SCIENTIFIC REPORT - SHORT TERM SCIENTIFIC MISSION (STSM)

(COST Action FA1403, POSITIVe)

STSM topic: Meta-analysis on flavonols

Grantee: Prof Maria Paula Pinto (Instituto Politécnico de Santarém, Escola Superior Agrária, Portugal (ESAS) and Instituto de Tecnologia Química e Biológica/Instituto de Biologia Experimental (ITQB/IBET, Oeiras, Portugal)

Host: Dr. Emilie Combet (The University of Glasgow, Glasgow, UK)

Period: 15/02/2016 to 26/02/2016

Reference code: COST-STSM-FA1403-32074

1. Aims

The aim of this Short Term Scientific Mission was to have training on meta-analysis methodology, which was applied to evaluate the effects of flavonol intake in several human cardiometabolic biomarkers, within the goals of WG2 of POSITIVe. One of the main objectives of this COST Action is to analyse and to understand the inter-individual variability in the response to the intake of bioactive compounds on specific cardio-metabolic biomarkers and to elucidate which factors affect this variability. Several subgroups were defined within WG2, each studying one particular bioactive. The current report refers to results of meta-analysis on flavonols, which is being coordinated by the above mentioned grantee.

Before this STSM, an extensive literature search was conducted on several databases, according to protocol defined within WG2, retrieving 520 unique papers that were distributed among flavonol team members for screening. The following criteria were applied for rejection of papers: in vitro and animal studies; studies without proper control, studies without flavonol or very low concentration comparing to other present polyphenols, studies not having the defined cardiometabolic outcomes and acute studies. After screening, 19 papers with randomized controlled trials were selected for data extraction, which was performed according to a pre-defined template in WG2 and used for meta-analysis, under the supervision of Dr. Emilie Combet from the University of Glasgow. This STSM comprised of two clearly defined objectives:

A) To acquire skills and knowledge of the tools needed for meta-analysis and application on flavonol extracted data.
B) To contribute to the current meta-analysis under development specifically evaluating the effects of flavanols and the assessment of inter-individual variability on specific cardio-metabolic biomarkers.

2. Description of the work carried out during the STSM and main results obtained

A. Meta-analysis flavonols/cardio-metabolic markers

a) Stratify the studies based on the specific outcome (biomarker) reported, as well as prepare a summary for each factor (age range, BMI, gender, ethnicity, health status, smoking, menopausal status, diet during the intervention, flavonols source, duration, etc.);
b) Define the quality of the study using the JADAD method;
c) Standardize the units for each outcome (biomarker). We carried out the conversions to the same estimators and units (mean and standard deviation, and preferably mmol/L for specific cardio-metabolic risk biomarkers);
d) Define which outcomes and which subgroups could be used for meta-analysis;
e) Insert the data for each outcome in the Comprehensive Meta-Analysis program including different data format:

- sample mean, SD pre and post, N, in each group, Pre/Post Corr.
- sample mean change, SD pre and post, N, in each group, Pre/Post Corr.
- sample size and *p*-value.

f) Run the analysis using the software Comprehensive Meta-Analysis V3 for each specific cardio-metabolic biomarker and make different comparisons between all selected factors in order to evaluate the effects of flavonols and the assessment of inter-individual variability.
g) Present the results obtained in a meeting with the group of Dr. Emilie Combet (anex)

B. Contribution to the meta-analysis on flavonols

a) Collaborate in the extraction of data from non-extracted papers;
b) Insert data for insulin in the Comprehensive Meta-Analysis software.

3. Follow up work on flavonol and flavanol meta-analysis

a) Register the meta-analysis protocol of Flavonols in Prospero (www.crd.york.ac.uk/Prospero, an international database of prospectively registered systematic reviews in health and social care) (we are currently writing the draft);
b) Perform all the remaining analysis on flavonols during the period of active licence on Comprehensive Meta-Analysis Software.
c) Present the results in a meeting of WG2 at Bucharest

4. Future collaboration with the host institution and foreseen publications/articles resulting from the STSM

This STSM has strengthened the collaboration between ESAS and ITQB/IBET and University of Glasgow within the COST Action POSITIVe to progress with the data analysis of some of the proposed systematic reviews as well as for the specific completion and

dissemination of the results of the flavonols and flavanols meta-analyses. The analyses carried out will be supervised by Dr. Emilie Combet from the University of Glasgow (host institution). Foreseen publications resulting from the STSM will be discussed in the next COST meeting in Bucharest.

