Resveratrol Prevents the Prohypertrophic Effects of Oxidative Stress on LKB1

Vernon W. Dolinsky, PhD; Anita Y.M. Chan, BSc; Isabelle Robillard Frayne, MSc; Peter E. Light, PhD; Christine Des Rosiers, PhD; Jason R.B. Dyck, PhD

Background—Master regulators of protein synthesis such as mammalian target of rapamycin (mTOR) and p70S6 kinase contribute to left ventricular hypertrophy. These prohypertrophic pathways are modulated by a number of kinase cascades, including the hierarchical LKB1/AMP-activated protein kinase (AMPK) energy-sensing pathway. Because oxidative stress inhibits the LKB1/AMPK signaling axis to promote abnormal cell growth in cancer cells, we investigated whether oxidative stress associated with hypertension also results in the inhibition of this kinase circuit to contribute to left ventricular hypertrophy.

Methods and Results—In the spontaneously hypertensive rat, a well-established genetic model of hypertension and subsequent cardiac hypertrophy, the development of left ventricular hypertrophy is associated with an increase in the electrophilic lipid peroxidation byproduct 4-hydroxy-2-nonenal (HNE). Using isolated cardiomyocytes, we show that elevated levels of HNE result in the formation of HNE-LKB1 adducts that inhibit LKB1 and subsequent AMPK activity. Consistent with inhibition of the LKB1/AMPK signaling pathway, the mTOR/p70S6 kinase system is activated, which is permissive for cardiac myocyte cell growth. Treatment of cardiomyocytes with resveratrol prevents HNE modification of the LKB1/AMPK signaling axis and blunts the prohypertrophic p70S6 kinase response. Furthermore, administration of resveratrol to spontaneously hypertensive rats results in increased AMPK phosphorylation and activity and reduced left ventricular hypertrophy.

Conclusions—Our data identify a molecular mechanism in the cardiomyocyte involving the oxidative stress–derived lipid peroxidation byproduct HNE and the LKB1/AMPK signaling pathway that contributes to the development of left ventricular hypertrophy. We also suggest that resveratrol may be a potential therapy for patients at risk for developing pathological cardiac hypertrophy by preventing this prohypertrophic process. (Circulation. 2009;119:1643-1652.)

Key Words: AMPK ■ hypertrophy ■ molecular biology ■ resveratrol ■ signal transduction

Pathological left ventricular hypertrophy (LVH) is a devastating condition that may lead to several serious cardiac events, including increased susceptibility to ischemia/reperfusion injury, arrhythmias, cardiomyopathies, heart failure, and sudden death (see elsewhere1 for review). Although one of the major contributors to the development of LVH in humans is increased hemodynamic load,1 alterations in signaling pathways also contribute to increased cardiac myocyte growth in conjunction with and in the absence of increased afterload.2,3 Because LVH is characterized by increased myocardial cell size, protein synthesis is a necessary mediator of this process.4 Indeed, a number of hierarchical kinase cascades and effector proteins have been identified as being involved in the regulation of cardiac myocyte protein synthesis (reviewed by Hedhli et al5). One major regulator of protein synthesis and cell growth is the mammalian target of rapamycin (mTOR)/p70S6 kinase (p70S6K) pathway, which can be regulated by several other kinase cascades.5,6

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AMP-activated protein kinase (AMPK) inhibits the mTOR/p70S6K pathway through its ability to regulate and activate tuberous sclerosis complex 2, mTOR, and eukaryotic elongation factor-2 kinase, thereby inhibiting protein synthesis and cell growth.7 Although activation of AMPK has been observed in perfused hypertrophic hearts,8,9 this may result from either ex vivo heart perfusion conditions that do not fully recapitulate the in situ setting or late-stage adaptations to compromised ATP supply, which may not describe the complex role of AMPK in the development of cardiac hypertrophy. Indeed, we have recently demonstrated that pharmacological and genetic activation of AMPK10–12 reduces cardiac myocyte cell growth and inhibits protein synthesis by decreasing p70S6K phosphorylation. Interestingly, tumor suppressors govern cell growth in part via the regulation of protein translation, evoking the concept that a
LKB1/AMPK/mTOR/p70S6K pathway may be one of the mechanisms by which LKB1 functions as a tumor suppressor. Because cancer and pathological hypertrophy both involve abnormal cellular growth, suppression of cardiac LKB1 activity may contribute to the abnormal growth of myocytes observed in pathological LVH.

