Calorie Restriction Prevents Hypertension and Cardiac Hypertrophy in the Spontaneously Hypertensive Rat

Vernon W. Dolinsky, Jude S. Morton, Tatsuiro Oka, Isabelle Robillard-Frayne, Mariel Bagdan, Gary D. Lopaschuk, Christine Des Rosiers, Kenneth Walsh, Sandra T. Davidge, Jason R.B. Dyck

Abstract—Because recent evidence demonstrated that calorie restriction (CR) has numerous beneficial cardiovascular effects, we investigated whether short-term CR could reduce hypertension and prevent cardiac hypertrophy inherent to the nonobese spontaneously hypertensive rat (SHR). After 5 weeks of either ad libitum feeding or short-term CR, SHRs subjected to short-term CR had lower systolic blood pressure (BP) and reduced left ventricular wall thickness as assessed by noninvasive tail-cuff BP measurements and echocardiography, respectively. In addition, ultrasound measurements of the femoral artery revealed that flow-mediated vasodilation was significantly improved in SHRs with CR compared to controls. Moreover, pressure myography of isolated mesenteric arteries and subsequent histological and biochemical analysis of these arteries demonstrated that short-term CR improved vascular compliance, increased endothelial nitric oxide synthase (eNOS) activity and nitric oxide bioavailability, and reduced vascular remodeling compared to ad libitum-fed SHRs. Although these effects are likely multifactorial, they were associated with elevated levels of the circulating adipokine, adiponectin, and enhanced AMP-activated protein kinase (AMPK) activity. To provide evidence that elevated adiponectin levels in the SHR is sufficient to prevent an increase in BP, adenoviral-mediated overexpression of adiponectin increased circulating levels of adiponectin, reduced BP, and activated the AMPK/eNOS pathway in the absence of CR. Overall, our findings provide compelling evidence that short-term CR exerts beneficial effects in the SHR via stimulation of an adiponectin/AMPK/eNOS signaling axis. As a result, CR may serve as an effective nonpharmacological treatment of hypertension, and targeting the adiponectin/AMPK/eNOS pathway may improve treatment of hypertension. (Hypertension. 2010;56:412-421.)

Key Words: adiponectin ■ calorie restriction ■ hypertension ■ hypertrophy ■ signal transduction

Despite the improvement in the treatment and management of systemic hypertension, a disturbingly high number of patients continue to have hypertension. Contributing to this may be the fact that 20% to 30% of hypertensive patients are resistant to blood pressure (BP) reduction with the maximum tolerated dose of at least 3 antihypertensive drugs. Because hypertension is associated with end-organ damage, arteriosclerosis, cardiac hypertrophy, and stroke, it is imperative that we achieve a better understanding of what causes hypertension and develop more effective strategies for its treatment. In preclinical testing, one nonpharmacological approach that was shown to be beneficial in a variety of cardiovascular (CV) diseases is long-term calorie restriction (CR). Although long-term CR and intermittent fasting have been shown to prevent increases in BP in nonobese hypertensive rats, little is known about the mechanisms responsible for these observations or whether shorter durations of CR have similar effects.

Typical CR protocols involve restricting calories by 40% of ad libitum-fed controls for at least 12 months. These long-term CR protocols have been shown to reduce risk factors for CV disease. Interestingly, recent evidence also showed the cardioprotective effects of short-term CR, which involves similar reductions in calories as long-term CR, but for only 4 to 5 weeks. A component of the beneficial CV effects of short-term CR are mediated via the adiponectin/AMP-activated protein kinase (AMPK) signaling axis. Adiponectin is a peptide hormone that is primarily secreted by adipose tissue and activates AMPK independently of metabolic stresses that deplete ATP. Additionally, AMPK phosphorylates and activates endothelial nitric oxide (NO) synthase (eNOS). Because NO synthesis promotes arterial vasodilation, short-term CR likely exerts its beneficial effects on the CV system via an adiponectin/AMPK/eNOS pathway. However, these preceding studies involved short-term CR of normotensive animals, and it is not known if short-term CR can improve vascular function in an animal model of hypertension.