Interindividual variation in response to consumption of plant food bioactives and determinants involved
COST Action (FA1403)

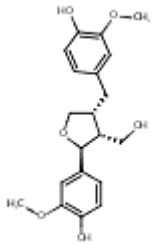
WG2 The variability in the response of humans to the intake of bioactive compounds: subgroup human studies

Inter-individual variability in response to the impact of flavanols on cardiometabolic biomarkers

systematic review and meta-analysis of randomized controlled human trials

(Poly)phenols

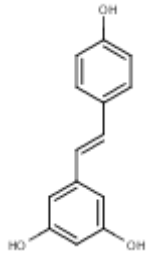
Lignans



Lariciresinol

Flavonoids

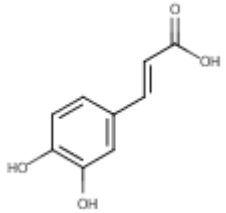
Stilbenes



resveratrol

Phenolic acids

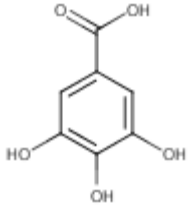
Hydroxycinnamic acids



Caffeic acid
[3-caffeoylquinic acid]

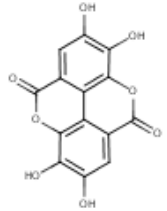
Hydroxybenzoic acids

monomers



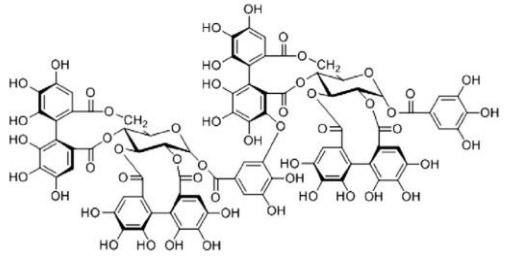
Gallic acid

dimers



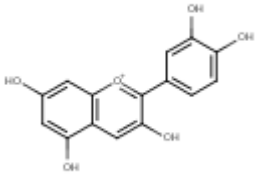
Ellagic acid

ellagitannins



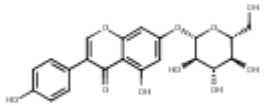
Sanguiin H6

Anthocyanins



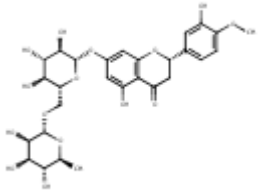
Cyanidin
[cyanidin-3-O-glucoside]

Isoflavonoids



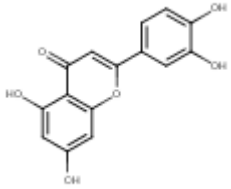
Genistin
[6’’-O-acetylgenistin]

Flavanones



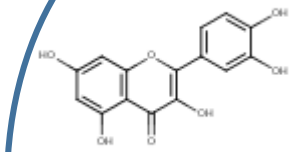
Hesperidin

Flavones



Luteolin
[Luteonin-7-O-diglucoronide]

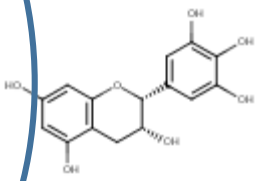
Flavonols



Quercetin
[Quercetin-3-O-rutinoside]

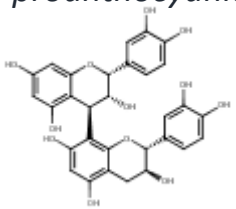
Flavanols

monomers



(-)-Epigallocatechin
[(-)-Epigallocatechin-3-O-gallate]

