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Sustained Activation of AMPK Ameliorates Age-Associated Vascular Endothelial Dysfunction via a Nitric Oxide-Independent Mechanism

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Abstract

Exercise restores endothelium-dependent dilation (EDD) in old mice by reducing oxidative stress and increasing nitric oxide (NO) bioavailability. Adenosine monophosphate protein kinase (AMPK) activation mimics some effects of exercise. Old (28–30 mo) B6D2F1 mice had reduced arterial AMPK expression and superoxide-mediated suppression of EDD vs. young (3–6 mo) controls. Pharmacological activation of AMPK by aminoimidazole carboxamide ribonucleotide (AICAR) for 2 weeks increased arterial AMPK and reversed this superoxide-induced impairment of EDD. The improvement in EDD was independent of NO or prostaglandin signaling, suggesting enhanced endothelium-dependent hyperpolarizing factor-related dilation. AMPK activation may represent a novel therapy for treating age-associated vascular dysfunction.

Keywords

endothelium-dependent dilation; prostaglandins; endothelium-dependent hyperpolarizing factor

Advancing age is the primary risk factor for cardiovascular diseases (CVD) (Lakatta and Levy, 2003). This is attributable in part to development of oxidative stress-mediated reductions in nitric oxide (NO) bioavailability and vascular endothelial dysfunction, the latter indicated by impaired endothelium-dependent dilation (EDD) (Brandes et al., 2005; Lakatta and Levy, 2003; Seals et al., 2011). As such, effective treatments for age-associated vascular endothelial dysfunction have potential for preventing CVD with aging (Seals et al., 2011).

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We have shown that aerobic exercise restores EDD in both older humans (DeSouza et al., 2000) and mice (Durrant et al., 2009) by ameliorating oxidative stress and restoring NO bioavailability (Durrant et al., 2009). However, most older adults cannot or will not exercise on a regular basis. As such, compounds with similar efficacy for treating vascular endothelial dysfunction with aging are needed.

Pharmacological activation of adenosine monophosphate protein-activated protein kinase (AMPK), a master regulator of metabolism, recently has been proposed to be an “exercise mimetic” (Narkar et al., 2008). Indeed, in young mice, pharmacological activation of AMPK induced some exercise-like metabolic changes (Winder et al., 2000) and increased exercise capacity (Narkar et al., 2008). In endothelial cell culture, pharmacological stimulation of AMPK induces antioxidant effects (Wang et al., 2011) and activates endothelial NO synthase, the enzyme responsible for NO production (Murakami et al., 2006).

Accordingly, here we tested the hypothesis that short-term treatment with the AMPK agonist aminoimidazole carboxamide ribonucleotide (AICAR) would reverse the age-associated impairment of EDD by reducing oxidative stress and increasing NO bioavailability.

Young (3–6 mo, n=19) and old (28–30 mo, n=16) B6D2F1 mice were fed normal chow and water ad libitum, maintained under a 12 h:12 h light:dark cycle and treated daily for 2 weeks with either saline vehicle or 0.25 mg/kg AICAR (s.q. 0.5 cc). The day after the final injection mice were euthanized by exsanguinations via cardiac puncture while maintained under isoflurane anesthesia. Aortas and carotid arteries were excised and used for assessment of AMPK activation by Western blot (Towler and Hardie, 2007) and ex vivo vascular testing (Durrant et al., 2009; Lesniewski et al., 2009; Lesniewski et al., 2011; Rippe et al., 2010), respectively. All animal procedures conformed to the Guide to the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 2010) and were approved by the University of Colorado at Boulder Animal Care and Use Committee.

Total expression ($P<0.01$) and the ratio of phosphorylated to total ($P=0.01$) AMPK- α 1/2 (Cell Signaling #2532 and 2535), the catalytic subunit of AMPK, were lower in aorta of old compared with young vehicle treated mice (Fig 1). AICAR restored total expression and activation (increased ratio of phosphorylated to total expression) of AMPK- α in old mice (both $P<0.05$), but had no effect on young mice. There were no differences in either total expression or phosphorylation of the β subunit of AMPK among the groups (not shown).

Maximal EDD to acetylcholine (ACh) was impaired ($P<0.01$) in old compared with young vehicle treated mice, and AICAR restored EDD in the old animals without influencing responses in the young controls (Fig 2A). There were no group differences in sensitivity to ACh, assessed by the concentration required to elicit the half maximal response (IC_{50}) (not shown). There were no group differences in maximal endothelium independent dilation to sodium nitroprusside, a measure of vascular smooth muscle sensitivity to NO (not shown, $P>0.30$).

