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Evidence That Chromium Modulates Cellular Cholesterol Homeostasis and ABCA1 Functionality Impaired By Hyperinsulinemia

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Abstract

Objective—Trivalent chromium (Cr^{3+}) is an essential micronutrient. Findings since the 1950s suggest that Cr^{3+} might benefit cholesterol homeostasis. Here we present mechanistic evidence in support of this role of Cr^{3+}.

Method and Results—High-density lipoprotein cholesterol generation in 3T3-L1 adipocytes, rendered ineffective by hyperinsulinemia, known to accompany disorders of lipid metabolism was corrected by Cr^{3+}. Mechanistically, Cr^{3+} reversed hyperinsulinemia-induced cellular cholesterol accrual and associated defects in cholesterol transporter ABCA1 trafficking and apolipoprotein A1-mediated cholesterol efflux. Moreover, direct activation of AMP-activated protein kinase (AMPK), known to be activated by Cr^{3+}, and/or inhibition of hexosamine biosynthesis pathway (HBP) activity, known to be elevated by hyperinsulinemia, mimics Cr^{3+} action.

Conclusion—These findings suggest a mechanism of Cr^{3+} action that fits with long-standing claims of its role in cholesterol homeostasis. Furthermore, these data implicate a mechanistic basis for the coexistence of dyslipidemia with hyperinsulinemia.

Keywords

Cholesterol; Chromium; Dyslipidemia; Hyperinsulinemia; Lipoprotein

Trivalent chromium (Cr^{3+}) is classified as an essential micronutrient for optimal carbohydrate and lipid metabolism. Although evidence relating Cr^{3+} deficiency and cardiovascular disease (CVD) is fragmentary, deficiency has been linked to reduced high-density lipoprotein cholesterol (HDL-C)[1]. A rate-limiting step in HDL-C generation entails cholesterol transporter ABCA1-mediated cholesterol efflux to lipid-poor apolipoprotein A1 (ApoA1). The HDL-C particle formed is preβ-1 HDL-C, a subclass which removes cholesterol from macrophages[2], a cardioprotective event. These findings raise the question of whether an essential mechanism of Cr^{3+} action involves ABCA1/ApoA1-mediated preβ-1 HDL-C generation. Importantly, ABCA1/ApoA1 dysregulation may represent an

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unappreciated basis of low HDL-C coexisting with metabolic derangements (e.g., hyperinsulinemia).

Methods

Insulin-sensitive and hyperinsulinemia-induced insulin-resistant 3T3-L1 adipocytes and Cr^{3+} in the picolinate form (CrPic) at a 1 μM dose were used as previously described[3-4]. Detailed methods and information on Cr^{3+} doses/forms tested are provided in online material (http://www.atcb.ahahournals.org).

Results

Examination of ABCA1 trafficking revealed that plasma membrane (PM) ABCA1 was diminished by hyperinsulinemic conditions relevant to disease[3], yet in the presence of Cr^{3+} this was prevented (Fig. 1A). Endosomal membrane (EM) ABCA1 was elevated by hyperinsulinemia and normalized by Cr^{3+} (Fig. 1B). Total ABCA1 protein was not changed (Fig. S1A). Mechanistically, ABCA1 is regulated by the EM-to-cytosol (Cyto) cycling of the GTPase Rab8. Hyperinsulinemia increased and decreased EM- and Cyto-Rab8, respectively, and these changes were normalized by Cr^{3+} (Figs. 1C, D).

Cholesterol accumulation has been implicated in EM ABCA1 sequestration in Niemann-Pick disease, type C (NPC)[5]. Like NPC, a substantial increase in EM cholesterol was found in cells cultured under hyperinsulinemic conditions that Cr^{3+} prevented (Fig. 2A). Interestingly, exercise is recognized to increase HDL-C levels, and like exercise, Cr^{3+} increases AMP-activated protein kinase (AMPK) activity[4], known to suppress cholesterol synthesis[6]. AICAR (5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside, an AMPK activator) and Cr^{3+} stimulated AMPK (Fig. 2B), and similarly to Cr^{3+}, AICAR lowered EM cholesterol (Fig. 2C) and corrected membrane Rab8/ABCA1 levels (Figs. S1B-E); however, a gain in Cyto-Rab8 was not seen; likely due to a shorter AICAR treatment duration not permitting a detectable level of Rab8 to accumulate in the dilute cytosol fraction. Importantly, Cr^{3+} and AICAR both prevented hyperinsulinemia-impaired ApoA1-mediated cholesterol efflux (Fig. 2D).

Contrasting AMPK, increased hexosamine biosynthesis pathway (HBP) activity has been implicated in cholesterol accrual induced by hyperinsulinemia[7]. Testing the effect of the HBP inhibitor 6-Diazo-5-Oxo-L-Norleucine (DON) revealed Cr^{3+}- and AICAR-like effects (Fig. 2D). Neither agent nor Cr^{3+} displayed any effect on control cells. Also, cholesterol lowering with methyl-β-cyclodextrin mimicked the protective effect on ApoA1-mediated cholesterol efflux (Fig. S1F).