Based on this rationale, the aim of the present study was to analyze the effects of short-term CR on the CV system of a...
well-characterized nonobese genetic model of hypertension and left ventricular hypertrophy (LVH), ie the spontaneously hypertensive rat (SHR). We hypothesized that short-term CR would prevent the typical increase in BP observed in the SHR by improving vascular function and, as a result, prevent the development of pathological LVH. Because we have recently shown that AMPK activity is impaired in the SHR heart,\textsuperscript{12} and because others observed that SHRs have low circulating adiponectin concentrations,\textsuperscript{13,14} we hypothesized that short-term CR would increase plasma adiponectin and restore AMPK/eNOS signaling in the vasculature of the SHR. Furthermore, we hypothesized that supplementation of adiponectin with gene therapy, in the absence of alterations in body weight, could prevent the elevation of BP in the SHR.

Materials and Methods

Animal Care and Diets

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 male SHRs and Wistar rats were obtained from Charles River Laboratories Canada. Ten-week old SHRs were housed in individual cages and fed the AIN93G standard chow diet (Research Diets) ad libitum for 5 weeks (control SHR). SHRs had CR (CR-SHR) according to established protocols,\textsuperscript{8,9} whereby animals received 90% of the average caloric intake of the control SHRs for 2 weeks, followed by 60% of the caloric intake of ad libitum-fed rats for the final 3 weeks of the experiment. CR diets were enriched in vitamins, minerals, and salts such that restricted animals were not nutrient-deficient or salt-deficient compared to the control animals. Analysis of tissues from 15-week-old SHRs or Wistar rats is described in the online Supplement (available online at http://hyper.ahajournals.org).

Materials

Antibodies utilized in this study were purchased from either Cell Signaling Technology or Santa Cruz Biotechnology. Most other cell culture reagents and chemicals were purchased from Sigma or Invitrogen. Production and injection of recombinant adenoviruses is described in the online Supplement.

In Vivo Assessment of Cardiac Function

Transthoracic echocardiography was performed on mildly anesthetized rats at 9 and 15 weeks of age using a Vevo 770 high-resolution imaging system equipped with a 30-MHz transducer. Detailed methodology can be found in the online Supplement.

BP Recordings

Noninvasive BP measurements were made using a tail-cuff system (IITC Life Science) previously described and were validated vs telemetry.\textsuperscript{15} After 3 days of training, each rat was assessed a minimum of 4 times per session.

Pressure Myography

Second-order mesenteric arteries were dissected from 15-week-old SHRs and mounted on a pressure myograph system. Vessels were exposed to a 10-minute increase in pressure to 80 mm Hg before being returned to 60 mm Hg. In another set of experiments, mesenteric arteries were constricted with phenylephrine in the presence or absence of NOS inhibitor (L-NAME), followed by a concentration–response curve to methacholine to assess endothelium-dependent relaxation. Passive characteristics of vessels were determined by mea-

### Table. Structural and Functional Parameters of SHR Hearts From Control and CR Rats Determined by In Vivo Echocardiography

<table>
<thead>
<tr>
<th>Cardiac Parameters</th>
<th>9 Weeks Pretreatment</th>
<th>15 Weeks Control</th>
<th>15 Weeks CR-SHR</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV mass, mg</td>
<td>332.6±13.9</td>
<td>827.3±18.3*</td>
<td>650.2±22.7†‡</td>
</tr>
<tr>
<td>Heart weight/body weight</td>
<td>ND</td>
<td>5.02±0.21</td>
<td>4.14±0.15‡</td>
</tr>
<tr>
<td>LV volume d, mL</td>
<td>177.1±11.2</td>
<td>252.9±12.2*</td>
<td>246.3±10.0†</td>
</tr>
<tr>
<td>LVIDd, mm</td>
<td>5.94±0.16</td>
<td>6.95±0.15*</td>
<td>6.84±0.12†</td>
</tr>
<tr>
<td>LVPWd, mm</td>
<td>1.25±0.01</td>
<td>2.01±0.05*</td>
<td>1.70±0.03†‡</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>68.2±0.8</td>
<td>70.8±3.7</td>
<td>68.8±1.3</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>335±17</td>
<td>376±8*</td>
<td>333±9†</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>22.6±0.5</td>
<td>27.2±0.7*</td>
<td>24.9±0.5</td>
</tr>
<tr>
<td>Myocardial performance index</td>
<td>0.50±0.01</td>
<td>0.74±0.03*</td>
<td>0.65±0.02‡‡</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *Significant difference (P<0.05) between 9-week-old SHR (n = 12) and 15-week-old control SHR (n = 10). †Significant difference between 9-week-old SHR and 15-week-old CR-SHR (n = 10). ‡Significant difference between 15-week-old control SHR and CR-SHR using 1-way analysis of variance with Bonferroni multiple comparisons test.

