RESEARCH PAPER

Cardiac dysfunction in adipose triglyceride lipase deficiency: treatment with a PPARα agonist

G Wölkart1, A Schrammel1, K Dörffel1, G Haemmerle2, R Zechner2 and B Mayer1

1Department of Pharmacology and Toxicology, Karl-Franzens-Universität Graz, Graz, Austria, and 2Department of Molecular Biosciences, Karl-Franzens-Universität Graz, Graz, Austria

BACKGROUND AND PURPOSE
Adipose triglyceride lipase (ATGL) has been identified as a rate-limiting enzyme of mammalian triglyceride catabolism. Deletion of the ATGL gene in mice results in severe lipid accumulation in a variety of tissues including the heart. In the present study we investigated cardiac function in ATGL-deficient mice and the potential therapeutic effects of the PPARα and γ agonists Wy14,643 and rosiglitazone, respectively.

EXPERIMENTAL APPROACH
Hearts isolated from wild-type (WT) mice and ATGL(-/-) mice treated with Wy14,643 (PPARα agonist), rosiglitazone (PPARγ agonist) or vehicle were perfused at a constant flow using the Langendorff technique. Left ventricular (LV) pressure–volume relationships were established, and the response to adrenergic stimulation was determined with noradrenaline (NA).

KEY RESULTS
Hearts from ATGL(-/-) mice generated higher LV end-diastolic pressure and lower LV developed pressure as a function of intracardiac balloon volume compared to those from WT mice. Likewise, passive wall stress was increased and active wall stress decreased in ATGL(-/-) hearts. Contractile and microvascular responses to NA were substantially reduced in ATGL(-/-) hearts. Cardiac contractility was improved by treating ATGL(-/-) mice with the PPARα agonist Wy14,643 but not with the PPARγ agonist rosiglitazone.

CONCLUSIONS AND IMPLICATIONS
Our results indicate that lipid accumulation in mouse hearts caused by ATGL gene deletion severely affects systolic and diastolic function, as well as the response to adrenergic stimulation. The beneficial effects of Wy14,643 suggest that the cardiac phenotype of these mice is partially due to impaired PPARα signalling.

Abbreviations
ATGL, adipose triglyceride lipase; CPP, coronary perfusion pressure; DEA/NO, 2,2-diethyl-1-nitroso-oxyhydrazine; FA, fatty acid; LVEDP, left ventricular developed pressure; LVSP, peak left ventricular systolic pressure; NA, noradrenaline; WT, wild-type; Wy14,643, [[4-chloro-6-[(2,3-dimethylphenyl)amino]-2-pyrimidinyl[thio]acetic acid

Introduction
Cardiomyopathy is a frequent and significant complication of metabolic disorders such as obesity, insulin resistance and diabetes mellitus. At an early, preclinical stage, cardiomyopathy is characterized by left ventricular (LV) diastolic filling impairments (Schannwell et al., 2002) and evolves over time to both systolic and diastolic dysfunction. Under physiological conditions, cardiomyocytes preferentially utilize long-chain fatty acids (FAs) for ATP generation...
Adipose triglyceride lipase (ATGL) has been identified as the key enzyme of triglyceride catabolism, which functions as a monoacyl hydrolase and catalyzes the initial, rate-limiting step of the triacylglycerol lipolysis cascade (Zimmermann et al., 2004). ATGL is predominantly expressed in adipose tissue but is also found to a lesser extent in cardiac muscle, skeletal muscle, testis and other tissues. Systemic deletion of the ATGL gene in mice resulted in massive accumulation of neutral lipids in most tissues (Haemmerle et al., 2008). ATGL(-/-) mice typically die from cardiac dysfunction at 9–10 weeks (comparable with an early diagnosis of neutral lipid storage disease in humans). Therefore, ATGL(-/-) mice (6–7 weeks) were subjected to three different treatment protocols to influence the progression of cardiac dysfunction. The control group received standard mouse chow (4% fat, 20% protein), the PPARα agonist-treated group was fed control diet mixed with 0.1% Wy14,643 (Cayman Chemical, Ann Arbor, MI, USA) for 3 weeks (Crabb et al., 2003), PPARα (Finck et al., 2002), or long-chain acyl-CoA synthetase A (Chiu et al., 2001) show severe steatosis, systolic dysfunction and hypertrophy, resembling a phenotype observed in end-stage metabolic cardiomyopathies.

