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Decreased plasma levels of select very long chain ceramide species Are associated with the development of nephropathy in type 1 diabetes

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ABSTRACT

Objective. Sphingolipid metabolism is altered in diabetes and we analyzed the plasma concentrations of sphingolipid species to investigate their association with the development of albuminuria in type 1 patients with diabetes.

Materials and Methods. Samples were collected from 497 type 1 diabetic patients during their enrollment into the Diabetes Control and Complications Trial (DCCT). We determined plasma concentrations of multiple ceramide species and individual sphingoid bases and their phosphates using high performance liquid chromatography-tandem mass spectrometry and investigated their association with the development of albuminuria during 14–20 years of follow-up.

Results. Patients exhibited normal albumin excretion rates (AER <40 mg/24 h) at the time of plasma sampling. Although the majority of patients (N = 291; 59%) exhibited normal levels of albuminuria throughout follow-up, 141 patients (28%) progressed to microalbuminuria (40 mg/24 h ≤ AER < 300 mg/24 h), while 65 (13%) progressed to macroalbuminuria (AER ≥300 mg/24 h). To test the association of log transformed plasma sphingolipid level with the development of albuminuria, generalized logistic regression models were used where normal, micro- and macroalbuminuria were the outcomes of interest. Models were adjusted for DCCT treatment group, baseline retinopathy, gender, baseline HbA1c %, age, AER, lipid levels, diabetes duration, and the use of ACE/ARB drugs.

Abbreviations: DCCT, Diabetes Control and Complications Trial; EDIC, Epidemiology of Diabetes Interventions and Complications; AER, Albumin excretion rate; ETDRS, Early treatment diabetic retinopathy study score; Cer, Ceramide; LC-ESI-MS/MS, High performance liquid chromatography-tandem mass spectrometry with an electrospray ion source; MRM, Multiple reaction monitoring; BMI, Body mass index; eGDR, Estimated glucose disposal rate; S1P, Sphingosine 1-phosphate; ACE/ARB, Angiotensin-converting enzyme inhibitors/Angiotensin II Receptor Blockers; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases.

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Increased plasma levels of very long, but not long chain ceramide species measured at DCCT baseline were associated with decreased odds to develop macroalbuminuria during the subsequent nineteen years (DCCT Baseline to EDIC year 8).

Conclusion. These studies demonstrate, prospectively, that decreased plasma levels of select ceramide species are associated with the development of macroalbuminuria in type 1 diabetes.

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1. Introduction

Diabetic nephropathy is the major cause of end-stage renal failure and one of the major causes of morbidity and mortality in diabetes [1]. Overt nephropathy is usually preceded by increased albuminuria [2] which is not only associated with the risk of developing renal insufficiency [3,4] but also with the development and progression of cardiovascular disease [5] in patients with diabetes. Thus, there is considerable interest in determining the mechanisms responsible for albuminuria and in identifying early markers that may be associated with this complication of diabetes.

The pathological mechanism(s) that are related to the development of increased urine albumin are not well defined. It has been suggested that dyslipidemia is a contributing factor to complications of diabetes and that modification in the metabolism of lipids may ultimately contribute to renal and other complications in diabetic subjects [6]. Circulating lipoproteins (HDL, LDL, and VLDL) and albumin are the carriers of sphingolipids in plasma. Sphingolipids are important constituents of cell membranes and in the last decade their role in cell signaling and activation has been extensively studied. Due to the complexity of the analyses and because of the expense of the technique and instrumentation necessary for quantitation of the different species of sphingolipids, efforts to measure blood sphingolipids and to use these measures as diagnostic and prognostic tools have been very limited. We [7–9] and others [10–12] have investigated human sphingolipidomics in plasma and in lipoproteins isolated from non-diabetic subjects using advanced mass spectroscopy techniques.

Although sphingolipids comprise only a small fraction of plasma lipids and hence, lipoprotein lipids, there is mounting evidence that sphingolipid metabolism is altered in diabetes and specific sphingolipid classes may contribute to diabetic complications [6]. In a cross sectional study of 326 type 1 diabetes patients enrolled in the Finnish Diabetic nephropathy Study (FinnDiane), plasma sphingomyelin levels emerged as a biochemical covariate of urinary albumin excretion rate [13]. Unfortunately, plasma ceramide levels were not investigated in that study. Most of the evidence demonstrating the role of sphingolipids in diabetes has arisen from studies using animal models of diabetes [14] with the focus on ceramide metabolism as it relates to insulin sensitivity [15–20]. The concentrations of plasma sphingosine also were reportedly elevated in a group of type 2 diabetic patients compared with levels in healthy control subjects suggesting that the rate of ceramide metabolism in the cells of diabetic patients was elevated [21]. Plasma ceramide levels are elevated in type 2 diabetic patients compared to levels determined in control subjects, with diabetic patients exhibiting elevated levels of the C18:0, C20:0, C24:1 ceramide species [20].

We investigated the potential association of the plasma concentrations of select ceramide species and of individual sphingoid bases and their phosphates with the future development of nephropathy in a subgroup of a well characterized group of type 1 diabetes patients, the DCCT/EDIC cohort.

2. Methods

2.1. Study subjects

The DCCT was a randomized, clinical trial of 1,441 patients who were 13–39 years of age and had type 1 diabetes for 1–15 years at study entry [22]. In this manuscript we studied a subgroup of patient from the DCCT who had diabetes for 1–15 years and normal albumin excretion rates (<40 mg/24 h) at entry into the study. Some of the patients were free from clinically evident retinopathy (Early Treatment Diabetic Retinopathy Study score, ETDRS = 1), some had mild to moderate non-proliferative diabetic retinopathy (ETDRS 2–9). The participants were randomized into groups that received either intensive or conventional insulin therapy and were followed for an average of 6.5 years. At the baseline DCCT examination, each participant received a complete physical examination which included a medical history, an electrocardiogram, and routine laboratory analyses to determine serum creatinine, lipid profile, and HbA_1c levels [22]. Four-hour urine collections for measurement of albumin excretion rate (AER) and creatinine clearance were also obtained during EDIC on alternate years [23]. The study was terminated early, in 1993, because of the observed major beneficial effect of intensive therapy on retinal, renal, and neurologic complications. In 1994, approximately 95% of the DCCT participants were enrolled into an observational study, the Epidemiology of Diabetes Interventions and Complications (EDIC) study. The goal of the EDIC study was to assess the long-term effects of prior separation of glycemic levels on micro- and macrovascular outcomes in type 1 diabetes [23]. During EDIC, all patients were under the care of their personal physicians and encouraged to practice intensive insulin therapy.

The present study was performed on plasma samples collected from type 1 diabetes patients at the time of their entry into the DCCT (Baseline) before they were randomized into one of the two study treatment arms. Our main aim was to determine whether plasma levels of ceramide species and of sphingoid bases and their phosphates measured at DCCT Baseline would be associated with the development of