Cardioprotective Effects of Short-Term Caloric Restriction Are Mediated by Adiponectin via Activation of AMP-Activated Protein Kinase

Ken Shinmura, MD, PhD; Kayoko Tamaki, BS; Kiyomi Saito, PhD; Yasuko Nakano, PhD; Takashi Tobe, PhD; Roberto Bolli, MD

Background—Overeating and obesity are major health problems in developed countries. Caloric restriction (CR) can counteract the deleterious aspects of obesity-related diseases and prolong lifespan. We have demonstrated that short-term CR improves myocardial ischemic tolerance and increases adiponectin levels. Here, we investigated the specific role of adiponectin in CR-induced cardioprotection.

Methods and Results—Adiponectin antisense transgenic (Ad-AS) mice and wild-type (WT) mice were randomly assigned to a group fed ad libitum and a CR group (90% of caloric intake of ad libitum for 3 weeks, then 65% for 2 weeks). Isolated perfused mouse hearts were subjected to 25 minutes of ischemia, followed by 60 minutes of reperfusion. CR increased serum adiponectin levels by 84% in WT mice. Gel filtration analysis of the oligomeric complex distribution showed that CR produced a marked increase in the high–molecular-weight complex of adiponectin in WT mice; in contrast, CR did not change serum adiponectin levels or their oligomeric pattern in Ad-AS mice. CR improved the recovery of left ventricular function after ischemia/reperfusion and limited infarct size in WT mice; these effects were completely abrogated in Ad-AS mice. CR also increased the phosphorylated form of AMP-activated protein kinase and acetyl-CoA carboxylase in WT but not in Ad-AS mice. Recombinant adiponectin restored CR-induced cardioprotection in Ad-AS mice, and inhibition of AMP-activated protein kinase phosphorylation completely abrogated CR-induced cardioprotection in WT mice.

Conclusion—The cardioprotective effects of short-term CR are mediated by increased production of adiponectin and the associated activation of AMP-activated protein kinase. (Circulation. 2007;116:2809-2817.)

Key Words: ischemia □ myocardial infarction □ nutrition □ reperfusion

More than 30% of American adults are obese (defined as a body mass index ≥30 kg/m²).1 The prevalence of obesity also is increasing in other developed countries. Obesity and overeating lead to the metabolic syndrome, resulting in increased cardiovascular disease.1–3 Novel nutritional approaches to control body weight and counteract the metabolic syndrome are becoming increasingly important.

In general, the daily caloric intake in animals subjected to CR has been restricted to 50% to 70% of the average food intake in animals eating ad libitum (AL). Mounting evidence indicates that CR profoundly affects the physiological and pathophysiological alterations associated with aging and markedly increases lifespan in several species, including mammals.4–6 Although the ability of CR to prolong the lifespan in humans has not been demonstrated conclusively, it now seems plausible that CR may attenuate visceral fat accumulation and counteract the deleterious aspects of obesity.

Lifelong CR has been reported to restore the cardioprotective effects of ischemic preconditioning in middle-aged (10 months)7 and aged (24 months)8 rats, indicating that CR enhances the innate defense mechanisms against ischemic stress. In addition, we have recently demonstrated that short-term (4-week) CR confers cardioprotection in both young and aged hearts without causing consequences sometimes associated with CR such as increased mortality.
and malnutrition. Clearly, the use of short-term CR is easier to incorporate into clinical practice than lifelong CR; moreover, the development of CR mimetics that can replicate the cytoprotective effects of CR would be much easier to incorporate into clinical practice than a strict CR protocol.

The adipose tissue has been recognized as an endocrine organ that secretes many peptides collectively referred to as adipokines. An impressive amount of evidence indicates that adipokines play an important role in the regulation of the cardiovascular system. CR decreases perigonadal adipose tissue and alters serum levels of several adipokines. We have found that CR significantly elevates serum levels of adiponectin and lowers those of leptin in both young and old rats and increases myocardial levels of phosphorylated AMP-activated protein kinase (AMPK)–α at baseline without affecting the myocardial AMP-to-ATP ratio. On the basis of these findings, we hypothesized that the increase in circulating adiponectin levels effected by CR activates myocardial AMPK, resulting in protection against ischemia.