Methods

Mice and experimental groups

Homozygous ATGL-deficient [ATGL(-/-)] mice of either sex and their corresponding wild-type (WT) littermates were used in this study. In our preliminary study (Haemmerle et al., 2006), the first impairments in cardiac function and lipid accumulation in ATGL(-/-) mice became evident at the age of 6 weeks (comparable with early diagnosis of neutral lipid storage disease in humans). Therefore, ATGL(-/-) and WT animals (6–7 weeks) were subjected to three different treatment protocols to influence the progression of cardiac dysfunction. The control group received standard mouse chow (4% fat, 20% protein), the PPARα agonist-treated group was fed control diet mixed with 0.1% Wy14,643 (Cayman Chemical, Ann Arbor, MI, USA) for 3 weeks (Crabb et al., 2001), and the PPARα agonist-treated group was fed control diet mixed with 0.013% rosiglitazone (Cayman Chemical) for 3 weeks (Kanda et al., 2009). This protocol was chosen because ATGL(-/-) mice typically die from cardiac dysfunction at 9–10 weeks. All groups were provided with food and water ad libitum. Food intake was not different between the experimental groups. Animals were housed in approved cages and kept on a regular 12 h dark/light cycle. All animal care and experimental procedures complied with the Austrian law on experimentation with laboratory animals (last amendment, 2004), which is based on the US National Institutes of Health guidelines, and were approved by the local ethical committee (C3) of the Ministry of Science and Education in Austria.

Langendorff heart perfusion

Mice were injected with heparin 1000 U·kg⁻¹ body weight (i.p.) and anaesthetized with urethane 1 g·kg⁻¹. Hearts were rapidly excised and arrested in ice-cold Krebs–Henseleit buffer. After cannulation of the aorta with a 20 gauge needle, retrograde perfusion was established at a constant flow of 20 mL·min⁻¹·g⁻¹ wet weight with a modified Krebs–Henseleit bicarbonate buffer, pH 7.4 (composition in mM: NaCl 118, NaHCO₃ 25, KH₂PO₄ 1.2, KCl 4.8, MgSO₄ 1.2, CaCl₂ 1.25,
glucose 11) using the ISO-HEART perfusion system (Hugo Sachs Elektronik, March-Hugstetten, Germany) as previously described (Brunner et al., 2001). The perfusate was filtered through a 5-μm filter before reaching the heart and continuously gassed with carbogen (95% O2, 5% CO2). Heart temperature, measured with a physitemp probe (Physitemp Instruments, Clifton, NJ, USA), was maintained at 37°C throughout the experiments. After removal of the left auricle, a tiny fluid-filled balloon made of a small square of polyethylene film was inserted into the left ventricle and connected to a pressure transducer via a 4F biluminal monitoring catheter (Vygon, Aachen, Germany) (Sutherland et al., 2003). The following cardiac parameters were monitored using the PLUGSYS data acquisition and control set-up for circulatory studies (Hugo Sachs Elektronik) and recorded using a PowerLab system (ADInstruments Ltd, Hastings, UK): LV end-diastolic pressure (LVDP), peak LV systolic pressure (LVSP), LV developed pressure (LVDevP), maximum rate of rise and fall of LV pressure (+dP/dt max, −dP/dt min), heart rate (obtained from the pressure signal using a differentiator and heart rate module, respectively) and coronary perfusion pressure (CPP; via a second pressure transducer attached to the aortic cannula).

**Experimental protocols**

Hearts were perfused for 30 min to establish stable baseline conditions. Thereafter, baseline pressure-volume relationships were determined for each heart. The balloon-volume (Vb) was set to the lowest possible volume at which minimal LV pressure tracings (<1 mmHg) could just be recorded. This volume was defined as zero volume. The balloon was then inflated in 5 μL increments using an airtight glass syringe (Hamilton Co., Whittier, CA, USA). LVEDP and LVSP were obtained 1 min after each increment when a new steady state was reached. LV volume was increased up to a value at which maximal LVDevP was reached and a further increase led to a decrease of LVDevP (optimal filling volume; Vmax). LVDP was then readjusted to 5 mmHg and baseline conditions re-established. Subsequently, noradrenaline (NA; 1 nM–3 μM; non-cumulative dosing) was added via a sidestream. At the end of each experiment, hearts were dissected and the different compartments weighed.

**Calculation of wall stress**

LV wall thickness and radius of the left ventricle were derived from the weight of the LV wall plus septum and the balloon volume, respectively, as described previously for rat hearts (Strömer et al., 1997; Wölkart et al., 2000). LV wall stress was then derived from Laplace’s law using the relation described by Mirsky (1979).

