using FreeStyle (Nipro, Osaka, Japan).

HSL activity. HSL activity was determined as neutral cholesteryl ester hydrolase (NCEH) activity using a cholesteryl [14C]oleate emulsion as described previously (22). Protein concentration was determined using the Bradford method.

Western blot analysis. Immunoblot analyses were performed as previously described using rabbit anti-HSL antibody or anti-ATGL antibody (Cell Signaling Technology, Beverly, MA) and analyzed with a FluorChem IS-8000 (Alpha Innotech, San Leandro, CA) (22).

Histological studies. Hearts were fixed in formalin (10% vol/vol), embedded, and sectioned. Tissue collagens were stained with picrosirius red as previously described with minor modifications (19).

RESULTS

HSL expression and activity in the heart of diabetic mice. To clarify the regulation of HSL in diabetic heart, C57BL/6 mice were injected with STZ to induce diabetes. Three weeks later, mRNA and protein expression of cardiac HSL were increased approximately twofold in diabetic hearts compared with control hearts (Fig. 1, A and B). In parallel, HSL activity was elevated 80% in the hearts of diabetic mice (Fig. 1C). These data indicate that cardiac HSL expression and activity are increased in diabetic mice.
Characterization of heart-specific HSL-overexpressing mice.

To explore the pathophysiological function of activated HSL in the development of diabetic cardiomyopathy, we planned to induce diabetes in the tetracycline-inducible, heart-specific HSL-overexpressing mice that we generated in a previous study (40). However, inducible HSL expression declined with increasing generations. Since constitutive activation of HSL did not appear to cause any detrimental effects on the fetus, we reconstructed a transgene that harbors rat HSL cDNA under a MHC-α promoter to create Tg mice with constitutive expression of HSL in the heart (Fig. 2A). Out of six founders, we established a Tg line, designated as MH-1, which showed a high level of HSL expression in a heart-specific manner (Fig. 2B, faint expression in lung is in pulmonary vessels). In parallel to protein expression, HSL activity in Tg heart was eight times higher than that in wild-type (Wt) heart (Fig. 2C). General appearance, growth and fertility of Tg mice were normal. The F6 or F7 heterozygous Tg mice and their Wt littermates were injected with STZ to induce diabetes, and 3 wk later, mice were killed for analysis.

Characterization of diabetic animals. Characterization and plasma parameters of control and diabetic Wt and Tg animals are summarized in Table 1. Plasma glucose concentration in STZ-treated mice was elevated over 500 mg/dl, insulin declined >80%, and body weight was decreased 24% in both genotypes. Interestingly, plasma TAG concentration was increased 73% with diabetes in Wt mice, whereas this elevation was not observed in Tg mice. To test the possibility that HSL Tg mice have increased cardiac VLDL metabolism in diabetes that might explain their reduced plasma TAG concentration, we investigated cardiac VLDL-TAG uptake by injecting 125I-labeled rabbit VLDL. The result showed that cardiac VLDL uptake was not altered with diabetes or HSL expression, whereas it was decreased twofold in liver in both genotypes (data not shown). Plasma concentrations of NEFA and T-Ch were not altered in either genotypes after 3 wk of diabetes; however, we documented an approximately twofold elevation of plasma NEFA concentration between 1 and 2 wk of the
experimental period (data not shown). Notably, the mortality during the experimental period was lower in diabetic Tg mice (12.2%) than in Wt mice (19.3%), and it was particularly apparent in the female group (Tg 9.4% vs. Wt 25.3%), providing strong evidence that HSL overexpression might be beneficial in diabetes. To investigate cardiac function, echocardiography was performed 3 wk after diabetes was documented (28). Although heart rate was decreased with diabetes in both conditions and genotypes (data not shown), basic systolic function seemed to be unaltered in diabetes. To study the effect of HSL overexpression on diabetic cardiomyopathy, we performed histological examinations on the left ventricle. Low magnification electron microscopy showed numerous lipid droplets were accumulated around clusters of mitochondria in cardiomyocytes of diabetic Wt mice (Fig. 3, B and F); however, Tg hearts accumulated virtually no lipid droplets in either control or diabetic conditions (Fig. 3, C, D, G, and H). Light microscopy with oil red-O staining also showed similar results (data not shown). No other morphological changes were observed in the hearts of Tg mice.

