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# Niacin bound chromium treatment induces myocardial Glut-4 translocation and caveolar interaction via Akt, AMPK and eNOS phosphorylation in streptozotocin induced diabetic rats after ischemia-reperfusion injury

Suresh Varma Penumathsa [a,1], Mahesh Thirunavukkarasu [a,1], Samson Mathews Samuel [a], Lijun Zhan [a], Gautam Maulik [b], Manashi Bagchi [c], Debasis Bagchi [c], Nilanjana Maulik [a,*]

[a] Molecular Cardiology and Angiogenesis Laboratory, Department of Surgery, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-1110, USA
[b] Department of Thoracic Surgery, Harvard Medical School, Boston, MA, USA
[c] InterHealth Research Center, Benicia, CA, USA

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ABSTRACT

Diabetes, one of the major risk factors of metabolic syndrome culminates in the development of Ischemic Heart Disease (IHD). Refined diets that lack micronutrients, mainly trivalent chromium ($Cr^{3+}$) have been identified as the contributor in the rising incidence of diabetes. We investigated the effect of niacin-bound chromium (NBC) during ischemia/reperfusion (IR) injury in streptozotocin induced diabetic rats. Rats were randomized into: Control (Con); Diabetic (Dia) and Diabetic rats fed with NBC (Dia+NBC). After 30 days of treatment, the isolated hearts were subjected to 30 min of global ischemia followed by 2 h of reperfusion. NBC treatment demonstrated significant increase in left ventricular functions and significant reduction in infarct size and cardiomyocyte apoptosis in Dia+NBC compared with Dia. Increased Glut-4 translocation to the lipid raft fractions was also observed in Dia+NBC compared to Dia. Reduced Cav-1 and increased Cav-3 expression along with phosphorylation of Akt, eNOS and AMPK might have resulted in increased Glut-4 translocation in Dia+NBC. Our results indicate that the cardioprotective effect of NBC is mediated by increased activation of AMPK, Akt and eNOS resulting in increased translocation of Glut-4 to the caveolar raft fractions thereby alleviating the effects of IR injury in the diabetic myocardium.



## 1. Introduction

The incidence of diabetes is associated with various risk factors such as obesity, increasing age, physical inactivity, autoimmune diseases, pancreatitis, viral infections etc. In addition to these risk factors, certain nutritional deficiencies have also been associated with a higher incidence of diabetes [1]. Chromium deficiency has been associated with elevated blood glucose levels and diabetes [1]. Chromium (Cr) is an essential micronutrient that is required for normal carbohydrate and lipid metabolism [1]. Highly refined diets that may be deficient in micronutrients, mainly trivalent chromium ($Cr^{3+}$) have been identified as the dominant factor in the rising incidence of diabetes. However, the exact mechanism by which $Cr^{3+}$ plays an important role in the regulation of glucose metabolism remains elusive. Furthermore, $Cr^{3+}$ seems to have a role in increasing the tyrosine kinase activity of the insulin receptor [2–5]. Different forms of trivalent chromium such as chromium chloride ($CrCl_3$) and chromium picolinate have been studied with respect to Type II diabetes or Non-Insulin Dependent Diabetes Mellitus (NIDDM) but not Type I diabetes or Insulin Dependent Diabetes Mellitus (IDDM) [5–7]. In addition, Trueblood et al. has shown that niacin protects the isolated heart from ischemia-reperfusion injury [8] by lowering the cytosolic redox state and increasing the lactate efflux rate, consistent with redox regulation of glycolysis.