**Statistical analysis**

All data are presented as mean values ± SEM of five experiments per experimental group. In WT hearts, the potency (EC50) of agonists was calculated by fitting individual dose-response curves to a Hill-type model. Concentration-response curves recorded with ATGL(-/-) hearts could not be fitted due to very minor effects of the agonists. ANOVA with post hoc Bonferroni–Dunn test was used for comparison between groups using StatView (Version 5.0) software (SAS Institute Inc., Cary, NC, USA). Significance was assumed at P < 0.05.

**Results**

**Body and heart weights**

At ~10 weeks of age, the body weight of ATGL(-/-) mice was slightly higher than that of WT, but the difference was not statistically significant (20.4 ± 0.9 g and 22.0 ± 0.3 g for untreated WT and ATGL(-/-), respectively). The body weight of ATGL(-/-) mice was significantly decreased after feeding the animals for 3 weeks with the PPARα agonist Wy14,643 (18.7 ± 0.2 g). In contrast, the PPARα agonist rosiglitazone increased body weight to 24.8 ± 0.3 g. In WT animals, Wy14,643 treatment also considerably decreased body weight (14.7 ± 0.3 g), while rosiglitazone was without effect. Triglyceride accumulation in cardiac muscle was already substantially more in untreated ATGL(-/-) mice, demonstrated by substantially higher heart wet weights (183 ± 6 mg) as compared with untreated WT (96 ± 2 mg; P < 0.05). Similar to body weights, heart weights were decreased in Wy14,643-treated (131 ± 4 mg) and increased in rosiglitazone-treated (215 ± 10 mg) ATGL(-/-) animals [P < 0.05 vs. untreated ATGL(-/-)]. A similar but less pronounced pattern was observed in WT animals. Thus, heart-to-body weight ratios were significantly increased in untreated ATGL(-/-) mice as compared with untreated WT (8.3 ± 0.4 and 4.7 ± 0.1, respectively). The decrease in body weight due to PPARα activation was more pronounced in WT (~28%) than in ATGL(-/-) (~18%) animals, while the decrease in heart weight was more pronounced in ATGL(-/-) (~28%) than in WT (~12%) mice. Hence, Wy14,643 treatment decreased the heart-to-body weight ratio in ATGL(-/-) but increased it in WT mice. Rosiglitazone treatment did not affect this parameter in WT or ATGL(-/-) animals (Table 1). Dissection of the hearts revealed that the twofold increase in relative weight was not due to proliferation of specific compartments, indicating whole heart hypertrophy (not shown).

**LV pressure–volume relationship and wall stress**

Untreated ATGL(-/-) hearts showed a steeper increase in LVDevP with increasing Vb. This pronounced diastolic dysfunction was ameliorated by treating ATGL(-/-) mice with Wy14,643, whereas rosiglitazone had no effect. In contrast, the LVDP pressure–volume curve was shifted leftwards in WT mice after Wy14,643 treatment (Figure 1A). LVSP was not significantly different between experimental groups at any balloon volume (Figure 1B), but LVDevP was used as indicator of systolic dysfunction rather than LVSP, because evaluating systolic pressure without considering diastolic preload does not reflect the in vivo situation. As shown in Figure 1C, ATGL(-/-) hearts generally exhibited a lower LVDevP than WT. Again, contractility of ATGL(-/-) hearts was improved by Wy14,643 but not by rosiglitazone. Optimal filling volume (Vmax) and maximal LVDevP were significantly higher in WT (42 ± 1 μL, 114 ± 11 mmHg) than in ATGL(-/-) hearts (19 ± 2 μL, 82 ± 9 mmHg). The latter two parameters were improved by Wy14,643 treatment [29 ± 2 μL, 101 ± 5 mmHg; P < 0.05 vs.
untreated ATGL(-/-), but unaffected by rosiglitazone treatment (23 ± 5 μL, 84 ± 5 mmHg). Wy14,643 treatment induced opposite effects in WT animals, that is, it reduced maximal LVDevP (98 ± 6 mmHg) and V_max (30 ± 4 μL). Importantly, evaluation of absolute pressure values negates differences in heart size and muscle mass. To compensate for differences in heart weights (and consequential wall thickness), wall stress was calculated for each filling volume V_B. As shown in Figure 2A, diastolic wall stress was increased in untreated ATGL(-/-) hearts over the whole V_s range, suggesting that reduced compliance of cardiac muscle caused by triglyceride accumulation was not effectively compensated for. Diastolic wall stress of ATGL-deficient hearts was not decreased by either of the two PPAR agonists, indicating that these drugs did not significantly affect myocardial compliance. In WT animals, diastolic wall stress was seemingly increased upon Wy14,643 treatment. However, when differences in the size of the ventricular cavities were taken into account by plotting diastolic wall stress versus V_s normalized by V_max, this effect in WT hearts was no longer evident and an improved diastolic function was revealed in Wy14,643-treated ATGL(-/-) hearts (Figure 2B).

