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Elevated Plasma Free Sialic Acid Levels in Individuals with Reduced Glomerular Filtration Rates

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N-acetylneuraminic acid (Neu5Ac, sialic acid) is a negatively charged monosaccharide, and the predominant form of sialic acid in human cells (1). Sialic acid is typically found as the terminal monosaccharide on glycoconjugates, where it plays a role in various physiologic and pathologic interactions (1). Sialylated glycoconjugates are critical contributors to the polyanionic component of the glomerular glycocalyx, contributing to size and charge selectivity for plasma macromolecules (1,2). Podocyte foot process morphology is maintained by the anionic charged sialic acid residues on glycoconjugates in podocyte membranes (2).

Free sialic acid is filtered but not reabsorbed by the human kidney, in a fashion similar to that for creatinine (3), but in contrast to the handling of other monosaccharides such as glucose, mannose, galactose, and fructose that are reabsorbed by tubular cells (4). Circulating sialic acid levels, both unbound and bound to glycoconjugates, have only been sporadically studied in different conditions, including some renal disorders (3,5−9); a possible causative link to reduced eGFRs has been seldom discussed or investigated. We designed this study to establish a correlation between eGFR and plasma free sialic acid across a range of subjects with glomerular disorders. This would not only emphasize this often-overlooked aspect of renal filtering of free sialic acid, but also inform our developmental program for glomerular diseases using the sialic acid precursor, N-acetylmannosamine (ManNAc) (10), which was expected to significantly increase plasma free sialic acid levels in subjects with reduced eGFR.

Peripheral blood was obtained from eight subjects enrolled in National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) natural history study 94-DK-0127 (ClinicalTrials.gov identifier, NTC00001392), "Pathogenesis of FSGS," and from eight subjects enrolled in NIDDK study 16-DK-0036 (ClinicalTrials.gov identifier, NCT02639260), "A Phase 1 Multiple Ascending Dose Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of ManNAc in Subjects with Primary Podocyte Diseases." To compare with pharmacokinetic parameters of subjects with normal kidney function, we used previously reported data that we collected with the same analytical assays from 19 subjects with GNE myopathy (11). All subjects provided informed consent before study participation. GFR (eGFR) was estimated using the CKD–Epidemiology Collaboration (CKD-EPI) creatinine/cystatin C equation (12) but, in GNE myopathy subjects, eGFR was calculated using the CKD-EPI cystatin C equation (12) because these individuals have muscle atrophy and therefore impaired creatinine production. Quantification of free Neu5Ac (sialic acid) in human plasma was performed using a validated liquid chromatography and tandem mass spectrometry method with an assay range of 25.0–10,000 ng/ml (Alliance Pharma, Malvern, PA) (13). For the development of the bioanalytical method, pooled normal human plasma (n=6; EDTA anticoagulant) was previously obtained from Bioreclamation (Westbury, NY) and was used to establish the normal range of Neu5Ac in human plasma. Among subjects with eGFR >90 ml/min per 1.73 m^2, the normal range for Neu5Ac was reported to be 100–200 ng/ml (13). Statistical analysis was performed using R version 3.6.2 and GraphPad Prism 5.

Plasma free sialic levels were determined in 16 proteinuric subjects with different glomerular diseases and a diverse eGFR range (8–92 ml/min per 1.73 m^2) and in 19 individuals with normal glomerular function (85–155 ml/min per 1.73 m^2) (Tables 1 and 2). First, our study established a plasma free sialic acid range of 109–206 ng/ml (mean 151±29 ng/ml) among subjects with normal eGFR (>90 ml/min per 1.73 m^2). This is consistent with previously reported normal free sialic acid ranges in plasma of 100–200 ng/ml (n=6), established using the same liquid chromatography and tandem mass spectrometry method as our assays (13), and a range in serum of 154–309 ng/ml (0.5–1.2 nmol/ml, n=9) (5) with a mean in serum of 68±6.7 ng/ml (n=50) (14), both established by a modified version of the thiobarbituric HPLC-based method of Warren.

Second, there was no apparent correlation of plasma free sialic acid levels to the particular glomerular diagnosis (Table 1), nor to proteinuria, sex, or age of the subjects in our study (Table 2). Previous work also reported that sex or age do not influence circulating free sialic acid (14).

