

Supplemental Information

**Massive Glutamine Cyclization to Pyroglutamic Acid in Human Serum
Discovered Using NMR Spectroscopy**

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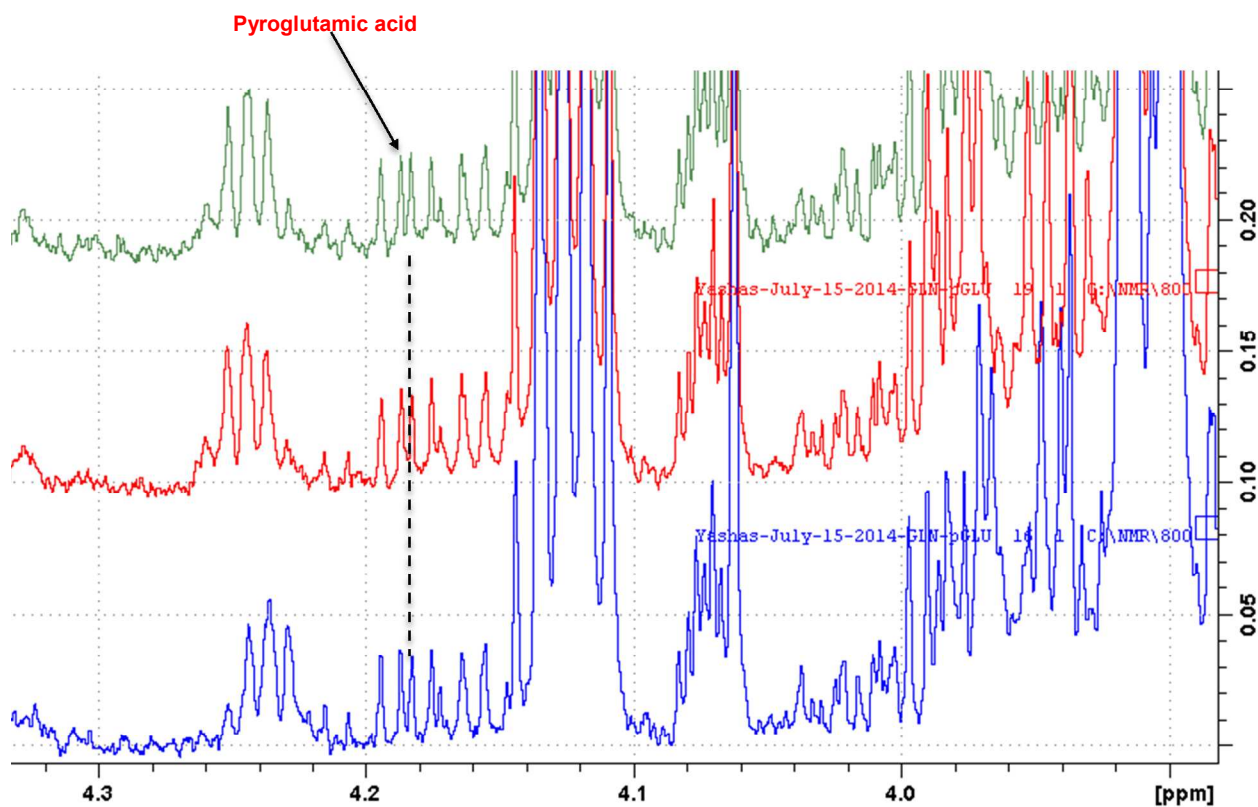