Finally, significantly decreased systolic wall stress in ATGL(-/-) hearts at all filling volumes suggests the development of cardiomyopathy in ATGL-deficient mice (Figure 2C). Wy14,643 increased systolic wall stress in WT and ATGL(-/-) hearts at V_s > 25 μL, while no effect was observed with rosiglitazone. Again, after normalization of wall stress to differences in the size of the ventricular cavity, the effect of Wy14,643 was not apparent in WT and more pronounced in ATGL-deficient hearts (Figure 2D).

Effects of NA
Cardiomyopathic impairments were further analysed by assessing pump function and heart rate in response to adrenergic stimulation. Diastolic pressure was adjusted to 5 mmHg to mimic cardiac preload under physiological conditions. In line with the data shown in Figure 1C, ATGL-deficient hearts showed significantly reduced LVDevP under basal conditions but also exhibited markedly impaired response to NA stimulation as compared with WT littermates (EC_{50} = 13 ± 7 nM; Figure 3A). This loss of the β-adrenoceptor response was not simply confined to reduced inotropy (reduced increase in LVDevP and dp/dt_{max}; Figure 3A and B, respectively), but also apparent as reduced lusitropy (reduced increase in dp/dt_{max}; Figure 3C), indicating severe systolic and diastolic dysfunc-

Discussion
Previous work has shown that progressing lipid accumulation and myocardial fibrosis in hearts of ATGL-deficient mice was accompanied by LV hypertrophy and impaired LV systolic function in vivo (Haemmerle et al., 2006). In the present study, we confirmed and extended these observations using an in vitro model of cardiac function that is independent of potential interference by circulating humoral and/or vegetative signals. In addition, we obtained a complete picture of the isovolumic pressure-volume relationship by assessing cardiac function on several points of the Frank–Starling mechanism and showed that PPARα activation improved cardiac contractility.

Diastolic dysfunction was apparent as reduced compliance of the left chamber, rendering ATGL(-/-) hearts more susceptible to even small changes in preload. Importantly, despite a considerably increased wall thickness, calculated passive wall stress was higher in ATGL(-/-) hearts, a condition that is observed in the transition from compensated to
decompensated cardiac hypertrophy (Veliotes et al., 2005). The sustained elevation of diastolic wall stress most likely triggered further fibrosis of cardiac muscle, eventually leading to congestive heart failure. Cardiomyopathy of ATGL(-/-) hearts was evident as compromised LV pressure development in response to varying LV filling volumes. The lower developed pressure in ATGL(-/-) hearts was associated with a lower active stress, showing that contractile dysfunction is due to impaired force development of the ventricular muscle, rather than increased muscle mass. Massive fibrosis and apoptosis may partially contribute to this pathology (Haemmerle et al., 2006). Interestingly, the response to inotropic stimulation by NA was also impaired in ATGL-deficient hearts, presumably due to reduced contractile reserve (cf. Figure 1C). Our observations showing a markedly decreased velocity of contraction and relaxation at both baseline condition and after NA stimulation (cf. Figure 3B,C) indicate increased stiffness of the cardiac muscle, most likely reflecting advanced fibrosis. Reduced muscle compliance might also, to some extent, account for the significantly less pronounced increase in heart rate in response to NA, possibly through increased occurrence of arrhythmias. Finally, decreased β-adrenoceptor density and/or receptor-effector coupling might have affected the response to NA in ATGL(-/-) hearts. However, the contractile response to NA was more severely affected by ATGL deficiency than the chronotropic effect (cf. Figures 3A and 4A), suggesting that impaired receptor-effector coupling may account for the reduced chronotropic response, whereas additional mechanisms, for example, fibrosis and/or mitochondrial dysfunction, appear to cause impaired contractility.

