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Functional and proteomic alterations of plasma high density lipoproteins in type 1 diabetes mellitus

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ABSTRACT

Objective. Higher HDL-cholesterol (HDL-C) is linked to lower cardiovascular risk but individuals with type 1 diabetes mellitus (T1DM) with normal or high HDL-C have higher cardiovascular events compared to age matched non-diabetic controls (ND). We determined whether altered HDL functions despite having normal HDL-C concentration may explain increased cardiovascular risk in T1DM individuals. We also determined whether irreversible posttranslational modifications (PTMs) of HDL bound proteins occur in T1DM individuals with altered HDL functions.

Methods. T1DM with poor glycemic control (T1D-PC, HbA1c ≥ 8.5%, n = 15) and T1DM with good glycemic control (T1D-GC, HbA1c ≤ 6.6%, n = 15) were compared with equal numbers of NDs, ND-PC and ND-GC respectively, matched for age, sex and body mass index (BMI). We measured cholesterol efflux capacity (CEC) of HDL in the serum using J774 macrophages, antioxidant function of HDL as the ability to reverse the oxidative damage of LDL and PON1 activity using commercially available kit. For proteomic analysis, HDL was isolated by density gradient ultracentrifugation and was analyzed by mass spectrometry and shotgun proteomics method.

Results. Plasma HDL-C concentrations in both T1DM groups were similar to their ND. However, CEC (%) of T1D-PC (16.9 ± 0.8) and T1D-GC (17.1 ± 1) were lower than their respective ND (17.9 ± 1, p = 0.01 and 18.2 ± 1.4, p = 0.02). HDL antioxidative function also was lower (p < 0.05). The abundance of oxidative PTMs of apolipoproteins involved in CEC and antioxidative functions of HDL were higher in T1D-PC (ApoA4, p = 0.041) and T1D-GC (ApoA4, p = 0.025 and ApoE, p = 0.041) in comparison with ND. Both T1D-PC and T1D-GC groups had higher abundance of amadori modification of ApoD (p = 0.002 and p = 0.041 respectively) and deamidation modification of ApoA4 was higher in T1D-PC (p = 0.025).

Conclusions. Compromised functions of HDL particles in T1DM individuals, irrespective of glycemic control, could be explained by higher abundance of irreversible PTMs of HDL proteins. These results lend mechanistic support to the hypothesis that HDL quality rather than quantity

Abbreviations: T1DM, type 1 diabetes mellitus; CVD, cardiovascular disease; HDL-C, HDL-cholesterol; ROS, reactive oxygen species; PTMs, posttranslational modifications; ND, non-diabetic controls; CEC, cholesterol efflux capacity; PON1, paraoxonase 1; T1D-PC, type 1 diabetes mellitus individuals with poor glycemic control; T1D-GC, type 1 diabetes mellitus individuals with good glycemic control; CV, coefficient of variation; FDRs, false discovery rates; A2MG, alpha 2 macroglobulin; ITIH2, inter-alpha (globulin) inhibitor 2; SAA2, serum amyloid a2; ITIH3, inter-alpha (globulin) inhibitor 3.

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determines HDL function in T1DM and suggest that measurements of concentrations of HbA1c and HDL-C are not sufficient as biomarkers of effective treatment to lower cardiovascular risk in T1DM individuals.

1. Introduction

Type 1 diabetes mellitus (T1DM) is a chronic condition with multiple complications, significantly affecting the quality of life and reducing the life span. Globally, 7% to 12% of the 415 million individuals with diabetes are estimated to have T1DM [1]. Improved insulin replacement therapies and meticulous glycemic control in particular have significantly decreased the microvascular complications associated with T1DM [2,3]. However, the effect of improved glycemic control on cardiovascular disease (CVD) complications in T1DM individuals is less impressive and the cause of persistently higher macrovascular disease in T1DM is not well understood [4,5]. Large population studies have linked higher HDL-cholesterol (HDL-C) concentrations to lower cardiovascular risk [6] and longer life span [7]. In contrast, in T1DM individuals, despite having normal or high HDL-C [8], CVD events are more common and occur earlier than in non-diabetic populations [9]. T1DM individuals have higher cardiovascular deaths than age matched non-diabetic individuals with an age-adjusted relative risk for CVD in T1DM being 10 times that of the general population. The biological mechanisms underlying this paradox remain to be fully understood [9]. There is emerging evidence indicating that elevated HDL-C levels may not always result in reduced CVD risk. Recent Mendelian randomization genetic studies have suggested that many genetic variants associated with increases in HDL-C are not correlated with a reduction in CVD risk [10] and latest clinical trials of various drug interventions to raise HDL-C levels have failed to show benefits in increased HDL-C levels in reducing the CVD mortality [11]. It has been proposed that the functional capacity of the HDL might be a more important determinant of cardiovascular risk than the HDL-C concentrations [12].