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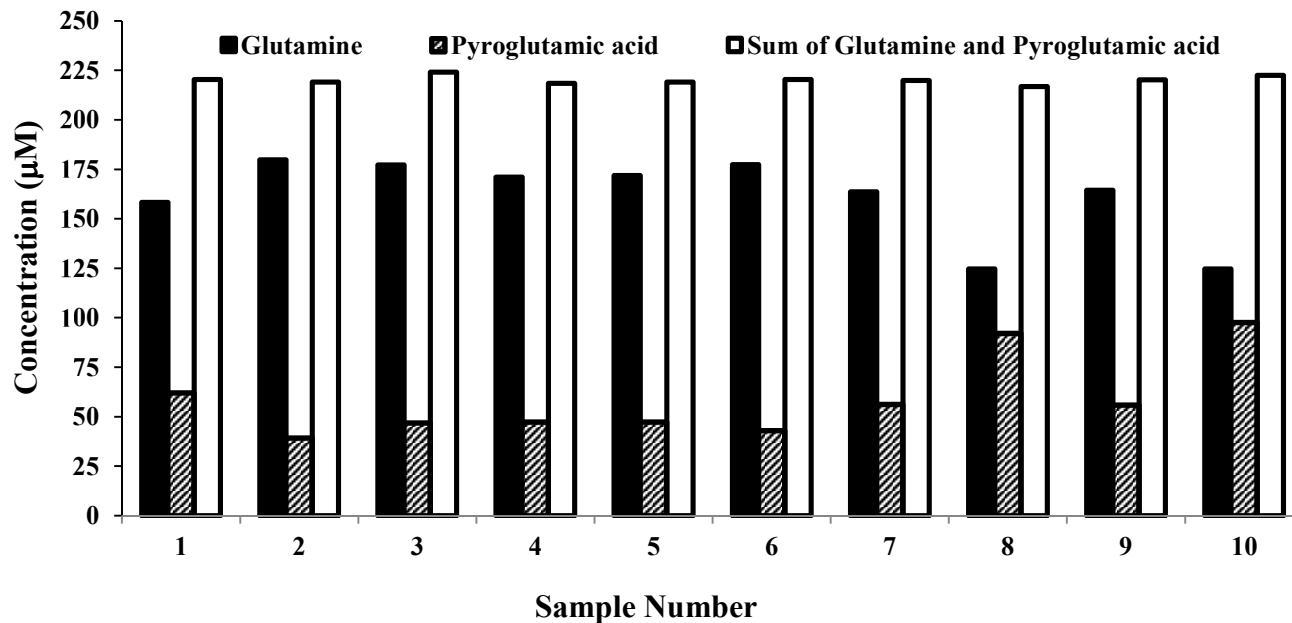
This file contains 6 pages. The first page is the Title page; page 2 contains Supplemental Table S1; pages 3, 4, 5 and 6 contain Supplemental Figures S1, S2, S3 and S4, respectively.