An obvious possibility is that heart function in ATGL(-/-) mice is affected by the disordered balance between carbohydrate and FA utilization for energy production. Typically, during development of cardiac hypertrophy and heart failure, myocardial metabolism switches from utilization of long-chain FAs to glucose because of the higher efficiency of glycolysis in terms of ATP generated mol⁻¹ O₂ consumed (Bishop and Altschuld, 1970). The effect of catecholamine stimulation on cardiac energy metabolism is less clear. An early study reported about twofold to threefold and threefold to fourfold increases, respectively, in the rates of glucose and triglyceride utilization during adrenergic stimulation in vitro (Williamson, 1964). Others have reported a reduced cardiac glucose uptake after adrenergic stimulation in vivo (Capaldo et al., 1992; Huang et al., 1997). Glucose uptake and utilization are increased in ATGL(-/-) cardiac muscle together with attenuated isoprenaline-stimulated FA mobilization in white adipose tissue (Haemmerle et al., 2006), even though gene expression studies suggest concerted down-regulation of oxidative pathways required for energy production from glucose in ATGL-deficient mice (Pinent et al., 2008). Since our

Figure 1
Effect of increasing balloon volume on LV end-diastolic (A), systolic (B) and corresponding developed (C) pressure of hearts isolated from WT and ATGL(-/-) mice treated with rosiglitazone, Wy14,643 or standard mouse chow. Data are mean values ± SEM of five hearts. *P < 0.05 versus untreated WT; †P < 0.05 versus untreated ATGL(-/-) (ANOVA).
observations of impaired mitochondrial respiration in ATGL-deficient hearts (submitted) indicate that the same applies to cardiac muscle, it is likely that glycolysis alone is not sufficient to provide enough primary energy for an adequate response to adrenergic stimulation.

We recently found that ATGL deficiency leads to severely impaired activation of PPARα and PPARδ target genes in cardiac and skeletal muscle, suggesting that ATGL-catalyzed triglyceride hydrolysis is essential for the supply of FA-derived PPAR ligands in these tissues (unpublished observations). The role of PPARs in cardiac function is not well understood (Zahradka, 2007; Robinson and Grieve, 2009). Activation of PPARγ was reported to inhibit hypertrophy of cardiac myocytes in response to mechanical strain (Yamamoto et al., 2001; Asakawa et al., 2002), whereas activation of PPARα, but not PPARγ, was shown to prevent apoptosis of cardiac myocytes after ischaemia/reperfusion injury (Yeh et al., 2006) and to regulate cardiomyogenesis from embryonic stem cells (Sharifpanah et al., 2008). In a rat coronary artery ligation model, a PPARα agonist attenuated the progression of heart failure by reducing fibrosis and heart weight, while PPARγ activation even exacerbated cardiac dysfunction (Linz et al., 2009). In support of the protective role of PPARα signalling, PPARα-deficient mice were reported to exhibit cardiac contractile dysfunction due to oxidative damage of myosin (Guellich et al., 2007).

In line with previous observations, we observed that PPARα activation of WT animals led to reduced body weight and substantial alterations in heart weight and function (Zungu et al., 2009). In our set-up, reduced contractility after

Figure 2
Dependence of LV end-diastolic (A,B) and systolic (C,D) wall stress on intracardiac balloon volume (Vb, left) and balloon volume normalized by optimal filling volume (Vmax, right) of hearts isolated from WT and ATGL(-/-) mice treated with rosiglitazone, Wy14,643 or standard mouse chow. Data are mean values ± SEM of five hearts. * P < 0.05 versus untreated WT; † P < 0.05 versus untreated ATGL(-/-) (ANOVA).
Wy14,643 treatment of WT mice was mainly due to reduced cardiac muscle mass (cf. Figure 2B,D), although additional mechanisms like impaired mitochondrial function (Keller et al., 1993) cannot be excluded. Despite this unfavourable
effect in WT animals, Wy14,643 reduced cardiac hypertrophy and improved diastolic and systolic function in ATGL-deficient mice, indicating that PPARα target genes are essential for maintaining normal cardiac function. Our data clearly indicate that this improvement was not simply caused by altered cardiac growth. As stimulation of PPARα triggers the expression of genes involved in lipid metabolism (Marx et al., 2004), treating the mice with Wy14,643 may have led to improved energy supply to the heart through increased rates of FA β-oxidation. Another possible explanation is improved mitochondrial function. Further experiments are needed to address this issue.

