Sirt1 acts in association with PPARα to protect the heart from hypertrophy, metabolic dysregulation, and inflammation

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Aims
A complex set of metabolic and inflammatory processes are involved in the development of cardiac hypertrophy. Accumulating evidence indicates an important role for Sirt1 in cardiac function, whereas peroxisome proliferator-activated receptor-α (PPARα) is a master controller of cardiac lipid metabolism and plays a protective role on cardiac hypertrophy. The objective of the present study was to explore the relationships between Sirt1 and PPARα in the control of hypertrophy, metabolism, and inflammation processes in the heart.

Methods and results
Neonatal cardiomyocytes (NCMs) were used for studies in vitro. Both the activation of Sirt1 with resveratrol (RSV) and overexpression of Sirt1 inhibited phenylephrine (PE)-induced NCM hypertrophy and prevented PE-induced down-regulation of fatty acid oxidation genes. Sirt1 also inhibited the PE-induced increase in mRNA levels of the pro-inflammatory cytokine monocyte chemoattractant protein-1 in NCMs and blocked the enhanced nuclear factor-kB (NF-kB) activity associated with exposure to PE. Importantly, inhibition of PPARα suppressed the beneficial effects of Sirt1 on hypertrophy, fatty acid metabolism, and inflammation. Co-immunoprecipitation studies revealed that overexpression of Sirt1 enhanced PPARα binding to the p65 subunit of NF-kB and led to p65-deacetylation in NCMs. Moreover, Sirt1 overexpression led to the deacetylation of the PPARα co-activator PGC-1α. Consistent with these observations in vitro, isoproterenol-induced cardiac hypertrophy, metabolic dysregulation, and inflammation in vivo were prevented by RSV in wild-type mice but not in PPARα-null mice.

Conclusions
Collectively, these findings reveal a major involvement of the Sirt1–PPARα interaction in the protective role of Sirt1 against cardiac hypertrophy.

Keywords
Cardiac hypertrophy • Sirt1 • PPARα • Metabolism • Inflammation

1. Introduction
Cardiac hypertrophy and subsequent progression to heart failure represent a major cause of morbidity and mortality in industrialized countries. The defining features of cardiac hypertrophy are an increase in cardiomyocyte size, enhanced protein synthesis, and a higher degree of sarcomere organization. These changes are preceded and accompanied by the re-induction of the so-called foetal cardiac gene programme, characterized by a pattern of altered gene expression that mimics those observed during embryonic heart development. Cardiac hypertrophy is also associated with a shift from fatty acids to glucose as energy source, an additional feature in common with the foetal heart.1

Sirt proteins (sirtuins) represent a highly evolutionarily conserved family of NAD-dependent deacetylases, some of which also possess ADP-ribosyltransferase activity. Seven Sirt subtypes (Sirt1–7) have been identified.2 Sirt1, the most studied member of the family, is a nuclear protein that plays a role in metabolic homoeostasis, increasing the consumption of oxygen in muscle fibres and inducing the expression of fatty acid oxidation genes, such as carnitine palmitoyltransferase I (mCPT-I), medium-chain acyl-CoA dehydrogenase (MCAD), and pyruvate dehydrogenase kinase 4 (PDK4).3 Moreover, Sirt1 is able to modulate peroxisome proliferator-activated receptors (PPARs) and PGC1-α activity in muscle.4 Studies using Sirt1-null mice have revealed a prominent role for Sirt1 in heart morphogenesis, whereas heart-specific over-expression of Sirt1 indicated a protective or damaging role of Sirt1 in relation to heart hypertrophy or fibrosis depending on the extent of Sirt1 overexpression.5

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Recent studies have indicated an important role for PPARs in cardiac disease. It has been shown that peroxisome proliferator-activated receptor-α (PPARα) is able to prevent the reduction in fatty acid metabolism gene expression and reverse the development of cardiac hypertrophy.6,7

It was recently reported that Sirt1 regulates fatty acid oxidation in the liver through positive regulation of PPARα action,8 revealing a new pathway through which Sirt1 controls metabolism.

In the present study, we analyse the role of Sirt1 during the development of cardiac hypertrophy in the heart. We show that the activation of Sirt1 prevents the development of cardiac hypertrophy and protects cardiac cells from metabolic disregulation and inflammation and these beneficial effects are mediated through the activation of the PPARα pathway.

