SCIENTIFIC REPORT- SHORT TERM SCIENTIFIC MISION (STSM)

(COST Action FA1403, POSITIVe)

STSM topic: Bioavailability of Aronia juice polyphenols as determinant of inter-individual variation in their effects on platelet function

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Background and objectives of the STSM

The aim of this STSM was to provide data on qualitative and quantitative composition of urine samples obtained in randomized, placebo controlled, three-arm, cross-over designed clinical trial, conducted in apparently healthy males and females at different levels of CVD risk, aiming to investigate the effects of 4-week long consumption of polyphenol-rich Aronia juice (100 mL in two intervention arms-High and Low Dose, compared to Placebo) on platelet function and other CVD risk factors. Prior to STSM, a team from Serbia conducted this RCT and spot samples of the total morning void were collected at each of six study visits, at fasting, in addition to blood sampling for determination of different biomarkers of platelet function, biochemical parameters, blood pressure and anthropometric measurements.

Further objective of this STSM was to focus on variations in bioavailability data between subjects and correlations with factors that could determine this variability (link to the objectives of Working Group 1-WG1 of the POSITIVe COST Action), as well as the effects of observed variations to the assessed risk factors of cardiovascular disease (CVD), with focus on platelet function (activation and aggregation), as a direct link to the objectives of Working Group 2 (WG2) of the POSITIVe COST action.

Description of the activities carried out during the STSM

- Training in advanced techniques, including LC-MS/MS techniques, all the preparatory techniques (mainly solid phase extraction, SPE), and methods for the analyses of data obtained (including introduction to Agilent MassHunter software)
- Analyses of urine samples using LC-MS/MS techniques, according to the validated and well-established protocols, available at Host institution (IFR). In order to identify and quantify present metabolites, reference standards of the known products of the breakdown

and microbal transformation of anthocyanins, procyanidins and phenolic acids (chlorogenic, neochlorogenic), as main phenolic compounds of Aronia juice, were used.

- Analyses carried out included:
  a) urine samples from 37 participants obtained before and after 4-week of High Dose juice consumption (High Dose=1000 mg of total polyphenols)
  b) urine samples from the same participants (n=35) before and after 4-week of Low Dose juice (250 mg of total polyphenols) consumption
  c) urine samples from 8 participants before and after 4-week of Placebo (no polyphenols detected) consumption

Main results obtained during the STSM

- From the 23 metabolites included in the method, 18 compounds were identified in urine samples (Table 1).

Table 1- Metabolites included in method and identified in samples

Metabolite	Identified
Protocatechuic acid (PCA)	YES
Phloroglucinaldehyde (PGA)	NO
3-Hydroxybenzoic acid	YES
4-Hydroxybenzoic acid	YES
Vanillic acid (VA)	YES
IsoVanillic acid (IVA)	YES
Methyl-3,4-dihydroxybenzoate	NO
PCA-glucuronide (3 and 4) *	YES
PCA- sulphate (3 and 4)	YES
Ferulic acid	YES
4-Hydroxy-phenylacetic acid	YES
3,4-dihydroxy-phenylacetic acid *	YES
4-hydroxy-benzaldehyde	YES
3, 4-dihydroxy-benzaldehyde	YES
4-methoxysalicylic acid	NO
Caffeic acid	YES
Cyanidin-3-glucoside	YES
5-(3',4'-dihydroxy-phenil)-γ-valerolactone *	YES
5-(3',4',5'-trihydroxy-phenil)-γ-valerolactone	NO
5-(3'-methoxy-4'-hydroxy-phenil)-γ-valerolactone	NO
5-(3',4'-dihydroxy-phenil)-γ-valerolactone-3-glucuronide *	YES
5-(3',4'-dihydroxy-phenil)-γ-valerolactone-4-glucuronide *	YES
Hippuric acid *	YES

*the increase in level was significant after the High Dose juice consumption

- Cyanidin (Cyanidin-3-glucoside used as a standard) was identified only in samples from 8 participants obtained after the High Dose juice consumption. In this purpose the HPLC method with DAD was applied