Flow-Mediated Vasodilation After Temporary Hind Limb Ischemia

Femoral arteries of 15-week-old male SHRs were visualized using ultrasound. Arterial diameter and Doppler flow measurements were obtained from longitudinal sections of the femoral arteries before and after 5 minutes of hind limb ischemia. Detailed methodology can be found in the online Supplement.

Statistical Analysis

Data are expressed as mean±SEM. Comparisons between treatment groups used the unpaired Student 2-tailed t test, or analysis of variance with a Bonferroni multiple comparisons test. P<0.05 was considered significant.

Results

Short-Term CR Prevents Hypertension in the SHR

Because recent work has established the CV benefits of short-term CR in healthy rodents,\textsuperscript{8,9} we investigated whether short-term CR is also beneficial in the nonobese SHR model of hypertension and cardiac hypertrophy.\textsuperscript{16,17} SHRs between 10 and 15 weeks of age utilized in this study had mild hypertension that advanced to pronounced hypertension that is accompanied by a compensatory increase in concentric LVH.\textsuperscript{16,17} To mimic the clinical treatment of hypertensive patients, we initiated CR in 10-week-old SHRs, which is an age when SHRs have mild hypertension but before development of increased left ventricular wall thickness (Table).
CR-SHRs had mild hypertension, 5 weeks of short-term CR improved compared to control SHRs (Figure 1A). No differences in body weight existed between SHRs randomized into ad libitum-fed (control SHR) or short-term (5 weeks) CR groups. The CR diet was formulated to ensure that CR-SHRs received the equivalent amount of salt, minerals, and vitamins as the control SHRs. As expected, 5 weeks of short-term CR reduced BP and increased vascular compliance, trichrome-stained mesenteric arteries (Figure 3D) from CR-SHRs had less perivascular collagen deposition (stained blue), a larger lumen diameter (Figure 3E), and smaller walls (stained red), as judged by the lower wall-to-lumen ratio (Figure 3F). Because inward remodeling of the artery wall is a typical feature of hypertension, the wall-to-lumen ratio of the CR-SHR is similar to that of arteries from normotensive animals. These observations suggest that CR improved the elasticity of the arteries from SHRs.

**Short-Term CR Improved Vascular Function in the SHR**

Because the arteries of SHRs are stiffer than those of normotensive controls and likely contribute to elevated BP, we investigated whether vascular function was improved by CR in vivo by monitoring femoral artery vasodilation and flow velocity using Doppler flow ultrasound. Although hyperemic vasodilation was unaffected by temporary ischemia (Figure 2C), femoral artery blood flow velocity after temporary ischemia was significantly higher in CR-SHRs compared with control SHRs (Figure 2D). To assess whether CR-induced vascular adaptations were also evident in the resistance arteries of SHRs, which could contribute to improved arterial flow in vivo, we studied mesenteric arteries from control and CR-SHRs. Five weeks of CR did not affect the sensitivity of mesenteric arteries to vasoconstriction by phenylephrine (Figure 3A). However, L-NAME increased the sensitivity of mesenteric arteries from CR-SHRs to phenylephrine-induced vasoconstriction compared to controls (Figure 3A), suggesting that the arteries of CR-SHRs may have an increased basal production of NO. To assess the vasodilation of mesenteric arteries, vessels were treated with methylcholine. Mesenteric arteries from CR-SHRs showed similar levels of relaxation as in controls when treated with increasing doses of methylcholine (Figure 3B). The methylcholine-induced relaxation of mesenteric arteries was inhibited by L-NAME to a similar level in control and CR-SHRs. Next, we measured the elasticity of mesenteric arteries in response to increasing pressure (passive curve). In response to increased pressure, the vascular compliance of mesenteric arteries from CR-SHRs was improved compared to control SHRs (Figure 3C). Consistent with lower BP and improved vascular compliance, trichrome-stained mesenteric arteries (Figure 3D) from CR-SHRs had less perivascular collagen deposition (stained blue), a larger lumen diameter (Figure 3E), and smaller walls (stained red), as judged by the lower wall-to-lumen ratio (Figure 3F). Because inward remodeling of the artery wall is a typical feature of hypertension, the wall-to-lumen ratio of the CR-SHR is similar to that of arteries from normotensive animals. These observations suggest that CR improved the elasticity of the arteries from SHRs.