Third, we identified a strong inverse relationship between eGFR and plasma free sialic acid levels

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Table 1. Summary of studied subjects

Condition	Number of Subjects	eGFR Range (ml/min per 1.73 m^2)	Plasma Neu5Ac (ng/ml)
FSGS	11	8–82	310–1260
Minimal change disease	1	92	204
Mesangial proliferative Glomerulonephritis	1	60	353
p-ANCA vasculitis	1	46	317
Membranous nephropathy	1	28	819
IgA nephropathy	1	20	546
GNE myopathy (11)	19	85–155	109–206
Normal control (13)	6	>90	100–200

Subjects included those with kidney disease who participated in this study (n=16), those with GNE myopathy (n=19), and controls (n=6) with normal kidney function who participated in our previously published studies. The same Neu5Ac bioanalytical liquid chromatography and tandem mass spectrometry assay was used. Neu5Ac, N-acetylneuraminic acid/sialic acid; p-ANCA, perinuclear ANCA.

(R^2=0.91) (Figure 1). Plasma free sialic acid levels start accumulating in individuals with eGFR <90 ml/min per 1.73 m^2 but did not rise above approximately 500 ng/ml in individuals with eGFR >45 ml/min per 1.73 m^2 (Figure 1). In subjects with marked decreased eGFR (<45 ml/min per 1.73 m^2), plasma free sialic acid levels were approximately threefold higher (431–1260 ng/ml; mean 557–1188 ng/ml) than the values seen across subjects with normal eGFR; varying from 431 ng/ml in a subject with eGFR of 27 ml/min per 1.73 m^2 to as high as 1260 ng/ml in a subject with eGFR of 8 ml/min per 1.73 m^2.

Not much information exists concerning the effects of chronic exposure to high circulating free sialic acid levels, as may occur in some subjects with CKD. Subjects with free sialic acid storage disease due to a defect in the lysosomal free sialic acid transporter SLC17A5 (15) exhibit chronically elevated serum free sialic acid levels, *i.e.*, 618–3059 ng/ml (n=8) (3), in the same range as glomerular disease subjects with eGFR <45 ml/min per 1.73 m^2. Free sialic acid storage disease is associated with developmental delay, coarse facies, ataxia, epileptic seizures, hepatosplenomegaly, and reduced life expectancy, and other organ systems, including the kidneys, are sporadically reported to be affected (15). These features appear predominantly attributed to lysosomal defects, as occurs in other lysosomal storage diseases, rather than elevated circulating free sialic acid levels. In two recent clinical trials for GNE myopathy, subjects were exposed to increased circulating free sialic acid levels. In a phase 3 trial (ClinicalTrials.gov identifier, NCT02377921), 45 subjects with GNE myopathy were exposed to 6 g/d oral extended-release sialic acid for 48 weeks. Their mean serum free sialic acid increased from 160 ng/ml at baseline to a steady state of approximately 300 ng/ml up to week 48, with no drug-related serious adverse events or noteworthy differences in vital signs and laboratory findings; low-grade gastrointestinal adverse events were reported, likely due to unabsorbed gastrointestinal sialic acid (16). Similarly, in a phase 1 trial of single oral doses of 3, 6, or 10 g of the sialic acid precursor ManNAc to subjects with GNE myopathy (ClinicalTrials.gov identifier, NCT01634750), plasma free sialic acid levels transiently increased up to 436 ng/ml after a single oral dose of 10 g ManNAc, and fell back to baseline within 48 hours (11). This study also reported low-grade gastrointestinal adverse events, likely associated with unabsorbed ManNAc in the gastrointestinal tract (11).

Based on these results, we consider chronically increased plasma free sialic acid levels up to approximately 500 ng/ml to be safe. Although no adverse events were identified related to plasma free sialic acid >500 ng/ml in our limited study population nor in the few subjects published in the literature, we recommend monitoring subjects with increased plasma free sialic acid >500 ng/ml for adverse events possibly related to increased free sialic acid. These recommendations are relevant for clinical studies that include dosing ManNAc or sialic acid to subjects with low eGFR, as in our planned clinical studies of ManNAc for subjects with glomerular diseases (10). We also anticipate that reducing or halting ManNAc or sialic acid supplementation will reduce free sialic acid levels in these subjects within days, based on previous pharmacokinetic parameters (11).

A variety of studies reported elevated circulating sialic acid levels in disorders without apparent renal involvement. Most of these studies report the total sialic acid levels, which include both sialic acid bound to glycoconjugates and free sialic acid. Elevated serum total sialic acid is often used as a marker for increased serum acute-phase response proteins, which are heavily sialylated, as reported for early-stage diabetic nephropathy (6,8), certain cancers (8), and rheumatic diseases (7,8). A few reports mention renal diseases with elevated total sialic acid (5), but without mention of free sialic acid or glomerular function. Serum free sialic acid levels are only occasionally reported, including increased serum free sialic acid in rheumatoid arthritis and SLE (7,8), again without mention of glomerular function, which could be affected in these disorders.