Oxidative stress induced by reactive oxygen species has an important role in regulating abnormal cell growth. Oxidation of membrane lipids can lead to the production of reactive aldehydes. Previous evidence has demonstrated that the electrophilic aldehyde lipid peroxidation byproduct 4-hydroxy-2-nonenal (HNE) is capable of directly modifying and inhibiting LKB1 activity in the human breast cancer cell line MCF-7. Interestingly, blood and cardiac levels of HNE are enhanced in the spontaneously hypertensive rat (SHR), a well-established genetic model of hypertension in which hypertension and LVH develop gradually in a manner similar to the human condition (see review by Bing et al). Because elevated cardiac levels of HNE may modify the LKB1/AMPK/mTOR/p70S6K signaling cascade, we hypothesized that elevated levels of HNE contribute to hypertrophy by creating a permissive environment for cardiac myocyte cell growth. Although it has previously been shown that reactive oxygen species, specifically 250 to 300 μmol/L hydrogen peroxide, activate AMPK in NIH 3T3 cells, this activation was found to be transient (4- to 5-fold after 5 minutes) while declining rapidly to reach basal values after 1 hour. Hence, it appears possible that the oxidative stress–derived aldehyde HNE may inhibit the LKB1/AMPK pathway by directly modifying this kinase cascade independently of reactive oxygen species. Therefore, the purpose of this study was to investigate whether HNE-LKB1 adduct formation contributed to LVH via inhibition of the LKB1/AMPK/mTOR/p70S6K pathway and to determine whether treatment strategies aimed at preventing this modification could prevent the development of pathological cardiac hypertrophy.

Methods

Animal Care

The University of Alberta Animal Policy and Welfare Committee adheres to the principles for biomedical research involving animals developed by the Council for International Organizations of Medical Sciences. All SHRs and Wistar rats were obtained from Charles River Laboratories (Pointe-Claire, Quebec). In some experiments, 12-week-old SHRs were administered either vehicle (50% ethanol) or 1% penicillin-streptomycin (Invitrogen). Radioisotopes were obtained from Perkin-Elmer (Shelton, Conn).

Materials

All primary antibodies used in this study were purchased from Cell Signaling Technology (Danvers, Mass), with the exception of the anti–HNE-protein adduct (Calbiochem, San Diego, Calif), mouse anti-FLAG M2 (Sigma Chemical Co, St Louis, Mo), mouse anti-LKB1 (Ley37D/G6), rabbit anti-LKB1 (M18), and anti-actin primary antibodies, as well as the secondary antibodies for goat anti-rabbit and donkey anti-goat (Santa Cruz Biotechnology, Santa Cruz, Calif). HNE was obtained from Cayman Chemicals (Ann Arbor, Mich). Most other cell culture reagents and chemicals were purchased from Sigma and Invitrogen (Carlsbad, Calif). Radioisotopes were obtained from Perkin-Elmer (Shelton, Conn).

Cell Culture

Newborn (1- to 3-day-old) rat hearts were isolated and neonatal cardiac myocytes were isolated and cultured as previously described. In some experiments, cells were infected with a combination of LKB1, STRADα, and MO25α adenoviruses, each at a multiplicity of infection of 7. Controls were infected with green fluorescent protein at a multiplicity of infection of 21. Twenty-four hours after infection, cells were harvested as described. Mouse embryonic fibroblasts (MEFs) isolated from wild-type and LKB1-null genotype mice (a gift from Drs R. Depinho and N. Bardeesy, Harvard Medical School, Boston, Mass) were cultured in DMEM containing 10% FBS (Sigma) and 1% penicillin-streptomycin (Invitrogen).