- Comparing the levels before and after the consumption of the High Dose juice, significant changes (Wilcoxon test $p<0.05$) were found for the (Figure 1):
  Protocatechuic acid-glucuronide (Pca-Glc),3,4-dihydroxy-phenylacetic acid, Hippuric acid, 5-(3',4'-dihydroxy-phenil)-γ-valerolactone (DHVL), 5-(3',4'-dihydroxy-phenil)-γ-valerolactone-3-glucuronide(DHVL-3-Glc),5-(3',4'-dihydroxy-phenil)-γ-valerolactone-4-glucuronide (DHVL-4-Glc).
  Increase was also found for the *3-hydroxy-benzoic acid* ($p=0.053$) and *Vanillic acid* ($p=0.067$), although not statistically significant.
- In case of Low Dose juice and Placebo, no significant increasess in metabolites' levels were observed after their consumption.

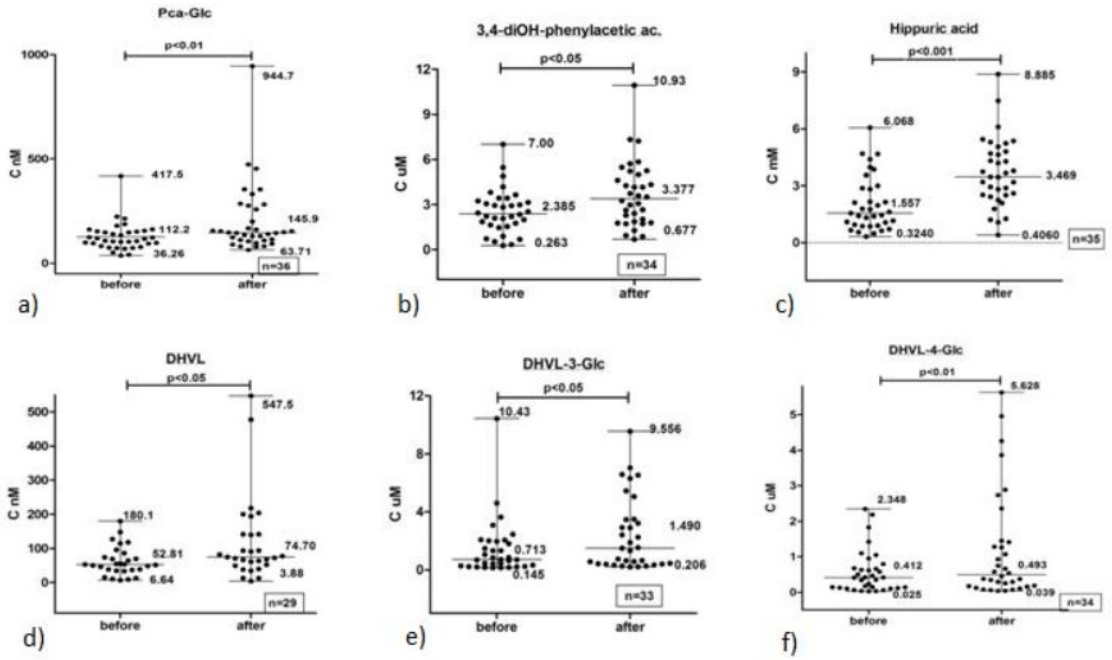

Figure 1. Levels of : a)Protocatechuic acid-glucuronide (Pca-Glc); b)3,4-dihydroxy-phenylacetic acid; c) Hippuric acid; d)5-(3',4'-dihydroxy-phenil)-γ-valerolactone (DHVL); e)5-(3',4'-dihydroxy-phenil)-γ-valerolactone-3-glucuronide(DHVL-3-Glc); f)5-(3',4'-dihydroxy-phenil)-γ-valerolactone-4-glucuronide(DHVL-4-Glc) before and after High Dose juice consumption;
Data are presented as median and range.

Future work and collaboration

Further analyses of obtained data are required in order to clarify observed inter-individual variations in bioavailability and possible factors that could determine this variability, as well as to investigate the potential effects of these variations to the assessed risk factors of CVD, mainly platelet function.

Both Home and Host Institution believe this STSM has been of great importance for strengthening the existing collaboration, as well as for reaching their and overall scientific objectives of the COST action POSITIVe.