The regulation of glucose uptake and its utilization is critical for the maintenance of glucose homeostasis. Insulin plays a significant role in controlling the rates of glucose uptake, glycogen synthesis and glycolysis in the cardiac muscle. It is well established that glucose uptake is regulated by glucose transporter (Glut-4) in the plasma membrane [9]. It has been reported that increased glucose uptake in ischemic cardiomyocytes is achieved primarily by the translocation of Glut-4 from its intracellular compartments to the caveolae of the plasma membrane [10–12]. The caveolae are small flask shaped invaginations (50–100 nm in diameter) which act as the membrane organizing centers and signaling microdomains of the plasma membrane in almost all cell types, predominantly in the endothelial cells, myocytes and the adipocytes [10,13–16]. The structural proteins (the caveolins, Cav-1, Cav-2, Cav-3), sphingolipids and cholesterol are the main components of the caveolae [13]. These structural proteins serve as scaffolds and regulators of many proteins [16]. The M-

* Corresponding author. Tel.: +1 860 679 2857; fax: +1 860 679 2825.
*E-mail address:* nmaulik@neuron.uchc.edu (N. Maulik).
[1] Both authors contributed equally.

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caveolin (Cav-3) is expressed in the muscle while Cav-1 is expressed in endothelial cells [17,18]. Studies in Cav-3$^{-/-}$ mice have shown that the absence of caveolin-3 leads to insulin resistance and increased adiposity [19]. It has also been reported that caveolin-1 acts like a physiological inhibitor of eNOS and that Cav-1 expression is upregulated in diabetic NOD mice [20]. eNOS derived NO is key enhancer of vascular functions, vessel relaxation and survival of vascular endothelial cells. The phosphorylation of eNOS at Ser1177 is said to render more activity to the protein. It is demonstrated that Akt/PKB phosphorylates eNOS at Ser1177 [21]. However, dominant negative Akt mutants were unable to block the phosphorylation of eNOS thereby showing an alternative mechanism for the activation of eNOS [22]. In this context, AMP-activated protein kinase (AMPK) has been shown to be involved in eNOS activation by enhancing its phosphorylation [23,24]. AMPK, a serine threonine kinase is known to be the metabolic master switch that senses and regulates the cellular energy status in various cell types [24,25]. Furthermore, phosphorylation of AMPK (pAMPK) and thus its activation has been associated with increased Glut-4 translocation and therefore increased glucose uptake in the myocardium [26,27]. We have demonstrated earlier that redox regulation of ischemic preconditioning is by differential activation of caveolin-1 and caveoin-3 and their association with eNOS and Glut-4 in which AMPK is also involved [10].

In conjunction with the previous reports including ours, in this study we investigated the effect of niacin-bound chromium (NBC) (commercially known as ChromeMate$^{R}$) against ischemia/reperfusion (IR) injury in streptozotocin induced diabetic rats. We also investigated the effect of NBC on the activation of Akt, AMPK and eNOS and its role in the regulation of Glut-4 translocation to the membrane by modulating the levels of Cav-1 and Cav-3, in the diabetic myocardium.

## 2. Materials and methods

### 2.1. Animals

This study was performed in accordance with the principles of laboratory animal care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institutes of Health (Publication No. 85-23, revised 1985). The experimental protocol was examined and approved by the Institutional Animal Care Committee of the Connecticut Health Center (Farmington, CT). All animals used in the study received humane care and treatment. Male Sprague Dawley rats (275–300 g) were used for the study. Experimental diabetes was induced in the animals by a one time intraperitoneal administration of streptozocin (STZ; Sigma, St Louis, MO) at a dosage of 65 mg kg$^{-1}$ in saline. Control rats received an equal volume of normal saline (i.p). Blood was drawn from the rats by tail snip, five days after STZ injection, and blood glucose levels were measured using glucose monitoring system (Thera Sense, Inc. Alameda, CA, USA). Rats with blood glucose concentrations ≥300 mg/dl were considered to be diabetic.

### 2.2. Experimental protocol

Rats were randomly divided into 3 groups ($n$=24 in each group): 1) Non-diabetic control rats (Con); 2) Diabetic rats (Dia); 3) Diabetic rats fed with diet containing Niacin-Bound Chromium (NBC) (Dia+NBC). Niacin bound chromium III complex commercially known as ChromeMate$^{R}$ was obtained from Inter Health Nutraceuticals. Benecia, CA, USA. The chromium dosage for an adult weighing 70 kg is 4 mg. For a rat weighing 250–275 g it is 0.0145 mg and considering a 5 fold faster metabolism in the case of rat [28] the daily chromium dose will be 5 times more i.e. 0.0727 mg (we calculated approximately as 0.1 mg due to the body weight changes during the treatment period of 30 days). A 250–275 g rat approximately consumes 25 g of chow/day; hence 4 mg of chromium (0.1/25×1000 g) was mixed with 1 kg of rat chow. After the treatment period of 30 days, rats were anesthetized; hearts were isolated and subjected to 30 min of ischemia followed by 2 h of reperfusion.