Compatible with the histological findings, cardiac TAG content was increased 45% with diabetes in Wt mice, whereas the elevation was not observed in Tg mice (Fig. 4A). Cardiac DAG content was 40% lower in Tg hearts than in Wt hearts under control conditions and tended to be lower in Tg hearts than in Wt hearts with diabetes (Fig. 4B). Cardiac content of free FA and F-Ch were not significantly altered with diabetes or HSL overexpression (Fig. 4, C and D), and only trace amounts of CE were found (data not shown). Importantly, cardiac content of lipid peroxides was twofold lower in Tg hearts than in Wt hearts in both control and diabetic conditions (Fig. 4E).

Gene expressions. We then analyzed cardiac mRNA expressions of genes related to FA metabolism by quantitative RT-PCR (Fig. 5, A–L). The expression of peroxisome proliferator-activated receptor-α (PPARα), a key regulator of FA oxidation and TAG synthesis, was increased greater than twofold with

<table>
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<td>Body weight before STZ, g</td>
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<td>Body weight 3 wk after STZ, g</td>
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<td>Heart weight, mg</td>
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<td>Heart/body weight, mg/g</td>
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<td>4.58±0.16</td>
<td>4.29±0.16</td>
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<td>Plasma glucose, mg/dl</td>
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<td>Insulin, pg/ml</td>
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<td>90±32*</td>
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<td>467±54</td>
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<td>TAG, mg/dl</td>
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<td>Total cholesterol, mg/dl</td>
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Values are means ± SE; n = 8–10 mice. Wt, wild type; Tg, transgenic; STZ, streptozotocin; NEFA, nonesterified fatty acids; TAG, triacylglyceride. *P < 0.05; †P < 0.01 between conditions. ‡P < 0.05 between genotypes.

Fig. 3. HSL overexpression inhibited cardiac steatosis in streptozotocin (STZ)-induced diabetic mice. Representative electron micrographs depict the histological appearance of left ventricles from Wt and Tg mice after 3 wk of diabetes (A–D, magnification ×2,740; E–H, magnification ×18,400). Diabetic Wt hearts (B and F) accumulated lipid droplets (arrowheads) around clusters of mitochondria, whereas no lipid droplets were observed in similar regions of the left ventricle from diabetic Tg mice (D and H). We used 5 animals per group and studied 6 positions per sample.
diabetes in Wt hearts, whereas this increase was completely absent in Tg hearts (Fig. 5A). The expression of genes involved in TAG synthesis, glycerol-3-phosphate acyltransferase, and phosphatidic acid phosphatases 2A and 2B displayed a tendency to be increased with diabetes in Wt hearts, whereas their expressions were significantly lower in Tg hearts compared with Wt hearts (Figs. 5, B–D). The expression of PPARγ was also increased with diabetes in Wt hearts, although the increase was blunted in Tg hearts (Fig. 5H). Among the lipases expressed in cardiomyocytes, lipoprotein lipase (LPL) was increased 80% with diabetes in Wt hearts, whereas this increase was completely absent in Tg hearts (Fig. 5E). The expression of endogenous HSL and ATGL were increased with diabetes in both Wt and Tg hearts (Fig. 5, F and G). Interestingly, ATGL protein expression was twofold lower in Tg hearts than in Wt hearts under control conditions, whereas its expression was similar in Wt and Tg hearts with diabetes (data not shown). The expression of fatty acid transport protein (FATP)-4 and -6 was lower in Tg hearts compared with Wt hearts, whereas fatty acid translocase (FAT)/CD36 was significantly increased with diabetes in Wt hearts (Fig. 5, I–K). Expression of nuclear receptor Nur77, which has been shown to regulate many genes for FA oxidation, including uncoupling protein-2 and -3 (UCP2 and UCP3) (26), was increased with diabetes in Wt hearts, in contrast, it was elevated in Tg hearts in both conditions (Fig. 5L).

We next analyzed the expression of genes involved in lipoprotein lipase and glucose-induced fibrosis. Among the genes related to lipoprotein lipase, the expression of the key molecules NF-κB and iNOS were significantly lower in diabetic Tg hearts compared with Wt hearts (Fig. 6, A and B). In contrast, expression of the ROS scavengers MT-1 and MT-2 were markedly increased in diabetic Tg hearts compared with Wt hearts. It is known that high-glucose stimulates TGF-β function and matrix metalloproteinase (MMP)-2 cleaves latent TGF-β to release the active form of the protein. As shown in Fig. 6, E and F, expression of both TGF-β and MMP-2 was decreased 40–50% in diabetic Tg hearts compared with Wt hearts. In parallel, expression of type I, III, and XV collagens were all decreased 70–80% in Tg hearts compared with Wt hearts with diabetes (Fig. 6, G–I).