Supplemental Table S1: Pooled human serum, standard solutions and sample treatments employed in the study.

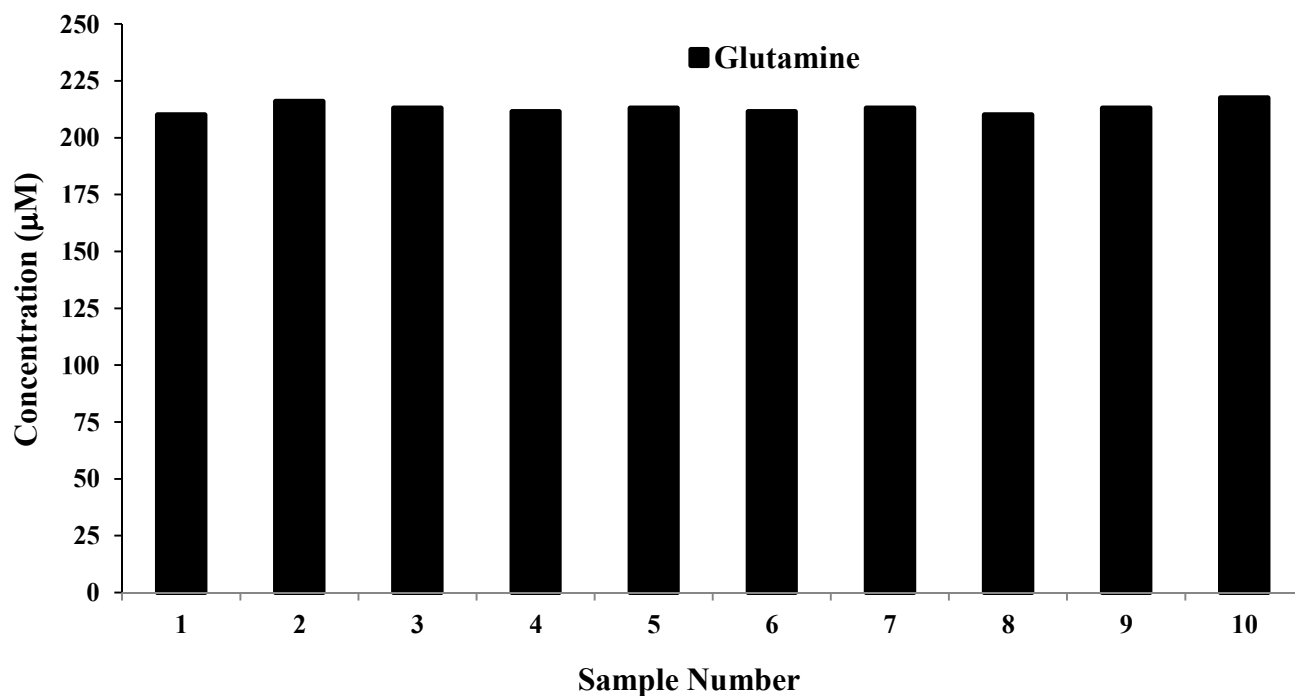
| Sample Processing method | Post-sample processing before NMR analysis | Number of replicates |
|--|---|-----------------------------|
| Methanol precipitation | Supernatant dried | 20 |
| | Supernatant not dried | 2 |
| Methanol precipitation after spiking with standard glutamine | Supernatant dried | 4 |
| Methanol precipitation before spiking with standard glutamine | Supernatant dried | 4 |
| Methanol precipitation after spiking with standard pyroglutamic acid | Supernatant dried | 4 |
| Ultrafiltration | Filtrate dried | 10 |
| | Filtrate not dried | 5 |
| Ultrafiltration after spiking with standard glutamine | Filtrate dried | 4 |
| Ultrafiltration before spiking with standard glutamine | Filtrate dried | 4 |
| Ultrafiltration after spiking with standard pyroglutamic acid | Filtrate dried | 4 |
| Intact serum | - | 10 |
| Intact serum spiked with standard pyroglutamic acid | - | 2 |
| Standard glutamine solution treated with methanol precipitation protocol | Supernatant dried | 2 |
| Standard glutamine solution treated with ultrafiltration protocol | Filtrate dried | 2 |



