Diabetic Complications Consortium

Application Title: Association of Plasma Deoxysphingolipids with Neuropathy in the DCCT/EDIC Cohort

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1. Project Accomplishments:

Neuropathy is the most common chronic complication of diabetes mellitus and clinically, diabetic neuropathy exhibits a pronounced similarity to the neuropathy of patients with hereditary sensory and autonomic neuropathy type 1 (HSAN1). HSAN1 patients exhibit elevated plasma levels of a newly identified sphingolipid class, deoxysphingolipids (DSL) which are generated when L-alanine or L-glycine is used for substrate instead of L-serine in the first biosynthetic step of the sphingolipid pathway. DSL have pronounced neurotoxic effects on neurite formation in cultured sensory neurons and plasma levels of DSL are elevated in Type 2 diabetes patients. Because no studies have reported plasma levels of DSL in Type 1 diabetes patients or in patients with diabetic neuropathy we determined the plasma levels of DSL in banked, frozen plasma obtained from Type 1 diabetes patients with diabetic neuropathy who were enrolled in the DCCT/EDIC cohort and compared the concentrations to those in plasma obtained form Type 1 diabetes patients without neuropathy. We also determined the plasma concentrations of free amino acids necessary for the generation of DSL in the two groups of diabetic patients.

2. Specific Aims:

Aim 1:
Compare the plasma sphingolipidomic profile, including the concentrations of deoxysphingolipids, from Type 1 diabetes patients who exhibit clinically confirmed and documented diabetic neuropathy with that of patients without neuropathy.

RESULTS:

The DCCT used a combination of self-reported symptoms, detailed neurological examinations, and nerve conduction studies to identify symptoms, signs, or electrophysiological evidence of distal symmetrical peripheral neuropathy. The primary neurological end point in the DCCT was the development of “confirmed clinical neuropathy” between baseline and the completion of the DCCT, whereas “definite clinical neuropathy” (symptoms and signs consistent with clinical neuropathy as determined by a board-certified neurologist) served as a secondary endpoint. Surveillance of neuropathy in the EDIC study differs from that in DCCT and is performed annually by the EDIC nurse coordinator or diabetologist using the Michigan

Neuropathy Screening Instrument (MNSI), a 15-item, self-administered patient questionnaire adapted from the Neuropathy Symptom Profile in combination with a structured foot examination. Thus, neuropathy was defined differently in the DCCT and the EDIC follow-up study. The DCCT definition of neuropathy was more specific than the definition based on the MNSI examination because it required the clinician to make a clinical judgment based on all available information about competing explanations for any identified signs. We selected patients exhibiting diabetic neuropathy either documented during DCCT using the more stringent guidelines or during EDIC using the MNSI tool and compared them to patients without documented neuropathy during the same period.

The plasma samples which were analyzed were obtained during the period 1996-1998 from DCCT/EDIC study patients who participated in a Program Project Grant (PPG) entitled “Markers and Mechanisms of Vascular Disease in Diabetes (Lopes-Virella, PI) which we were awarded to study macrovascular and microvascular disease development in diabetic patients. As part of this PPG, we collected samples from approximately 1051 patients already enrolled in the EDIC cohort who agreed to participate in our PPG. We also collaborate with the DCCT/EDIC Research Group of investigators and they provided us with clinical data on all study participants as part of our ongoing collaboration.

For our study, “cases” were defined as those patients who exhibited any symptomology consistent with neuropathy prior to sample pull date, i.e., those who had clinically determined neuropathy at any point during DCCT or during EDIC, those who met the MNSI Patient or Clinical Questionnaire threshold prior to the date of sample pull. This classification resulted in there being 57 cases and 23 “normals” without neuropathy symptoms; matching between cases and controls was not employed during these analyses.