Immunoprecipitations and In Vitro Kinase Activity Assays

AMPK activity was measured with the in vitro AMPK substrate activity assay as described previously. Exogenous FLAG-tagged LKB1 was immunoprecipitated from 100 μg cellular protein with anti-FLAG M2-agarose (Sigma) and assayed for LKB1 activity with the LKB1 substrate as previously described or subjected to SDS-PAGE and immunoblot analysis. For immunoprecipitation of LKB1 from rat heart tissue, 500 μg protein homogenate was incubated at 4°C for 16 hours on a rotating platform with 40 μL protein G-Sepharose covalently conjugated to 5 μg anti-LKB1 (Ley37D/G6) antibody. The immunoprecipitates were washed twice with 0.5 mL LKB1 lysis buffer containing 0.5 mmol/L NaCl and twice with 0.5 mL buffer A (50 mmol/L Tris · HCl, pH 7.4, 0.1 mmol/L EGTA) and subjected to SDS-PAGE and immunoblot analysis.

Analysis of Heart Tissue

SHRs and Wistar rats 7 and 15 weeks of age were euthanized with an intraperitoneal injection of euthanyle (0.5 mL/kg body weight). Heart tissue was homogenized, and the protein concentration was assayed with Bradford protein reagent. Fifteen to 20 μg protein was used for SDS-PAGE and immunoblot analysis.

Quantification of ATP, AMP, and HNE-Protein Adducts in Rat Heart

Frozen rat heart samples (20 mg) were homogenized, and ATP and AMP concentrations were determined by high-performance liquid chromatography as described previously. HNE-protein adducts were quantified from 100 mg frozen rat heart samples with gas chromatography–mass spectrometry as described.

In Vivo Assessment of Cardiac Function and Blood Pressure

Transthoracic echocardiography was performed on mildly anesthetized rats with a Vevo 770 High-Resolution Imaging System equipped with a 30-MHz transducer (RMV-707B; VisualSonics, Toronto, Ontario, Canada), and cardiac function was determined as previously described. Myocardial performance index or Tei index was calculated using the following equation: (isovolumic contraction time + isovolumic relaxation time)/ejection time. Noninvasive blood pressure measurements were made with the CODA 2 tail cuff system (Kent Scientific Corp, Torrington, Conn).

Statistical Analysis

Data are expressed as mean±SEM. Comparisons between rat strains or treatment groups were performed with the unpaired Student t test or the Mann-Whitney U test to compare the differences in scores between groups when appropriate. A value of P<0.05 was considered significant. The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.
Results

Reductions of Cardiac AMPK and LKB1 Activities Are Associated With the Development of Cardiac Hypertrophy

To investigate whether AMPK activity is altered in the SHR heart, phosphorylation of AMPK at its activation site, threonine 172 (P-AMPK), was measured in hearts from SHRs and normotensive Wistar control rats at 7 and 15 weeks of age. At 7 weeks of age, before the development of cardiac hypertrophy in the SHR, AMPK activity was similar in both groups (Figure 1A). Interestingly, at 15 weeks of age, when blood pressure and heart mass are increased in the SHR,20,29,30 AMPK activity was significantly reduced as assessed by P-AMPK levels and an in vitro kinase assay (Figure 1B and 1C). Because AMPK activity is often regulated by cellular energy status, we examined whether the AMP/ATP ratio was different between SHRs and Wistar rats at 15 weeks of age. Our data show that the ratio of AMP to ATP was not significantly different in the SHR hearts compared with hearts from age-matched Wistar rats (Figure 1D), suggesting that energy status does not explain the lower cardiac AMPK activity observed in the SHRs.

Because LKB1 is largely responsible for the phosphorylation and activation of AMPK in the heart,11,31 reduced LKB1 activity was investigated as an alternative mechanism explaining the inhibition of AMPK activity in the SHR hearts. As a surrogate marker of LKB1 activity, we performed immunoblot analysis using P-AMPK and phosphorylated LKB1 (P-LKB1). Because recent reports suggest that both the level of AMPK phosphorylation and LKB1 activity are correlated with the level of phosphorylation at its serine 428 (S428) site,32,33 we measured total and P-LKB1 (S428) levels in addition to P-AMPK in the 15-week-old rat hearts. Consistent with our hypothesis, a significantly reduced P-LKB1 (Figure 1E) correlates with reduced P-AMPK (Figure 1B) levels in the SHR hearts compared with the Wistar control rat hearts. Together, these observations suggest that reduced LKB1 activity could promote LVH via reduced AMPK activity.