**Short-Term CR Increased Arterial eNOS and AMPK Activities in the SHR**

We observed that short-term CR reduced BP and increased vascular compliance in the SHR; therefore, we hypothesized that endogenous NO production by the vasculature was higher in CR-SHR. To investigate this, we measured phosphorylation levels of eNOS and vasodilator-stimulated phosphoprotein at serine 1177 and serine 239, respectively, and used these as surrogate markers of increased NO production. Increased phosphorylated eNOS at serine 1177 (Figure 4A) and phosphorylated vasodilator-stimulated phosphoprotein at serine 239 (Figure 4B) were observed in the mesenteric arteries isolated from CR-SHRs compared with controls, reflecting increased NO bioavailability in the CR-SHRs. Because AMPK phosphorylation of eNOS at serine 1177 correlates with increased NO production, we investigated whether AMPK activity was altered in the SHR vasculature by CR. As predicted, phosphorylation of AMPK at threonine 172, was increased in the CR-SHR mesenteric arteries compared to those of control SHRs (Figure 4C). This result is consistent with the concept that AMPK activation by CR increases vascular NO production. AMPK is also an upstream regulator of p70S6 kinase (p70S6K), which regulates protein synthesis and cellular hypertrophy. Because smooth muscle cell...
Hypertrophy in the arterial wall is a hallmark of hypertension and vascular disease that also involves elevated p70S6K activity. We investigated the effects of CR on the activity of p70S6K, as determined by its phosphorylation at threonine-421/serine-424. Consistent with our previous work, stimulation of AMPK by CR lowered phosphorylated threonine-421/serine-424 of p70S6K in the mesenteric arteries of CR-SHRs compared to controls (Figure 4D). The CR-mediated decrease in p70S6K activity was consistent with a reduced hypertrophy of vascular smooth muscle cells and reduced arterial wall thickness in CR-SHR arteries (Figure 3D–F).

Short-Term CR Prevented LVH in the SHR
Because CR induced a significant reduction in BP via improved vascular function, we assessed whether CR also prevented the development of LVH associated with the SHR model. Short-term CR produced a substantial reduction in total cardiac mass as determined by gross morphometric assessment (Figure 5A) and reduced heart weight/tibia length ratio (Figure 5B) compared to control SHRs. In addition, echocardiographic assessment revealed that short-term CR prevented thickening of the left ventricular posterior wall (Figure 5C–E) and intraventricular septum (Figure 5F) and decreased heart rate (Table), but it did not alter chamber size.
CR Increased Cardiac AMPK Activity in Accordance With Reduction of Total Cardiac Mass

To investigate whether AMPK activity was also altered in the SHR heart, phosphorylation of AMPK at threonine 172 was measured in hearts from SHRs at 15 weeks of age, when SHRs have LVH compared to age-matched Wistar rats. Although phosphorylated AMPK is significantly reduced in SHR hearts compared to hearts from Wistar rats, 5 weeks of CR increased cardiac phosphorylated AMPK in the SHR (Figure 6A) to a level that was observed in the age-matched Wistar controls (data not shown). Furthermore, in SHRs, short-term CR also increased the phosphorylation of acetyl-CoA carboxylase at serine 79, which is a downstream target of AMPK and an in vivo marker of AMPK activation (Figure 6B). In agreement with our concept that increased AMPK activity reduces cardiac mass,20 the activity of the p70S6K, as determined by its phosphorylation (phosphorylation at threonine-421/serine-424), was lower in short-term CR-SHR hearts compared to that of controls (Figure 6D). This result suggested that short-term CR reduced protein synthesis in the myocardium of the SHR. The activity of Akt was unchanged in the SHR heart by short-term CR (Figure 6D), suggesting that the inhibition of p70S6K is mediated by elevated AMPK activity and not via reduced signaling through Akt.