2. Methods

2.1 Animals

Neonatal (1–2 days old) Sprague–Dawley rats were used for the isolation of neonatal cardiac myocytes (NCMs). Cardiac hypertrophy in vivo was induced by isoproterenol (ISO) administration (Sigma, St Louis, MO, USA). Four-day-old neonates from wild-type (wt) and PPARα-null mice obtained from Jackson Laboratory (USA) were subjected to intra-peritoneal injection of ISO (15 mg/kg/day) alone or combined with resveratrol (RSV, 50 mg/kg/day; Sigma) for 5 days. Control animals received saline solution. All experiments were performed according to the European Community Council directive 86/609/EEC and approved by the Institutional Animal Care and Use Committee of the University of Barcelona.

2.2 Cell culture

NCMs were isolated and cultured as described by de Vries et al.,9 using differential plating to separate myocytes from non-myocytes. Briefly, 1–2-day-old Sprague–Dawley rats were decapitated and their hearts were removed. Hearts were digested with a collagenase solution (Collagenase Type I, Gibco) followed by differential plating. Cells were plated at a density of 2.5 × 10^4 cells/well in six-well plates coated with 1% gelatine and cultured overnight in plating medium [DMEM supplemented with 10% horse serum, 5% newborn calf serum, 50 mg/L gentamicine, and 0.25 mM-L-carnitine, 0.25 mU/mL insulin, and 1% bovine serum albumin]. ARA C was added to non-exposed cells to suppress the growth of the remaining fibroblasts. Sixteen hours after isolation, NCMs were incubated in serum-free medium consisting of 0.25 mM-L-carnitine, 0.25 mU/mL insulin, and 1% bovine serum albumin. Ara C was present at all times during cell culture.

NCMs were stimulated with the α1-adrenergic agonist phenylephrine (PE, 10 μM), a hypertrophic growth factor, or the pro-inflammatory agent lipopolysaccharide (LPS, 10 ng/mL; Sigma). After treating for 24 h, cells were harvested for RNA isolation. Where indicated, cells were pretreated with RSV, sirtinol, or the PPARα antagonist GW6471 (Sigma) for 24 h prior to PE/LPS exposure. Appropriate vehicles served as controls. None of the agents induced significant cell loss at the concentrations used.

2.3 RNA isolation and real-time reverse transcription-polymerase chain reaction

Total RNA was extracted using Tripure (Roche, Indianapolis, IN, USA). Reverse transcriptase was performed using random hexamers primers (Applied Biosystems, Foster City, CA, USA) and 0.5 μg of RNA in a total reaction volume of 20 μL. Polymerase chain reaction was conducted in duplicate for increased accuracy. TaqMan Gene Expression Assays were used, and each 25 μL of reaction mixture contained 1 μL of cDNA, 12.5 μL of TaqMan Universal PCR Master Mix (Applied Biosystems), 250 nM probes, and 900 nM primers from Assays-on-Demand Gene Expression Assay Mix or Assays-by-Design Gene Expression Assay Mix (Applied Biosystems). Each sample was run in duplicate and the mean value was used to calculate the mRNA expression of the gene of interest and the housekeeping reference gene (cyclophilin A, PPIA). The mRNA level for the gene of interest in each sample was normalized to that of the reference control using the comparative (2^ΔΔCT) method following the manufacturer’s instructions.

2.4 Adenoviral infection

A recombinant adenovirus expressing murine Sirt1 cDNA was constructed (Ad5-CMV-Sirt1, CEBATEG, Barcelona, Spain). Prior to treatment with RSV or sirtinol, NCMs were infected with the Sirt1 adenoviral vector (AdSirt1) or an AdCMV-GFP control vector (AdGFP) at 10 IFU/cell for 24 h in serum-free medium. Based on an assessment of GFP fluorescence, this treatment led to an infection efficiency of ~90%. Adenoviral infection at 10 IFU induced no significant cell death.

2.5 Transient transfection

Four hours after adenovirus infection, NCMs were transfected with 0.5 μg of a nuclear factor-κB (NF-κB)-luciferase reporter vector (pNF-κB) or empty pTAL vector (control) (Clontech) using the transfection reagent FuGENE 6 (Roche). The pRL-CMV expression vector for the sea pansy (Renilla reniformis) luciferase was used as an internal control for transfection efficiency (Promega, Madison, WI, USA). After 16 h of transfection, the medium was replaced with experimental medium and cells were exposed to PE (10 μM). Cells were harvested 1 h later and immediately processed for the determination of reporter activity. Firefly luciferase and Renilla luciferase activities were measured in a Glomax 96 Microplate Luminometer using the Dual Luciferase Reporter Assay System Kit (Promega). Cells were lysed by suspending 100 μL of passive lysis buffer and agitating for 20 min. NF-κB-dependent luciferase activity was measured in 20 μL of homogenates and normalized for variations in transfection efficiency using Renilla luciferase activity as an internal standard.