Figure 1. Analysis of cardiac AMPK expression in 7- and 15-week-old SHRs and Wistar rats. Immunoblot analysis was performed on homogenates from ventricles isolated from 7-week-old (A) and 15-week-old (B and E) SHRs and Wistar rats. Levels of P-AMPK were quantified by densitometry and normalized against total AMPK. AMPK activity (C) and ratio of AMP to ATP levels (D) in ventricles isolated from 15-week-old SHRs and Wistar rats are shown. Levels of P-LKB1 were quantified by densitometry and normalized against total LKB1 (E). Values are the mean±SEM of 5 rat hearts in each group. *Significant difference (P<0.05) between SHRs and Wistar rats.

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activity in the 15-week-old SHR hearts. In accordance with this, the master regulators of protein synthesis, mTOR and p70S6K, exhibited higher activities as assessed by their phosphorylation status (P-mTOR serine 2448 and P-p70S6K threonine 421/serine 424, respectively) in the 15-week-old SHR hearts compared with hearts from age-matched Wistar rats (Figure 2A and 2B). Interestingly, although phosphorylation of mTOR at serine 2448 is mediated by Akt, hearts from 15-week-old SHRs did not have increased Akt activity (Figure 2C). However, because commercial antibodies are not available to directly assess the phosphorylation of mTOR at the site of AMPK action (threonine 2446) and because of the competitive inhibition of phosphorylation at these 2 sites, increased phosphorylation at serine 2448 in the absence of elevated Akt activity serves as a surrogate marker for decreased AMPK phosphorylation of mTOR.

LKB1-AMPK Signaling Is Inhibited by HNE in Rat Cardiac Myocytes

Because previous studies have shown a strong correlation between elevated levels of HNE and LVH in the SHR, we examined the direct effects of HNE on LKB1 and AMPK activities in isolated neonatal rat cardiac myocytes. Cardiac myocytes expressing the epitope-tagged proteins comprising the active AMPK kinase complex (LKB1/MO25α/STRADα) were treated with either vehicle or 40 μmol/L HNE. This concentration of HNE is similar to that of HNE-protein adducts found in whole blood of SHRs and almost identical to the concentration used by Wagner et al, who initially showed that HNE modifies LKB1 in cancer cells. Treatment with HNE resulted in significantly reduced levels of P-LKB1 (Figure 3A and 3B) and a 3-fold decrease in total LKB1 levels (Figure 3A and 3C). In agreement with a significant reduction in the ratio of P-LKB1 to LKB1 in HNE-treated cardiac myocytes (Figure 3A and 3D) as well as reduced LKB1 protein levels, there was also a significant 5-fold decrease in LKB1 activity (Figure 3E). Consistent with an inactivation of LKB1 and in agreement with our findings in hearts from SHRs, treatment of cardiac myocytes with HNE also significantly reduced P-AMPK levels (Figure 4A) and resulted in a 3-fold inhibition of AMPK activity (Figure 4B). The ability of HNE to reduce the activation of the LKB1/AMPK signaling pathway also was evident in freshly isolated adult rat cardiac myocytes (data not shown). In addition, p70S6K phosphorylation was increased 4-fold by HNE treatment of cardiac myocytes (Figure 4C), demonstrating that the treatment of cardiac myocytes with HNE induced changes in the signaling pathways that control protein synthesis similar to those observed in the hypertrophic SHR heart.