It remains to be determined whether glycemic control is a determinant of HDL function since good glycemic control has less impact on cardiovascular risks in T1DM than on microvascular complications. Glycemic control is assessed by measurement of glycated hemoglobin (HbA1c), but T1DM individuals have greater glucose variability irrespective of their HbA1c levels as HbA1c levels identify states of sustained hyperglycemia but are unaffected by hypoglycemic episodes and short lived glucose spikes [13–15] thus revealing the shortcomings of HbA1c as the "gold standard" indicator of metabolic control. In addition, it is well recognized that glucose data in T1DM are not normally distributed and thus HbA1c has limited correlation with glucose control in the disorder [16]. Oscillating glucose levels observed in T1DM individuals with poor and good glycemic controls are associated with increase in reactive oxygen species (ROS) and hyperglycemic episodes could induce oxidative stress resulting in diabetic complications through several mechanisms [4]. Increased oxidative stress and increased resting O_2 consumption during insulin deprivation have been reported in T1DM [17]. In this context, higher O_2 consumption is associated with inefficient mitochondrial coupling [18] and accelerated oxidative damage to ApoA1 protein, a major constituent of HDL [19] and this damage may critically affect protein quality. The functional capacity of HDL is in turn determined by its proteome quality, which could be impaired by irreversible posttranslational modifications (PTMs) caused by hyperglycemia and oxidative stress.

In the current study, we sought to determine HDL function and protein quality differences between non-diabetic controls (ND) and T1DM individuals with good glycemic control and poor glycemic control. We hypothesized that higher oxidative stress and glycemic variability that occur in T1DM individuals, irrespective of their glycemic control as assessed by HbA1c levels, would cause irreversible PTMs to HDL proteins, resulting in diminished functional capacity. To test this, we measured three major functions of serum HDL particles: cholesterol efflux capacity (CEC), antioxidative capacity and paraoxonase 1 (PON1) activity. We also analyzed the HDL proteome for PTMs.

2. Methods

2.1. Study Participants

The study protocol was approved by the Mayo Foundation Institutional Review Board and conformed to the principles outlined in the Declaration of Helsinki. Informed written consent was obtained from all the participants. We studied 15 T1DM individuals with poor glycemic control (T1D-PC, HbA1c ≥ 8.5% (69.4 mmol/mol)) and 15 T1DM individuals with good glycemic control (T1D-GC, HbA1c ≤ 7.0% (53 mmol/mol)). We matched the 30 T1DM individuals, at enrollment, to 30 ND individuals (fasting glucose ≤ 100 mg/dL; 15 ND per each group, ND-PC and ND-GC respectively) for age, sex and body mass index (BMI). All study participants underwent detailed history, physical examination, and hematological and biochemical profiling. All participants were nonsmokers, with normal systolic and diastolic blood pressures and with total leukocyte counts within normal limits of the physiological range (to rule out any acute/chronic inflammatory conditions). Participants that fit any of the following criteria were excluded from the study: active kidney disease, liver disease, debilitating chronic diseases, serum creatinine >1.4 mg/dL for women or >1.5 mg/dL for men, serum transaminase elevation ≥3 times the upper limit of normal range, participants with type 2 diabetes mellitus or children under the age of 18.

2.2. Study Protocol

All study participants were placed on a standard weight-maintaining diet (protein/carbohydrate/fat, 15%:55%:30% by calories) by the Mayo Clinic Clinical Research Unit for 3 consecutive outpatient days. On the third day, the participants were admitted to the Clinical Research Unit at 17:00 h