Table 1 details the characteristics of the Type 1 diabetic patients enrolled in the DCCT/EDIC cohort whose plasma samples were analyzed during our investigation. There were no statistically significant differences between the group of patients with neuropathy symptoms compared to the group which exhibited no symptoms of neuropathy with respect to age, weight, BMI, plasma lipid concentrations, systolic and diastolic blood pressure, HbA1c level, or the percentages of subjects enrolled in the Intensive Treatment group or who were a part of the Primary Prevention Retinopathy Cohort during the DCCT. Similarly, there were no significant differences between the two groups in the percentages of patients who were treated with ACE inhibitor medications. There was a marginally significant increase in the albumin excretion rate ($p = 0.07$) in the group of patients which exhibited symptoms of neuropathy, but there were no significant differences in the rates of creatinine clearance between the two groups of patients.

TABLE 1 - Characteristics of Type 1 diabetic patients enrolled in the DCCT/EDIC cohort who exhibited symptoms of neuropathy compared to patients with no neuropathy sympthoms

Characteristic	Overall n=80	Symptoms n=57	No Symptoms n=23	P-Value
Age (y)	40.7 (6.9)	41.1 (7.1)	40.0 (6.5)	0.433
Intensive Treatment Group % (n)	36.3 (29)	33.3 (19)	43.5 (10)	0.393
Primary Prevention Retinopathy Cohort % (n)	40.0 (32)	38.6 (22)	43.5 (10)	0.687
Weight (kg)	80.7 (14.9)	82.3 (14.9)	76.8 (14.5)	0.242
BMI (kg/m^2)	26.8 (4.4)	27.0 (4.6)	26.3 (3.8)	0.759
Total Cholesterol (mg/dl)	176 (19)	176 (20)	175 (16)	0.755
LDL Cholesterol (mg/dl)	101 (16)	102 (16)	98 (14)	0.134
HDL Cholesterol (mg/dl)	61 (13)	60 (13)	63 (12)	0.350
Triglycerides (mg/dl)	68 (24)	68 (24)	68 (26)	0.916
Diastolic Blood Pressure (mm)	74 (8)	74 (8)	73 (10)	0.823
Systolic Blood Pressure (mm)	118 (12)	119 (12)	117 (10)	0.563
HbA1c (%)	8.2 (1.4)	8.3 (1.4)	7.8 (1.3)	0.281
Albumin Excretion Rate [AER] (mg/24 h)	48.9 (154.4)	64.0 (181.0)	11.3 (12.7)	***0.070***
Creatinine Clearance (ml/min)	130.3 (29.9)	129.4 (27.1)	132.5 (36.4)	0.907
ACE Inhibitor Use % (n)	11.3 (9)	10.5 (6)	13.0 (3)	0.712

P-Values for continous characteristics or assay measures are conducted using a Wilcoxon Rank Sum test statisti
P-Values for categorical characteristics are measured using Pearson Chi-Square test statistic
Values reported are Mean ± SD unless otherwise indicated

We determined the complete sphingolipidomics profile in banked plasma samples obtained from the Type 1 diabetes patients with and without neuropathy symptoms. The plasma concentrations of individual ceramide species and the sphingoid bases and their phosphates for the two groups of patients are summarized in **TABLE 2**.

TABLE 2 - Plasma concentrations of individual ceramide species and the sphingoid bases and their phosphates in the DCCT/EDIC cohort who exhibited symptoms of neuropathy compared to patients with no neuropathy symptoms

