Decomposition of ^{1}H NMR spectra of blood plasma for identification of complex metabolic response to glucose uptake

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Introduction:

High resolution ^{1}H Nuclear Magnetic Resonance (NMR) spectroscopy of blood plasma has a potential to characterize a variety of metabolic processes. These spectra contain resonances from diverse groups of molecules, resulting in broad shapes (proteins and lipid micelles) and sharp peaks (small molecules). Analysis of blood plasma spectra is quite challenging and a traditional solution for metabolomic investigation of blood is segmenting predetermined regions of the spectra and integrating the signal in each segment, called binning. Here we present a technique where an entire spectral region is analyzed and represented as a mixture of several spectral shapes and their fractional contributions to the original spectrum. The identification of the metabolites in each shape, as well as their kinetic behavior over the course of the experiment can be directly related to metabolic responses of the system.

Methods:

Seven blood plasma samples from five horses were analyzed using NMR spectroscopy. The samples were collected at 30 minute intervals for 3 hours following an oral glucose dose of 0.25g/kg. NMR data was acquired on Varian 600MHz spectrometer with relaxation-edited spin-lock and water-suppression acquisition sequence. The methyl resonance of the alanine (1.46 ppm) was utilized for global alignment of the data. Further, the position of individual peaks were locally adjusted [1]. The spectral data between [0.5-4.5] ppm was decomposed, using cNMF [2]. This algorithm is based on nonnegative matrix factorization, where the recovered spectral patterns and their corresponding amplitudes must be positive, thus these recovered spectral patterns are physically realizable. Without the preprocessing and local alignment this decomposition would be unsuccessful. The entire analysis was performed by software tool, *HiRes* [3], which is freely available for research purposes at http://hatch.cpmc.columbia.edu /highresmrs.html.

Results and Discussion:

The results of our analysis are presented in Figure 1 and they depict a detailed time-related metabolic response to the glucose uptake. The results underscore the power of the decomposition approach, when applied in combination with correct and rigorous pre-processing of the data, to identify underlying basic spectral shapes within a complex set of spectra, and to connect them quantitatively with the biological end point measurements. This approach has promise for deconvolving complex metabolic responses from disease, drugs and toxins and should find application to identification of biomarker combinations for disease diagnosis and for monitoring the beneficial and adverse effects of pharmaceutical compounds.

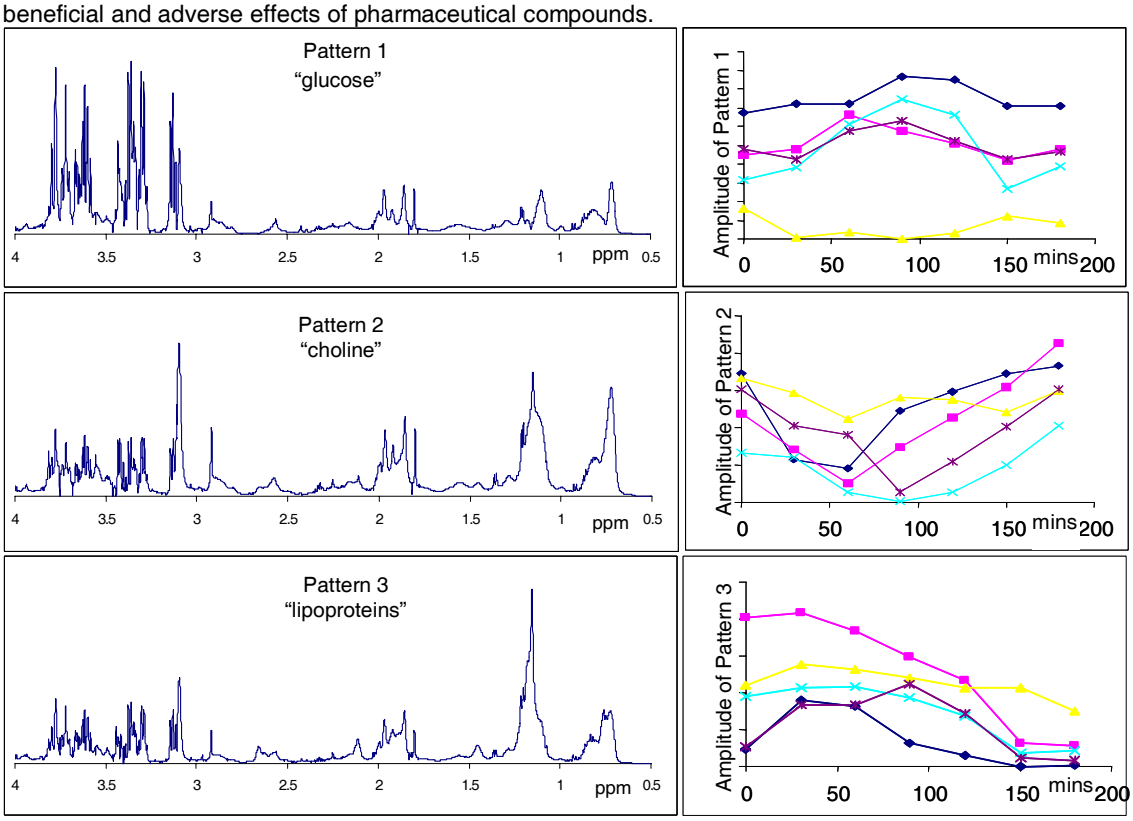

Figure 1: Recovered spectral patterns (left) and their corresponding amplitudes (right) in each horse's blood samples. The first pattern is related mainly to the glucose metabolites and its time-related behavior is quite uniform and it roughly reflects the peaking behavior of a physiological response to a dose of insulin. The plot also shows the muted response in one of the horses (yellow series), who did not receive a full dose. These results were confirmed by traditional glucose assays. The second pattern is related mainly to choline and some lipo-/glycoproteins and has reciprocal behavior to the first one. And finally the third pattern highlights specific lipoproteins and is depleted by the end of the three hours.

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Reference: [1] Stoyanova R., Nicholls A.W., et al, J. of Mag. Res., 170, 329-335, 2004; [2] Sajda, P., *et al*, *IEEE Trans. Med. Imaging,* 23, 1453-1465, 2004; [3] Zhao Q., Stoyanova R. et al, Bioinformatics, 22, 2562-2564, 2006