Adiponectin Is Involved in the Regulation of BP in the SHR by Short-Term CR

Because multiple molecular mechanisms could be responsible for the CV benefits of short-term CR in the SHR, we examined several pathways that could be involved. First, we measured nucleotide levels in the heart and in agreement with the results of Shinmura et al.,8 we found that the AMP-to-ATP ratio was not different between controls and CR-SHRs (Figure 7A). Because we previously identified a mechanism whereby high levels of the lipid peroxidation product volume or ejection fraction when compared to controls (Table). Because we did not observe any changes in systolic function, we also investigated diastolic function. The isovolumic relaxation time was slightly, although not significantly, reduced by CR (Table). However, isovolumic relaxation time is dependent on heart rate; therefore, the ratio of the peak mitral flow velocity (E-wave) to the peak velocity of the late filling wave of atrial contraction (A-wave), termed E/A ratio, was a better measure of diastolic function (Figure 5H). Indicative of diastolic dysfunction, control SHRs had an abnormally low E/A ratio (Figure 5G), whereas short-term CR improved diastolic function, as judged by the increased E/A ratio (Figure 5G). To assess overall systolic and diastolic cardiac function in the SHR, the myocardial performance index was calculated and revealed improved function of CR-SHR hearts compared to control SHR hearts (Table).
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4-hydroxy-2-nonenal (HNE) observed in SHR contributed to the development of LVH via reduced LKB1/AMPK signaling.\(^{1,2}\) We hypothesized that short-term CR could reduce HNE levels in the SHR and contribute to the increase in AMPK activity. However, the levels of HNE were not significantly different in the control and short-term CR-SHR hearts (Figure 7B). Because CR affects circulating levels of adipokines, we measured leptin and adiponectin in the plasma of SHRs. As expected, short-term CR significantly reduced plasma leptin by \(\approx 3\)-fold (control SHR, 7.48\(\pm\)1.31 ng/mL vs CR-SHR, 2.37\(\pm\)0.39; \(P<0.001\)). Although reduced leptin could exert several positive effects on the CV system, we hypothesized that increased adiponectin was responsible for the CV benefits of short-term CR in the SHR because: (1) circulating adiponectin levels were low in control SHRs compared to normotensive Wistar rats\(^{13,14}\) (Figure 7C); (2) adiponectin levels were increased in the SHR by short-term CR to similar levels as observed in the Wistar rats (Figure 7C); and (3) treatment of C2C12 cells with exogenous adiponectin increased AMPK phosphorylation and reduced p70S6K phosphorylation\(^{23}\) in a manner similar to CR. Furthermore, adiponectin replenishment reduced obesity-related hypertension and increased eNOS expression in obese mice overexpressing the agouti gene.\(^{24}\) To test whether the restoration of normal adiponectin levels in the circulation of the SHR by short-term CR was responsible for the CV benefits of CR in the absence of changes in body weight, adiposity, and leptin, SHRs were injected with the recombinant adenovirus harboring adiponectin (Ad.APN) to transiently increase circulating adiponectin levels in the SHR. Five days after injection of Ad.APN, plasma adiponectin concentrations were significantly increased (Figure 7D) and systolic BP and diastolic BP were significantly lower than Ad.green fluorescent protein (GFP)-injected controls (Figure 7E). Consistent with the reduction in BP and elevated adiponectin levels in the Ad.APN-treated SHRs, phosphorylated AMPK at threonine 172 (Figure 7F), phosphorylated eNOS at serine 1177 (Figure 7G), and phosphorylated vasodilator-stimulated phosphoprotein levels at serine 239 (Figure 7H) were also increased in mesenteric arteries of Ad.APN-treated SHRs compared to Ad.GFP-treated SHRs. Although Ad.APN reduced BP, we did not observe changes in heart weight/tibia length (Figure 7I), suggesting that 5 days was not sufficient to affect cardiac structure. In addition, heart rate (Figure 7J), fasting blood glucose, and body weight (not shown) were not different between Ad.GFP and Ad.APN-treated SHRs. Therefore, adiponectin replenishment in the SHR recapitulates the decrease in BP associated with CR, even in the absence of changes in whole-body metabolism or body weight and leptin. Together, these data imply that increasing plasma adiponectin levels is sufficient to reduce BP, activate the AMPK/eNOS signaling axis, and increase NO bioavailability in the SHR.

**Discussion**

In the present study, we provide evidence that short-term CR prevented an increase in BP in the SHR when initiated at a time when the rats were had mild hypertension (Figure 2A,
B). Although we have shown that short-term CR can reduce BP in the SHR, we cannot be certain that this response occurs in all models of hypertension. Then again, given the fact that short-term CR also reduces BP in the 2-kidney 1-clip mouse model of hypertension (data not shown), short-term CR appears to be effective in preventing an increase in BP in more than one rodent model of hypertension. Therefore, the ability of short-term CR to prevent an increase in BP may involve mechanisms that target fundamental pathways involved in pathological increases in BP such as vascular remodeling. Unfortunately, initiation of short-term CR in older SHRs with more pronounced hypertension (systolic BP, 202 ± 8 mm Hg) did not significantly reduce BP after 5 weeks of short-term CR (systolic BP, 190 ± 11 mm Hg). Therefore, the beneficial effects observed with short-term CR may be restricted to a time when vascular remodeling has only been initiated and not irreversibly remodeled.