proanthocyanidins



Proanthocyanidin B1

Bioactive

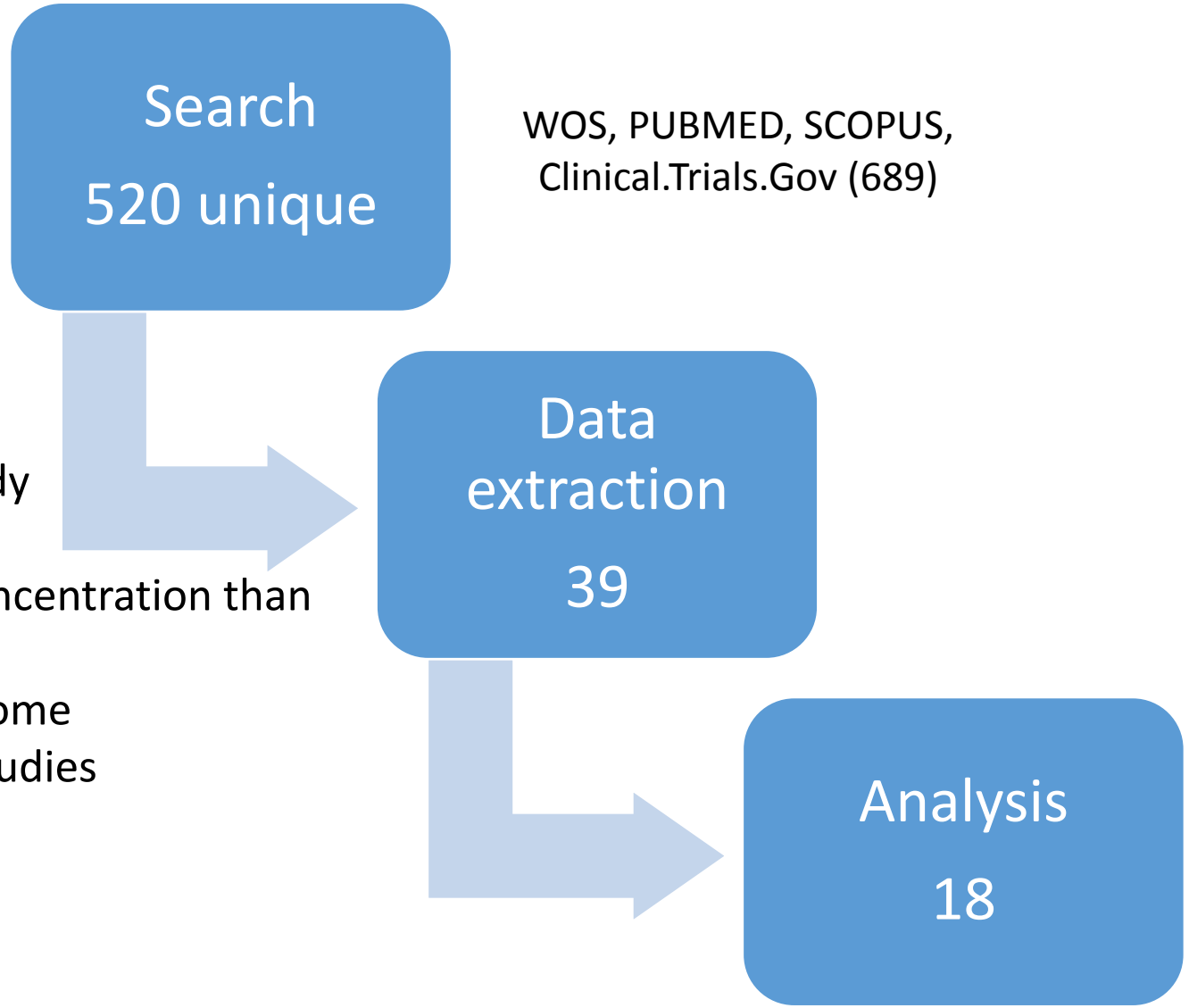
polyphenol* OR flavonoid* OR flavonol* OR quercetin* OR kaempferol* OR galangin* OR isorhamnetin* OR jaceidin* OR kaempferide* OR morin* OR myricetin* OR patuletin* OR rhamnetin* OR spinacetin

Foods rich in the bioactive

spice* OR caper* OR saffron* OR caraway* OR clove* OR oregano* OR onion* OR shallot* OR broccoli* OR spinach* OR asparagus* OR "asparagus officinalis" OR bean* OR "phaseolus vulgaris" OR "chilli pepper" OR berry* OR "black chokeberry" OR "aronia melanocarpa" OR "American cranberry" OR lingonberry* OR "vaccinium vitis-idaea

Outcome

FMD OR "flow-mediated dilation" OR "flow-mediated vasodilation" OR "flow-mediated vasodilatation" OR "endothelial function" OR "endothelial dysfunction" OR "blood pressure" OR hypertens* OR "mean arterial pressure" OR "pulse pressure" OR cholesterol* OR LDL* OR HDL* OR "exercise capacity" OR "exercise performance" OR "aerobic capacity" OR platelet* OR antiplatelet* OR CD62P OR P-sel* OR GPIIbIIIa OR GPIIb-IIIa OR GPIbIX OR GPIb-IX OR LTA OR PFA-100 OR aggregometry* OR "thromboxane B2" OR "BMI*" OR "body mass index" OR "waist*" OR HOMA-IR OR HOMA2 OR "homeostatic model assessment" OR insulin* OR QUICKI OR "impaired sensitivity" OR "Syndrome X" OR glucose OR glycemia OR "glycemic control" OR HbA1c OR "glycosylated haemoglobin" OR "glycated haemoglobin" OR "haemoglobin A1c" OR "euglycemic clamp" OR dyslipidemia* OR hyperlipidemia* OR hypertriglyceridemia* OR triglyceride* or triacylglycer*


Search
520 unique
WOS, PUBMED, SCOPUS,
Clinical.Trials.Gov (689)
Excluded
• In vitro or animal study
• No proper control
• No flavonol/lower concentration than other (poly)phenols
• Not the defined outcome
• Postprandial/acute studies
Data extraction
39
Analysis
18