The inotropic and chronotropic effects of NA were markedly decreased in ATGL-deficient hearts. Based on the observation that targeted gene deletion of PPARα causes down-regulation of β-adrenoreceptors (Loichot et al., 2006), Wy14,643 may have restored the chronotropic response by increasing NA receptor density. However, the explanation for this finding is probably more complex because β-adrenoreceptor-mediated contractility was not improved by Wy14,643 in ATGL(-/-) mice and unaltered in WT mice (in terms of Δ increase). The reduced contractile response to NA may reflect attenuated FA mobilization in ATGL-deficient cardiac muscle, as observed previously in white adipose tissue of ATGL(-/-) mice (Haemmerle et al., 2006). Thus, even though the PPARα agonist improved mitochondrial function, glycolysis is apparently not sufficient to supply enough primary energy for a NA-triggered contraction. The observation that the increase in coronary flow in response to NA was reduced in ATGL(-/-) hearts suggests that ATGL deficiency may affect microvascular function. This possibility is currently being investigated in our laboratory.

Rosiglitazone had no cardioprotective effects at all. As the survival rate of PPARα-deficient mice is extremely low (Duan et al., 2007), the role of PPARα signalling in the heart is only poorly understood and highly controversial. Animal in vitro studies suggest a cardioprotective role of glitazones in ischaemia/reperfusion injury (Shimabukuro et al., 1996; Khandoudi et al., 2002; Molavi et al., 2006), while recent clinical surveys indicate that the risk of heart failure is even increased by rosiglitazone (Nissen and Wolski, 2007; Home et al., 2009). Notably, in mice overexpressing lipoprotein lipase, another model of lipotoxic cardiomyopathy, PPARγ (but not PPARα) agonists were shown to reduce cardiac lipid levels and markers of cardiomyopathy (Vikramadithyan et al., 2005). However, in that model, the protective effects of PPARγ activation may have been simply due to re-allocation of triglycerides and FAs from the heart to adipose tissue.

In summary, the present study shows that severe cardiac dysfunction due to lipid accumulation in ATGL-deficient mice is improved by activation of PPARα, suggesting that treatment with fibrates may be beneficial in human neutral lipid storage disease.

Acknowledgements

This work was supported by the Fonds zur Förderung der Wissenschaftlichen Forschung in Austria (SFB F30 Lipotox; R. Zechner, B. Mayer).

Conflict of interest

None declared.

References

Asakawa M, Takano H, Nagai T, Uozumi H, Hasegawa H, Kubota N et al. (2002). Peroxisome proliferator-activated receptor-γ plays a critical role in inhibition of cardiac hypertrophy in vitro and in vivo. Circulation 105: 1240–1246.

Barbier O, Torra JP, Duguay Y, Blanquat C, Fruchart JC, Glineur C et al. (2002). Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis. Arterioscler Thromb Vasc Biol 22: 717–726.

Bishop SP, Altschuld RA (1970). Increased glycolytic metabolism in cardiac hypertrophy and congestive failure. Am J Physiol 218: 153–159.

Borradaile NM, Schaffer JE (2005). Lipotoxicity in the heart. Curr Hypertens Rep 7: 412–417.

Brunner F, Andrew P, Wolkart G, Zechner R, Mayer B (2001). Myocardial contractile function and heart rate in mice with myocyte-specific overexpression of endothelial nitric oxide synthase. Circulation 104: 3097–3102.

Campagna F, Nanni L, Quagliarini F, Pennisi E, Michailidis C, Pierelli F et al. (2008). Novel mutations in the adipose triglyceride lipase gene causing neutral lipid storage disease with myopathy. Biochem Biophys Res Commun 377: 843–846.

Capaldo B, Napoli R, Di Marino L, Sacca L (1992). Epinephrine directly antagonizes insulin-mediated activation of glucose uptake and inhibition of free fatty acid release in forearm tissues. Metabolism 41: 1146–1149.

Chiu HC, Kovacs A, Ford DA, Hsu FF, Garcia R, Herrero P et al. (2001). A novel mouse model of lipotoxic cardiomyopathy. J Clin Invest 107: 813–822.

Chiu HC, Kovacs A, Blanton RM, Han X, Courtois M, Weinheimer CJ et al. (2005). Transgenic expression of fatty acid transport protein 1 in the heart causes lipotoxic cardiomyopathy. Circ Res 96: 225–233.