In addition, our data show that lower BP (Figure 2A, B) and a subsequent reduction in total cardiac mass (Figure 5) were associated with significantly reduced arterial stiffness and vascular remodeling in short-term CR-SHRs compared to control SHRs (Figures 2D, 3C, 3D). In agreement with the prevention of vascular remodeling and subsequent increases in BP in the SHR by short-term CR, our findings using the femoral and mesenteric arteries of SHRs suggest that improved arterial function in the CR-SHR is likely a consequence of its effects on endothelial and smooth muscle cells. Indeed, improved in vivo blood flow (Figure 2D), increased...
arterial compliance (Figure 3C), and reduced arterial wall thickness (Figure 3D, E) suggested that short-term CR affected the smooth muscle cells of SHRs. However, increased basal NO levels (as suggested by Figures 3A, 4A, 4B) imply that short-term CR has beneficial effects on SHR endothelium. Although we have not fully investigated the involvement of endothelial cells vs smooth muscle cells in the effects of short-term CR, it is clear from our data that short-term CR prevented inward remodeling of the artery wall and improved the elasticity of the arteries from SHRs (both of which likely contribute to the antihypertensive effects of short-term CR).

In addition to the physiological benefits of short-term CR, the depressed vascular AMPK/eNOS signaling pathway observed in control SHRs was restored by short-term CR (Figure 4) and increased adiponectin appeared to mediate these effects (Figure 7). Furthermore, adiponectin replenishment also prevented an increase in BP (Figure 7E) and these effects (Figure 7). Furthermore, adiponectin replenishment also prevented an increase in BP (Figure 7E) and these effects (Figure 7). Furthermore, adiponectin replenishment also prevented an increase in BP (Figure 7E) and these effects (Figure 7). Furthermore, adiponectin replenishment also prevented an increase in BP (Figure 7E) and these effects (Figure 7). Furthermore, adiponectin replenishment also prevented an increase in BP (Figure 7E) and these effects (Figure 7). Furthermore, adiponectin replenishment also prevented an increase in BP (Figure 7E) and these effects (Figure 7). Furthermore, adiponectin replenishment also prevented an increase in BP (Figure 7E) and these effects (Figure 7). Furthermore, adiponectin replenishment also prevented an increase in BP (Figure 7E) and these effects (Figure 7).

In addition to the physiological benefits of short-term CR, the depressed vascular AMPK/eNOS signaling pathway observed in control SHRs was restored by short-term CR (Figure 4) and increased adiponectin appeared to mediate these effects (Figure 7). Furthermore, adiponectin replenishment also prevented an increase in BP (Figure 7E) and restored the vascular AMPK/eNOS signaling pathway in control-fed SHRs (Figure 7F, G), even in the absence of changes in whole-body metabolism or body weight. That said, one of the limitations of our study was that we could not prevent the increase in adiponectin in the CR-SHRs to confirm that the antihypertensive effects of CR are mediated by adiponectin in the SHR. However, taken together, our data suggest that short-term CR increases plasma adiponectin levels and restores AMPK/eNOS signaling in the vasculature of the SHR, thus preventing vascular remodeling and a subsequent increase in BP.

Although multiple molecular mechanisms are likely involved in the CV benefits of short-term CR in the SHR, our results are consistent with several reports that demonstrated the involvement of increased adiponectin in the cardioprotective benefits of CR. However, in this study, we did not ascertain whether the effect on cardiac growth was mediated via the reduction of BP or whether direct effects on the growth of the cardiac myocyte were also involved. That said, we have previously shown that adiponectin-null mice are significantly more susceptible to the development of pressure overload cardiac hypertrophy and that supplementation of adiponectin markedly attenuated LVH.