2.6 Assessment of cell size

NCMs were grown on laminin (10 μg/mL)-coated glass cover slips. Immunolocalization was performed in vivo by incubating for 30 min with the membrane marker WGA-Oregon red (1:1000), to stain the cellular membrane, and DAPI (1:1000), to stain the nucleus. Binding reactions were performed at 4 °C (for 30 min) to avoid internalization of WGA. Next, cells were washed with phosphate-buffered saline and fixed with 3% paraformaldehyde. Immunofluorescence was visualized using a laser-scanning confocal fluorescence microscope (Olympus Fluoview FV500). Photographic images were collected from five randomly selected fields. Cardiomyocyte surface area (μm²) was determined from randomly selected fields from three independent cell cultures using ImageJ.

2.7 Co-immunoprecipitation

Preparation of nuclear extracts and immunoprecipitation procedures have been described previously.11 Nuclear extracts were immunoprecipitated by using a PPARα antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) and analysed by western blot using antibodies against Sirt1 (Upstate Biotechnology, Inc., Lake Placid, NY, USA), p65 and PGC-1α (Santa Cruz Biotechnology), acetylated-p65, and acetylated-Lysine (Cell Signaling Technology, Inc., Beverly, MA, USA) antibodies.

2.8 Statistics

Cell culture experiments were conducted in triplicate on at least three independent cardiomyocyte isolations. We used five mice/group in the in vivo experiments.
experiments. Results are presented as mean ± SEM. Data were analysed by one-way ANOVA, followed by Bonferroni’s or Dunnet’s post hoc tests, as appropriate, using GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA). A P-value of <0.05 was considered to be statistically significant.

3. Results

3.1 Sirt1 activation by RSV and Sirt1 overexpression inhibit cardiac hypertrophy

PE treatment induced an increase in the hypertrophic markers atrial natriuretic factor (ANF) and α-skeletal actin (α-SKA) mRNA levels (Figure 1A), which was completely prevented by RSV. In contrast, the Sirt1 inhibitor sirtinol was completely ineffective in preventing PE-induced up-regulation of ANF and α-SKA mRNA. AdSirt1 infection significantly attenuated the induction of hypertrophic markers by PE, with or without added RSV. ANF mRNA levels in AdGFP- and AdSirt1-infected cells tended to be lower and higher, respectively, than those in non-infected cells, but the differences were not statistically significant. Treatment of NCMs with PE significantly increased cardiomyocyte size, an indication of hypertrophy (Figure 1B). Overexpression of Sirt1 by AdSIRT1 infection attenuated the PE-induced cell enlargement in the presence or absence of RSV. Collectively, these data indicate that activation or overexpression of Sirt1 in vitro is capable of preventing cardiac hypertrophy.
3.2 Effects of Sirt1 on fatty acid oxidation genes in cardiomyocytes

One of the features of cardiac hypertrophy is the reversion from fatty acids to glucose as the main energy source. To explore whether regulation of this metabolic switch was part of Sirt1’s protective effects against hypertrophy, we analysed mRNA levels of the fatty acid oxidation genes PDK4, mCPT-I, and MCAD. Exposure of NCMs to PE showed significantly decreased PDK4, mCPT-I, and MCAD expression levels, and this effect was abrogated by RSV and by infection with AdSirt1. RSV, in the absence of PE, only enhanced PDK4 mRNA expression levels significantly (Figure 2). In summary, Sirt1 modulates PE-induced cardiac hypertrophy and prevents the associated down-regulation of fatty acid oxidation genes as part of its protective effects.