Crabb DW, Pinaire J, Chou WY, Sissom S, Peters JM, Harris RA (1992). Epinephrine and in vivo critical role in inhibition of cardiac hypertrophy and congestive failure. Am J Physiol 218: 153–159.

Feuvray D, Darmellah A (2008). Diabetes-related metabolic perturbations in cardiac myocyte. Diabetes Metab 34 (Suppl. 1): 3–9.

Finck BN, Lehman JJ, Leone TC, Welch MJ, Bennett MJ, Kovacs A et al. (2002). The cardiac phenotype induced by PPARα overexpression mimics that caused by diabetes mellitus. J Clin Invest 109: 121–130.

Fischer J, Lefèvre C, Morava E, Mussini JM, Laforêt P, Negre-Salvayre A et al. (2007). The gene encoding adipose
triglyceride lipase (PNPLA2) is mutated in neutral lipid storage disease with myopathy. Nat Genet 39: 28–30.

Guellich A, Damy T, Lecarpentier Y, Conti M, Claes V, Samuel JL (2007). Role of oxidative stress in cardiac dysfunction of PPARα−/− mice. Am J Physiol 293: H93–H102.

Haemmerle G, Lass A, Zimmermann R, Gorkiewicz G, Meyer C, Rozman J et al. (2006). Defective lipolysis and altered energy metabolism in mice lacking adipose triglyceride lipase. Science 312: 734–737.

Hirano Ki, Ikeda Y, Zaima N, Sakata Y, Matsumiya G (2008). Triglyceride deposit cardiomyovasculopathy. N Engl J Med 359: 2396–2398.

Home PD, Pocock SJ, Beck-Nielsen H, Curtis PS, Gomis R, Hanefeld M et al. (2009). Rosiglitazone evaluated for cardiovascular outcomes in oral agent combination therapy for type 2 diabetes (RECORD): a multicentre, randomised, open-label trial. Lancet 373: 2125–2135.

Huang MT, Lee CF, Dobson GP (1997). Epinephrine enhances glycogen turnover and decreases glucose uptake in vivo in rat heart. FASEB J 11: 973–980.

Kanda T, Brown JD, Orasanu G, Vogel S, Gonzalez FJ, Sartoretto J et al. (2009). PPARα in the endothelium regulates metabolic responses to high-fat diet in mice. J Clin Invest 119: 110–124.

Keller BJ, Bradford BU, Marsman DS, Cattley RC, Popp JA, Bojes HK et al. (1993). The nongenotoxic hepatocarcinogen Wy-14,643 is an uncoupler of oxidative phosphorylation in vivo. Toxicol Appl Pharmacol 119: 52–58.

Khandoudi N, Delerive P, Berrebi-Bertrand I, Buckingham RE, Staels B et al. (2005). Peroxisome proliferator-activated receptor-α−/− mice. Am J Physiol 293: H93–H102.

Linz W, Wohlhart B, Baader M, Breitschopf K, Falk E, Schäfer HL et al. (2009). The peroxisome proliferator-activated receptor-α (PPAR-α) agonist, AVE8134, attenuates the progression of heart failure and increases survival in rats. Acta Pharmacol Sin 30: 935–946.

Loichot C, Jesel L, Tesea A, Tabernero A, Schoonjans K, Roul G et al. (2006). Deletion of peroxisome proliferator-activated receptor-α induces an alteration of cardiac functions. Am J Physiol 291: H161–H166.

Marx N, Duez H, Fruchtart JC, Staels B (2004). Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. Circ Res 94: 1168–1178.

Mirskey I (1979). Elastic properties of the myocardium: a quantitative approach with physiological and clinical applications. In: Berne R, Sperelakis N, Geiger S (eds). Handbook of Physiology – The Cardiovascular System, Vol. I (The Heart). American Physiological Society: Bethesda, MD, pp. 497–531.

Molavi B, Chen J, Mehta JL (2006). Cardioprotective effects of rosiglitazone associated with selective overexpression of type 2 angiotensin receptors and inhibition of p42/44 MAPK. Am J Physiol 291: H687–H693.

Neely JR, Rovetto MJ, Oram JF (1972). Myocardial utilization of carbohydrate and lipids. Prog Cardiovasc Dis 15: 289–329.

Nissen SE, Wolski K (2007). Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. N Engl J Med 356: 2457–2471.

Pinet M, Hackl H, Burkard TR, Prokesch A, Papak C, Scheidel M et al. (2008). Differential transcriptional modulation of biological processes in adipocyte triglyceride lipase and hormone-sensitive lipase-deficient mice. Genomics 92: 26–32.