Consistent with this, the present study also demonstrated that activation of an adiponectin/AMPK signaling axis was associated with suppression of hypertrophic signaling pathways in the heart (Figure 6). Together, these findings suggest short-term CR may have direct effects on cardiac growth in addition to the benefits of BP-lowering in SHR. Given the fact that we observed changes in intracellular signaling pathways in the heart of short-term CR-SHRs (Figure 6), it is likely that short-term CR may reduce cardiac mass via a reduction in BP and by preventing direct molecular signaling mechanisms involved in the induction of LVH. However, based on the significant reduction in BP in the short-term CR-SHRs, the reduction in cardiac mass is most likely attributable to this phenomena and less so to direct alterations in cardiac signaling.
Our data show that short-term CR lowers BP in hypertensive SHRs, improves vasodilatory function and reduces vascular remodeling and left ventricular mass compared to ad libitum-fed control SHRs. In addition, short-term CR improved vascular compliance in resistance arteries and increased eNOS signaling, which was associated with elevated levels of adiponectin and increased AMPK activity. Together, these data provide clear evidence for the potential benefit of CR in hypertensive patients. However, despite the positive CV benefits of short-term CR reported herein, compliance remains a major impediment because the lifestyle changes associated with CR require considerable patient commitment. Based on our findings, gene therapy or pharmacological treatment strategies that stimulate the adiponectin/AMPK/eNOS signaling axis could provide an alternative approach for the treatment of hypertension.

Figure 7. Changes in circulating adiponectin levels are associated with reduced blood pressure (BP) in spontaneously hypertensive rats (SHR). Ratio of cardiac ATP/AMP levels (A), cardiac HNE protein adducts (B), and circulating adiponectin levels (C). Circulating adiponectin levels before and 5 days after adiponectin gene delivery (D). Tail-cuff systolic and diastolic blood pressure (BP) of SHRs 5 days after adenoviral gene delivery (E). Immunoblot analysis was performed on homogenates of mesenteric arteries isolated from Ad.GFP and Ad.APN-treated SHRs. Phosphorylated threonine-172 of AMPK (P-AMPK) was quantified by densitometry and normalized against total AMPK (F). Phosphorylated serine-1177 of eNOS (P-eNOS) was quantified by densitometry and normalized against total eNOS (G). Phosphorylated serine-239 of VASP (P-VASP) was quantified by densitometry and normalized against total VASP (H). Heart weight/tibia length (HW/TL) ratio (I). Heart rate (J). Values are mean±SEM. *Significant difference (P<0.001) between control SHRs (n=10) and both the CR-SHRs (n=10) and Wistar rats (n=6) using a 1-way analysis of variance (ANOVA) with a Bonferroni multiple comparisons test. #Significant difference (P<0.05) between Ad.GFP (n=4) and Ad.APN (n=4) using a 1-way ANOVA with a Bonferroni multiple comparisons test.
Antihypertensive Effects of Caloric Restriction

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Disclosures
None.

References
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Calorie Restriction Prevents Hypertension and Cardiac Hypertrophy in the Spontaneously Hypertensive Rat

Dolinsky et al. Anti-hypertensive effects of calorie restriction

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SUPPLEMENTAL METHODS

Analysis of Tissues- 15 week-old SHR or Wistar rats were euthanized with an intraperitoneal injection of euthanyl (0.5ml/kg body weight). Tissues were homogenized and the protein concentration was assayed using Bradford protein reagent. 15-20 µg of protein was used for SDS-PAGE and immunoblot analysis. For quantification of ATP and AMP, frozen rat heart samples (20 mg) were homogenized and concentrations were determined by HPLC as described previously1. HNE-protein adducts were quantified from 100 mg of frozen rat heart samples using gas chromatography-mass spectrometry (GCMS) as we have described in detail elsewhere2. Histology of tissues performed by the Alberta Diabetes Institute Histology Core, University of Alberta.

In Vivo Assessment of Cardiac Function- Transthoracic echocardiography was performed on mildly anesthetized rats (sedated with 3% isofluorane & 1.0 L/min oxygen and maintained at 1-1.5% isofluorane &1L/min oxygen) at 9 and 15 weeks of age using a Vevo 770 High-Resolution Imaging System equipped with a 30-MHz transducer (RMV-716; VisualSonics, Toronto). Rodent paws were taped to ECG metal strips on a rat handling platform (P/N 11437, VisualSonics Inc, Toronto) to obtain ECG tracings simultaneously with the cardiac images. Cardiac functional parameters were determined by a trained and blinded research animal echocardiographer, as previously described3. Briefly, two-dimensional M-mode recordings were obtained from the short axis view at the level of the papillary muscles. Ventricular dimensions were obtained from M-mode measurements of at least (3 to 6 cardiac cycles and %Ejection Fraction and % Fractional Shortening were determined. Doppler tissue imaging from the apical four chamber view was used to assess mitral valve annular velocities, E` and A`. In addition, pulse wave Doppler of the mitral E and A wave velocities were taken from the four chamber view. Also, the isovolumic relaxation time (IVRT), isovolumic contraction time (IVCT) and aortic ejection time (ET) were measured from these waveforms to determine myocardial performance Index. The myocardial performance index was calculated using the equation (IVRT+ IVCT)/ET.