3.3 Sirt1 inhibits PE-induced pro-inflammatory gene expression and NF-κB activation

There is close relationship between hypertrophy and inflammation in the cardiomyocyte; moreover, Sirt1 has been reported to interact with NF-κB in other cellular systems. Thus, to elucidate the molecular mechanisms responsible for the anti-hypertrophic effects of Sirt1, we investigated the involvement of the inflammatory pathway, focusing on the hypertrophy marker ANF and the pro-inflammatory cytokine and NF-κB target, monocyte chemoattractant protein-1 (MCP-1). First, NCMs were treated either with PE or with the pro-inflammatory agent LPS for 12 and 24 h (Figure 3A). Both agents up-regulated the hypertrophy marker ANF after 12 h, but only PE induced an increase in ANF mRNA levels that was sustained for 24 h. MCP-1 was also significantly up-regulated in all cases. Overexpression of Sirt1 or activation of Sirt1 by RSV completely abrogated the induction of MCP-1 mRNA expression levels by PE and strongly suppressed the enhanced expression of MCP-1 induced by LPS in NCMs (Figure 3B). Finally, NCMs were co-transfected with an NF-κB-responsive promoter-reporter construct and AdSirt1. PE exposure induced a significant (two-fold) increase in NF-κB transcriptional activity (Figure 3C), which was significantly reduced by Sirt1 overexpression.

3.4 The protective effects of Sirt1 on cardiomyocytes require an active PPARα pathway

We next investigated whether the PPARα pathway is involved in the effects of Sirt1 on cardiac hypertrophy, fatty acid oxidation, and inflammation. NCMs were infected with either AdSirt1 or AdGFP (control) prior to stimulation with PE and further treatment with the synthetic PPARα inhibitor GW6471 (1 μM). The protective effects of Sirt1 overexpression on PE-induced cell hypertrophy (Figure 4A) and the expression of the hypertrophy markers ANF and α-SKA (Figure 4B) were lost when the cells were co-incubated with GW6471, indicating that the protective action of Sirt1 requires an active PPARα pathway (Figure 4A and B). We also analysed MCAD, PDK4, and mCPT-I mRNA levels under the same conditions (Figure 4C). Blockade of the PPARα pathway completely abrogated the beneficial effects of Sirt1 overexpression on fatty acid oxidation genes. Finally, down-regulation of the inflammatory marker MCP-1 after AdSirt1 and PE treatment was prevented when the cells were co-treated with the PPARα antagonist (Figure 4D). Collectively, these findings indicate that the protective effects of Sirt1 on cardiac hypertrophy, fatty acid metabolism, and inflammation require an active PPARα pathway.

3.5 Sirt1 overexpression promotes the interactions among Sirt1, PPARα, and p65 and causes p65 and PGC-1α deacetylation

To explore the interaction of Sirt1 and PPARα in cardiomyocytes and assess the involvement of both proteins in the inhibition of the pro-inflammatory pathway, we performed co-immunoprecipitation studies. In accordance with reports in other cell systems demonstrating that Sirt1 and PPARα may interact, western blotting detected Sirt1 in PPARα immunoprecipitates from extracts of NCMs, and
The Sirt1 signal was enhanced in cells overexpressing Sirt1 (Figure 5A). The p65 subunit of NF-κB has been shown to physically interact with PPARα.17 We found that overexpression of Sirt1 increased the association of PPARα and p65, indicating that Sirt1 promotes the formation of a complex between p65 and PPARα (Figure 5B and C). Because Sirt1 acts as a protein deacetylase, we explored the acetylation state of p65. PE stimulation induced a marked increase in p65 acetylation, and overexpression of Sirt1 reversed PE-induced p65 acetylation completely. Finally, to explore the relationship between Sirt1 and the PPARα-dependent increase of fatty acid oxidation genes, we explored the acetylation status of PGC-1α, a major co-activator of PPARα known to be expressed in the heart and target of deacetylation by Sirt1 in other cell systems (Figure 5D and E).18 No changes were observed in PGC-1α levels in immunoprecipitates of NCMs with PPARα antibody, either treated or not treated with PE or after overexpression of Sirt1. However, Sirt1 overexpression resulted in a marked reduction in the signal corresponding to deacetylated PGC-1α in the immunoprecipitate.

3.6 RSV prevents cardiac hypertrophy, metabolic dysregulation, and inflammatory response in vivo in wt mice, but not in PPARα−/− mice