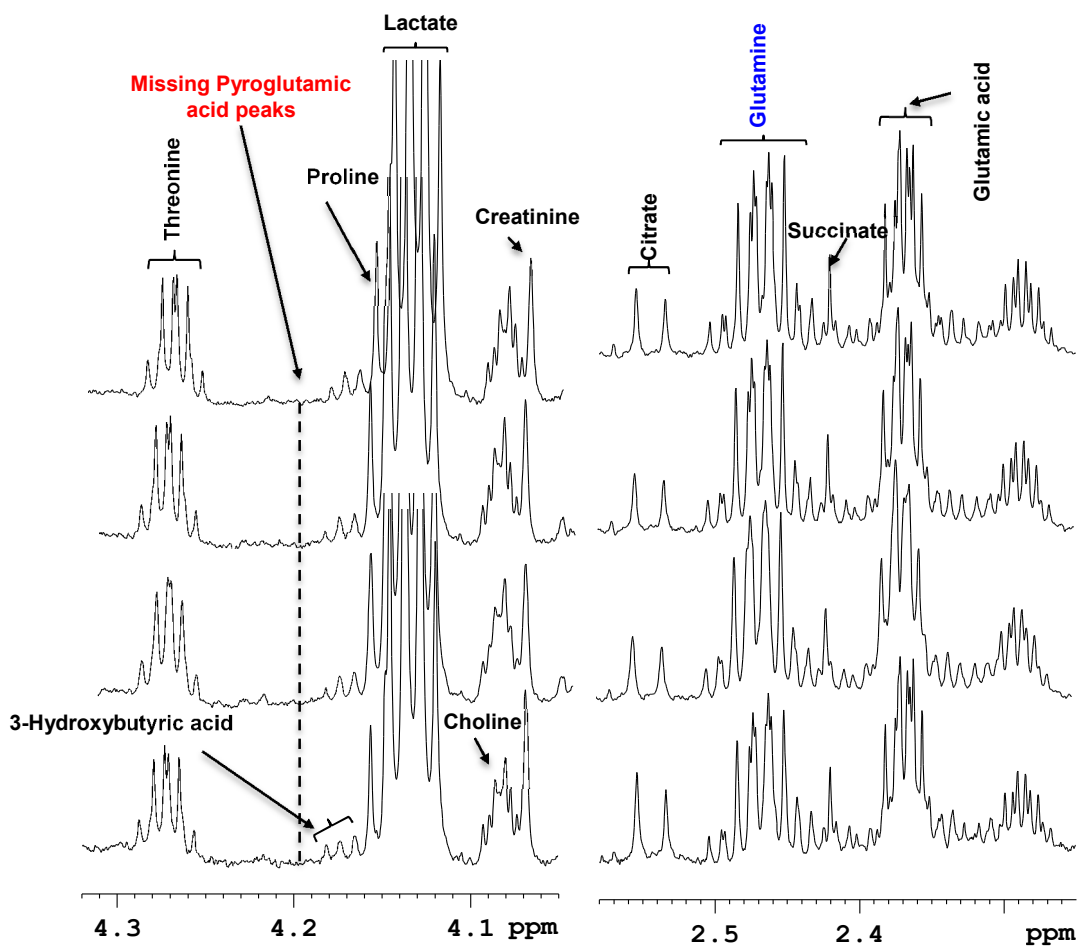
Supplementary Figure S1: Regions of 800 MHz ^1H NMR spectra of different sample portions from the same pool of human serum obtained after ultrafiltration, under virtually identical conditions, using 3kDa molecular weight cut-off filters. Filtrates were directly used for NMR analysis, without drying. Note the cyclization of glutamine to pyroglutamic acid in different samples.



Supplementary Figure S2: Plots of typical concentrations of glutamine, pyroglutamic acid and their sum as determined by 800 MHz ^1H NMR spectroscopy in different portions of samples from the same pooled human serum obtained after ultrafiltration. Note that while glutamine cyclizes to pyroglutamic acid, the sum of glutamine and pyroglutamic acid is the same in all the portions of serum samples (CV < 3%).



Supplementary Figure S3: Plots of typical concentrations of glutamine as determined (with reference to the internal alanine concentration in protein precipitated serum) by 800 MHz ^1H NMR spectroscopy in different portions of intact samples from the same pooled human serum. No glutamine cyclization to pyroglutamic acid was observed in the intact serum and the concentration of glutamine in intact serum is virtually the same as the sum of glutamine and pyroglutamic acid as observed in protein precipitated and ultrafiltered serum (see Figure 5 and Supplementary Figure S2).



Supplementary Figure S4: Parts of 800 MHz ^1H NMR spectra of different portions of intact serum taken from the same pooled human serum, highlighting the absence of glutamine cyclization unlike that observed in ultrafiltered and protein precipitated serum. Note the absence of pyroglutamic acid and the undiminished peak intensity for glutamine in all the spectra. The concentration of glutamine determined from these spectra is equal to the sum of apparent glutamine and pyroglutamic acid concentrations as determined in protein precipitated or ultrafiltered serum (see Figure 5 and Supplementary Figure S2).