SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: COST Action FA1403 - 37843, POSITIVe
STSM title: Gene expression regulation in human trials following intervention with bioactive compounds: a literature survey.
STSM start and end date: 03/06/2017 to 29/06/2017
Grantee name: Biljana Pokimica

PURPOSE OF THE STSM:

Human intervention studies suggest that various plant bioactives and food can affect the regulation of gene expression. However, reported data are very heterogenous, since the studies have been performed on diverse human cells and tissues, using different plants, ingested or applied topically. Additionally, studies differ in design and statistical analysis of data, so the role of plant bioactives in human gene regulation has not been established yet.

Therefore, this STSM has 2 purposes:

1. To analyse the quality of design and data presentation of performed studies;
2. To clarify the possible molecular mechanisms of diverse plants on gene expression in different human cells and organs.

DESCRIPTION OF THE WORK CARRIED OUT DURING THE STSMS

Before the STSM started, the WG2 team had collected the articles and made a template in Excel with the main information that should be extracted from the articles (authorship, year, title, bioactive compound and tissue examined). The collected articles were published from 1994. till 2017, all being about the influence on plant food or bioactives on human gene expression, analysed with RT-PCR.

The working plan consistsed of :

1. Completing the data extraction using Excel tables (with detailed information on each article);
2. Updating literature data with the most recent studies (published during June 2017);

3. Designing and completing the Word tables (that will be published in the article, or as a supplementary material);
4. Writing a review.

During June 2017 I have been reading the selected articles (63) and extracting data from them into Excel and Word tables.

The Excel tables enable us to obtain useful information on:

1. Participants (age: minimum, maximum, mean, range; gender; ethnicity; status: health, menopausal, smoking; use of: vitamins, minerals, medications);
2. Study design (registration number, blinding, randomization, washout period, intervention duration, number and description of arms, number of time points, diet baseline and during study, compliance);
3. Analysis of metabolites (type of sample, type of compound or metabolite and its concentration detected in each group before and after intervention);
4. Administration and content of treatment and control;
5. RNA isolation and storage (type of sample, sampling protocol, processing time until RNA extraction, sample storage, RNA extraction protocol, RNA concentration, method for checking RNA purity and its values, RNA pooling, RNA storage);
6. RT-PCR analysis (type of RT-PCR, endogenous control, number of samples for each group before and after intervention, analysis system, data analysis, criteria for significance, data results presentation, main results, information on variability);
7. Protein confirmation and other responses of interest;
8. Problems and comments;
9. The most important points for quality check (number of analysed samples before and after intervention, use of placebo/control, number of time-points, dose-response analysis, quality samples/RNA, reporting significance of the results, confirmation protein/activity levels, other responses related, level of evidence).

I have also worked and prepared three main Word tables that collect the most interesting data from all the articles. The Tables summarize information about :

1. Participants (health status, gender);
2. Study design (randomized, controlled, crossover, parallel, acute);
3. Product description (bioactive compounds, administration form, total dose per day, duration in days);
4. Gene(s) (change attributed to treatment, number of compared samples, time points);
5. Data presentation (expression levels; change: ratio, FC, %; % of individuals with a change;value: mean, median, individual data; variability: SD, SEM, range, 95% CI, % of individual with a change, data in figure);
6. Compliance with metabolites or protein;
7. RT-PCR protocol (sample description, RNA quality, reference gene(s)).

Every day, I had the opportunity to discuss with Dr Mayte Garcia Conesa the work I had carried out, together we improved data extraction and tables design, and prepared ideas for the review. She also explained to me the characteristics, advantages and disadvantages of each study and thus I have learned a lot about gene expression regulation as well as how to work to prepare a review. These aspects will be very valuable for my PhD work.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

After reading 63 articles, we have found that the best design for this type of study should be the crossover (only 18 articles of the selected ones), enabling comparison between the same individuals before and after different treatments, which is of great importance due to inter-individual variability. Information about variability have not been reported in 4 articles, while in the rest the variability have been expressed using at least one of the following: SEM (25 articles), SD (22 articles), range (4 articles), 95 % CI (6 articles), figures

(4 articles).

We consider that the quality of RNA should be checked prior to RT-PCR and values reported in the article, as we have found in 5 articles. Additionally, we conclude that the endogenous control should be tested, and the explanation why the endogenous control was chosen should be in the text, as found in 3 articles. According to us, the results would be more reliable if the gene expression was supported by the protein expression (18 articles), and related to the plant metabolite presence in the tissue or blood samples (7 articles).

Comparing all the articles, we found that 12 of them have investigated the influence of olive (as oil, leaf or water waste extract) on expression of various genes, all in mononuclear blood cells except one in adipose tissue.

Another well represented plant product in the articles is broccoli (7 articles), the influence of which has been investigated mostly on *HO-1* and *NQO1* expression, in breast tissue and blood and nasal lavage cells

A widely investigated single compound was resveratrol (7 articles), in various tissues (skeletal muscle, adipose, colon) and mononuclear blood cells, on different genes.

In the majority of the articles (9) the expression of *HO-1* gene has been investigated and found to be increased upon broccoli in cells from nasal lavage, but not changed in mononuclear blood cells. Also upon broccoli, transcripts were detected in breast tissue. Coffee has been shown to downregulate *HO-1* in lymphocytes, while curcumin has not changed it in lymphocytes and monocytes. The same as coffee, flaxseed has downregulated *HO-1*, but in different type of cells- buccal swabs.

The expression of *NQO1* has been investigated in 7 articles, in 3 with broccoli, and it has been upregulated in cells from nasal lavage, detected in breast tissue, and non significantly downregulated in blood. The bilberry has increased *NQO1* expression, while resveratrol has not changed it, both in mononuclear blood cells. The coffee and flaxseed have not changed the *NQO1* expression in lymphocytes and buccal swabs, respectively.

The expression of *COX2* has been investigated in 7 articles, and shown to be upregulated in white blood cells upon olive oil, but downregulated in mononuclear blood cells upon olive leaf extract, while not changed in: lymphocytes, leukocytes and muscle upon quercetin, blood upon mixed fruit and vegetables, gastric antrum upon curcumin, and prostate tissue upon lycopene or fish oil.

FUTURE COLLABORATIONS (if applicable)

At the end of this STSM Dr Mayte García-Conesa and me agreed to continue working on review, keeping in touch via e mail and skype. We defined our future objectives related to this STSM:

1. To double-check the extracted data in Excel and Word reading all the articles again;
2. To try to determine which studies are best designed (in order to give recommendation on design);
3. To try to give a conclusion about gene expression variability in human samples;
4. To decide if the review should be organized according to same genes or same plants (bioactive plant derived compounds);
5. To read a few articles on microarrays and about gene expression in cells and in animal studies, in order to compare them with human studies on RT-PCR;
6. Dr Mayte Garcia Conesa will present our results on the next COST meeting in Thessaloniki in September 2017.

This STSM has strengthened the collaboration between CEBAS-CSIC (Murcia, Spain) and CENM (University of Belgrade, Serbia) within the COST Action POSITIVe, and has given me a great opportunity to learn about gene expression regulation and review preparation.