To identify potential adverse effects of HSL overexpression, we studied cardiac mRNA expression of TNF-α, interleukin-6, atrial and brain natriuretic peptides, and MHC-β/α. However, expression of none of these genes was significantly altered in Tg mice compared with Wt mice. In addition, we studied potential macrophage infiltration in cardiac muscle, analyzing scavenger receptor type A1 and A2. Again, the expression was similar between Tg and Wt mice (data not shown).

Cardiac fibrosis. Since mRNA expression of collagens was markedly decreased in diabetic Tg hearts, we investigated cardiac fibrosis by histological examination with collagen staining. As shown in Fig. 7, A–D, interstitial collagens were significantly increased in diabetic Wt hearts. In contrast, this interstitial fibrosis was absent in diabetic Tg hearts. A morphometric analysis showed that the interstitial collagen mass, which includes cross-linked collagens, was increased 2.3-fold with diabetes in Wt mice. In contrast, the increase was totally absent in Tg heart (Fig. 7E). In addition, cardiac content of soluble collagens, which represents recently synthesized collagen pool, was increased ~30% in diabetic Wt mice, whereas this elevation was not observed in diabetic Tg mice (Fig. 7F). To test the effect of HSL overexpression on collagen degradation pathways, we analyzed mRNA expressions of MMP-9 and tissue inhibitor of metalloproteinase-1 and -2. However, these genes were not affected in any groups. In addition, the expression of aldose reductase, which is a key regulator in the polyol pathway, was not altered (data not shown).

**DISCUSSION**

In the current study we have shown that cardiac overexpression of HSL is able to prevent histological, biochemical, and molecular changes associated with diabetic cardiomyopathy. It appears that an increase (~2-fold) in cardiac HSL expression and activity in diabetic heart (Fig. 1) is a self-adjusting mechanism in response to an energy shift from glucose to FA. In preliminary experiments we have documented that the maximum increase in cardiac HSL activity under physiological conditions was up to threefold when mice were refed after a 24-h fast. In contrast, the HSL activity in our Tg mice seems to be superphysiological (8-fold increased), which could have overcome diabetes-induced TAG accumulation, resulting in beneficial consequences as related to cardiomyopathy.
Several laboratories, including ours, have reported the phenotypes of tissue-specific HSL-overexpressing mice. In macrophage-specific HSL-overexpressing mice, increased NCEH activity in macrophages led to an increase in atherosclerotic lesions with a paradoxical increase in cellular CE content (10). In Tg mice with \textit{/H9252}\textsuperscript{-cell-specific HSL overexpression, reduction of ATP and increased UCP2 led to impaired insulin secretion (47). Interestingly, adipose HSL-overexpressing mice showed impaired catecholamine-induced lipolysis (25). Thus FA toxicity might have exerted adverse effects in each of these HSL Tg mouse models. On the basis of these reports, we expected to observe some evidence of cardiac lipotoxicity in the current Tg mice. However, none of the genes related to lipotoxicity was significantly altered in Tg hearts. Instead, cardioprotective genes MTs were markedly increased. These data provide evidence that cardiac HSL overexpression is beneficial rather than detrimental in diabetes.

Interestingly, cardiac HSL overexpression caused systemic effects in mortality and plasma TAG concentration. The mortality during STZ treatment was significantly reduced in female Tg mice. Since female mice are STZ resistant, they required more STZ injections than male mice. The average number of the STZ injections to induce diabetes was 1.6 \pm 0.13 for male mice and 3.4 \pm 0.15 for female mice. When the present data were analyzed in male and female groups separately, the female group tended to show more significant differences between genotypes compared with the male group. Although it is difficult to clarify the precise mechanism for this difference, we speculate that increased STZ injections might have exerted some cardiotoxicity on the top of the lipo- and glucotoxicity in female mice and that HSL overexpression ameliorated overall toxicity by decreasing ROS and increasing MTs. The cardioprotective function of estrogen also might have affected the female mortality (27).