	Overall n=80				Symptoms n=57				No Symptoms n=23				
SPHINGOLIPID (nM)	Mean	SD	Median	Range	Mean	SD	Median	Range	Mean	SD	Median	Range	P Value
Sph	24.8	11.2	22.8	10.0-65.4	25.4	11.4	22.8	10.0-65.4	23.5	10.6	23.2	11.7-62.4	0.560
Sph_1P	371.2	174.1	312.2	104.1-1061.0	359.3	181.4	295.6	104.1-1061.0	400.5	154.1	376.2	203.3-749.0	0.178
dhC16_Cer	55.6	76.6	37.5	9.0-591.0	51.9	83.7	34.6	9.0-591.0	64.6	55.6	47.2	10.2-226.7	***0.028***
dhSph	15.5	7.7	13.5	4.5-53.7	16.6	8.1	14.1	4.5-53.7	12.8	5.8	11.7	5.0-28.0	***0.038***
dhSph_1P	76.2	38.9	63.6	17.5-216.6	74.0	40.3	58.4	17.5-216.6	81.5	35.5	68.3	36.3-154.7	0.301
C14_Cer	77.9	28.4	79.1	22.9-149.5	78.9	28.6	78.6	22.9-149.5	75.7	28.4	79.7	27.7-135.3	0.703
C16_Cer	135.4	64.6	124.2	52.5-403.3	126.9	69.7	107.2	52.5-403.3	156.6	44.2	152.2	78.4-270.7	***0.002***
C18:1_Cer	39.6	20.7	33.9	7.6-120.0	40.7	21.9	35.6	10.1-120.0	37.0	17.7	32.6	7.6-82.7	0.597
C18_Cer	25.9	12.6	23.3	7.4-68.4	27.9	13.2	24.9	11.0-68.4	20.8	9.5	18.5	7.4-39.6	0.028
C20:1_Cer	5.1	3.1	4.5	1.5-16.8	5.4	3.4	4.6	1.7-16.8	4.5	2.0	4.3	1.5-10.6	0.459
C20_Cer	53.1	29.1	44.8	15.5-144.8	55.9	30.9	47.3	16.5-144.8	46.0	23.0	39.4	15.5-101.5	0.246
C22:1_Cer	103.5	32.0	99.2	44.9-222.3	104.8	35.0	98.1	44.9-222.3	100.2	23.4	100.4	54.8-151.8	0.924
C22_Cer	688.9	236.6	675.0	300.2-1382.3	673.0	236.4	669.0	300.2-1382.3	728.3	237.8	718.1	341.6-1298.0	0.404
C24:1_Cer	1209.6	348.7	1212.1	524.9-2103.2	1185.8	357.2	1119.7	524.9-2103.2	1268.4	326.6	1314.4	535.5-1839.5	0.296
C24_Cer	2822.3	905.2	2877.9	880.1-5156.8	2773.6	967.4	2831.7	880.1-5156.8	2943.1	734.1	3020.7	1529.2-4473.4	0.459
C26:1_Cer	52.4	18.1	51.8	15.7-105.7	51.6	18.3	51.7	15.7-105.7	54.5	18.0	54.1	16.4-86.8	0.546
C26_Cer	110.9	38.1	110.0	29.0-210.6	108.4	39.8	108.7	29.0-209.1	117.0	33.6	114.7	49.6-210.6	0.363
Total_Cer	5324.6	1528.6	5333.0	2044.4-9134.8	5232.9	1583.9	5249.2	2044.4-9134.8	5551.9	1389.0	5724.3	2667.9-8365.2	0.486

There was a significant decrease in the plasma concentrations of the ceramide precursor dihydroceramide (dhC16_Cer) in the group of patients which presented with

neuropathy symptoms compared to the patients without symptoms but there were no significant differences in the plasma concentrations of any individual ceramide species except C16_Cer or of total ceramide between the two groups. There also was a significant increase in the plasma concentration of dihydrosphingosine (dhSph), an intermediate to ceramide synthesis via the *de novo* pathway, but no other significant differences in the concentrations of the sphingosine species between the two groups including that of sphingosine-1-phosphate (S1P).

We determined for the first time the concentrations of the hexosyl- and lactosylceramide species in plasma samples obtained from the Type 1 diabetes patients with and without neuropathy symptoms. The plasma concentrations of the individual hexosylceramide species in plasma samples from the two groups of patients are summarized in **TABLE 3**.