This report emphasizes the role of renal function in determining circulating free sialic acid levels. When encountering increased plasma or serum total sialic acid levels in certain disease conditions, the contribution of decreased eGFR should be considered, especially in disorders associated with kidney disease. While encountering high circulating free sialic acid in subjects with reduced eGFR, some of these subjects may have reduced sialic acid on glomerular glycoconjugates (2,10). A possible correlation between circulating free sialic acid and glomerular hyposialylation remains to be investigated.

Table 2. Plasma free sialic acid (Neu5Ac) levels in subjects with diverse eGFR

Subject	Age(yr)	Sex	eGFR[a](ml/min per 1.73 m^2)	Plasma Free Neu5Ac		Serum Creatinine (mg/dl)	Serum Albumin (g/dl)	Urine Creatinine (mg/dl)	Urine Protein (mg/dl)	Urine Albumin (mg/L)	UPCR (mg/mg)	UACR (mg/g)
				(ng/ml)	(μM/L)							
MCD1	28	M	92	204	0.660	1.09	5.1	158	12	14.9	0.076	9.4
FSGS1	47	F	82	310	1.002	0.84	3.5	176	673	5030	3.824	2858
FSGS2	36	M	79	369	1.193	1.32	3.6	104	358	3034	3.442	2917
FSGS3	58	M	61	345	1.116	1.28	3.3	33	117	847	3.545	2567
GN1[b]	46	M	60	353	1.141	1.39	4.4	39	14	85.9	0.359	220.3
FSGS4	42	F	56	344	1.112	1.35	3.5	34	44	293	1.294	862
ANCA1[c]	27	F	46	317	1.025	1.52	4.1	223	327	3188	1.668	1429.6
FSGS5	74	M	35	629	2.034	1.85	3.6	44	381	2670	8.659	6068
FSGS6	37	M	30	677	2.189	2.91	4.3	84	175	1361	2.083	1620.2
FSGS7[d]	45	M	29	603	1.950	2.75	3.7	121	509	3776	4.207	3120
MN1	65	M	28	819	2.648	2.87	1.5	170	1810	12662	10.647	7448.2
FSGS8	39	M	27	431	1.394	2.34	3.6	71	673	4654	9.479	6555
FSGS9	49	M	26	509	1.646	2.68	3.9	45	131	923	2.911	2051.1
IgA1	68	F	20	546	1.765	2.35	4.5	48	49	293.4	1.021	611.3
FSGS10	74	F	14	851	2.752	3.48	4.5	95	305	1934	3.211	2035.8
FSGS11	53	M	8	1260	4.074	8.36	4.8	61	74	403.4	1.213	661.3
Normal range			>90	100–200	0.32–0.65	0.5–1.2	3.5–5.2	15–392	0–14	0–20.9	0.001–0.16	<30
GNEM-1	37	M	155	179	0.579	—	—	—	—	—	—	—
GNEM-2	32	M	149	114	0.369	—	—	—	—	—	—	—
GNEM-3	47	M	146	138	0.446	—	—	—	—	—	—	—
GNEM-4[e]	36	F	143	121	0.391	—	—	—	—	—	—	—
GNEM-5[e]	36	F	143	175	0.566	—	—	—	—	—	—	—
GNEM-6	32	M	141	134	0.433	—	—	—	—	—	—	—
GNEM-7[e]	49	M	137	129	0.417	—	—	—	—	—	—	—
GNEM-8	32	M	135	116	0.375	—	—	—	—	—	—	—
GNEM-9	30	F	131	129	0.417	—	—	—	—	—	—	—
GNEM-10	47	F	119	109	0.352	—	—	—	—	—	—	—
GNEM-11[e]	51	F	116	206	0.666	—	—	—	—	—	—	—
GNEM-12[e]	51	F	110	167	0.534	—	—	—	—	—	—	—
GNEM-13	53	F	108	173	0.559	—	—	—	—	—	—	—
GNEM-14	54	M	105	140	0.453	—	—	—	—	—	—	—
GNEM-15[e]	49	M	105	141	0.456	—	—	—	—	—	—	—
GNEM-16	35	F	105	166	0.537	—	—	—	—	—	—	—
GNEM-17	30	F	95	121	0.391	—	—	—	—	—	—	—
GNEM-18	65	M	95	180	0.582	—	—	—	—	—	—	—
GNEM-19	50	F	95	200	0.647	—	—	—	—	—	—	—
GNEM-20	44	F	92	142	0.459	—	—	—	—	—	—	—
GNEM-21	39	F	90	182	0.588	—	—	—	—	—	—	—