Comprehensive meta analysis - [C:\Users\Asus\Desktop\

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Run analyses →

	Study name	Subgroup within study	Comparison
1	Brull 2015	Blank	overweight
2	Karlsen 2012	Blank	mixed
3	Egert 2009	Blank	overweight
4	Dower 2015	Blank	mixed
5	Lee 2011	Blank	mixed
6	Chen 2015	Blank	mixed
7	Kim 2015	Blank	mixed
8	Lu 2015	Blank	mixed
9	Larmo 2009	Blank	mixed
10	Edwards 2009	pre-hypertensive	overweight
11	Edwards 2006	hypertensive	overweight
12	Choi 2015	Blank	mixed
13	Pfeuffer 2013	ApoE3	overweight
14	Pfeuffer 2013	ApoE4	overweight
15	Zahedi 2013	Blank	mixed
16	Conquer 1998	Blank	mixed

Click on the icons to select the data entry format

- Dichotomous (number of events)
- Continuous (means)
  - Unmatched groups, post data only
  - Unmatched groups, pre and post data
    - Means, SD pre and post, N, in each group, Pre/Post Corr
    - Means, SD difference, N, in each group, Pre/Post Corr
    - Means pre and post in each group, t within groups, N
    - Means pre and post in each group, p within groups, N
    - Means pre and post in each group, F for difference between changes, N
    - Mean change, SD pre and post, N, in each group, Pre/Post Corr
    - Mean change, SD difference, N, in each group, Pre/Post Corr
    - Mean change in each group, t within groups, N
    - Mean change in each group, p within groups, N
    - Mean change in each group, F for difference between changes, N
    - F for difference between changes, N
  - One group (pre-post) and matched groups
  - Computed effect sizes
- Correlation
- Rates (events by person years)

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Study name	Subgroup within study	Comparison	Outcome	Data format	Treated Pre Mean	Treated Pre SD	Treated Post Mean	Treated Post SD	Treated Sample size	Placebo Pre Mean	Placebo Pre SD	Placebo Post Mean	Placebo Post SD
Brull 2015	Blank	overweight	TAG	Means, SD in each group	1,810	1,090	1,830	1,370	34	1,760	1,250	1,720	1,370
Karlsen 2012	Blank	mixed	TAG	Mean change, SD difference in each group									
Egert 2009	Blank	overweight	TAG	Means, SD in each group	1,820	0,980	1,940	1,310	47	1,950	0,990	1,830	0,990
Dower 2015	Blank	mixed	TAG	Means, SD in each group	1,200	0,600	1,300	0,600	18	1,300	0,500	1,300	0,500
Lee 2011	Blank	mixed	TAG	Means, SD in each group	1,850	0,990	1,770	1,120	49	2,090	1,040	1,930	1,370
Chen 2015	Blank	mixed	TAG	Mean change, SD difference in each group									
Kim 2015	Blank	mixed	TAG	Means, SD in each group	1,120	0,400	1,070	0,330	18	1,230	0,500	1,320	0,490
Lu 2015	Blank	mixed	TAG	Means, SD in each group	1,540	0,270	1,570	0,320	12	1,420	0,320	1,620	0,470
Larmo 2009	Blank	mixed	TAG	Mean change, SD difference in each group									
Edwards 2009	pre-hypertensive	overweight	TAG	Means, SD in each group	2,000	1,050	1,760	0,520	10	1,820	1,050	1,930	0,920
Edwards 2006	hypertensive	overweight	TAG	Means, SD in each group	2,320	1,830	2,430	1,590	11	2,370	1,590	2,200	1,360
Choi 2015	Blank	mixed	TAG	Means, SD in each group	1,290	0,510	1,260	0,430	34	1,410	0,580	1,570	0,710
Pfeuffer 2013	ApoE3	overweight	TAG	Means, SD in each group	1,080	0,480	1,420	0,520	10	1,080	0,480	1,450	0,480
Pfeuffer 2013	ApoE4	overweight	TAG	Means, SD in each group	1,270	0,550	1,320	0,540	15	1,270	0,550	1,560	0,880
Zahedi 2013	Blank	mixed	TAG	Means, SD in each group	2,240	0,230	2,100	0,200	34	1,710	0,110	1,950	0,060
Conquer 1998	Blank	mixed	TAG	Means, SD in each group	1,270	1,140	1,150	1,250	13	1,410	1,190	1,400	1,090

Comprehensive meta analysis - [Analysis]