To test this hypothesis, we investigated in the present study the role of adiponectin in CR-induced cardioprotection using adiponectin antisense (Ad-AS) transgenic mice. We analyzed the oligomeric state of circulating adiponectin (which consists of 3 forms: the high–molecular-weight [HMW] form, the hexameric form, and the trimeric form) because, among the 3 oligomeric complexes, the HMW complex appears to be the most active and protective form, independent of total adiponectin levels. Our results demonstrate that increased production of adiponectin is essential for CR-induced cardioprotection against ischemia and suggest that the HMW form of adiponectin is likely to account for this effect by activating AMPK in the myocardium.

Methods

An expanded Methods section can be found in the online-only Data Supplement.

Ad-AS Transgenic Mice

Transgenic mice expressing an Ad-AS oligonucleotide were created as described previously. Briefly, an Ad-AS expression vector was constructed by inserting an inverted fragment of the mouse adiponectin cDNA into the unique EcoRI site between the cytomegalovirus immediate early enhancer–chicken β-actin promoter and the 3′-flanking sequence of the rabbit β-globin gene of the pCAGGS expression vector. A Basic Local Alignment Search Tool (BLAST) analysis (National Center for Biotechnology Information, National Library of Medicine, Bethesda, Md) of the GenBank nucleotide database indicated that this antisense sequence showed no significant homology to any other mouse genes.

CR Protocols

CR was performed as described previously. Briefly, 8-week-old male Ad-AS and wild-type (WT) mice were housed in individual cages and fed AL for 3 weeks. After weaning, mice were randomly allocated into 2 groups. AL mice continued to be fed AL using control diet A for the subsequent 5 weeks. CR mice were fed 90% of the average caloric intake during the AL period for 3 weeks (10% restriction), followed by 65% of that for 2 weeks (35% restriction).

Ischemia/Reperfusion Protocol and Measurement of Infarct Size

Under anesthesia, the hearts were excised quickly and perfused with modified Krebs-Henseleit buffer according to the Langendorff procedure, as described previously.

All hearts were subjected to 25 minutes of global no-flow ischemia, followed by 60 minutes of reperfusion. Infarct size (percent of the left ventricle [LV]) was quantified as described previously. The perfusate was collected during reperfusion, and total creatine kinase (CK) activity released into the perfusate was measured with commercially available spectrophotometric assays (Sigma-Aldrich, St Louis, Mo).

Measurement of Serum Adiponectin Levels

Mice were fasted overnight, and blood samples were collected from the chest cavity when the hearts were excised. Serum levels of adiponectin were measured with a commercially available ELISA kit (R&D Systems, Minneapolis, Minn). Gel filtration analysis of the oligomeric complex distribution of adiponectin was performed with serum samples from each group (n=4) as described previously.

Western Immunoblotting

Standard SDS-PAGE Western immunoblotting was performed with 40 μg protein sample as described previously. Densitometric values (arbitrary density units) of the phosphorylated protein were normalized to the total amount of protein detected and expressed as a percentage of the corresponding values in AL WT mice. Polyonal antibodies against AMPK, phosphorylated AMPK–α at the Thr172 residue, acetyl-CoA carboxylase (ACC), and phosphorylated ACC at the Ser79 residue were purchased from Cell Signaling (Beverly, Mass).

Metabolites Analysis

Myocardial glycogen content was determined from methanol precipitates of KOH-digested tissue using the amyloglucosidase method. ATP and creatine phosphate contents were determined spectrophotometrically from neutralized perchloric acid extracts of tissue samples as described previously.

Rescue and AMPK Inhibition Experiments

An Alzet micro-osmotic pump (model 1007D, DURECT, Cupertino, Calif) was implanted subcutaneously in the intrascapular region of Ad-AS CR mice 1 week before their death. The reservoir of each pump was preloaded with 96 μL sterile Tris-buffered saline or murine recombinant adiponectin (rAd; 1.55 μg/μL, BioVision, Mountain View, Calif). CR was continued for 1 additional week, and 5 hearts from each group were subjected to the same ischemia/reperfusion protocol as described above.

WT mice were treated with adenine 9-D arabinofuranoside (AraA, Sigma-Aldrich), an AMPK inhibitor. Thirty minutes before the mice were killed, 2 μg/g AraA or vehicle was injected intravenously. Four hearts from each group were used for Western immunoblotting. Six hearts from WT mice treated with AraA or vehicle were perfused with modified Krebs-Henseleit buffer containing 10 μU/mL insulin, 0.4 mmol/L oleate, and 1% BSA to assess the physiological role of AMPK. Hearts were subjected to the ischemia/reperfusion protocol as described above.