TABLE 3 - Plasma concentrations of individual hexosylceramide species in the DCCT/EDIC cohort who exhibited symptoms of neuropathy compared to patients with no neuropathy symptoms

	Overall				**Symptoms**				**No Symptoms**				
	n=80				**n=57**				**n=23**				
SPHINGOLIPID (nM)	Mean	SD	Median	Range	Mean	SD	Median	Range	Mean	SD	Median	Range	P Value
C14_HxCer	42.4	12.5	41.8	17.0-88.1	42.2	13.4	40.1	22.9-88.1	43.0	10.0	43.3	17.0-63.0	0.347
C16_HxCer	1454.5	410.5	1417.3	571.7-2714.5	1443.6	443.2	1413.1	571.7-2714.5	1481.5	322.4	1421.7	1011.9-2416.1	0.575
C18:1_HxCer	148.1	52.7	140.0	50.7-332.2	142.7	51.3	136.8	50.7-332.2	161.4	54.9	146.5	101.1-319.8	0.178
C18_HxCer	91.1	37.0	79.8	28.8-209.5	88.2	34.6	76.5	28.8-199.4	98.3	42.3	93.7	29.4-209.5	0.386
C20:1_HxCer	38.2	17.4	36.1	11.0-123.0	36.7	15.6	35.4	11.0-81.2	41.8	21.2	39.8	18.2-123.0	0.311
C20_HxCer	41.3	24.4	37.1	9.6-165.0	41.0	25.9	34.0	13.4-165.0	42.1	20.8	41.0	9.8-91.5	0.440
C22:1_HxCer	158.8	79.7	153.2	24.5-374.9	143.9	78.0	132.7	24.5-374.9	195.7	73.0	177.9	102.3-357.4	***0.013***
C22_HxCer	1266.4	510.0	1103.6	277.0-2870.6	1249.9	505.4	1098.0	277.0-2850.7	1307.2	530.3	1253.4	560.3-2870.6	0.604
C24:1_HxCer	1809.9	608.6	1714.8	711.8-3589.5	1821.4	629.8	1708.2	711.6-3589.5	1781.3	564.9	1725.6	935.7-3422.6	0.857
C24_HxCer	3320.7	1158.6	3139.4	858.6-7207.2	3267.1	1277.6	3071.0	858.6-7207.2	3453.5	799.7	3257.8	1763.5-4936.5	0.165
C26_HxCer	81.4	63.8	62.1	7.3-303.7	93.6	70.3	74.3	7.3-303.7	51.3	26.1	50.5	14.7-121.3	***0.005***
Total_Hex_Cer	8452.7	2471.5	7966.6	3125.6-15436.3	8370.3	2613.2	7922.6	3125.9-15436.3	8657.0	2119.4	8010.6	5027.9-14485.2	0.519

We determined that there was a significant increase in the plasma concentration of the C26_hexosylceramide species (C26_HxCer), but a significant decrease in the concentration of C22:1_hexosylceramide in the group of patients with neuropathy symptoms. There were no significant differences observed in the concentrations of total hexosylceramide in plasma between the two groups of patients.

We also determined the plasma concentrations of the individual lactosylceramide species in samples from the two groups of patients and the results of these analyses are summarized in **TABLE 4**. There were significant increases in the plasma concentrations of the C18:1, C18, C20:1, C22, and C24_lactosylceramide species in plasma from the group of diabetic patients with neuropathy symptoms compared to levels in the group with no symptoms. There were no significant differences in the total concentrations of lactosylceramides in plasma between the two groups of patients.

TABLE 4 - Plasma concentrations of individual lactosylceramide species in the DCCT/EDIC cohort who exhibited symptoms of neuropathy compared to patients with no neuropathy symptoms