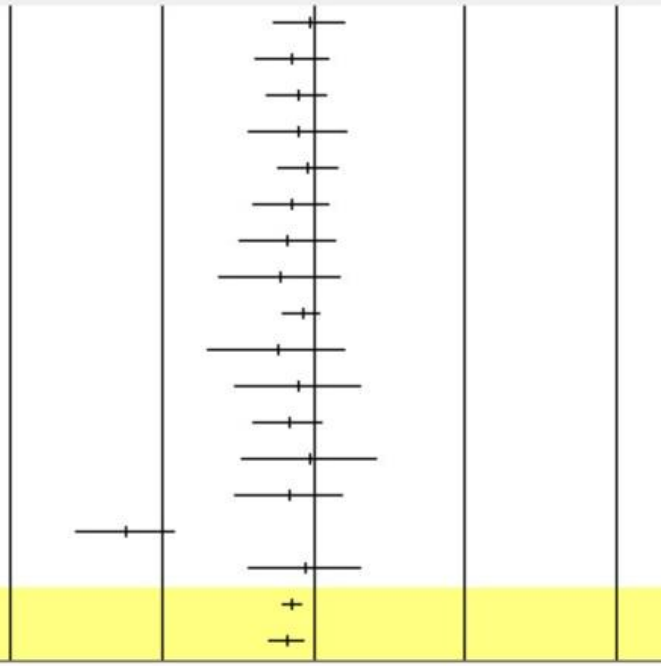
File Edit Format View Computational options Analyses Help

Data entry | Next table | High resolution plot | Select by ... | Effect measure: Std diff in means

Model	Study name	Subgroup within study	Comparison	Outcome	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
	Brull 2015	Blank	overweight	TAG	-0,044	0,243	0,059	-0,519	0,432	-0,181	0,857
	Karlsen	Blank	mixed	TAG	-0,280	0,255	0,065	-0,780	0,221	-1,096	0,273
	Egert 2009	Blank	overweight	TAG	-0,206	0,208	0,043	-0,614	0,201	-0,993	0,321
	Dower 2015	Blank	mixed	TAG	-0,181	0,339	0,115	-0,845	0,484	-0,533	0,594
	Lee 2011	Blank	mixed	TAG	-0,064	0,209	0,044	-0,474	0,345	-0,308	0,758
	Chen 2015	Blank	mixed	TAG	-0,298	0,260	0,067	-0,807	0,211	-1,148	0,251
	Kim 2015	Blank	mixed	TAG	-0,333	0,331	0,110	-0,982	0,316	-1,007	0,314
	Lu 2015	Blank	mixed	TAG	-0,423	0,413	0,170	-1,232	0,386	-1,024	0,306
	Larmo 2009	Blank	mixed	TAG	-0,148	0,132	0,018	-0,408	0,111	-1,119	0,263
	Edwards	pre-hyperte	overweight	TAG	-0,476	0,466	0,217	-1,389	0,438	-1,021	0,307
	Edwards	hypertensiv	overweight	TAG	-0,189	0,427	0,183	-1,027	0,648	-0,443	0,658
	Choi 2015	Blank	mixed	TAG	-0,320	0,238	0,056	-0,785	0,146	-1,345	0,179
	Pfeuffer	ApoE3	overweight	TAG	-0,060	0,460	0,211	-0,961	0,841	-0,130	0,896
	Pfeuffer	ApoE4	overweight	TAG	-0,329	0,368	0,135	-1,049	0,392	-0,894	0,371
	Zahedi	Blank	NR	TAG	-2,473	0,338	0,114	-3,136	-1,810	-7,309	0,000
	Conquer	Blank	mixed	TAG	-0,094	0,385	0,149	-0,849	0,661	-0,244	0,807
Fixed					-0,284	0,066	0,004	-0,413	-0,154	-4,299	0,000
Random					-0,351	0,125	0,016	-0,596	-0,107	-2,820	0,005

Statistics for each study

Std diff in means and 95% CI



Comprehensive meta analysis - [Publication bias]

