Clustering human metabolites resulting from TOTUM•070 absorption (a plant-based, polyphenol-rich hypocholesterolemic ingredient) improve lipid metabolism in human hepatocytes: lessons from an original ex vivo clinical trial

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Purpose
Hypcholesterolemia is a well established reversible risk factor of atherosclerotic cardiovascular diseases. TOTUM•070 is a patented polyphenol-rich compound which has shown hypocholesterolemic properties in preclinical studies. The objective of this study is to characterize the mechanisms of action of TOTUM•070 metabolites in human using an original ex vivo clinical approach that considers the physiological digestive processes of absorption and metabolism.

Methods
A pool of 10 healthy men volunteered for this study. To determine kinetic of TOTUM•070 bioactive molecules profile in the serum, volunteers fasted for 12 h were given 5g of TOTUM•070. Venous blood was collected from the cubital vein before the ingestion and every 20 minutes for 240 minutes after the ingestion. Human circulating metabolites from polyphenols were quantified and characterized by UPLC-MS. Once the absorption peak was determined, volunteers were called back for the collection of the enriched serum fraction. For this second clinical phase, volunteers who fasted for 12 h were given 5g of TOTUM•070. Venous blood was drawn from the cubital vein before the ingestion for the collection of a naive serum and at the maximum absorption peak (Tmax) for TOTUM•070 metabolites enriched serum collection.

Clinical phase: Dose 5g of TOTUM•070 in 8 capsules

Ex vivo phase: human HepG2 hepatoma

Step 1: Results of kinetic profile of TOTUM•070 absorption in humans

The digestion and absorption profile of the extract was monitored through a kinetic of apparition of the metabolites following TOTUM•070 ingestion to determine the time frame of the absorption peak. We found 20 detectable human metabolites. Circulating human metabolites include cholesterergic acid, cycryl, four ferulic acid sulfate isomers, luteolin, three luteolin glucuronides isomers, four oleuropein glucuronides isomers, tyrosol sulfate, three hydroxy-tyrosol sulfate isomers and two hydroxy-tyrosol glucuronides isomers. Thus, these data strongly evidence an efficient absorption and bio-availability of the product. The Tmax (time to reach the maximum concentration observed in serum) ranged from 40min to 120min post absorption depending on the type of metabolites. Consequently, enriched serum TOTUM•070 metabolites was collected at 60 min post ingestion for optimized metabolite diversity.

Step 2: Effects of TOTUM•070 metabolites ex vivo on human hepatocytes metabolism under lipotoxic stress

Clustering human metabolites resulting from TOTUM•070 absorption reduced the protein level of FA synthase enzyme (p<0.01) as well as decreased activity of the enzyme HMG-CoA Reductase (p<0.0001). This pattern indicates inhibition of the novo synthesis of fatty acid and cholesterol, respectively, which is in line with the observed reduction of lipid content in the cells. Interestingly, TOTUM•070 enriched serum increased activity of the LCAT enzyme that exerts anti-atherogenic properties.

Conclusion
These results demonstrate that TOTUM•070 metabolites circulating in human serum protect the HepG2 cells from palmitate overload evidenced by reduction of intracellular triglycerides and cholesterol content. Various metabolic-related genes and enzymes regulating cholesterol homeostasis in hepatocytes were regulated with TOTUM•070 enriched sera. All together, these data provide biochemical insights into the beneficial mechanisms of action of TOTUM•070.

RNA sequencing analysis

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