Plasma TAG concentration was elevated with diabetes in Wt mice, whereas it remained normal in Tg mice. We first hypothesized that Tg hearts might have increased VLDL-TAG uptake with diabetes; however, the results of tracer experiments ex-

Fig. 5. Cardiac expression of genes related to TAG synthesis (A–D), lipolysis (E–G), and FA uptake/oxidation (H–L). The mRNA expressions were analyzed by RT-qPCR and normalized to GAPDH. The relative expressions are presented against control Wt mice. A: peroxisome proliferator-activated receptor-\(\alpha\) (PPAR\(\alpha\)). B: glycerol-3-phosphate acyltransferase (GPAT). C: phosphatidic acid phosphatase 2A (PAP2A). D: PAP2B. E: lipoprotein lipase (LPL). F: endogenous HSL. G: adipose triglyceride lipase (ATGL). H: PPAR\(\gamma\). I: fatty acid transport protein-4 (FATP4). J: FATP6. K: fatty acid translocase (FAT)/CD36. L: nuclear receptor Nur77. *\(P < 0.05\) between genotypes or conditions as indicated. Values are means \pm SE for 5 mice in each group.
It has been reported that HSL-deficiency is cardioproteective. Park et al. (30) have shown that HSL knockout (KO) mice on a high-fat diet have increased cardiac glucose uptake and decreased TAG content compared with Wt mice (30). We interpret that these finding are due to impaired adipose lipolysis and resultant lower plasma FA concentrations in HSL KO mice. Indeed, we have documented 60% lower plasma FA concentrations in HSL KO mice (39a). Thus HSL KO mice appear to exhibit phenotypes related to impaired adipose lipolysis, masking the adverse effects on cardiomyocytes. Cardiac overexpression of HSL-deficiency is cardioprotective. Other possible explanations might involve increased function of systemic or cardiac LPL and decreased hepatic VLDL production/secretion. Since interorgan cross talk has been reported (24, 44), cardiac HSL overexpression might have affected function of the tissues or molecules involved in plasma TAG metabolism. These possibilities remain to be investigated.

It has been reported that HSL-deficiency is cardioprotective. Park et al. (30) have shown that HSL knockout (KO) mice on a high-fat diet have increased cardiac glucose uptake and decreased TAG content compared with Wt mice (30). We interpret that these finding are due to impaired adipose lipolysis and resultant lower plasma FA concentrations in HSL KO mice. Indeed, we have documented 60% lower plasma FA concentrations and cardiac TAG content in the heart of fasted HSL KO mice (39a). Thus HSL KO mice appear to exhibit phenotypes related to impaired adipose lipolysis, masking the effects in other tissues expressing HSL. In contrast, since our animal model has HSL overexpression exclusively in the heart, we were able to identify the heart-specific effects of HSL overexpression in the setting of diabetes.

PPARα is a ligand-gated nuclear transcription factor that directly or indirectly regulates the expression of many proteins and enzymes modulating FA uptake and oxidation. The transcriptional regulation of PPARα is not completely understood; however, it has been reported that PPARα mRNA expression is increased by FA in the liver of STZ-diabetic rats (1) and by retinol in cultured adipocytes (45). Although PPARα is a key regulator of FA metabolism in the heart, recent studies have shown that excess PPARα (and PPARγ) function can have adverse effects on cardiomyocytes. Cardiac overexpression of PPARα caused lipotoxic cardiomyopathy (11), whereas PPARα KO mice were resistant to diabetic cardiomyopathy (12). Recently, it was reported that cardiac overexpression of PPARγ also leads to steatotic cardiomyopathy (38). A PPARα agonist led to cardiomyocyte necrosis due to increased FA oxidation and oxidative stress (34) and to stimulated cardiac fibrosis after ischemia-reperfusion (8). In the current study, heart-specific HSL Tg mice did not show a diabetes-induced increase in mRNA of PPARα and TAG synthetic genes, some of which are known to be PPARα regulated (Fig. 5A). Thus blocking the upregulation of PPARα (and PPARγ) is likely to be one of the mechanisms how HSL overexpression inhibits cardiac steatosis. In addition, since HSL hydrolyzes retinyl ester, and given that its product, retinol, which is a ligand of retinoid X receptors, plays an important role in the development and function of cardiomyocytes (31), accelerated retinol ester metabolism might be also involved in the altered expression and function of PPARα in HSL Tg heart.

