Development of a QconCAT internal standard for absolute quantification of apolipoproteins

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Introduction. The proteomics field has evolved tremendously throughout the last decade. New implementations in sample preparation, data acquisition and data analysis allowed a deeper understanding of protein biology in complex systems. This trend is also valid in the lipoprotein field, in which apolipoproteins are linked to several inflammatory diseases. In this context, the high-density lipoprotein (HDL) is a key player, due to its diverse proteome composed of dozens of proteins. Mass spectrometry-based proteomics has made it possible to gain insights into fundamental questions of the HDL biology, such as understanding the composition of different HDL subclasses and their composition and abundance changes in healthy and disease states. However, a challenge that remains is to move from a relative to an absolute protein quantification. To overcome some technical challenges imposed by absolute quantification, a strategy known as QconCAT (Quantification Concatamer) or PCS (Peptide Concatenated Standard) has been previously developed by independent research groups. This internal standard combines the primary sequences of tryptic peptides from several target protein sequences into a single polypeptide. This polypeptide is expressed in bacteria, purified and used for absolute protein quantification.

Methodology. Using previous data from our lab and the literature, we in silico designed a polypeptide containing 53 peptides from 29 protein groups that are important in HDL biology, due to their roles in lipid metabolism, inflammation and lipoprotein structure. The gene encoding this protein was inserted into a plasmid for heterologous expression in Escherichia coli and protein expression was induced using the lac operon in light or heavy labeled media. We quantified our protein standard using synthetic peptides by mass spectrometry. Protein was digested using trypsin, injected onto the mass spectrometer in a data-independent acquisition mode and peptide abundances were analyzed using the Skyline software.

Results and Discussion. We spiked our heavy labeled QconCAT internal standard into isolated HDL and analyzed a few analytical parameters of this protein. Of note, trypsin hydrolysis was efficient in standard overnight condition and peptide peaks were distributed across the whole chromatogram range. Quantification was precise (coefficient of variation below 20%) in 47 out of the 53 analyzed peptides in a HDL matrix. Impressively, 23 of those peptides presented coefficients of variation as low as 10%. With those results, we made some improvements in the sequence of the standard protein and developed a new sequence (five quantotypic peptides were removed from analysis and eight peptides were substituted).

Conclusion. Our results point to a promising role of using QconCAT technology to perform absolute protein quantification in lipoproteins, especially HDL. More experiments are required to evaluate the extent by which this standard could be useful in clinical and mechanistic studies.

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