Finally, we analysed the interaction between Sirt1 and PPARα, inducing cardiac hypertrophy in vivo in wt and PPARα−/− mice. The increased heart weight/body weight ratio (ΔHW/BW), defined as the difference between the HW/BW ratio from each animal treated vs. the HW/BW ratio from corresponding control mice, was significantly higher in wt and PPARα−/− mice after 5 days of ISO administration (Figure 6A). Administration of RSV prevented HW/BW increase in the wt mice but not in the PPARα−/− mice. Consistent with heart enlargement, the cardiac hypertrophy markers ANF and α-SKA were also induced in the ISO-treated mice (Figure 6B and C). RSV significantly attenuated the hypertrophy markers in the wt mice but not in the PPARα−/− mice. Expression of the fatty acid oxidation-associated gene PDK-4 (Figure 6D) was significantly down-regulated after the induction of cardiac hypertrophy in wt mice, and this reduction was prevented by RSV administration. PDK-4 expression levels in the PPARα−/− mice were already lower in the control situation and were unchanged under the different conditions. Finally, we observed that the mRNA levels of the pro-inflammatory marker gene MCP-1 were significantly increased in hearts treated with ISO in both mouse models (Figure 6E). However, RSV only prevented this up-regulation in the wt mice but not in the PPARα−/− mice. Collectively, these in vivo data confirm our in vitro findings and clearly involve the PPARα pathway in the RSV protective effects on cardiac hypertrophy.

4. Discussion

In the present study, we demonstrate that Sirt1 prevents the development of cardiac hypertrophy and we propose a model in which Sirt1 interacts with PPARα, favouring the deacetylation of PGC-1α, and promoting by this means the transcription of fatty acid oxidation...
genes. Moreover, the Sirt1-PPARα complex also binds and deacetylates the p65 subunit of NF-kB, thereby inhibiting NF-kB pro-inflammatory pathway. Both processes—PPARα activation and NF-kB inhibition—contribute to preventing the hypertrophic state.

4.1 Sirt1 and hypertrophy
The current findings indicate that Sirt1 exerts anti-hypertrophic effects on the cardiac muscle. Adenoviral-mediated overexpression of Sirt1 as well as activation of Sirt1 by RSV blunted the increase in ANF and α-SKA mRNA levels and inhibited cell size enlargement of PE-stimulated NCMs. Moreover, wt mice treated for 5 days with the hypertrophic agent ISO showed elevated mRNA levels of hypertrophy markers and an increased heart weight/body weight ratio, which was prevented by RSV. These results are in line with previous in vivo and in vitro studies, which demonstrated a protective role of Sirt1 against ageing and oxidative stress.19,20 The direct or indirect effect of RSV on Sirt1 activation is controversial,21 but a recent study showed a direct dose-dependent increase in Sirt1 activity elicited by RSV in cardiomyocytes.22 The same authors demonstrated the protective role of RSV on angiotensin II-induced cardiovascular damage. Moreover, the protective role of RSV against cardiac hypertrophy has been shown in hypertensive rats23 and in pressure overload-induced cardiac hypertrophy.24 Taken together, our current results and these earlier observations indicate a prominent role for Sirt1 in cardiac development and function.

4.2 Cardiac hypertrophy and fatty acid metabolism
It is still a matter of controversy whether changes in intracellular substrate and metabolite levels in NCMs are the cause or the consequence of cardiac hypertrophy. However, several studies support a role for altered cardiac metabolism in the development of cardiac hypertrophy.25 – 27 In the present study, we showed how overexpression/activation of Sirt1 prevents PE-dependent down-regulation of the fatty acid oxidation genes. Moreover, RSV prevented the decreased expression of key genes of fatty acid oxidation in wt mice subjected to cardiac hypertrophy in vivo. The capacity of Sirt1 to reverse the cardiac metabolic switch characteristic of hypertrophy appears to be a relevant component of the protective effects of Sirt1 against hypertrophy.

4.3 Cardiac hypertrophy and inflammation
Accumulating evidence indicates that chronic inflammation plays a prominent role in cardiac disease. Our demonstration that a hypertrophic agonist (PE) and a pro-inflammatory agent (LPS) are able to activate inflammatory and hypertrophic pathways in concert in cardiomyocytes supports this notion. Furthermore, both PE- and LPS-induced increases in MCP-1 mRNA levels were prevented by Sirt1 overexpression/activation in NCMs, indicating an important role for Sirt1 in the inflammatory process. This intricate relationship between inflammation and hypertrophy suggests the existence of common factors that determine these two processes. Heart failure in patients is associated with significant myocardial NF-kB activation,28 and several studies have shown that NF-kB inhibition is able to block or attenuates the hypertrophic response either in vitro29 – 32 or in vivo.33 Accordingly, NF-kB might serve as the common factor linking the inflammatory and hypertrophy pathways.
in the cardiac muscle cell. In support of this, we observed increased NF-κB-responsive promoter activity after PE stimulation, an effect that was mitigated by overexpression of Sirt1. Collectively, these observations point to a pivotal role for Sirt1 in limiting the inflammatory response.