### 2.3. Isolated working heart preparation

Rats were given an intraperitoneal bolus of heparin (500 IU kg$^{-1}$) and were anesthetized by the intraperitoneal administration of pentobarbital sodium (80 mg kg$^{-1}$, Abbot, Baxter Health Care, Deep Field, IL). After ensuring a sufficient depth of anesthesia, a thoractomy was performed; the hearts were rapidly excised and transferred into container having chilled Krebs Henseleit Bicarbonate perfusion Buffer (KHB, 118 mM NaCl, 4.7 mM KCl, 1.7 mM $CaCl_2$, 25 mM $NaHCO_3$, 1.2 mM $KH_2PO_4$, 1.2 mM $MgSO_4$, 10 mM glucose). The aorta was cannulated, the hearts were subjected to retrograde perfusion on a Langendorff perfusion system at a hydrostatic pressure of 100 cm of $H_2O$ where the buffer was continuously oxygenated and maintained at a constant temperature of 37 °C [29,30]. The pulmonary vein was cannulated to another cannula to continue with the isolated working heart procedure. The Langendorff preparation was switched to the antegrade working mode, following a brief washout period, by switching the supply of the perfusate (maintained at a hydrostatic pressure of 17 cm of $H_2O$) from the aorta to the left atrium. After the attainment of steady state, cardiac baseline functional parameters were recorded. The circuit was then switched back to the retrograde mode and the hearts were perfused for 5 min with KHB buffer, and were then subjected to 30 min of global ischemia followed by 2 h of reperfusion in antegrade working heart mode and the functional parameters were recorded at 30, 60, 90 and 120 min of reperfusion [29,30].

### 2.4. Cardiac function

Aortic pressure was measured using a pressure transducer (Micro-Med, Inc.,) and the signal was amplified using a HPA-400 (Micro-Med, Inc, USA). The Heart Rate (HR), Left Ventricular Developed Pressure (LVDP), and ($dp/dt_{max}$) were all derived from the continuously obtained pressure signal. Aortic flow (AF) was measured using a calibrated flow meter (Gilmont Instrument Inc., Barrington, IL, USA) and coronary flow (CF) was measured by timed collection of the coronary effluent dripping from the heart [29,30].

### 2.5. Measurement of infarct

At the end of reperfusion, the heart was perfused with a 1% (w/v) solution of triphenyl tetrazolium chloride in phosphate buffer through the aortic cannula for 1 min at 37 °C. To quantify the areas of interest in pixels, Scion image analysis software was used. The infarct size was quantified and was expressed in percentage [29,30].

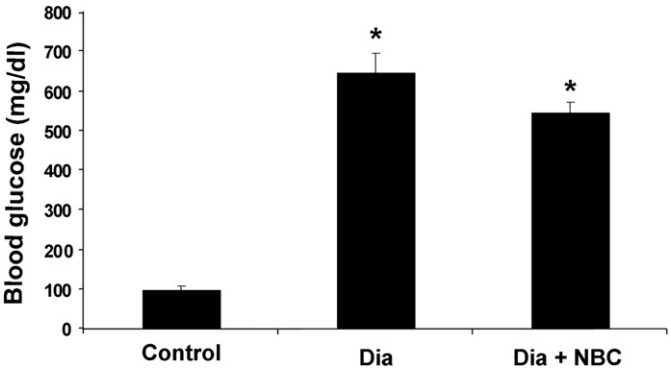


**Fig. 1.** Effect of NBC on blood glucose levels. *$p$<0.05 compared with control.