Statistical Methods

Data are reported as mean±SEM. Serum adiponectin levels, cardiac parameters, infarct size, total CK activity, and densitometric measurements of Western immunoblots were analyzed by 2-way ANOVA (WT versus Ad-AS, AL versus CR), followed by Scheffé’s test for post hoc comparisons. One-way ANOVA was used when the effect of recombinant vehicle or adiponectin supplementation was compared among 3 groups. Differences were considered significant at P<0.05.


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

#### Effect of CR on Body Weight and Serum Adiponectin Levels

No difference existed in body weight at baseline between WT and Ad-AS mice or between AL and CR mice (Table 1). Five weeks of CR decreased body weight to a similar extent in both strains. No mouse died during CR period in either strain. Serum levels of adiponectin were lower in Ad-AS AL than in WT AL mice (Figure 1A). CR significantly increased serum adiponectin levels in WT mice. However, CR did not change these levels significantly in Ad-AS mice (0.05 < P < 0.1).

Gel filtration analysis of the oligomeric complex distribution of adiponectin showed an increase in all fractions with CR in WT mice; the increase was particularly pronounced for the HMW form (first peak) (Figure 1B). In contrast, the increase in the HMW form of adiponectin was not significant in Ad-AS CR mice (Figure 1C).

#### Effect of CR on Myocardial Ischemia/Reperfusion Injury

Although CR significantly reduced LV weight in both strains, no difference was observed in LV weight between WT AL and Ad-AS AL mice or between WT CR and Ad-AS CR mice (Table 1). In addition, no difference was present in LV function at baseline between WT and Ad-AS mice (Table 1). In WT mice, CR significantly improved the recovery of LV developed pressure (LVDP), peak positive dP/dt, and peak negative dP/dt throughout reperfusion compared with WT AL mice (Figure 2A, 2C, and 2D). In Ad-AS mice, CR improved percent recovery of dP/dt and −dP/dt at 30 and 40 minutes after reperfusion, but this effect was transient, and the difference between AL and CR was no longer statistically significant 50 minutes after reperfusion (Figure 2C and 2D).

In WT mice, CR significantly reduced infarct size as detected by 2,3,5-triphenyltetrazolium chloride (TTC) staining (Figure 3A and 3B). In contrast, infarct size in Ad-AS CR mice was equivalent to that in Ad-AS AL mice, indicating that the cardioprotective effect of CR is abrogated in Ad-AS

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Table 1. Morphological and Cardiac Parameters at Baseline in Each Group

<table>
<thead>
<tr>
<th></th>
<th>WT Mice</th>
<th>WT AL</th>
<th>WT CR</th>
<th>Ad-AS Mice</th>
<th>Ad-AS AL</th>
<th>Ad-AS CR</th>
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<tbody>
<tr>
<td>Morphological</td>
<td></td>
<td></td>
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<tr>
<td>BW at baseline, g*</td>
<td>24.1±0.4</td>
<td>24.6±0.5</td>
<td>23.9±0.5</td>
<td>24.3±0.4</td>
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<tr>
<td>BW before death, g*</td>
<td>27.4±0.7</td>
<td>21.5±0.6§</td>
<td>27.2±0.7</td>
<td>21.2±0.6§</td>
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<td>Change in BW, %</td>
<td>13.7±1.5</td>
<td>−12.6±1.1§</td>
<td>13.8±1.4</td>
<td>−12.8±1.0§</td>
<td></td>
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<tr>
<td>LWW (mg)†</td>
<td>76.6±2.4</td>
<td>66.7±1.9§</td>
<td>76.1±2.3</td>
<td>66.0±1.8§</td>
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<tr>
<td>LWW/BW (%)†</td>
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<td>0.31±0.01§</td>
<td>0.28±0.01</td>
<td>0.31±0.01§</td>
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<tr>
<td>Cardiac‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, bpm</td>
<td>502±17</td>
<td>525±11</td>
<td>505±22</td>
<td>485±22</td>
<td></td>
<td></td>
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<tr>
<td>LVDP, mm Hg</td>
<td>91±4</td>
<td>85±4</td>
<td>86±2</td>
<td>90±3</td>
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<tr>
<td>dP/dt</td>
<td>3760±140</td>
<td>3780±160</td>
<td>3550±290</td>
<td>3830±260</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−dP/dt</td>
<td>3380±110</td>
<td>3470±330</td>
<td>3280±210</td>
<td>3430±330</td>
<td></td>
<td></td>
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<td>RPP×10²</td>
<td>507±20</td>
<td>499±20</td>
<td>487±31</td>
<td>491±34</td>
<td></td>
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</tbody>
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BW indicates body weight; LWW, LV weight; HR, heart rate; and RPP, rate-pressure product.