Covalent Modification of Cardiac Myocyte LKB1 by HNE

To determine whether the ability of HNE to inhibit AMPK activity was mediated via LKB1, wild-type and LKB1-null MEFs were treated with vehicle or HNE (40 μmol/L). HNE treatment dramatically reduced levels of P-AMPK in wild-type MEFs yet had much less of an effect in LKB1-null MEFs (Figure 5A), suggesting that LKB1 may be a direct target of HNE. The fact that the effect of HNE on P-AMPK was not completely prevented in the LKB1-null MEFs also suggests that HNE may act on other AMPK kinases such as Ca2+/calmodulin-dependent protein kinase kinase. As we have recently reported that resveratrol can activate AMPK in the cardiac myocyte, we also speculated that resveratol may prevent the modification of LKB1 by HNE. To address this, homogenates from cells treated with HNE in the presence or absence of resveratol (100 μmol/L) were subsequently immunoblotted with an antibody that recognizes HNE-protein adduct formation. Because LKB1 protein levels are lower in HNE-treated cardiac myocytes than controls, we loaded relative amounts of LKB1 from all treatment groups to allow direct comparisons. Consistent with HNE acting directly on LKB1, HNE-LKB1 adduct formation was increased in car-
myocytes treated with HNE but prevented when myocytes were cotreated with resveratrol (Figure 5B). Simultaneous treatment of cardiac myocytes with resveratrol and HNE also prevented the HNE-induced inhibition of P-LKB1 (Figure 5C) and P-AMPK (Figure 5D).

To investigate the mechanism by which resveratrol may regulate LKB1 activity, LKB1 was immunoprecipitated from cardiac myocytes after treatment with HNE (Figure 5E). Although HNE treatment reduced the amount of AMPK that coimmunoprecipitated with LKB1 (consistent with the reduction of LKB1 levels), resveratrol restored the ability of LKB1 to interact with AMPK (Figure 5E). Because the HNE-induced decrease in P-LKB1 and P-AMPK is prevented with resveratrol (Figure 5C and 5D), activation of p70S6K by HNE also is prevented (Figure 5F), suggesting that resveratrol may inhibit the prohypertrophic signaling pathways by preventing the HNE-mediated reduction of LKB1 levels (Figure 5C) and its activity (Figure 3E). Of importance, when HNE was analyzed from media coincubated with HNE and resveratrol using gas chromatography–mass spectrometry, we found no evidence for chemical modifications of HNE (data not shown), suggesting that the effects of resveratrol on the

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**Figure 3.** HNE inhibits LKB1 protein levels and activity in rat cardiac myocytes. Immunoblot analysis was performed on homogenates from cultured neonatal rat cardiac myocytes expressing the epitope-tagged proteins comprising the active AMPK kinase complex (LKB1/MO25α/STRADα) and incubated for 1 hour with vehicle (control) or 40 μmol/L HNE. Levels of P-LKB1 (A and B) and total LKB1 (A and C) were quantified by densitometry and normalized against actin as a control for protein loading. Levels of P-LKB1 also were normalized to the levels of total LKB1 protein (D). Immunoprecipitated LKB1 activity in myocytes treated with vehicle or HNE (E). Values are the mean±SEM of 4 to 8 separate neonatal rat myocyte preparations in each group. *Significant difference (P<0.05) between control and HNE-treated groups.

**Figure 4.** HNE inhibits AMPK activity and increases p70S6K activity in rat cardiac myocytes. Immunoblot analysis was performed on homogenates from cultured neonatal rat cardiac myocytes incubated for 1 hour with vehicle (control) or 40 μmol/L HNE. Levels of P-AMPK were quantified by densitometry and normalized against total AMPK as a control for protein loading (A). AMPK activity in myocytes treated with vehicle or HNE (B). Levels of phosphorylated p70S6K (P-p70S6K) (P-p70S6K) were quantified by densitometry and normalized against actin (C). Values are the mean±SEM of 6 to 8 separate neonatal rat myocyte preparations in each group. *Significant difference (P<0.05) between control and HNE-treated groups.
actions of HNE are mediated intracellularly. Consistent with this, cardiac myocytes pretreated with resveratrol (6 hours), washed with resveratrol-free media, and then treated with HNE also show almost identical effects on P-LKB1 (Figure 5G) and P-AMPK (Figure 5H) levels as when HNE was coincubated with resveratrol, further supporting an intracellular action of resveratrol.