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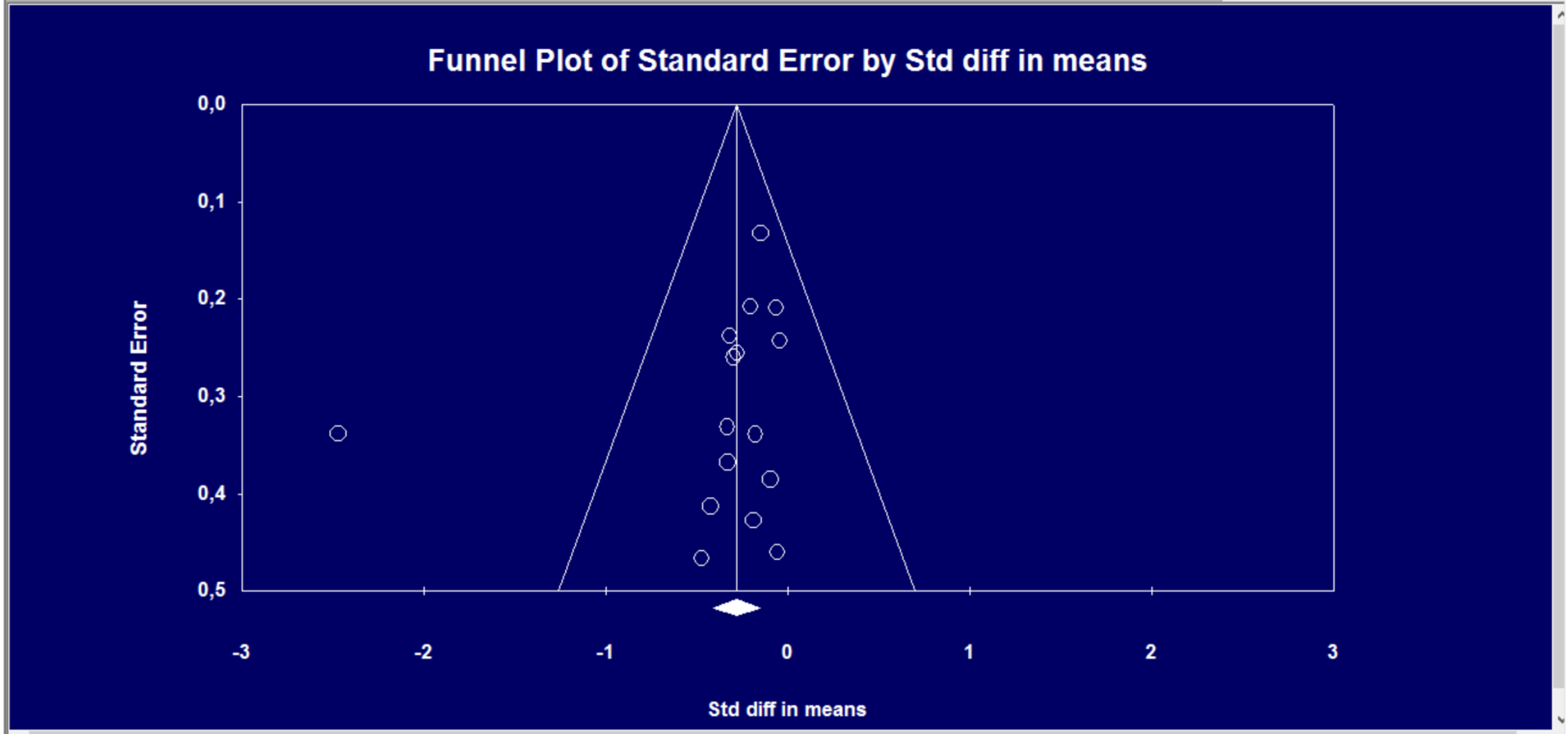
Core analysis | Table | Funnel plot | Plot observed studies only | Plot standard error | Center line | Guidelines | Color for screen



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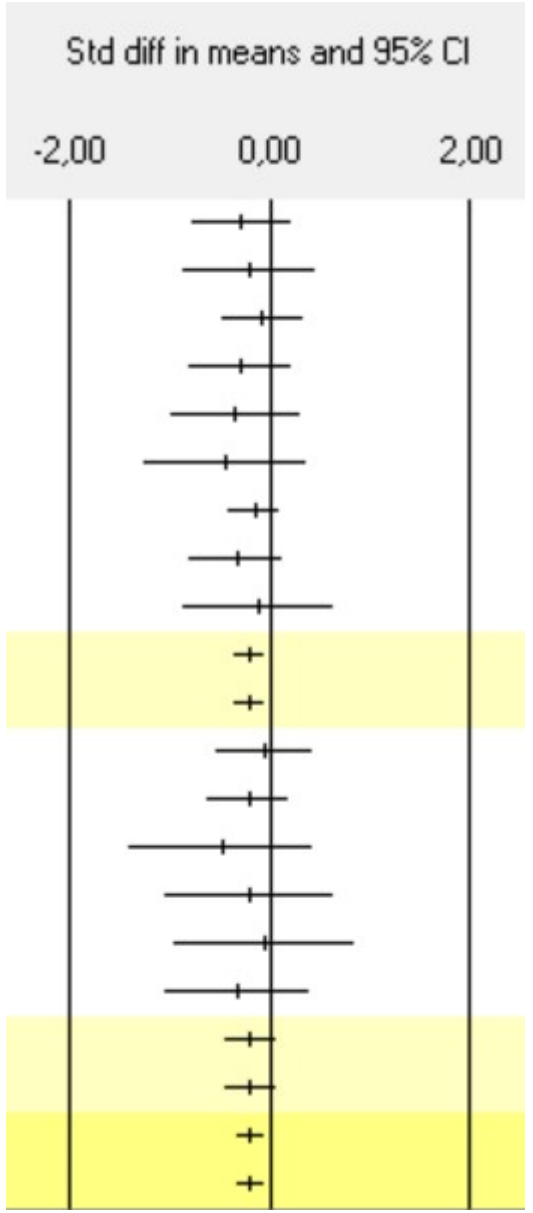
Model	Group by Comparison	Study name	Std diff in means	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
	mixed	Karlsen	-0,280	0,255	0,065	-0,780	0,221	-1,096	0,273
	mixed	Dower 2015	-0,181	0,339	0,115	-0,845	0,484	-0,533	0,594
	mixed	Lee 2011	-0,064	0,209	0,044	-0,474	0,345	-0,308	0,758
	mixed	Chen 2015	-0,298	0,260	0,067	-0,807	0,211	-1,148	0,251
	mixed	Kim 2015	-0,333	0,331	0,110	-0,982	0,316	-1,007	0,314
	mixed	Lu 2015	-0,423	0,413	0,170	-1,232	0,386	-1,024	0,306
	mixed	Larmo 2009	-0,148	0,132	0,018	-0,408	0,111	-1,119	0,263