Expression of LPL, which is also known to be a PPARα target gene, and FATPs was also altered in parallel to PPARα. Cardiac LPL hydrolyzes TAG in circulating lipoproteins, and FATPs facilitate FA uptake into cardiomyocytes, playing a role in TAG accumulation in cardiomyocytes. Given the function of these molecules and the reports showing that overexpression of cardiac LPL or FATP1 can cause steatotic cardiomyopathy (6, 49), suppression of these genes might also contribute to the inhibition of steatosis in HSL Tg mice. The question then is, how does HSL overexpression inhibit PPARα upregulation in diabetes despite an expected increased FA availability mediated by the action of HSL? One possibility would be that in HSL Tg heart, FA oxidation is activated so that released FA are immediately oxidized before they stimulate PPARα expression. Supporting this hypothesis, mRNA expression of Nur77 was significantly increased (Fig. 5L) and that of UCP2 was 60% increased in HSL Tg hearts (significant only in the female group; data not shown). Another possibility is that FA released by HSL action might be rapidly diffused out of the cells via a
transmembrane flip-flop mechanism (15). The modest increase in cardiac FA content in Tg mice (Fig. 4C) might support the participation of this process. The metabolic fates of FA released by HSL should be investigated.

MTs are potent antioxidant proteins that scavenge free radicals and toxic metal ions in many tissues, including heart (5). Overexpression of MT2 has been shown to protect cardiomyocytes from apoptosis by inhibiting caspase-3 activation in STZ-diabetic mice (48) and to rescue doxorubicin or high fat-induced cardiomyopathy by scavenging ROS (9, 21). In the current study, mRNA of both MT1 and MT2 were markedly increased in diabetic Tg hearts (Fig. 6, C and D). Thus the upregulation of MTs might have contributed to the inhibition of cardiac fibrosis and protection against oxidative stress.

TGF-β is known to be a key regulator inducing cardiac fibrosis in diabetes, hypertension, and ischemic heart disease (7, 36). It has been reported that TGF-β expression is increased in the hearts of diabetic mice (46). In the diabetic HSL Tg mice, expression of MMP2 and TGF-β were decreased, and this was paralleled by a marked decrease in collagen expression and content (Figs. 6 and 7). These findings are quite similar to those in STZ-diabetic mice treated with an angiotensin receptor blocker (46). In our HSL Tg mice, a reduction in lipid peroxide content due to increased actions of HSL and MTs might have suppressed the MMP2/TGF-β pathway.

TGF-β can be regulated by several other mechanisms. Diabetic mice have been shown to have cardiac DAG accumulation and evidence of PKCα activation, which stimulates the TGF-β pathway (18). Given these findings, we studied membrane-bound DAG-sensitive PKCs by performing Western blotting. However, the DAG-sensitive PKCα, PKCβ, and PKCe were not altered between Wt and Tg hearts (data not shown). Although a longer period of diabetes might have shown greater DAG accumulation and significant activation of PKCs (20), the results suggest that lipid peroxides are likely to have a greater role than PKCs in stimulating TGF-β-induced fibrosis in this animal model.

Figure 7G summarizes a working hypothesis how HSL overexpression inhibits cardiac fibrosis in diabetic animals. HSL overexpression hydrolyzes intracellular acylglycerides,
leading to a decrease in toxic lipid peroxides. Increased MTs also support scavenging ROS/lipid peroxide. As a result, MMP2 and TGF-β are decreased, leading to inhibition of collagen expression, synthesis, and finally, fibrosis. It has been reported that the PPARγ agonist rosiglitazone improves diabetic cardiomyopathy by reducing cardiac TAG content in Zucker diabetic rats (50). In that report, rosiglitazone also improved plasma glucose and FA concentrations. In the current study, our animal model shows that decreasing intracellular acylglycerides has a protective effect against fibrosis, even in the presence of a high glucose concentration. Although lipotoxicity is only one possible mechanism, and others might exist, the current study supports the importance of controlling lipotoxicity for preventing the development of diabetic cardiomyopathy. Since HSL has been shown to exert beneficial effects without FA toxicity in the heart, there is a potential for cardiac HSL to be a clinical target to control or prevent diabetic cardiomyopathy.

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