To determine if the age-related impairment of EDD and AICAR rescue of EDD in old mice were oxidative stress-dependent, arteries were pretreated for 60 min ex vivo with the superoxide dismutase mimetic, TEMPOL (1 mmol l⁻¹). In control (vehicle treated) mice, TEMPOL restored maximal EDD in old animals, while having no effect on young mice (Fig 2B), suggesting excessive superoxide is mediating vascular endothelial dysfunction with aging. Importantly, TEMPOL had no effect on maximal EDD in old (or young) AICAR treated mice, suggesting that AICAR restored maximal EDD in old animals by ameliorating vascular oxidative stress. TEMPOL had no effect on sensitivity to ACh within any group (not shown). Consistent with these functional results, nitrotyrosine abundance, a marker of

oxidative stress, was markedly increased with age in aorta of untreated mice ($P=0.05$) and reversed by AICAR treatment (Fig 2E, $n=4-6/\text{group}$, both $P<0.01$).

To determine if aging and AICAR exerted their differential effects on EDD by influencing NO bioavailability, EDD to ACh was assessed in the presence of the nitric oxide synthase (NOS) inhibitor, *N*^G-nitro-L-arginine methyl ester (L-NAME, 0.1 mmol l⁻¹, 30 min). Pre-constriction to phenylephrine did not differ between untreated and L-NAME treated arteries in any group (all $P>0.22$). In contrast to aerobic exercise (Durrant et al., 2009), the AICAR-associated increase in maximal EDD in old animals persisted after NOS inhibition ($P<0.05$) (Fig 3A), with the contribution of NO to EDD not different in the 2 conditions (Fig 3B). These results indicate that an increase in NO bioavailability was not responsible for the improvement in function.

ACh dose responses were repeated after combined inhibition of NOS and cyclooxygenase (COX) (indomethacin, 0.1 mmol l⁻¹, 60 min), as endothelium-synthesized prostanoids can contribute to EDD (Koller et al., 1993). The AICAR-associated increase in maximal EDD in old mice remained after combined NOS and COX inhibition (Fig 3A, $P<0.05$), indicating that the contribution of prostanoids to maximal dilation did not differ in old mice after AICAR (Fig 3B, $P=0.17$). This finding also suggests that the improvement in maximal EDD after AICAR in old mice resulted from an increased contribution of the 3rd and final possible influence on EDD, i.e., endothelium derived hyperpolarizing factor/s (EDHF) (Fig 3B, $P<0.05$). One candidate EDHF responsible for the improved dilation is hydrogen peroxide (H_2O_2) (Feletou and Vanhoutte, 2006). To assess this possibility, we measured H_2O_2 in aortic lysates from the untreated and treated mice using a commercially available ELISA (Enzo Life Sciences, #ADI-907-015). The concentration of H_2O_2 was not different with aging in vehicle treated mice (YV: $52.0\pm6.1\mu\text{M}$ vs. OV: $65.4\pm3.9\mu\text{M}$, $P=0.18$, $N=7/\text{group}$) or after AICAR treatment in either young ($52.8\pm10.9\mu\text{M}$, $P=0.94$, $N=7$) or old ($65.8\pm4.2\mu\text{M}$, $P=0.97$, $N=6$) mice, suggesting that increased H_2O_2 bioavailability was not responsible for the increased EDHF contribution to EDD in old mice after AICAR. Although H_2O_2 does not appear to be the EDHF responsible for AICAR-associated increases in ACh-induced dilation of the carotid arteries of old mice, cytochrome P450 epoxygenases and their metabolites; the 15-lipoxygenase metabolite, 11,14,15-trihydroeoicosatrienoic acid; and the putative EDHF, C-type natriuretic peptide, are all candidates that are deserving of future investigation (Feletou and Vanhoutte, 2006).

Pretreatment of carotid arteries with the superoxide dismutase mimetic, TEMPOL, improved maximal ACh-mediated dilation in the absence and presence of L-NAME (Fig 3C, both $P<0.05$) in old vehicle, but not old AICAR treated mice. In old vehicle mice, TEMPOL increased the contribution of NO to maximal dilation ($P<0.05$), decreased the prostanoid contribution ($P<0.05$) and increased the EDHF contribution to maximal dilation ($P<0.05$) (Fig 3D). In old AICAR mice, TEMPOL was without effect on the contribution of NO ($P=0.4$) or COX products ($P=0.6$), but increased the EDHF component ($P=0.05$) (Fig 3D). Overall, these results suggest that AICAR restored EDD in old mice by reducing oxidative stress, which, in turn, lead to enhanced EDHF-mediated dilation.