	Overall n=80				Symptoms n=57				No Symptoms n=23				P Value
SPHINGOLIPID (nM)	Mean	Std	Median	Range	Mean	Std	Median	Range	Mean	Std	Median	Range	
C14_LactCer	115.2	49.6	106.5	37.1-251.1	113.3	50.4	105.6	37.1-245.0	120.1	48.1	110.2	41.6-251.1	0.446
C16_LactCer	4030.9	1782.3	3925.5	770.7-10315.3	3896.2	1862.5	3534.2	770.7-10315.3	4364.7	1553.7	4108.8	1359.8-7765.4	0.149
C18:1_LactCer	103.1	47.2	98.5	22.0-276.6	97.2	50.1	84.6	22.0-276.6	117.7	36.0	120.0	46.8-173.9	***0.028***
C18_LactCer	156.4	64.6	147.5	40.7-349.5	145.4	67.1	129.4	40.7-349.5	183.8	49.3	175.4	99.3-274.2	***0.005***
C20:1_LactCer	14.4	11.5	11.1	1.0-65.0	14.0	12.9	9.1	1.02-65.0	15.5	6.9	13.6	6.5-31.8	***0.034***
C22:1_LactCer	10.5	6.9	8.7	0.8-35.0	9.8	6.8	8.6	0.8-33.7	12.4	7.1	10.0	5.2-35.0	0.071
C22_LactCer	45.5	27.4	36.8	7.3-153.2	43.3	30.2	34.5	7.3-153.2	50.9	18.3	51.6	20.7-97.7	***0.040***
C24:1_LactCer	130.6	82.7	110.3	24.7-483.2	124.8	88.1	94.5	24.7-483.2	145.0	67.2	144.9	39.0-311.6	0.064
C24_LactCer	117.0	52.8	109.2	36.7-301.1	110.2	55.5	99.9	36.7-301.1	133.9	41.9	134.4	60.8-200.5	***0.013***
Total_LactCer	4723.7	2014.0	4628.7	957.7-11243.6	4554.2	2107.3	4179.8	957.7-11243.6	5143.9	1732.8	4815.1	1690.0-8767.9	0.138

While there is a substantial body of knowledge regarding intracellular metabolism of LacCer and HexCer in kidney disease, information regarding the transport and metabolism of these complex sphingolipids in plasma is lacking. Glycosphingolipids have numerous roles in regulating cellular processes including cell proliferation, apoptosis, inflammation and cellular signaling. Dysregulation of glycosphingolipid metabolism leads to the accumulation of particular species of glycosphingolipid and induces several different pathologies as shown by knockout studies in mice. Data of the transport of these complex sphingolipids in plasma and lipoproteins is limited and this is the first report of the analysis of plasma from diabetic patients. These data provide critical baseline data for future investigations of glycosphingolipid composition and transport in plasma from diabetes patients.

While ceramide may be considered the central molecule in sphingolipid metabolism, sphingomyelin (SM), which is derived from ceramide, is the most abundant sphingolipid in plasma lipoproteins. Sphingomyelin is localized predominantly in the hydrophilic outer layer of the lipoprotein particle together with free cholesterol and phospholipids. Studies of intracellular sphingolipid metabolism have exhaustedly documented the potential metabolic interconversion of ceramide and sphingomyelin but the fatty acid composition of the sphingomyelin and ceramide species in plasma differ significantly, presumably resulting from the multiple potential origins of ceramide. Thus, because the composition of plasma and lipoprotein sphingomyelin species composition cannot be inferred from analysis of ceramide species composition, we undertook the task of analyzing the distribution and composition of the species of sphingomyelin in plasma from the two groups of patients. No studies have examined the impact of diabetes or diabetic neuropathy on plasma sphingomyelin composition because the technology to adequately quantitate sphingomyelin species is recent and not widespread. The concentrations of the species of sphingomyelin in plasma from Type 1 diabetic patients with and without neuropathy are summarized in **TABLE 5**.

TABLE 5 - Plasma concentrations of individual sphingomyelin species in the DCCT/EDIC cohort who exhibited symptoms of neuropathy compared to patients with no neuropathy symptoms

