3 INHIBITION OF THE RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS RESTORES CHOLESTEROL EFFLUX IN THP-1 HUMAN MACROPHAGES EXPOSED TO PLASMA FROM TYPE 1 DIABETES MELLITUS PATIENTS

JM Corletto,^{1,3*} S Accacha,^{1,3} I Voloshyna,^{2,3} N Siegart,^{2,3} L Kasselmann,^{2,3} MC Magana,¹ J DeLeon,^{2,3} AB Reiss^{2,3}. ¹Pediatric Endocrinology, Winthrop University Hospital, Mineola, NY, United States; ²Medicine, Winthrop University Hospital, Mineola, NY, United States; ³Winthrop Research Institute, Winthrop University Hospital, Mineola, NY, United States

10.1136/jim-2016-000080.3

Purpose of Study Advanced glycation end products (AGE), proteins formed by nonenzymatic glycation, are prevalent in patients with diabetes mellitus (DM) and contribute to the development of atherosclerosis. We have demonstrated that plasma from patients with type 1 DM (T1DM) decreases expression of cholesterol efflux proteins in cultured THP-1 macrophages compared to healthy control (HC) plasma. Here we explore a mechanism that restores cholesterol efflux in T1DM plasma through blockade of the receptor for AGE (RAGE).

Methods Used Carboxy-methyl-lysine-modified proteins (CML-MP), the most prevalent AGE *in vivo*, were measured in the plasma of 20 pediatric T1DM patients and 20 sex and age-matched HC by ELISA. THP-1 macrophages ($10^6/\text{ml}$) were incubated for 18 h in RPMI media in the presence of 10% plasma from each enrolled patient in triplicate $\pm$ anti-RAGE antibody. Cholesterol efflux proteins: ATP binding cassette transporter (ABC)A1 and 27-hydroxylase were quantified by real-time RT-PCR using specific primers for each gene.

Summary of Results The level of CML-MP was significantly elevated in T1DM plasma (1.65 ± 1.3 ng/ml) versus HC plasma (1.05 ± 0.4 ng/ml) ($P < 0.05$, $n = 20$). ABCA1 expression was significantly lower in macrophages exposed to T1DM plasma (1.108 ± 0.8 U) versus HC plasma (1.624 ± 0.6) ($P < 0.05$, $n = 20$). Exposure of THP-1 macrophages to T1DM plasma downregulated 27-hydroxylase mRNA to 1.94 ± 0.9 U in T1DM versus 3.4 ± 2.9 U in HC ($P < 0.01$, $n = 20$). Inactivation of RAGE before exposure to T1DM plasma increased ABCA1 expression by 20%. However, we observed no effect on the level of 27-hydroxylase.

Conclusions We demonstrate that elevated AGE in plasma of T1DM patients inhibits cholesterol efflux and suppresses intracellular cholesterol processing via 27-hydroxylase and ABCA1 in naïve macrophages. RAGE inactivation restores mRNA level of the ABCA1 transporter, but not the 27-hydroxylase enzyme. These findings impart new targets for prevention of cardiovascular disease in DM and suggest that factors other than AGE may impact cholesterol transport.