Our novel findings are that advancing age is associated with a reduction in arterial activation of the α subunit of AMPK and that pharmacological activation of AMPK by AICAR can restore EDD in old mice. Interestingly, this does not occur by increasing NO bioavailability as is observed with exercise. It is possible that the present AICAR treatment does not achieve the same magnitude of AMPK activation as exercise. However, in preliminary studies a higher dose (0.5 mg/kg) of AICAR was not tolerated by old mice. It also is possible that AMPK activation is insufficient to induce increases in NO in the absence of exercise-induced increases in shear stress. Finally, increases in NO and/or EDHF

bioavailability after AICAR-mediated activation of AMPK may differ by artery size or location.

In conclusion, here we demonstrate that sustained AMPK activation by short-term treatment with AICAR completely restores vascular endothelial function (EDD) in old mice by reducing oxidative stress. Overall, our findings provide the first evidence that chronic pharmacological activation of AMPK may be an effective therapeutic strategy to reverse arterial aging and prevent age-associated CVD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ACh	acetylcholine
AICAR	aminoimidazole carboxamide ribonucleotide
AMPK	adenosine monophosphate protein kinase
COX	cyclooxygenase
CVD	cardiovascular disease
EDD	endothelium dependent dilation
EDHF	endothelial derived hyperpolarizing factor
L-NAME	NG-nitro-L-arginine methyl ester
NO	nitric oxide
NOS	nitric oxide synthase

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Highlights

- Aging is associated with decreased arterial AMPK expression;
- Short-term pharmacological AMPK activation with AICAR ameliorates age-associated vascular endothelial dysfunction by reducing oxidative stress;
- AMPK activation is a potential therapy for treating arterial aging and reducing cardiovascular disease risk.

Figure 1.

Total expression (white bars) and ratio of phosphorylated to total expression (hashed bars) of the alpha subunit of adenosine monophosphate protein kinase (AMPK) in aortas from young (Y) and old (O) vehicle (V) and AICAR (A) treated mice (N=6–9/group). Representative blot images are shown below the summary graph. Total protein expression is presented relative to own GAPDH to account for differences in protein loading and expressed relative to the young vehicle mean within the given blot and exposure. Phosphorylated proteins were assessed on identical lysates run on a second gel/membrane, normalized to own GAPDH measured after stripping of the membrane and presented as a ratio to the total protein expression normalized to own GAPDH. Data presented as mean $\pm$ S.E.M, * denotes difference from YV; † denotes difference from OV. $P < 0.05$.

Figure 2.

Vasodilation to increasing doses of acetylcholine in isolated carotid arteries from young (Y, black symbols) and old (O, white symbols) vehicle (V, circles) and AICAR (A, triangles) treated mice (N=7–10/group) in the absence (Panel A, Control) or presence (Panel C, TEMPOL) of the superoxide dismutase mimetic, TEMPOL. Maximal carotid artery dilation to acetylcholine in the absence (B) and presence (D) of TEMPOL in YV, OV, YA and OC mice. Nitrotyrosine abundance (E) in aortic lysates from YV, OV, YA and OA mice. Data presented as mean $\pm$ S.E.M, * denotes difference from YV; † denotes difference OV vs. OA. ‡ denotes difference OV Control vs. OV TEMPOL. $P < 0.05$.

Figure 3.

Vasodilation to increasing doses of acetylcholine in isolated carotid arteries from old vehicle (black) and AICAR (white) treated mice without (Panel A, Control) and with (Panel C, TEMPOL) prior incubation with the superoxide dismutase mimetic, TEMPOL, and in the absence (circles) or presence of the nitric oxide synthase inhibitor, L-NAME (triangles) and in vitro combined treatment with L-NAME and indomethacin (squares), a cyclooxygenase inhibitor (N=7–10/group). Contribution of nitric oxide (NO), prostanoid dilators (COX) and endothelium derived hyperpolarizing factor (EDHF) to maximal acetylcholine-induced dilation in carotid arteries from OV and OA mice in the absence (Panel B) and presence (Panel D) of TEMPOL. Data presented as mean $\pm$ S.E.M, * denotes difference in maximal dilation from paired response in OV; † denotes difference from OV. ‡ denotes difference Control vs. TEMPOL. $P < 0.05$.