	Overall n=80				Symptoms n=57				No Symptoms n=23				
SPHINGOLIPID (μM)	Mean	Std	Median	Range	Mean	Std	Median	Range	Mean	Std	Median	Range	P Value
C14_SM	14.9	5.7	13.8	6.1-37.6	15.9	6.1	14.8	6.8-37.6	12.4	3.7	12.5	6.1-22.2	***0.012***
C16_SM	95.0	18.9	95.0	54.1-154.5	98.0	16.4	95.9	66.0-154.5	87.7	22.8	78.4	54.1-127.0	***0.035***
C18:1_SM	3.7	0.7	3.6	2.3-5.7	3.7	0.7	3.7	2.4-5.0	3.6	0.9	3.5	2.3-5.7	0.568
C18_SM	6.2	1.1	6.1	4.0-9.4	6.2	1.1	6.0	4.3-9.4	6.1	1.1	6.2	3.9-8.6	0.882
C20:1_SM	1.6	0.3	1.6	1.0-2.8	1.6	0.3	1.6	1.1-2.4	1.6	0.4	1.5	1.0-2.8	0.422
C20_SM	4.2	0.8	4.1	2.8-6.3	4.2	0.9	4.1	2.8-6.3	4.1	0.6	4.1	2.8-5.1	0.924
C22:1_SM	8.1	1.6	8.0	5.2-11.7	8.1	1.6	8.0	5.2-11.7	8.0	1.6	8.0	5.5-11.1	1.000
C22_SM	10.4	2.6	10.4	5.1-16.4	10.2	2.7	10.1	5.1-16.4	11.1	2.2	11.3	7.6-15.4	0.117
C24:1_SM	14.4	2.9	14.1	9.1-23.2	14.3	2.7	14.0	9.4-21.5	14.7	3.4	14.2	9.1-23.2	0.759
C24_SM	8.0	2.3	7.9	2.7-13.3	7.7	2.2	7.7	2.7-12.5	9.0	2.4	9.0	5.7-13.3	***0.041***
C26:1_SM	8.6	2.7	8.5	2.4-16.2	8.7	2.8	8.5	2.4-16.2	8.4	2.4	7.2	5.6-13.1	0.519
Total_SM	175.0	25.4	173.5	122.3-275.4	178.4	24.9	177.0	136.7-275.4	166.6	25.2	156.6	122.3-220.5	***0.045***

NOTE - plasma concentration is expressed in micromolar (μM)

Basic knowledge of SM species composition in plasma, but especially isolated lipoproteins, may prove pivotal to furthering our understanding of cell-lipoprotein interactions. Cellular membrane lipid rafts may be considered as concentrating platforms for individual cell surface receptors and they are defined by their cholesterol- and sphingomyelin-rich nature. The contribution of lipoprotein sphingomyelin to cellular sphingomyelin remains to be determined but the HDL receptor, SR-BI, plays a pivotal role in the exchange of lipoprotein-lipids with cells, including lipoprotein-mediated, S1P delivery, and it is localized in lipid rafts in the cell membrane. The fatty acid chain length and degree of unsaturation of membrane sphingomyelins have a distinct biophysical impact on membrane organization, fluidity, and lipid rafts. Thus, increased saturated sphingomyelin acyl chains within circulating lipoprotein particles may contribute to alterations in metabolism in peripheral tissues. These data provide the first report of the distribution and concentration of sphingomyelins in plasma from diabetic patients with neuropathy and this knowledge will increase our understanding of cell-lipoprotein interactions.

Sphingolipid *de novo* synthesis is initiated by the molecular condensation reaction mediated by the enzyme serine palmitoyltransferase (SPT) during which an activated fatty acid (predominantly palmitoyl-CoA) and L-serine combine to form sphinganine. In patients with hereditary sensory and autonomic neuropathy type 1 (HSAN1), the enzyme SPT shows a shift in substrate specificity to alanine which results in the formation of an atypical class of sphingolipids, the deoxysphingolipids. The deoxysphingolipids class is devoid of the C1-hydroxyl group and therefore, are neither metabolized to complex sphingolipids nor degraded by the regular sphingolipid catabolism since S1P, a required catabolic intermediate, cannot be formed from deoxysphingolipids. No data was previously available regarding the concentrations of deoxysphingolipids in patients with diabetic neuropathy who exhibit symptoms similar to patients with hereditary HSAN1. Therefore, we determined the plasma concentrations of deoxysphingolipids in Type 1 diabetes patients with neuropathy and compared them to those determined in patients without symptoms of neuropathy and a summary of our findings is reported in **TABLE 6**.

