Evidence That Chromium Modulates Cellular Cholesterol Homeostasis and ABCA1 Functionality Impaired by Hyperinsulinemia—Brief Report

Whitney Sealls, Brent A. Penque, Jeffrey S. Elmendorf

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\(^{3+}\).

Methods and Results—High-density lipoprotein cholesterol generation in 3T3-L1 adipocytes, which are rendered ineffective by the hyperinsulinemia that is 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 ATP-binding cassette transporter-A1 trafficking and apolipoprotein A1–mediated cholesterol efflux. Moreover, direct activation of AMP-activated protein kinase, which is known to be activated by Cr\(^{3+}\), or inhibition of hexosamine biosynthesis pathway activity, which is 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 imply a mechanistic basis for the coexistence of dyslipidemia with hyperinsulinemia.

Key Words: ABC transporter ■ apolipoproteins ■ diabetes mellitus ■ lipoproteins ■ chromium

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 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 pre-β-1 HDL-C, a subclass that 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 pre-β-1 HDL-C generation. Importantly, ABCA1/ApoA1 dysregulation may represent an unappreciated basis of low HDL-C coexisting with metabolic derangements (eg, hyperinsulinemia).

Methods

Insulin-sensitive and hyperinsulinemia-induced insulin-resistant 3T3-L1 adipocytes and Cr\(^{3+}\) in the picolinate form at a 1 \(\mu\)mol/L dose were used as previously described.\(^{3,4}\) Detailed methods and information on Cr\(^{3+}\) doses and forms tested are provided in the supplemental material, available online at http://atvb.ahajournals.org.

Results

Examination of ABCA1 trafficking revealed that plasma membrane ABCA1 was diminished by hyperinsulinemic conditions relevant to disease,\(^{3}\) yet in the presence of Cr\(^{3+}\), this was prevented (Figure 1A). Endosomal membrane (EM) ABCA1 was elevated by hyperinsulinemia and normalized by Cr\(^{3+}\) (Figure 1B). Total ABCA1 protein was not changed (Supplemental Figure IA).

Mechanistically, ABCA1 is regulated by the EM-to-cytosol (Cyto) pathway activity, which is known to be elevated by hyperinsulinemia, and Cr\(^{3+}\) mimics Cr\(^{3+}\) action. Cholesterol accumulation has been implicated in EM ABCA1 sequestration in Niemann-Pick disease, type C.\(^{5}\) As in Niemann-Pick disease, type C, a substantial increase in EM cholesterol was found in cells cultured under hyperinsulinemic conditions that Cr\(^{3+}\) prevented (Figure 2A). Interestingly, exercise is recognized to increase HDL-C levels, and like exercise, Cr\(^{3+}\) increases AMP-activated protein kinase (AMPK) activity,\(^{6}\) which is known to suppress cholesterol synthesis.\(^{7}\) 5-Aminomidazole-4-carboxamid-1,β-d-ribofuranoside (AICAR), an AMPK activator, and Cr\(^{3+}\) stimulated AMPK (Figure 2B), and similarly to Cr\(^{3+}\), AICAR lowered EM cholesterol (Figure 2C) and corrected membrane Rab8/ABCA1 levels (Supplemental Figure IB to IE); however, a gain in Cyto-Rab8 was not seen, likely because of 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 (Figure 2D).

In contrast to AMPK, increased hexosamine biosynthesis pathway activity has been implicated in cholesterol accrual induced by
hyperinsulinemia. Testing the effect of the hexosamine biosynthesis pathway inhibitor 6-diazo-5-oxo-l-norleucine revealed Cr\textsuperscript{3+} and AICAR-like effects (Figure 2D). Neither agent nor Cr\textsuperscript{3+} displayed any effect on control cells. Also, cholesterol lowering with methyl-\(\beta\)-cyclodextrin mimicked the protective effect on ApoA1-mediated cholesterol efflux (Supplemental Figure IF).

**Discussion**

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

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

Mechanistically, the 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, the importance of adipose tissue cholesterol in the generation of HDL-C has recently been recognized.\(^12,13\) In particular, the generation of pre-beta-1 HDL-C appears to be critical in mediating cholesterol efflux from cholesterol-laden macrophages. The idea that Cr\textsuperscript{3+} could have an indirect effect on cholesterol handling by macrophages is of interest. Testing this possibility, as well as characterizing any direct effect that Cr\textsuperscript{3+} may have on macrophage cholesterol metabolism, is warranted.