* n=18 each; † n=14 each; ‡ n=6 each.

§ P<0.05 vs AL.

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![Figure 1](https://example.com/figure1.png)

**Figure 1.** Serum adiponectin levels (A) and gel filtration analysis of the oligomeric complex distribution of adiponectin (B and C). A and B, A global increase, particularly pronounced for the fractions containing the HMW form, was observed in WT mice treated with CR. A and C, In contrast, serum adiponectin levels were less in Ad-AS mice, and no increase occurred in any fraction in Ad-AS mice treated with CR. Data are mean±SEM. * P<0.05 vs AL, ** P<0.05 vs corresponding group in WT.
mice. The results obtained with TTC staining were substantiated by the measurement of total CK activity released into the perfusate (Figure 3C). Attenuation of CK release during reperfusion was not observed in Ad-AS mice subjected to CR.

Effect of CR on AMPK and ACC Phosphorylation
Myocardial expression levels of total AMPK protein were similar between WT and Ad-AS mice and between AL and CR mice (Figure 3A). At baseline, myocardial levels of AMPK-α phosphorylated at the Thr172 residue increased significantly with CR in WT mice (Figure 3A and 3B), suggesting that CR activated AMPK in WT hearts. In contrast, no increase occurred in myocardial levels of phosphorylated ACC at the Ser79 residue in Ad-AS CR compared with Ad-AS AL mice. As expected, myocardial levels of phosphorylated ACC at the Ser79 residue increased significantly with CR in WT mice, but no increase in phosphorylated ACC occurred in Ad-AS CR mice (Figure 4C and 4D). Prolonged ischemia caused a robust increase in the expression levels of phosphorylated AMPK-α in both strains; no difference was present in phosphorylated AMPK-α levels between AL and CR in either strain (Figure 4A and 4B).

Effect of CR on Myocardial Metabolites
Myocardial glycogen content at baseline was similar between AL and CR in WT mice (Table 2). In contrast, myocardial glycogen content in Ad-AS CR mice was less than that in Ad-AS AL mice, suggesting that activated AMPK plays a role in maintaining myocardial glycogen content under CR. No difference existed in myocardial ATP and creatine phosphate content between AL and CR in both strains.

Restoration of CR-Induced Cardioprotection by Exogenous Adiponectin in Ad-AS Mice
The delivery of rAd via implanted micro-osmotic pumps in Ad-AS CR mice resulted in circulating levels of adiponectin similar to those observed in WT mice treated with CR (Figure 5A). Consequently, the recovery of LV function after ischemia/reperfusion was significantly improved and the infarct size was reduced in Ad-AS CR mice implanted with micro-osmotic pumps delivering rAd compared with Ad-AS mice implanted with pumps delivering vehicle (Figure 5B and 5C). These results indicate that administration of exogenous adiponectin can restore the cardioprotective effect of CR in Ad-AS mice.
Treatment With an AMPK Inhibitor Abrogates CR-Induced Cardioprotection

Administration of AraA decreased the expression levels of phosphorylated AMPK in response to CR in WT mice (Figure 6A and 6B). The dose of AraA chosen in the present study did not affect LV function at baseline in either WT AL or WT CR mice (data not shown). The recovery of LVDP after ischemia/reperfusion was significantly better in WT mice with administration of AraA in itself did not completely abrogate the cardioprotective effect of CR in WT AL or WT CR mice (data not shown). The recovery of LVDP after ischemia/reperfusion was significantly better in WT mice with administration of AraA in itself did not completely abrogated the cardioprotective effect of CR in WT mice, although administration of AraA in itself did not exacerbate the degree of ischemia/reperfusion injury (Figure 6C and 6D).

Discussion

This study provides 4 major findings: (1) short-term CR confers protection against myocardial ischemia/reperfusion injury in WT mice; (2) the increase in circulating adiponectin levels associated with CR is necessary for the cardioprotective effects of CR; (3) increased production of the HMW form of adiponectin during CR is associated with activation of AMPK (as indicated by the increase in phosphorylated AMPK-α); and (4) activation of AMPK plays an obligatory role in the cardioprotection afforded by short-term CR.