	mixed	Choi 2015	-0,320	0,238	0,056	-0,785	0,146	-1,345	0,179
	mixed	Conquer	-0,094	0,385	0,149	-0,849	0,661	-0,244	0,807
Fixed	mixed		-0,202	0,079	0,006	-0,358	-0,047	-2,546	0,011
Random	mixed		-0,202	0,079	0,006	-0,358	-0,047	-2,546	0,011
	overweight	Brull 2015	-0,044	0,243	0,059	-0,519	0,432	-0,181	0,857
	overweight	Egert 2009	-0,206	0,208	0,043	-0,614	0,201	-0,993	0,321
	overweight	Edwards	-0,476	0,466	0,217	-1,389	0,438	-1,021	0,307
	overweight	Edwards	-0,189	0,427	0,183	-1,027	0,648	-0,443	0,658
	overweight	Pfeuffer	-0,060	0,460	0,211	-0,961	0,841	-0,130	0,896
	overweight	Pfeuffer	-0,329	0,368	0,135	-1,049	0,392	-0,894	0,371
Fixed	overweight		-0,184	0,127	0,016	-0,432	0,064	-1,452	0,147
Random	overweight		-0,184	0,127	0,016	-0,432	0,064	-1,452	0,147
Fixed	Overall		-0,197	0,067	0,005	-0,329	-0,065	-2,928	0,003
Random	Overall		-0,197	0,067	0,005	-0,329	-0,065	-2,928	0,003