TABLE 6 - Plasma concentrations of individual deoxysphingolipid species in the DCCT/EDIC cohort who exhibited symptoms of neuropathy compared to patients with no neuropathy symptoms

	Overall n=80				Symptoms n=57				No Symptoms n=23				
SPHINGOLIPID (nM)	Mean	SD	Median	Range	Mean	SD	Median	Range	Mean	SD	Median	Range	P Value
DeoxyC22:1_Cer	53.9	22.4	50.8	24.3-125.7	56.1	22.4	53.2	24.7 - 122.2	48.5	21.7	41.0	24.3-125.7	0.127
DeoxyC24:1_Cer	379.0	141.3	357.6	132.1-745.8	372.3	147.8	342.4	132.1-744.6	395.8	125.3	365.8	225.2-745.8	0.316
DeoxyC24_Cer	11.0	4.1	10.6	4.0-21.3	10.6	4.2	9.8	4.0-21.3	11.8	3.7	10.7	6.1-20.9	0.198
DeoxyC26:1_Cer	10.6	4.1	10.0	4.0-24.6	10.3	4.2	9.7	4.0-24.6	11.3	3.8	10.3	6.6-23.2	0.233
DeoxyC26_Cer	12.0	7.5	10.2	3.4-51.8	11.8	7.5	10.2	5.0-51.8	12.4	7.7	10.0	3.4-33.7	0.604
DeoxyC18:1_Cer	13.0	5.7	12.2	4.8-30.6	12.3	6.0	10.9	4.8-30.6	14.8	4.2	14.4	5.3-25.1	***0.008***
DeoxyDHSph	116.2	49.2	105.6	21.8-268.7	121.9	51.9	117.3	21.8-268.7	101.9	39.1	91.9	57.8-227.7	0.083
DeoxySph	65.1	79.4	44.8	20.7-521.1	54.0	45.1	45.4	20.7-320.0	92.6	127.9	39.3	27.5-521.1	0.975
Total_Deoxy	479.5	171.3	444.6	183.4-949.0	473.4	177.9	429.3	183.4-938.7	494.7	156.5	455.9	287.8-949.0	0.410
Total_Sat_Deoxy	23.0	10.5	20.5	10.1-70.9	22.4	10.5	19.8	10.1-70.9	24.3	10.8	20.7	13.0-54.5	0.398
Total_Unsat_Deoxy	456.6	162.8	425.8	172.9-911.8	451.0	169.5	411.9	172.9-891.8	470.5	147.8	429.1	272.4-911.8	0.428

There were no significant differences in the plasma concentrations of any deoxysphingolipids species except that of deoxyC18:1-Cer between the group of Type 1 diabetes patients with neuropathy compared to that with no symptoms of neuropathy. The plasma concentrations of total deoxysphingolipids as well as those of the saturated and unsaturated deoxysphingolipids species did not differ between the two groups.

Aim 2: Compare the concentrations of free amino acids in plasma from patients with Type 1 diabetes who exhibit documented diabetic neuropathy to the levels determined in plasma from patients without documented neuropathy

RESULTS:

Free amino acids are required for the condensation reaction catalyzed by SPT which is a part of physiologic sphingolipid biosynthesis and for the synthesis of DSL as detailed above. Previous investigations determined that both plasma DSL concentrations and also plasma amino acid levels were altered in Type 2 diabetic patients compared to those in non-diabetic, control subjects. It is not known if plasma free amino acid concentrations and distribution in Type 1 diabetes patients who exhibit diabetic neuropathy differ from those in patients without diabetic neuropathy. Therefore, we determined the plasma concentrations of free amino acids in the same banked samples used in Specific Aim 1 to determine if amino acid concentration and distribution differ in Type 1 diabetes patients with, compared to those without, diabetic neuropathy. These results are summarized in **TABLE 7**.

