# Response to Heiner-Fokkema et al.

To the Editor:

The authors of "The multiple faces of urinary glucose tetrasaccharide as a biomarker for patients with hepatic glycogen storage diseases" report their experience of monitoring the urinary glucose tetrasaccharide biomarker, $Glc_4$, in patients with glycogen storage disorder (GSD) III.[1] Their findings of consistently elevated $Glc_4$ in patients with GSD III, and generally higher values than in age-matched Pompe disease populations, is in agreement with published studies.[2,3]

The authors described one infant demonstrating a decrease in $Glc_4$, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) between 0.66 and 1.27 years of age, and this was suggested to reflect an improvement in liver enzymes in response to dietary treatment. Historically, decreases in liver size and transaminases in children with GSD III were interpreted as evidence for an improvement in the liver disease with age. However, we have proposed that these trends over time may alternatively be explained by a progression of the liver disease,[2,4] similar to other progressive liver diseases and conditions such as hepatitis C virus (HCV) and nonalcoholic fatty liver disease (NAFLD). In our GSD III pediatric patient cohort, aged 2–22 years ($n = 26$), we demonstrated a negative correlation with age for ALT, AST, and $Glc_4$ levels. While these trends could be interpreted as an improvement in hepatic function, as suggested by Heiner-Fokkema et al., the finding of liver fibrosis in all eight of our patients in whom a biopsy was available (median age 1.7 years, range: 0.8 to 20), contradicts that hypothesis. Further supporting our findings, the naturally occurring GSD IIIa dog model demonstrated increased ALT, AST, and $Glc_4$ over the first 10 to 24 months of life, and thereafter decreased, correlating with a decrease in hepatic glycogen and histological evidence of progressive liver fibrosis. Hence, progressive liver fibrosis cannot be ignored as a possible cause of the combined reduction in $Glc_4$ and the transaminases in GSD III.

Further evidence that $Glc_4$ may decrease secondary to tissue loss is available from $Glc_4$ trends in patients with Pompe disease. A patient with infantile Pompe disease treated as part of a clinical trial with alglucosidase alfa showed an increase in $Glc_4$ followed by a decrease over three years, corresponding with a clinical decline in the patient.[5] This can be explained by a reduction of glycogen secondary to muscle loss and fibrosis, similar to the decline in CK that occurs in Duchenne muscular dystrophy (DMD) with disease progression.[6] In addition, measurement of the proton density fat fraction via whole-body muscle magnetic resonance image (MRI) studies in late-onset Pompe disease demonstrated fat infiltration, likely resulting from muscle fibrosis.[7] Comparing the muscle fat fraction with urine $Glc_4$, patients with normal $Glc_4$ consisted of two distinct groups. The first had minimal disease progression demonstrated by a low intramuscular fat fraction and good performance on physical therapy (PT) assessments, whereas the second had significant disease progression with a high fat fraction and poor performance on PT. We surmised that patients approach a "burn-out" phase in the later stages of the disease, where there is insufficient healthy muscle in which to accumulate glycogen.

The international study referenced by the authors reported adverse liver outcomes, including hepatic cirrhosis, adenomas, and/or hepatocellular carcinoma (HCC), in only 11% of patients.[8] Histological and imaging data were not reported in this study, and hence the extent of liver involvement was not systematically evaluated in these patients. The liver regenerative capacity and extent of liver disease may vary among individuals, and not all GSD III patients display signs of fibrosis. In our study, we emphasized the need to monitor liver fibrosis in GSD III, and to cautiously interpret the decreases in AST, ALT, and $Glc_4$[2] over the first two decades of life. We agree with the authors that there is a need for more biomarkers and noninvasive tools to better understand and monitor disease progression in this disorder.

In the context of the pediatric patient presented by the authors, it will be important to follow this patient over time. During the short observation period, it is possible the decreases in biomarkers reflect a reduction in hepatic glycogen storage in response to dietary management. However, the patient was not evaluated for liver fibrosis by histology or elastography, and the length of follow up was very short.

Finally, it was speculated that "$Glc_4$ may be a good biomarker for muscle disorders in general". Our experience indicates that $Glc_4$ is not a sensitive biomarker for muscular disorders in which glycogen is not a primary accumulating substrate. We previously reported that $Glc_4$ has high specificity when used as a diagnostic biomarker in patients evaluated for Pompe disease.[9] However, $Glc_4$ was not correlated with CK in patients with a myopathy in whom Pompe disease had been ruled out by enzyme testing.[9] The evidence for $Glc_4$ elevations in DMD is derived from a report of an increased rate of $Glc_4$ excretion in 20 patients with DMD (aged 5–20 years), ranging from 3.3 to 12 mg/24 hours, compared with 0.1 to 2.5 mg/ 24 hours in 20 controls (aged 9–20).[10] It should be noted that although the age ranges were reasonably matched, no information was provided on the age distribution in the control group. As urine output increases as a function of body weight (pediatric

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urinary output: 1–2 mL/kg/hour), a carefully age-matched control group is needed to properly interpret increases in the rate of $Glc_4$ excretion. Thus, the association of elevated $Glc_4$ in DMD warrants further study.

To summarize, our studies emphasize the importance of correlating serum and urine biomarkers trends with histological and imaging studies, to better understand and interpret these biomarkers in GSD III.

## DISCLOSURE

P.S.K. has received research/grant support from Sanofi Genzyme, Valerion Therapeutics, Shire Pharmaceuticals, Amicus Therapeutics, Pfizer, Alexion Pharmaceuticals, and Ultragenyx, and consulting fees and honoraria from Sanofi Genzyme, Shire Pharmaceuticals, Alexion Pharmaceuticals, Amicus Therapeutics, Vertex Pharmaceuticals, Ultragenyx, and Asklepios Biopharmaceutical, Inc. (AskBio). P.S.K. is listed as an inventor on a licensed Duke University patent for the use of rhGAA in the treatment of GSDIII and other GSDs excluding GSDII. To date, neither Duke University nor the inventor has received any money from the commercialization of rights associated with this patent. Stephanie Austin received consulting fees from Ultragenyx. S.P.Y. works for a laboratory that offers clinical testing for urinary Glc4. She has received grant support from Sanofi Genzyme, Amicus Therapeutics, Biomarin Pharmaceutical, PTC Therapeutics, and Valerion Therapeutics and has consulted for Amicus Therapeutics, Sanofi Genzyme, and PTC Therapeutics. The other authors declare no conflicts of interest.



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