Table 2. (Continued)

Subject	Age(yr)	Sex	eGFR[a](ml/min per 1.73 m^2)	Plasma Free Neu5Ac		Serum Creatinine (mg/dl)	Serum Albumin (g/dl)	Urine Creatinine (mg/dl)	Urine Protein (mg/dl)	Urine Albumin (mg/L)	UPCR (mg/mg)	UACR (mg/g)
				(ng/ml)	(μM/L)							
GNEM-22	52	M	85	164	0.530	—	—	—	—	—	—	—

Neu5Ac, N-acetylneuraminic acid/sialic acid; UPCR, urine protein-creatinine ratio; UACR, urine albumin-creatinine ratio; MCD, minimal change disease; M, male; F, female; MN, membranous nephropathy; IgA, IgA nephropathy; GNEM, GNE myopathy; CKD-EPI, CKD Epidemiology Collaboration; p-ANCA, perinuclear ANCA.
[a]Note that eGFR was estimated using the CKD-EPI creatinine/cystatin C equation for subjects with glomerular disease, and using the CKD-EPI cystatin C equation for subjects without kidney disease (12).
[b]Mesangial proliferative GN.
[c]p-ANCA vasculitis.
[d]Collapsing FSGS.
[e]In three subjects, plasma free Neu5Ac and eGFR were assessed twice, at least 3 mo apart. Although these three samples were from the same subjects, they were included in our assessment because they were collected under different circumstances. The following subject numbers represent the same individual: GNEM-4 and GNEM-5; GNEM-11 and GNEM-12; GNEM-7 and GNEM-15.

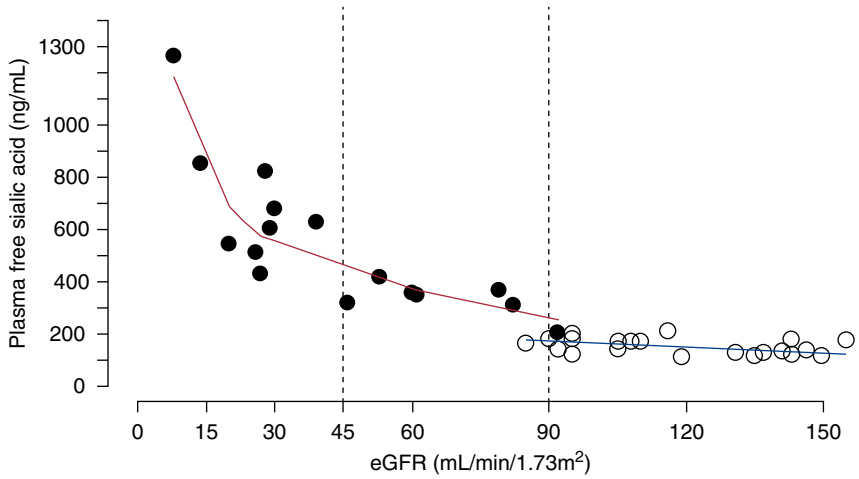

Figure 1. | **Inverse relationship between eGFR and plasma free sialic acid levels.** Plasma free sialic acid (Neu5Ac) levels are shown for subjects with glomerular disease (filled circles) and subjects without kidney disease (open circles), showing a significant inverse relationship between eGFR and plasma free sialic acid among subjects with glomerular disease (R^2=0.91). Note that eGFR was estimated using the CKD–Epidemiology Collaboration (CKD-EPI) creatinine/cystatin C equation for glomerular disease subjects, and using the CKD-EPI cystatin C equation for subjects without kidney disease (12).

Disclosures

All authors have nothing to disclose.

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Author Contributions

J. Blake, N. Carrillo, F. Fuentes, W. Gahl, M. Huizing, J. Kopp, and P. Leoyklang were responsible for investigation; J. Blake, N. Carrillo, F. Fuentes, M. Huizing, and J. Kopp were responsible for data curation; J. Blake and M. Huizing were responsible for project administration; N. Carrillo, F. Fuentes, M. Huizing, and K. Wilkins were responsible for visualization; N. Carrillo, W. Gahl, and J. Kopp were responsible for funding acquisition; F. Fuentes and M. Huizing were responsible for formal analysis; F. Fuentes, M. Huizing, and K. Wilkins wrote the original draft; W. Gahl, M. Huizing, and P. Leoyklang conceptualized the study; W. Gahl and J. Kopp were responsible for resources; M. Huizing provided supervision; M. Huizing, P. Leoyklang, and K. Wilkins were responsible for methodology; K. Wilkins was responsible for software; and all authors reviewed and edited the manuscript.