CR is currently the only known intervention that significantly prolongs the maximal lifespan in mammals.4–6 It is speculated to be of possible relevance in delaying the deleterious effects of aging in humans.4–6 However, the exact mechanisms by which CR prolongs lifespan and reverses senescent changes have not been clarified. Lifelong CR significantly attenuates tissue oxidative damage and decreases apoptosis.4–6 Recent reports suggest that CR provokes a mild stress response, resulting in enhanced cell defenses, probably coordinated by the endocrine system (called hormesis).5,6 This concept is analogous to the well-known phenomenon of preconditioning, in which a sublethal stress greatly enhances the tolerance of the organ to a subsequent more severe stress.26,27 Accordingly, we hypothesized that short-term CR preconditions organs and improves ischemic tolerance. As predicted by this hypothesis, short-term CR improved myocardial ischemic tolerance in rats9 and in mice. It is likely that multiple mechanisms account for the protective effect of CR against myocardial ischemia/reperfusion injury. However, on the basis of our previous work, we hypothesized that activation of AMPK, accompanied by changes in adipocyte-derived cytokines, is mainly responsible for CR-induced cardioprotection.9

CR markedly changes adipokine production9,12,13,28; specifically, circulating levels of adiponectin increase and those of leptin decrease during CR.9,13 Higami et al.28 demonstrated that CR profoundly decreases mRNA levels of leptin in adipose tissue (≈1/10th) and increases those of adiponectin (1.5-fold) by CR. Thus, it is plausible that

Table 2. Myocardial Glycogen, ATP, and Creatine Phosphate Content at Baseline in Each Group

<table>
<thead>
<tr>
<th></th>
<th>AL</th>
<th>CR</th>
<th>AL</th>
<th>CR</th>
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<tbody>
<tr>
<td><strong>Glycogen, μmol/g wet weight</strong></td>
<td>13.6±1.0</td>
<td>12.8±1.1</td>
<td>13.4±1.0</td>
<td>10.0±0.9*</td>
</tr>
<tr>
<td><strong>ATP, μmol/g wet weight</strong></td>
<td>4.3±0.4</td>
<td>4.2±0.4</td>
<td>4.4±0.3</td>
<td>4.0±0.4</td>
</tr>
<tr>
<td><strong>CRP, μmol/g wet weight</strong></td>
<td>6.0±0.5</td>
<td>6.6±0.4</td>
<td>6.1±0.4</td>
<td>5.6±0.4</td>
</tr>
</tbody>
</table>

*P<0.05 vs AL.
adipose tissue modulates the effects of CR by secreting humoral factors that promote health and prevent the aging process. However, direct evidence that adipose-derived factors are essential for the beneficial effect of CR is lacking.

To address this crucial issue, we investigated the role of adiponectin in CR-induced cardioprotection using mice expressing an Ad-AS gene. We chose heterozygous littermates because we sought to evaluate the specific role of adiponectin in CR using animals that were as healthy as possible. Although heterozygous Ad-AS mice showed a slight decrease in serum adiponectin levels, no differences were evident between WT and Ad-AS mice with respect to LV weight, baseline LV function, and the extent of myocardial ischemia/reperfusion injury (Table 1 and Figures 2 and 3). In contrast, the increase in adiponectin production by CR was completely suppressed in Ad-AS mice, suggesting that we successfully separated the role of adiponectin during CR from that under physiological conditions. Shibata et al reported that infarct size after in vivo ischemia/reperfusion was significantly increased in adiponectin knockout mice compared with WT mice, indicating that endogenous production of adiponectin has a protective effect against myocardial ischemia/reperfusion injury. In contrast, in the present study, infarct size in Ad-AS transgenic mice was similar to that in WT mice. The maintenance of normal circulating adiponectin levels at baseline in Ad-AS mice could explain the discrepancy between the results obtained with adiponectin knockout mice and results in Ad-AS mice. Our present findings that CR-induced cardioprotection is abrogated in Ad-AS mice and that exogenous administration of recombinant adiponectin (rAd; Table 1 and Figures 2 and 3) achieved circulating levels of adiponectin similar to those observed in WT mice treated with CR. Consequently, percentage recovery of LVDP was significantly improved and infarct size was reduced in Ad-AS CR+rAd mice. Data are mean±SEM. *P<0.05 vs AL, †P<0.05 vs corresponding vehicle (v) group.