Molecular Changes Associated With Resveratrol Treatment of SHRs

To determine whether the beneficial effects of resveratrol observed in culture could be mimicked in vivo, 12-week-old SHRs with established hypertension were treated with either vehicle or 2.5 mg/kg body weight resveratrol daily for 2 weeks. Treatment of SHRs with resveratrol significantly reduced cardiac levels of HNE-protein adducts by 30% (Figure 6A), lessened the amount of HNE-LKB1 adduct (Figure 6B), and significantly increased P-LKB1 levels (Figure 6C). Consistent with elevated levels of P-LKB1 and likely LKB1 activity, P-AMPK also was dramatically increased in

Figure 5. HNE inhibits the LKB1/AMPK signaling axis via formation of LKB1-HNE adducts. Wild-type (WT) and LKB1-null MEFs were treated for 1 hour with vehicle (control) or 40 μmol/L HNE, and levels of P-AMPK and total AMPK were measured (A). Immunoblot analysis was performed on FLAG-LKB1 immunoprecipitates from homogenates of cultured neonatal rat cardiac myocytes expressing the epitope-tagged proteins comprising the active AMPK kinase complex (LKB1/MO25α/STRADβ) and incubated for 1 hour with vehicle (−) or HNE (+) in the absence (control) or presence of 100 μmol/L resveratrol (Resv). Equal amounts of LKB1 protein were loaded onto the SDS-PAGE, and HNE adducts attached to the immunoprecipitated LKB1 were detected with the anti-HNE adduct antibody (B). Immunoblot analysis was performed on homogenates of cells treated as above, and the levels of P-LKB1 and total LKB1 (G), as well as P-AMPK and total AMPK (H), are shown.
Discussion

Although the primary determinant of hypertrophy in the SHR is likely increased hemodynamic load on the LV wall, the molecular development of LVH is multifactorial in nature and likely also involves load-independent mediators. Indeed, increased levels of reactive oxygen species are associated with chronically elevated blood pressure in humans, which may also contribute to the stimulation of cardiac growth. Because reactive oxygen species promote the production of specific reactive aldehydes, it is possible that products such as HNE may play an integral role in the promotion of LVH in certain pathological conditions. Consistent with this, oxidative stress and lipid peroxidation products such as HNE are elevated in the failing myocardium of human patients and may contribute to hypertrophic growth in cardiac myocytes. In addition, levels of HNE are elevated in hearts of aortic-banded rats, indicating that the involvement of HNE in the regulation of LVH may not be restricted to only the SHR model but could also play an important role in other models of pressure overload–induced LVH.

Although the SHR is an established model of genetic hypertension and LVH, the molecular mechanisms underlying the development of cardiac hypertrophy in this animal are only beginning to be characterized. It is well established that between 7 and 22 weeks of age, considerable change occurs in hearts of the SHR as the rat transitions from normal blood pressure to established hypertension. In response to pressure overload, active hypertrophic growth occurs between 12 and 20 weeks that is accompanied by elevated protein synthesis and cardiac mass. During this same time frame, elevated levels of oxidative stress and increased myocardial HNE-protein adducts also have been identified. Consistent with this, we provide evidence that HNE forms covalent adducts with LKB1, resulting in the inhibition of this kinase.

We also show that an important consequence of decreased LKB1 activity is the subsequent inactivation of AMPK and, using LKB1-null MEFs, demonstrate that the effect of HNE on AMPK is mediated via LKB1. Because we and others have established that AMPK activation prevents cardiac myocyte hypertrophy, it is possible that the converse is true. Indeed, we show that a major effect of HNE is the reduction in AMPK activity and subsequent changes in the mTOR/p70S6K signaling pathway that regulate protein synthesis in the heart. As such, our data provide information that may help explain one of the mechanisms that link elevated HNE-protein adduct levels in the SHR heart with the increased myocardial protein synthesis that also has been reported in this model. Despite our rationale, however, we do not present data to support this concept, and it is still unproven as to whether reduced LKB1/AMPK signaling contributes directly to accelerated protein synthesis and cardiac myocyte growth in vivo.

