Effect Of Chronic AMPK Activation On Blood Pressure In Normal And Hyperlipidaemic Mice

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Adenosine monophosphate-activated protein kinase (AMPK) has been likened to a cellular fuel gauge which regulates cellular energy homeostasis in response to changes in the balance of adenosine monophosphate (AMP) and adenosine triphosphate (ATP). Cellular stressors such as hypoxia, inflammation, oxidised LDL and hyperglycaemia can activate AMPK in an attempt to restore energy balance. Activation of AMPK may be beneficial in hyperlipidaemic or atherosclerotic animals by enhancing reverse cholesterol transport (1), decreasing neutrophil myeloperoxidase activity (2), inhibiting macrophage proliferation (3) and reducing blood pressure in hypertensive animals (4). However, to date no study has examined the effect of chronic AMPK activation *in vivo* in normal and hyperlipidaemic mice and the effect this has on blood pressure, AMPK activity and function and plasma myeloperoxidase activity.

Male C57BL/6 mice were administered daily i.p. injections of either the AMPK activating agent AICAR (400mg/kg) or an equivalent volume of saline vehicle for 14 days. Age-matched ApoE$^{-/-}$ mice were fed on a high fat diet for one month prior to injection of either saline or AICAR as per the C57 group. After 14 days, blood pressure was measured by cannulation of the carotid artery in mice anaesthetised with isoflurane (1% in oxygen) and heart rate was derived from this. Blood was collected by cardiac puncture for measurement of plasma MPO content using an ELISA kit (Hycult Biotech). Expression and phosphorylation of AMPK and its downstream target acetyl-coenzymeA carboxylase (ACC) were studied using Western blotting (4) in arterial and organ homogenates with GAPDH as a loading control. Body weight measurements were analysed by an unpaired Student's t-test and other data using a two-way ANOVA with Bonferroni's post hoc test.

One month of high fat diet significantly raised mean arterial blood pressure (MAP) in ApoE$^{-/-}$ mice compared to C57 controls (94.1±1.5mmHg vs. 112.2±1.5mmHg; n=8; $p<0.05$). Chronic AICAR treatment had no effect on MAP in C57 mice but significantly lowered it in ApoE$^{-/-}$ mice (112.2±1.5mmHg vs. 98.5±1.8mmHg; n=8; $p<0.05$). Heart rate was also significantly raised in ApoE$^{-/-}$ mice but chronic AICAR had no effect on heart rate in either group. Both groups of C57 mice gained weight during the 14 day study period while ApoE$^{-/-}$ mice lost weight. AICAR significantly increased weight loss in ApoE$^{-/-}$ mice. In homogenised samples of aorta, the expression of phosphorylated AMPKα and phosphorylated ACC was increased in C57 mice treated with AICAR with no effect on total AMPK. In ApoE$^{-/-}$ mice, expression of total AMPK and ACC in the aorta was lower compared to controls and chronic AICAR had no significant effect in increasing phosphorylation. Plasma MPO was dramatically increased in fat-fed ApoE$^{-/-}$ mice compared to C57 controls. Treatment with AICAR significantly increased MPO activity in ApoE-/- (598.2±55.0ng/mL vs. 762.7±71.1ng/mL; n=9; $p<0.05$) but had no effect in C57 mice.

In summary, chronic activation of AMPK using AICAR can lower MAP in hyperlipidaemic mice and this corresponds to increased activation of AMPK and its downstream target ACC within the arterial wall.

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(4) Ford *et al.* (2012) *J Hypertens* **30**: 725-733.