References

1. Schauer R: Sialic acids as regulators of molecular and cellular interactions. *Curr Opin Struct Biol* 19: 507–514, 2009
2. Quaggin SE: Sizing up sialic acid in glomerular disease. *J Clin Invest* 117: 1480–1483, 2007
3. Seppala R, Renlund M, Bernardini I, Tietze F, Gahl WA: Renal handling of free sialic acid in normal humans and patients with Salla disease or renal disease. *Lab Invest* 63: 197–203, 1990
4. Wright EM, Hirayama BA, Loo DF: Active sugar transport in health and disease. *J Intern Med* 261: 32–43, 2007
5. Ozben T: Elevated serum and urine sialic acid levels in renal diseases. *Ann Clin Biochem* 28: 44–48, 1991
6. Yokoyama H, Jensen JS, Jensen T, Deckert T: Serum sialic acid concentration is elevated in IDDM especially in early diabetic nephropathy. *J Intern Med* 237: 519–523, 1995
7. Chrostek L, Cylwik B, Gindzienska-Sieskiewicz E, Gruszewska E, Szmitkowski M, Sierakowski S: Sialic acid level reflects the disturbances of glycosylation and acute-phase reaction in rheumatic diseases. *Rheumatol Int* 34: 393–399, 2014
8. Sillanaukee P, Pönniö M, Jääskeläinen IP: Occurrence of sialic acids in healthy humans and different disorders. *Eur J Clin Invest* 29: 413–425, 1999
9. Gruszewska E, Cylwik B, Panasiuk A, Szmitkowski M, Flisiak R, Chrostek L: Total and free serum sialic acid concentration in liver diseases. *BioMed Res Int* 2014: 876096, 2014
10. Huizing M, Yardeni T, Fuentes F, Malicdan MCV, Leoyklang P, Volkov A, Dekel B, Brede E, Blake J, Powell A, Chatrathi H, Anikster Y, Carrillo N, Gahl WA, Kopp JB: Rationale and design for a phase 1 study of N-acetylmannosamine for primary glomerular diseases. *Kidney Int Rep* 4: 1454–1462, 2019
11. Xu X, Wang AQ, Latham LL, Celeste F, Ciccone C, Malicdan MC, Goldspiel B, Terse P, Cradock J, Yang N, Yorke S, McKew JC, Gahl WA, Huizing M, Carrillo N: Safety, pharmacokinetics and sialic acid production after oral administration of N-acetylmannosamine (ManNAc) to subjects with GNE myopathy. *Mol Genet Metab* 122: 126–134, 2017
12. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, Kusek JW, Manzi J, Van Lente F, Zhang YL, Coresh J, Levey AS; CKD-EPI Investigators: Estimating glomerular filtration rate from serum creatinine and cystatin C [published correction appears in *N Engl J Med* 367: 2060, 2012]. *N Engl J Med* 367: 20–29, 2012
13. Shi Y, Xu X, Fang M, Zhang M, Li Y, Gillespie B, Yorke S, Yang N, McKew JC, Gahl WA, Huizing M, Carrillo-Carrasco N, Wang AQ: Quantitative hydrophilic interaction chromatography-mass spectrometry analysis of N-acetylneuraminic acid and N-acetylmannosamine in human plasma. *J Chromatogr B Analyt Technol Biomed Life Sci* 1000: 105–111, 2015
14. Hangloo VK, Kaul I, Zargar HU: Serum sialic acid levels in healthy individuals. *J Postgrad Med* 36: 140–142, 1990
15. Barmherzig R, Bullivant G, Cordeiro D, Sinasac DS, Blaser S, Mercimek-Mahmutoglu S: A new patient with intermediate severe salla disease with hypomyelination: A literature review for salla disease. *Pediatr Neurol* 74: 87–91.e2, 2017
16. Lochmüller H, Behin A, Caraco Y, Lau H, Mirabella M, Tournev I, Tarnopolsky M, Pogoryelova O, Woods C, Lai A, Shah J, Koutsoukos T, Skrinar A, Mansbach H, Kakkis E, Mozaffar T: A phase 3 randomized study evaluating sialic acid extended-release for GNE myopathy. *Neurology* 92: e2109–e2117, 2019

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