Figure 5. Serum adiponectin levels (A), percentage recovery of LVDP (B), and infarct size (C) in Ad-AS mice implanted with osmotic pumps. A, The delivery of recombinant adiponectin via implanted micro-osmotic pumps in Ad-AS CR (Ad-AS CR+rAd) achieved circulating levels of adiponectin similar to those observed in WT mice treated with CR. B and C. Consequently, percentage recovery of LVDP was significantly improved and infarct size was reduced in Ad-AS CR+rAd mice. Data are mean±SEM. *P<0.05 vs AL, †P<0.05 vs corresponding vehicle (v) group.

Figure 6. Western immunoblotting for AMPK (A and B), percentage recovery of LVDP (C), and infarct size (D) in WT CR mice treated with AraA. A, Representative Western immunoblots showing the expression of total and phosphorylated AMPK-α (Thr172) at the Thr172 residue. B, Densitometric analysis of phosphorylated AMPK-α (Thr172) signals. A and B, The increase in myocardial levels of phosphorylated AMPK-α in WT with CR was abrogated by pretreatment with AraA. C and D, The cardioprotection afforded by CR was completely blocked by the inhibition of AMPK activation. Data are mean±SEM. *P<0.05 vs AL, †P<0.05 vs corresponding vehicle group (AraA[+]).
Adiponectin in Ad-AS mice restores CR-induced cardioprotection provide direct evidence that demonstrates, for the first time, a necessary role of increased adiponectin production in the beneficial effects of CR. Saito et al.\(^{23}\) reported a remarkable decrease in body weight and adipose tissue during 3 days of starvation in Ad-AS mice. In the present study, the decrease in body weight in Ad-AS mice was similar to that in WT mice, and no mice in either strain died during CR. However, myocardial glycogen content was less in Ad-AS mice treated with CR, associated with attenuated AMPK activation by CR (Table 2 and Figure 4B). These results further support the previous conclusion that adiponectin plays an important role in maintaining energy homeostasis under energy depletion in mammals.\(^{18}\)

The mechanisms by which adiponectin protects myocardium from ischemia/reperfusion injury have not been fully elucidated. Shibata et al.\(^{29}\) have demonstrated that adiponectin alleviates ischemia/reperfusion injury via AMPK- and cyclooxygenase-2–dependent mechanisms. We could not find any increase in cyclooxygenase-2 protein in CR hearts, and the expression levels of cyclooxygenase-2 remained at low levels (data not shown). Thus, it is unlikely that cyclooxygenase-2 mediates the cardioprotective effect of short-term CR. Recently, Tao et al.\(^{30}\) reported that disruption of adiponectin gene exacerbates myocardial ischemia/reperfusion injury as a result of increased oxidative/nitrative stress via enhanced induction of inducible nitric oxide synthase and gp\(^{91}\)phox protein. CR reduces oxidative stress,\(^{4–6}\) but the involvement of the inducible nitric oxide synthase/gp\(^{91}\)phox system in CR-induced cardioprotection remains to be clarified.

It is still controversial whether activation of AMPK is detrimental or protective to the ischemic heart.\(^{23,31–34}\) Most reports demonstrate that activation of AMPK improves myocardial ischemic tolerance, resulting in attenuated myocardial ischemia/reperfusion injury.\(^{23,32,34}\) However, AMPK-dependent acceleration of fatty acid oxidation during reperfusion has the potential to be detrimental in the setting of ischemia/reperfusion.\(^{33}\) In the present study, CR confers protection in isolated heart perfused with or without insulin and free fatty acids. Before the induction of ischemia, marked activation of AMPK was present in WT mice treated with CR. These results strongly suggest that activation of AMPK before ischemia is protective against myocardial ischemia/reperfusion injury. The loss of CR-induced cardioprotection by AraA treatment before ischemia supports this concept, although this compound is not specific for AMPK, and limitations of pharmacological inhibition should be taken into account when these results are interpreted. Interestingly, AMPK was activated in CR hearts, but myocardial glycogen content and high-energy phosphate content were similar to those in AL hearts. These results are consistent with previous reports on the energy metabolites in the CR heart.\(^{35}\) \(^{35}\) and suggest that activated AMPK may compensate for the limited supply of energy during CR by increasing the uptake of substrates and glycolysis rather than by increasing glycolgenolysis. The decreased myocardial glycogen content in Ad-AS mice with CR may contribute, at least in part, to greater damage after ischemia/reperfusion compared with WT mice treated with CR because glycogenolysis is protective against ischemia/reperfusion injury until the accumulation of deleterious metabolites (lactate, H\(^+\), NADH, and inorganic phosphate) outweighs the benefit of ATP production.\(^{36}\)