Robinson E, Grieve DJ (2009). Significance of peroxisome proliferator-activated receptors in the cardiovascular system in health and disease. Pharmacol Ther 122: 246–263.

Schumm-Danae M, Schneppenheim R, Perings S, Pfehn G, Strauer BE (2002). Left ventricular diastolic dysfunction as an early manifestation of diabetic cardiomyopathy. Cardiology 98: 33–39.

Schiffrin EL (2005). Peroxisome proliferator-activated receptors and cardiovascular remodeling. Am J Physiol 288: H1037–H1043.

Sharifpanah F, Wartenberg M, Hannig M, Picher HM, Sauer H (2008). Peroxisome proliferator-activated receptor-α agonists enhance cardiomyogenesis of mouse ES cells by utilization of a reactive oxygen species-dependent mechanism. Stem Cells 26: 64–71.

Sharma S, Adrogue JV, Gofman L, Uray I, Lemm J, Youker K et al. (2004). Intramyocardial lipid accumulation in the failing human heart resembles the lipotoxic rat heart. FASEB J 18: 1692–1700.

Shimabukuro M, Higa S, Shinzato T, Nagamine F, Komiya I, Takasu N (1996). Cardioprotective effects of troglitazone in streptozotocin-induced diabetic rats. Metabolism 45: 1168–1173.

Strömer H, Cittadini A, Szynska G, Apstein CS, Morgan JP (1997). Validation of different methods to compare cardiac function in isolated hearts of varying sizes. Am J Physiol 272: H501–H510.

Takasu N (1996). Cardioprotective effects of troglitazone in streptozotocin-induced diabetic rats. Metabolism 45: 1168–1173.

Touyz RM, Schiffrin EL (2005). Peroxisome proliferator-activated receptors in vascular biology: molecular mechanisms and clinical implications. Vasc Pharmacol 45: 19–28.

Veillette B, Halko M, Horvath K, Halko J, Horvath G et al. (2000). Aldosterone receptor blockade prevents the transition to cardiac pump dysfunction induced by beta-adrenoreceptor activation. Hypertension 45: 914–920.

Vikramadityan RK, Hirata K, Yagyu H, Hu Y, Augustus A, Honma S et al. (2005). Peroxisome proliferator-activated receptor agonists modulate heart function in transgenic mice with lipotoxic cardiomyopathy. J Pharmacol Exp Ther 313: 586–593.

Williamson JR (1964). Metabolic effects of epinephrine in the isolated, perfused rat heart. I. Dissociation of the glycogenolytic from the metabolic stimulatory effect. J Biol Chem 239: 2721–2729.

Wittels B, Spann JF Jr (1968). Defective lipid metabolism in the failing heart. J Clin Invest 47: 1787–1794.

Wölkart G, Strömer H, Brunner F (2000). Calcium handling and role of endothelin-1 in monocrotaline right ventricular hypertrophy of the rat. J Mol Cell Cardiol 32: 1995–2005.

Yagyu H, Chen G, Yokoyama M, Hirata K, Augustus A, Kako Y et al. (2003). Lipoprotein lipase (LPL) on the surface of cardiomyocytes increases lipid uptake and produces a cardiomyopathy. J Clin Invest 111: 419–426.

Yamamoto K, Okhi R, Lee RT, Ikeda U, Shimada K (2001). Peroxisome proliferator-activated receptor γ activators inhibit cardiac hypertrophy in cardiac myocytes. Circulation 104: 1670–1675.

Yeh CH, Chen TP, Lee CH, Wu YC, Lin YM, Lin PJ (2006). Cardiomyocytic apoptosis following global cardiac ischemia and
reperfusion can be attenuated by peroxisome proliferator-activated receptor α but not γ activators. Shock 26: 262–270.

Zahradka P (2007). Cardiovascular actions of the peroxisome proliferator-activated receptor-alpha (PPARα) agonist Wy14,643. Cardiovasc Drug Rev 25: 99–122.

Zimmermann R, Strauss JG, Haemmerle G, Schoiswohl G, Birner-Gruenberger R, Riederer M et al. (2004). Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. Science 306: 1383–1386.

Zungu M, Young ME, Stanley WC, Essop MF (2009). Chronic treatment with the peroxisome proliferator-activated receptor α agonist Wy-14,643 attenuates myocardial respiratory capacity and contractile function. Mol Cell Biochem 330: 55–62.