Discussion

The role of Cr^{3+} in health and disease is complex. While patients with diabetes on Cr^{3+} supplementation see improvement in hyperglycemia, benefits on raising HDL-C remain unclear[8]. An emerging appreciation is that total HDL-C measurements are misleading in understanding its cardioprotective actions, as the ABCA1-generated preβ-1 HDL-C particle likely represents the “functional” subfraction[2]. Therefore, study demonstrating that Cr^{3+} enhances this ABCA1-mediated event in cells cultured in a diabetic milieu is significant.

As the serum concentration of the preβ-1 HDL-C accounts for only a small fraction of total HDL-C, trials designed to assess the benefits of Cr^{3+} on total HDL-C may have had an inherent flaw in understanding Cr^{3+}’s effect. In addition, Cr^{3+} deficiency in humans is expected to be slight, if any[9], thus measurement of a supplemental effect may be negligible. Nevertheless, analyses reveal popular weight loss diets provide Cr^{3+} at suboptimal levels[10].

Mechanistically, observation that AMPK stimulation ramps up ABCA1/ApoA1 functionality is interesting, given the appreciated benefits of exercise, a known stimulant of AMPK activity, on the prevention of metabolic syndrome and its consequences. In this regard, skeletal muscle and adipose tissue contain more cholesterol than any other organ[11]. In fact, a new importance of adipose tissue cholesterol in the generation of HDL-C has recently been recognized[12-13]. In particular, the generation of preβ-1 HDL-C appears critical in mediating cholesterol efflux from cholesterol-laden macrophages. The idea Cr^{3+} could have an indirect effect on cholesterol handling by macrophages is of interest. Testing this possibility as well as characterizing any direct effect Cr^{3+} may have on macrophage cholesterol metabolism is warranted.

In closing, these data suggest low circulating HDL-C, resulting from metabolic disorder, may arise from hyperinsulinemia/HBP-mediated peripheral tissue cholesterol accrual (Fig. 2E). This is associated with an EM sequestration of Rab8/ABCA1, and low preβ-1 HDL-C. Data also implicate that Cr^{3+} suppresses cholesterol synthesis/accrual via AMPK and this improves Rab8/ABCA1 functionality and HDL-C generation. Whether this cell-based model explains the benefits of Cr^{3+} and/or exercise in humans with diabetes remains to be validated.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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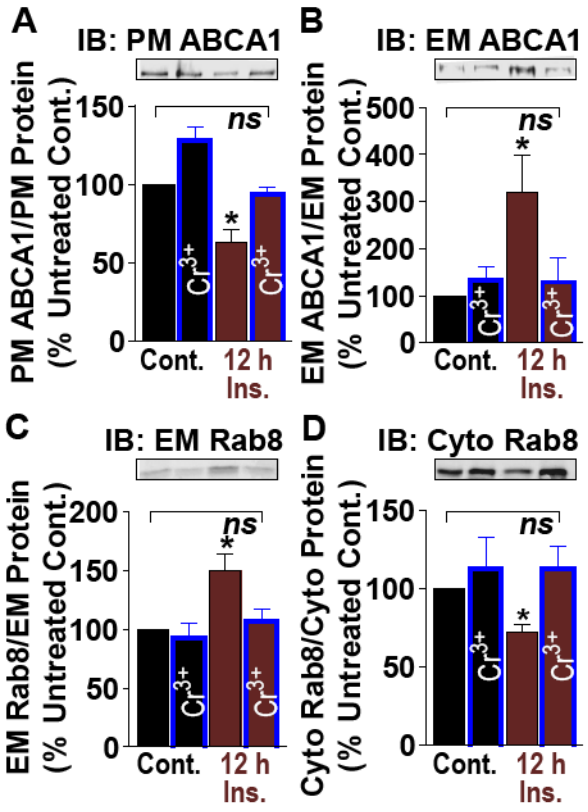

Fig. 1.

Control (Cont.) or hyperinsulinemic (12h Ins.) cells were treated without or with Cr^{3+}. (**A**) Plasma membrane (PM) ABCA1, (**B**) Endosomal membrane (EM) ABCA1, (**C**) EM Rab8, and (**D**) Cytosolic Rab8. n = 4-11. *$P < 0.05$ versus untreated control.

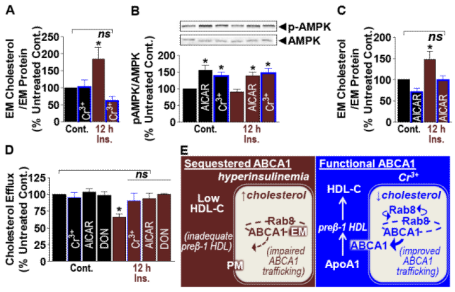

Fig. 2.

Control (Cont.) or hyperinsulinemic (12h Ins.) cells were treated without or with Cr^{3+}, AICAR, or DON. (**A, C**) EM cholesterol, (**B**) pAMPK, and (**D**) ApoA1-mediated cholesterol efflux. n = 4-11. *$P < 0.05$ versus untreated control. (**E**) Model of Cr^{3+} protection against (Blue) hyperinsulinemia-induced cholesterol-associated impairment in (Red) Rab8/ABCA1 trafficking and ApoA1-mediated cholesterol efflux.