The increase in the cellular AMP-to-ATP ratio is a major regulator of AMPK activity.\(^{32,37,38}\) but recently, adipocyte-derived hormones also have been reported to activate AMPK.\(^{10,11,38}\) Most of the beneficial effects of adiponectin appear to be mediated by AMPK-associated signaling.\(^{10,11,14,16,17,29,39}\) In the present study, AMPK-α phosphorylated at the Thr\(^{172}\) residue, the activated form of AMPK,\(^{37}\) was increased by CR in WT mouse hearts, and the magnitude of cardiac AMPK phosphorylation during CR appears to correlate with the distribution of the HMW oligomers. Different oligomeric forms of adiponectin bind to the specific adiponectin receptors adipo R1 and adip R2 in a distinct manner, activating different signaling pathways and exerting distinct functions on the target tissues.\(^{10,11,14,16,17,29,39}\) Tsao et al.\(^{17}\) reported that the globular domain of adiponectin, which can form only trimers, is more potent than other forms in activating AMPK in rat skeletal muscle. In contrast, Pajvani et al.\(^{20}\) showed that the HMW complex is the most active form of adiponectin in lowering blood glucose levels in mice. Furthermore, only the HMW form can protect endothelial cells from apoptosis. These considerations support the concept that increased production of the HMW complex by CR activates AMPK, resulting in cardioprotection against ischemia.

Further studies are necessary to determine whether the increase in the HMW form of adiponectin also contributes to the various effects of lifelong CR. Whether the metabolic adaptation to lifelong CR in the heart is related to changes in AMPK activity is controversial.\(^{35}\) It seems reasonable to assume that lifelong CR switches off ATP-consuming pathways and switches on ATP-saving pathways. Long-term CR experiments using Ad-AS mice might resolve the role of the adiponectin-AMPK signaling pathway in lifelong CR.

In summary, this study demonstrates a cause–effect relationship between increased adiponectin production and cardioprotection by short-term CR. In addition, the present data demonstrate that inhibition of AMPK activation, which occurs before ischemia in the CR heart, completely abrogates the cardioprotection afforded by CR. Therefore, we conclude that short-term CR preconditioning the myocardium against lethal ischemic injury by enhancing adiponectin production and activating AMPK. In this connection, activation of AMPK has been shown to occur in the setting of ischemic preconditioning.\(^{40}\) The present results also suggest that activators of adiponectin-mediated signaling may be potentially useful as a novel class of cardioprotective agents (CR mimetics).

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Disclosures

None.

References

CLINICAL PERSPECTIVE

Overeating and obesity are major health problems in advanced countries. They lead to the metabolic syndrome, resulting in the increased incidence of cardiovascular disease. Accumulation of visceral fat is proposed to be a fundamental pathology because adipokines secreted from visceral fat are closely related to the development of obesity-related diseases; among these adipokines, adiponectin is important because it has antiarteriosclerotic and cardioprotective properties. Caloric restriction (CR) has been widely investigated as a powerful intervention that can prevent and reverse aging-related changes. CR has recently attracted additional interest as a means of controlling body weight and counteracting the metabolic syndrome. Given the adverse effects of obesity, it is plausible that CR provides health benefits by decreasing fat mass. However, the exact mechanisms by which CR prolongs lifespan and reverses the deleterious aspects of obesity have not been clarified. Using the adiponectin antisense transgenic mouse, this study demonstrates that short-term CR confers cardioprotection and that the increase in circulating adiponectin levels, especially the increase in the high-molecular-weight form of adiponectin, associated with CR is necessary for CR-induced cardioprotection. Furthermore, downstream activation of AMP-activated protein kinase plays an obligatory role in this cardioprotection. These findings provide novel therapeutic approaches for managing obese patients with advanced arteriosclerosis. In addition to CR, activators of adiponectin-mediated signaling may be potentially useful as a novel class of cardioprotective agents (CR mimetics). The data also suggest that the increase in the high-molecular-weight form of adiponectin may be a marker of successful CR.
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