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S1. Quantitative Proteomics

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COMPARATIVE STUDY OF TWO PROTEOMIC QUANTITATIVE METHODS, DIGE AND ITRAQ USING 2D GEL- OR LC-ESI QTOF

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The quantification of differences between two or more physiological states of a biological system is among the most important but also most challenging technical tasks in proteomics.

Classical methods perform an initial separation step at the protein level, usually a high resolution 2D electrophoresis, where quantitation takes place. A major limitation of the technique is that mainly soluble abundant proteins are separated and detected. A second step is needed to identify the proteins of interest. Over the past few years, mass spectrometry-based quantification methods have gained increasing popularity. This is, probably, because by working at the peptide level they overcome the two important shortcomings of classical proteomic quantification methods: the limited protein range that can be examined and the two-step strategy requirement. However, mass spectrometry is not inherently quantitative because proteolytic peptides exhibit a wide range of physicochemical properties affecting their mass spectrometric response. Thus, most of de mass spectrometry-based methods employ differential stable isotope labeling to create a specific mass tag that can be recognized by a mass spectrometer and provide the basis for quantification.

The optimal strategy to perform quantitative proteomic analyses to obtain the most informative data sets is undefined and it, probably, depends on the systems under study.

In the present study we have compared two quantitative methods: a gel-based DIGE approach and a mass spectrometry-based iTRAQ approach using LC-ESI QTOF for advancing our understanding of proteomic changes in hypertensive heart disease.