4.4 Mutual interactions among Sirt1, PPARα, PGC-1α, and NF-κB

The above results showed that Sirt1 prevents cardiac hypertrophy, promotes expression of fatty acid oxidation genes, and blocks the NF-κB pro-inflammatory pathway. A critical factor linking these processes is the nuclear receptor PPARα. Inhibition of PPARα blocked the protective action of Sirt1 overexpression in PE-stimulated NCMs, preventing Sirt1-mediated reductions in cell size and expression of cardiac hypertrophy markers. Moreover, the protective effects of Sirt1 overexpression on fatty acid oxidation genes and the pro-inflammatory marker MCP-1 were lost after PPARα inhibition, indicating that PPARα is a common factor that regulates these diverse processes. Recent studies have reported that Sirt1 interacts with and deacetylates NF-κB. Furthermore, it has been previously shown that PPARα inhibits NF-κB by a trans-repression mechanism involving protein–protein interaction. In this study, we demonstrated for the first time that Sirt1 promotes the formation of a complex involving PPARα, NF-κB, and Sirt1 in cardiac cells. Moreover, PE-induced cardiac hypertrophy was associated with enhanced acetylation of the p65 subunit of NF-κB, which was

![Figure 5](image-url)

**Figure 5** Physical association of PPARα with total and acetylated NF-κB p65 subunit, and with total and acetylated PGC-1α, in Sirt-1 over-expressing NCMs. (A) Nuclear extracts from NCMs transduced with AdSirt1 or the AdGFP control vector (10 IFU/cell) were immunoprecipitated using an anti-PPARα antibody coupled with protein A/G-agarose beads and immunoblotted with an anti-Sirt1 antibody. Nuclear extracts from PE-treated or untreated NCMs transduced with AdSirt1 were immunoblotted with anti-p65, anti-acetylated-p65 (B), an anti-PGC-1α and anti-acetylated-Lysine (Ac-K) antibodies (D). Bars are means ± SEM of densitometric data from the immunoblot signal from at least three separate experiments (*P < 0.05 compared with control, #P < 0.05 compared with PE-stimulated cells). Representative examples are shown in (C) and (E), respectively. n.s., non-specific band.
Sirt1, PPARα, and cardiac hypertrophy

Figure 6 RSV prevents cardiac hypertrophy, metabolic dysregulation, and inflammatory response in vivo in wt mice, but not in PPARα−/− mice. wt (white bars) and PPARα−/− mice (black bars) were subjected to ISO injection for 5 days to induce cardiac hypertrophy alone or combined with RSV as indicated. (A) ΔHeart weight/body weight ratio (HW/BW) expressed in milligrams per gram. (B–D) mRNA expression levels of marker genes: hypertrophy markers ANF (B) and α-SKA (C); metabolic marker PDK-4 (D) and inflammatory marker MCP-1 (E). Results are expressed as mean ± SEM (*p < 0.05 compared with corresponding control (CT) mice; #p < 0.05 compared with corresponding ISO-treated mice; $p < 0.05 compared with wt CT mice; n = 5 mice/group).

Prevented by formation of the Sirt1–PPARα–NFκB complex. On the other hand, the observation that Sirt1 promotes the deacetylated status of the PGC-1α associated with PPARα in NCMs provides an explanation for the enhanced transcription of fatty acid oxidation genes. Previous studies in skeletal muscle have shown that Sirt1 activates PGC-1α through deacetylation, and by this means Sirt1 activates fatty acid catabolism.34 In the light of our present findings, it is likely that a similar process takes place in the heart for the action of Sirt1 promoting fatty acid oxidation. Finally, to confirm these in vitro findings, we induced cardiac hypertrophy in vivo in wt and PPARα−/− mice. We observed that all the protective effects of Sirt1 activation by RSV in the heart were lost in the PPARα−/− mice, therefore confirming that Sirt1-protective actions on cardiac hypertrophy involve the PPARα pathway.

In summary, we provide evidence that the positive effects of Sirt1 on cardiac hypertrophy, fatty acid oxidation genes, and the inflammatory pathway appear to involve PPARα, demonstrating that the beneficial effects of Sirt1 are abrogated by inhibition of this nuclear receptor. From a biomedical viewpoint, the possibility of stopping or reversing pathologial cardiac hypertrophy, and thereby slowing the development of heart failure, is a topic of considerable interest. Collectively, the findings reported in this study suggest that the convergence of Sirt1 and PPARα activation deserve further research as therapeutic candidates in the management of cardiac hypertrophy.
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