In closing, these data suggest that low circulating HDL-C, resulting from metabolic disorder, may arise from hyperinsulinemia/hexosamine biosynthesis pathway-mediated peripheral tissue cholesterol accrual (Figure 2E). This is associated with an EM sequestration of Rab8/ABCA1 and low pre-beta-1 HDL-C. Data also imply that Cr\textsuperscript{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\textsuperscript{3+} or exercise in humans with diabetes remains to be validated.

**Acknowledgments**

We thank Nutrition 21 for providing the Cr\textsuperscript{3+} in the picolinate form.

**Sources of Funding**

This work was supported by National Institutes of Health Grants AT001846, DK082773, and DK082773-01S1 (to J.S.E.) and Indiana Center for Vascular Biology Grant HL079995 (to W.S.).

**Disclosures**

None.

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Arterioscler Thromb Vasc Biol. 2011;31:1139-1140; originally published online February 10, 2011;
doi: 10.1161/ATVBAHA.110.222158
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/31/5/1139

Data Supplement (unedited) at:
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**Supplemental Material**

**Fig. I**

**Fig. S1.** Control (Cont.) or hyperinsulinemic (12h Ins) cells were treated without or with Cr³⁺, AICAR or βCD. (A) Whole cell ABCA1, (B) PM ABCA1, (C) EM ABCA1, (D) EM Rab8, (E) Cyto Rab8, and (E) ApoA-1-mediated cholesterol efflux. N=3-4. *P<0.05 versus untreated control.
Supporting Online Material for

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

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This PDF file includes:

Materials and Methods

Fig. S1

References
MATERIALS AND METHODS

Cell Culture. Murine 3T3-L1 preadipocytes were purchased from Dr. Howard Green (Harvard Medical School) and used as previously described S1. Briefly, cells were cultured in DMEM containing 25 mM glucose and 10% calf serum at 37°C in an 8% CO₂ atmosphere. Confluent cultures were induced to differentiate into adipocytes as previously described S2. All studies were performed on adipocytes which were between 8 and 12 days post-differentiation. Groups supplemented with Cr³⁺ were treated with 1 µM Cr³⁺ picolinate (CrPic) for 16 h as previously described S2. Note that numerous control experiments we have performed have consistently shown similar cholesterol lowering action by other forms of chromium (e.g. chloride-bound, CrCl; niacin-bound, CrN) and at pharmacologically relevant doses. Because the use of CrPic best allows for comparisons with our previous studies and the work of others as this is the most commonly used form in such studies, the current study used CrPic, initiated 4 h before chronic exposure to insulin. The AICAR (1 mM) treatment was performed following the 12 h insulin exposure for 45 min, and the DON (20 µM) and βCD (300 µM) treatments were for 12 h during the overnight insulin exposure. Note the low βCD dose used has previously been documented to effectively lower endosomal membrane cholesterol content S3.

Cholesterol Efflux. ApoA1-mediated cholesterol efflux was determined as described elsewhere S4. Briefly, adipocytes were labeled with 0.5 µCi/mL ³H-cholesterol (Sigma Aldrich) for 24 hours in 25 mM glucose DMEM containing 0.2% BSA. Cells were then washed and incubated in the absence or presence of 5 nM insulin to induce insulin resistance (as described above). Cells were then incubated in 25 mM glucose DMEM containing 0.2% BSA and 10 µg/mL lipid-free
ApoA1 for 4 hours. This was followed by measuring \(^3\)H-cholesterol in the medium and in the cells. The percentage of acceptor-specific efflux was calculated using the following equation: 
\[
\text{medium}/(\text{medium} + \text{cells})
\]
Values obtained in the absence of acceptor were subtracted to account for non-specific \(^3\)H-cholesterol efflux/leakage.

**Subcellular Fractionation and Western Blotting.** Plasma membrane (PM), endosomal membrane (EM), and whole cell lysate fractions were isolated as described in \(^5\). After addition of 1% NP40 detergent to the prepared fractions, total protein recovered was determined by the Bradford method. Proteins were separated by SDS-PAGE and immunoblotted with either an ABCA1 (Abcam), Rab8 (BD Biosciences), or AMPK (Cell Signaling) antibody, followed by an IRDye™ 700DX or 800DX conjugated secondary (Rockland). Immunoblots were analyzed by Li-COR Odyssey infrared imaging quantification. For subcellular fractions, protein loading was normalized to Ponceau staining and quantified by ImageJ software. For whole cell lysate, blots were normalized to total AMPK or \(\beta\)-actin. Using a portion of the EM fraction, cholesterol was also measured as previously described \(^2\).

**Statistical Analyses.** Values are presented as means ±SE. Differences between two groups were analyzed by the Student’s \(t\)-test for independent samples. GraphPad Prism 4 software was used for all analyses. \(P < 0.05\) was considered significant.
SUPPLEMENTAL REFERENCES