There were no statistically significant differences in the plasma total concentration of free amino concentration in the group of Type 1 diabetes patients with neuropathy symptoms compared to the group with no symptoms. While there were significant differences in the plasma concentrations of cysteine, phenylalanine, and tyrosine, the plasma concentration of the free amino acids most closely associated with altered deoxysphingolipids metabolism, alanine and glycine, did not differ between the two groups of patients.

TABLE 7 - Plasma concentrations of free amino acids in the DCCT/EDIC cohort who exhibited symptoms of neuropathy compared to patients with no neuropathy symptoms

	Overall n=80				Symptoms n=57				No Symptoms n=23				
AMINO ACID (μM)	Mean	SD	Median	Range	Mean	SD	Median	Range	Mean	SD	Median	Range	p Value
Alanine	5.6	4.9	3.6	1.7-27.1	6.1	5.5	3.9	1.7-27.1	4.2	2.5	3.2	2.2-12.7	0.195
Arginine	61.9	75.1	24.3	2.7-348.0	70.3	81.6	33.4	2.7-348.0	41.3	51.8	16.3	6.5-199.7	0.459
Aspartine	12.5	8.0	9.6	5.9-56.3	13.4	9.0	10.0	6.7-56.3	10.2	4.0	8.9	5.9-23.9	0.110
Cysteine	1.5	0.8	1.3	0.6-4.3	1.7	0.8	1.5	0.6-4.3	1.1	0.3	1.1	0.6- 1.8	***0.001***
Glutamine	58.3	28.7	53.7	6.1-181.2	56.0	29.3	53.5	6.1-181.2	63.9	26.8	53.9	25.0-127.6	0.206
Glycine	9.3	9.0	7.0	2.0-61.3	10.1	10.2	7.1	2.0-61.3	7.5	4.2	6.7	2.1-18.7	0.465
Histidine	1.0	1.0	0.4	0.2-4.0	1.1	1.0	0.6	0.2-4.0	0.6	0.6	0.3	0.2-2.2	0.141
Leucine	379.6	150.1	322.1	251.9-1202.9	397.2	169.3	330.5	251.9-1202.9	335.9	72.3	316.2	276.9-600.3	0.068
Lysine	30.7	19.8	24.6	6.7-120.5	30.4	20.8	24.1	6.7-120.5	31.4	17.4	25.3	12.1-66.6	0.440
Methionine	15.4	7.6	13.1	7.9-58.7	15.6	8.5	12.4	7.9-58.7	14.9	4.8	13.6	9.4-31.0	0.428
Phenylalanine	100.2	34.6	89.2	67.8-283.5	105.6	38.6	92.0	67.8-283.5	86.7	15.2	81.5	70.4-136.9	***0.006***
Proline	63.3	44.7	43.8	20.7-199.7	65.8	47.5	48.3	20.7-199.7	57.3	37.2	43.2	23.6-157.0	0.849
Serine	15.3	11.5	11.0	6.3-68.9	16.3	12.9	11.2	6.3-68.9	12.7	6.6	10.1	6.5-33.5	0.664
Threonine	15.6	11.4	10.6	6.5-64.7	16.5	12.7	11.1	6.5-64.7	13.4	6.9	10.4	7.8-36.3	0.857
Tyrosine	92.6	35.7	80.5	56.8-266.8	98.5	39.5	84.8	56.8-266.8	77.9	17.4	72.2	62.6-138.3	***0.003***
Valine	128.1	67.8	100.0	72.2-491.3	133.7	75.9	109.9	72.2-491.3	114.3	39.6	97.5	80.7-249.8	0.808
Total AA	990.8	461.8	781.4	569.3-3171.4	1038.2	510.3	852.6	569.3-3171.4	873.5	287.5	748.9	624.3-1777.6	0.392

3. Publications:

The data presented above were finalized only at the beginning of this month. Thus, the data analyses we present are basic and very preliminary. We have not yet analyzed the data while employing more sophisticated statistical methods but plan to do so. Thus, no publications have been produced by this study as of this time but they are planned for the future.