Although LKB1 has generally been thought to be constitutively active, LKB1 expression and/or activity have been shown to be reduced in the hypertrophied hearts of various hearts from SHRs. Because resveratrol was able to prevent the inhibition of the LKB1/AMPK signaling axis normally observed in hearts from SHRs (Figure 6E). In addition, in agreement with what was previously observed after long-term treatment of cardiac myocytes with resveratrol, phosphorylated Akt was not altered by treatment of cardiac myocytes (Figure 6E). It is well established that HNE forms covalent adducts with LKB1, resulting in the inhibition of this kinase.

Table. Physical and Cardiac Parameters of SHRs Treated With Vehicle or Resveratrol

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n=5)</th>
<th>Resveratrol (n=5)</th>
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</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>288.75±6.7</td>
<td>279.83±4.5</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.39±0.06</td>
<td>1.21±0.04*</td>
</tr>
<tr>
<td>Tibia length, mm</td>
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<td>39.4±0.1</td>
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<tr>
<td>Heart weight/tibia length×100</td>
<td>3.51±0.13</td>
<td>3.07±0.07*</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>4.30±0.16</td>
<td>4.29±0.14</td>
</tr>
<tr>
<td>LVWd, mm</td>
<td>7.20±0.18</td>
<td>6.95±0.14</td>
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<tr>
<td>LVPW, mm</td>
<td>2.91±0.04</td>
<td>2.67±0.04*</td>
</tr>
<tr>
<td>IVS, mm</td>
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<td>2.68±0.04*</td>
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<tr>
<td>Ejection fraction, %</td>
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<tr>
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<td>23.52±0.75</td>
</tr>
<tr>
<td>Tei index</td>
<td>0.70±0.01</td>
<td>0.65±0.01*</td>
</tr>
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LVIDd indicates systolic LV internal diameter; LVIDd, diastolic LV internal diameter; LVWd, LV posterior wall thickness; IVS, intraventricular septal wall thickness; and IVRT, isovolumic relaxation time. Values are mean±SEM. *Significant difference (P<0.05) between vehicle-treated SHRs (control) and resveratrol-treated SHRs.
transgenic mouse models. As such, inhibition of LKB1 activity may be an important contributor to the hypertrophic process and may be a more universal mechanism underlying the development of LVH than previously thought. Indeed, when SHRs with established hypertension were administered resveratrol, inhibition of LKB1/AMPK signaling was prevented, and septal and posterior wall thickness and overall cardiac mass in SHRs were all significantly reduced. These findings are consistent with previous reports showing that resveratrol reduces LV mass in various rodent models of pressure overload–induced hypertrophy. Importantly, resveratrol-treated rats remained hypertensive, demonstrating that factors other than changes in hemodynamic load were involved in the beneficial effects of resveratrol. We propose that this mechanism involves the reduction of HNE adduct formation on LKB1. Indeed, administration of resveratrol to SHRs significantly decreased the levels of cardiac HNE-protein adducts, reduced HNE-LKB1 adduct formation, and significantly enhanced the LKB1/AMPK signaling pathway, thereby preventing dysregulation of the molecular cascades that control protein synthesis.