Pressure myography- Pressure myography was performed essentially as described elsewhere4. Briefly, second order mesenteric arteries were dissected from 15 week-old SHRs and mounted on a pressure myograph system (Living Systems, Burlington). Vessels were bathed in PSS, composition 10mM HEPES, 5.5 mM glucose, 1.56 mM calcium chloride, 4.7 mM potassium chloride, 142 mM sodium chloride, 1.17 mM magnesium sulfate, 1.18 mM KH2PO4, pH 7.5 warmed to 37 °C. Following a 20 minute equilibration period, vessels were exposed to a 10 minute increase in pressure to 80 mmHg before being returned to 60 mmHg. Vessel diameters were measured using a calibrated microscope eyepiece.

Mesenteric arteries were constricted with phenylephrine (PE; 10^-8 - 10^-4M) in the presence or absence of a nitric oxide synthase (NOS) inhibitor (N^G nitro-L-arginine methyl ester, L-NAME; 10^-4M). The concentration required to
produce 80% of the maximal response to PE was calculated. This concentration was then used to preconstrict the vessels and a concentration–response curve to methylcholine (MCh; \(10^{-10}–10^{-5}\)M), was conducted to assess endothelium-dependent relaxation in the presence or absence of L-NAME (\(10^{-4}\)M). Finally, passive characteristics of the vessels were determined by measuring changes in arterial diameter in response to increasing pressures (0-140 mmHg).

*Flow-mediated vasodilation after temporary hindlimb ischemia*—Male 15 week-old SHR were mildly anesthetized with isoflurane (4% induction and 2.5% maintenance), and kept stable by performing the examination on a heated examination table with warming lamps directed at the animal. The femoral artery was visualized with a 30-MHz transducer (RMV-707B; VisualSonics, Toronto, Canada). Following a procedure, adapted from others\(^5\), the femoral artery was identified by its characteristic flow pattern and the position of the probe was optimized to show clear vessel wall/lumen interfaces, the settings were optimized, and the probe was fixed in a stand and not changed throughout the investigations. Ultrasound diameter and Doppler-flow measurements were obtained from longitudinal sections of the FA before and after 5 min of hindlimb ischemia \((n = 5)\). Reproducible ischemia and reperfusion of the hindlimb were achieved with an arterial loop occluder that was positioned upstream of the site to be visualized, around the common iliac artery, through a trans-abdominal access. This approach was chosen to minimize movement artifacts and to ensure complete ischemia of the hindlimb. The loop occluder consisted of a 5-0 prolene filament around the artery and passed through a 15-cm PE-90 tubing, which was externalized and skin closed with clips. Hindlimb ischemia was achieved by pulling on the filament through the tubing and clamping with a hemostat clamp. After a 15-min equilibration period, baseline readings were taken and the common iliac artery was occluded with the loop occluder. Flow arrest was confirmed by abrogation of the Doppler signal. After 5 min of ischemia, the hindlimb was reperfused by release of the occluder. Reactive hyperemia was monitored by flow velocity and diameter of the FA at 0, 0.5, 1, 1.5, 2, 3, 4, and 5 min.

*Recombinant adenoviruses*—Production of the adenovirus producing the full-length mouse adiponectin is described elsewhere\(^6\). Expression of adiponectin is under the control of the mammalian CAG promoter. A single dose of adenovirus \((5 \times 10^9\) plaque-forming units) harboring GFP (Ad.GFP) or mouse adiponectin (Ad.APN; a gift from K. Walsh, Boston University) was injected into male SHRs (12 weeks-old) via the tail vein. Blood pressure was measured prior to injection and five days post-injection. Prior to injection as well as five days postinjection, rats were fasted (16 h) and a sample of blood was drawn from the tail for determination of circulating adiponectin levels. Levels of plasma adiponectin were measured using ELISA (Millipore).
REFERENCES


