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 (Cr3+) is an essential micronutrient. Findings since the 1950s suggest that Cr3+ might benefit cholesterol homeostasis. Here we present mechanistic evidence in support of this role of Cr3+.

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 Cr3+. Mechanistically, Cr3+ 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 Cr3+, or inhibition of hexosamine biosynthesis pathway activity, which is known to be elevated by hyperinsulinemia, mimics Cr3+ action.

Conclusion—These findings suggest a mechanism of Cr3+ 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 (Cr3+) is classified as an essential micro-nutrient for optimal carbohydrate and lipid metabolism. Although evidence relating Cr3+ deficiency and cardiovascular disease is fragmentary, deficiency has been linked to reduced high-density lipoprotein cholesterol (HDL-C). A rate-limiting step in HDL-C generation entails cholesterol transporter ABCA1-mediated cholesterol efflux to lipopoor apolipoprotein A1 (ApoA1). The HDL-C particle formed is pre-β-1 HDL-C, a subclass that removes cholesterol from macrophages, a cardioprotective event. These findings raise the question of whether an essential mechanism of Cr3+ 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 Cr3+ in the picolintone form at a 1 μmol/L dose were used as previously described. Detailed methods and information on Cr3+ 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, yet in the presence of Cr3+, this was prevented (Figure 1A). Endosomal membrane (EM) ABCA1 was elevated by hyperinsulinemia and normalized by Cr3+ (Figure 1B). Total ABCA1 protein was not changed (Supplemental Figure IA). 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 Cr3+ (Figure 1C and 1D).

Cholesterol accumulation has been implicated in EM ABCA1 sequestration in Niemann-Pick disease, type C. As in Niemann-Pick disease, type C, a substantial increase in EM cholesterol was found in cells cultured under hyperinsulinemic conditions that Cr3+ prevented (Figure 2A). Interestingly, exercise is recognized to increase HDL-C levels, and like exercise, Cr3+ increases AMP-activated protein kinase (AMPK) activity, which is known to suppress cholesterol synthesis. 5-Aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR), an AMPK activator, and Cr3+ stimulated AMPK (Figure 2B), and similarly to Cr3+, 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, Cr3+ 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...
hypermagnesemia.7 Testing the effect of the hexosamine biosynthesis pathway inhibitor 6-diaz o-5-oxo-L-norleucine revealed Cr3++.

The role of Cr3++ in health and disease is complex. Although patients with diabetes on Cr3++ 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-β 1-HDL-C particle likely represents the “functional” subfraction.2 Therefore, study demonstrating that Cr3++ enhances this ABCA1-mediated event in cells cultured in a diabetic milieu is significant.

As the serum concentration of the pre-β 1-HDL-C accounts for only a small fraction of total HDL-C, trials designed to assess the benefits of Cr3++ on total HDL-C may have had an inherent flaw in understanding Cr3++’s effect. In addition, Cr3++ 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 Cr3++ 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-β 1-HDL-C appears to be critical in mediating cholesterol efflux from cholesterol-laden macrophages. The idea that Cr3++ could have an indirect effect on cholesterol handling by macrophages is of interest. Testing this possibility, as well as characterizing any direct effect that Cr3++ 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-β 1-HDL-C. Data also imply that Cr3++ 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 Cr3++ or exercise in humans with diabetes remains to be validated.

Discussions

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Disclosures

None.

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Fig. S1. Control (Cont.) or hyperinsulinemic (12 h 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. *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 \(^{51}\). Briefly, cells were cultured in DMEM containing 25 mM glucose and 10% calf serum at 37°C in an 8% CO\(_2\) atmosphere. Confluent cultures were induced to differentiate into adipocytes as previously described \(^{52}\). All studies were performed on adipocytes which were between 8 and 12 days post-differentiation. Groups supplemented with Cr\(^{3+}\) were treated with 1 µM Cr\(^{3+}\) picolinate (CrPic) for 16 h as previously described \(^{52}\). 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 \(^{53}\).

Cholesterol Efflux. ApoA1-mediated cholesterol efflux was determined as described elsewhere \(^{54}\). Briefly, adipocytes were labeled with 0.5 µCi/mL \(^3\)H-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: medium/(medium+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 β-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