Study Limitations

Although we provide evidence that resveratrol mediates the LKB1/AMPK signaling axis in cultured cardiac myocytes and in hearts from SHRs in vivo, some limitations to our study need to be addressed. For example, the ability of exogenous HNE to inhibit LKB1 activity in cardiac myocytes appears to be due to both decreased phosphorylation of LKB1 and reduced expression of LKB1 protein, the latter of which is not recapitulated in SHRs. Although these divergent results may simply be due to the intrinsic differences in the 2 experimental model systems (ie, cultured cells versus whole heart), we cannot provide data explaining why LKB1 protein levels are not decreased in hearts from SHRs. Despite this, the fact that HNE-protein adducts and HNE-LKB1 adducts are reduced in whole-heart tissue from SHRs treated with resveratrol demonstrates that the LKB1/AMPK signaling axis can be restored by resveratrol in vivo independently of changes in LKB1 protein expression. Another limitation to this study is that we do not provide data showing what percent of total LKB1 is modified by HNE adduct formation and how this directly relates to AMPK phosphorylation. Although the cumulative data presented in this study support the notion that the proportion of HNE-modified LKB1 is of sufficient quantity to produce a physiological effect (given the reduced phosphorylation of AMPK in hearts that have HNE-LKB1 adducts present), we cannot rule out alternative responses such as changes in protein phosphatase expression, altered energetic status (ie, a trend to lower ratio of AMP to ATP) or additional factors that may contribute to impaired AMPK phosphorylation in hearts from SHRs. Furthermore, although we conclude that the restoration of this signaling pathway by resveratrol prevents hypertrophic growth in the SHR, we cannot be certain that resveratrol is not also acting via additional pathways. Indeed, given the multiple kinase pathways that resveratrol appears to mediate, it is possible that other signaling cascades, in addition to those we describe here, are involved in mediating the antihypertrophic effects of resveratrol. Moreover, because resveratrol has effects other than regulating kinase signaling processes such as changing the oxidative status of the cell, these cellular changes may also be involved in mediating the antihypertrophic effects of resveratrol. In fact, potential explanations for the reduced HNE-protein adducts in hearts from resveratrol-treated SHRs include decreased lipid peroxidation or improved enzymatic removal by aldehyde dehydrogenase, alcohol dehydrogenase, and glutathione-s-transferase. All of these additional effects of resveratrol warrant further investigation in our model.

Conclusions

We provide evidence that HNE-mediated inhibition of the LKB1/AMPK signaling axis in a model of hypertension allows the activation of mTOR and p70S6K, thereby assisting in the hypertrophic process. The identification of this previously unidentified molecular mechanism provides considerable insight into the pathogenesis of LVH and suggests that pharmacological targeting of this pathway may be a potential therapy for pathological cardiac hypertrophy. The finding that resveratrol can prevent the inhibition of the LKB1/AMPK energy-sensing pathway and subsequently influence master regulators of protein synthesis to prevent LVH is of significant clinical importance. In addition, our results suggest that resveratrol or analogues of resveratrol could be a potential adjunct therapy for hypertensive patients at risk for developing pathological cardiac hypertrophy.

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Disclosures

None.

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6. Moschella PC, Rao VU, McDermott PJ, Kuppuswamy D. Regulation of mTOR and S6k1 activation by the nPKC isoforms, PKCepsilon and...


35. Deleted in proof.


39. Deleted in proof.


CLINICAL PERSPECTIVE
Pathological left ventricular hypertrophy (LVH) positively correlates with coronary artery disease and all-cause mortality. Although LVH is a compensatory mechanism for increased afterload in the setting of hypertension and aortic valve stenosis, LVH also involves the inhibition of cardiomyocyte antihypertrophic molecular signals. One of these antihypertrophic signals involves the hierarchical LKB1/AMPK energy-sensing pathway, which is able to inhibit the protein synthesis that is necessary for the development of LVH. Here, we provide evidence that inhibition of the LKB1/AMPK signaling axis is associated with LVH in a well-established rat model of hypertension and cardiac hypertrophy. We also show that this inhibition is due to a direct modification of LKB1 by the electrophilic lipid peroxidation byproduct 4-hydroxy-2-nonenal (HNE). Because HNE levels are elevated during hypertension, our study identifies a novel molecular mechanism that may contribute to the pathogenesis of LVH. Furthermore, we provide evidence that pharmacological targeting of this pathway may be a potential therapy for pathological LVH. Indeed, we show that administration of resveratrol to hypertensive rats reduces cardiac HNE levels, prevents the inhibition of the LKB1/AMPK signaling pathway, and subsequently inhibits signals controlling protein synthesis. As a result, cardiac hypertrophy is lessened in these hypertensive rats even in the absence of any decreases in blood pressure or LV performance. Overall, our results suggest that resveratrol, analogues of resveratrol, or possibly any agent that targets the LKB1/AMPK signaling axis could be a potential adjunct therapy to improve LV remodeling in hypertensive patients or in patients with established LVH.
Resveratrol Prevents the Prohypertrophic Effects of Oxidative Stress on LKB1
Vernon W. Dolinsky, Anita Y.M. Chan, Isabelle Robillard Frayne, Peter E. Light, Christine Des Rosiers and Jason R.B. Dyck

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