Sphingolipid variations between hypertensive and normotensive patients elucidated by MRMS

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Introduction
Hypertension is one of the major worldwide causes of death. Although the precise cause-effect relationship is controversial, many studies have proposed that endothelial dysfunction may contribute to emergence of hypertension [1]. Sphingolipids are involved in the regulation of both vascular growth and vascular tone. Several reports have shown that in hypertension essentially sphingolipids levels are altered. The profiling of sphingolipids is usually carried out by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). As a alternative to LC-MS/MS, which suffers from long analysis time and lack of reproducibility due to retention time drift, direct infusion magnetic resonance mass spectrometry (DI-MS/MS) provides short analysis time for sample screening including high mass accuracy as well as iso-topic fine structure and metabolic identification, allowing analysis without the front-end separation step and derivatization. This MRMS workflow maximizes high throughput in metabolic profiling. In this study sphingolipid levels among healthy and hypertensive patients measured by DI-MS/MS were investigated.

Methods
Extraction
Twenty-four patients with hypertension (defined as DBP ≥90 mmHg and/or SBP ≥140 mmHg or on the basis of use of hypertensive medication) and 9 healthy donor control subjects (non-smokers and non-diabetic) without previous cardiovascular events and not on statin therapy, belonging to the Campania Salute Network Registry, were studied. The database generation of the Campania Salute Network was approved by the Federico II University Hospital Ethic Committee. Signed informed consent was obtained from all the participants to use data for scientific purposes. Plasma samples were thawed and extracted with methanol (MeOH) (70% v/v) and derivatization. This MRMS workflow maximizes high throughput in metabolic profiling. In this study sphingolipid levels among healthy and hypertensive patients

Results
Total lipid extracts from 33 human plasma samples were analysed in this study. The analysis of plasma lipid extracts showed a very complex profile. Roughly 200 lipids (considering both positive and negative ionization) were tentatively annotated, with very good mass accuracy (average error ≤ 0.1 ppm) and detected lipids belonging to different classes. Among them as shown in figure 1 different sphingolipids showed alteration between healthy and hypertensive subjects (patients).

This is shown in figure 1 reporting the fold change of many sphingolipids. Both groups, healthy and hypertensive, could be clearly separated in the statistic plot shown in figure 2. In particular the ceramide level in the medium-length ceramides such as Cer (18:1-4E/16:0) showed a increase of hypertensive patients. This data is validated by a growing number of publications of hypertensive studies [2].

Conclusions
- DI-MS/MS is a fast and reliable method for sphingolipids profiling.
- Several sphingolipids levels alter by hypertensive disease when comparing healthy and hypertensive patients.
- This data further highlights the role of ceramides associated to endothelium dysfunction.

Figure 2: Both groups (healthy, CTRL, red) and hypertensive (HT, green), could be clearly separated with high significance in the statistic plot.

Figure 1: Fold change of mainly sphingolipids by hypertension - ratio of case (hypertension) to control(normotensive) is shown

Figure 3: Fold change of mainly sphingolipids by hypertension - ratio of case (hypertension) to control(normotensive) is shown

References

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