Groups		Effect size and 95% confidence interval					Test of null (2-Tail)		Heterogeneity			
Group	Number Studies	Point estimate	Standard error	Variance	Lower limit	Upper limit	Z-value	P-value	Q-value	df (Q)	P-value	I-squared
Fixed effect analysis												
mixed	9	-0,202	0,079	0,006	-0,358	-0,047	-2,546	0,011	1,600	8	0,991	0,000
overweight	6	-0,184	0,127	0,016	-0,432	0,064	-1,452	0,147	0,966	5	0,965	0,000
Total within									2,565	13	0,999	
Total between									0,015	1	0,901	
Overall	15	-0,197	0,067	0,005	-0,329	-0,065	-2,928	0,003	2,581	14	1,000	0,000
Mixed effects analysis												
mixed	9	-0,202	0,079	0,006	-0,358	-0,047	-2,546	0,011				
overweight	6	-0,184	0,127	0,016	-0,432	0,064	-1,452	0,147				
Total between									0,015	1	0,901	
Overall	15	-0,197	0,067	0,005	-0,329	-0,065	-2,928	0,003				

High resolution plot

Mixed BMI

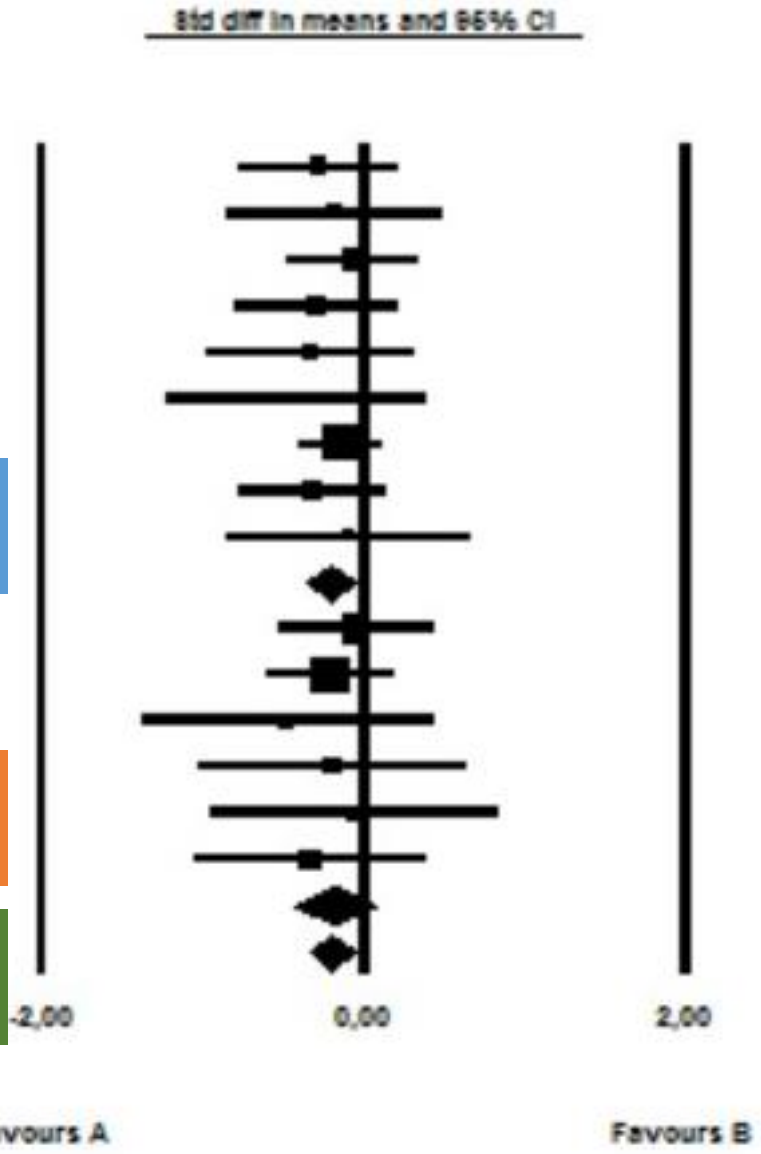
-0,202 (-0,358;-0,011)
P=0,017

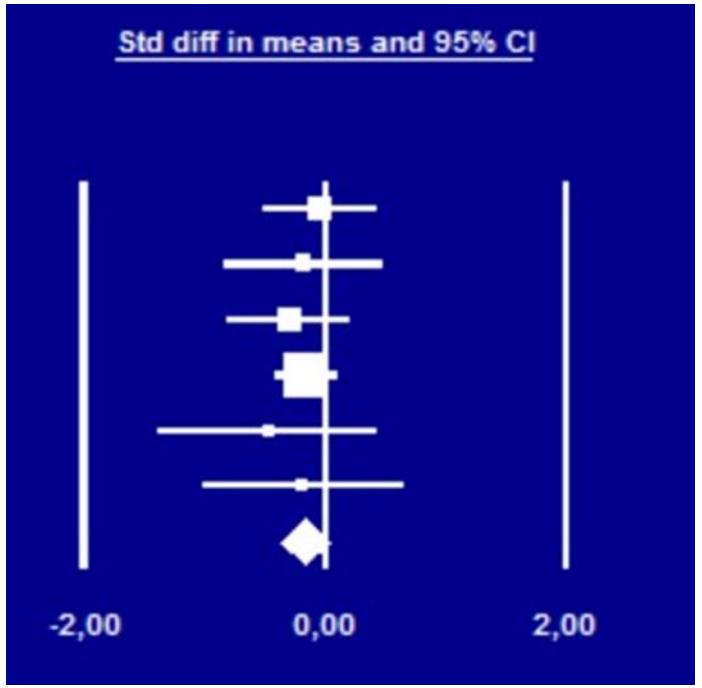
Overweight

-0,184 (-0,432;0,064)
P=0,147

Overall

-0,197 (-0,329;0,065)
P=0,003





Overall
Top quality (score 8-10)

-0.171 (-0.360, 0.018)
P=0.076

Quality of studies

Selection bias	Random sequence generation	Yes No or unclear	1 0
	Allocation concealment	Yes No or unclear	1 0
Performance bias	Blinding (participants, researchers, statitians)	Yes No or unclear	1 for each 0
	Compliance measure	Yes, biomarker Yes, counting or self reporting No or unclear	1 0.5 0
Attrition bias	Flow of participants	Yes No or unclear	1 0
Other bias	Industry funding	Yes No or unclear	0 1
	Baseline comparability	Yes No or unclear	1 0
	Data quality	Central measure and dispersion Anything missing	1 0

Low quality : below 5	**Medium quality: 5 to 7**	**High quality: 8 to 10**

Definition of subgroups for comparison

Study comparisons			
Dose of flavonol	Low: < 200 mg	Medium: ≥200 <500	High: ≥500
Study duration	Acute: < 1day	Chronic: ≥ 2 days	
Duration of chronic studies	Short: up to 4 weeks	Medium: 5 to 10 weeks	Long: more than 10
Compound	pure	Extract	food
Quality	Low: below 5	Medium: 5 to 7	High: 8 to 10

Definition of subgroups for comparison

Interindividual variation	
BMI	Normal weight, overweight, obese, mixed
Health status	Helthy, with disease, at risk of CVD With medication, without medication
Age	Young, middle age adults, old, mixed
Gender	Male, Female, mixed
Country	Asian, European, USA, Arabian
Smoking	Smoker, no smoker, mixed
Genetic polymorphisms	ApoE3, ApoE4

Other factors: diet background, lifestyle, bioavailability, microbiota, waist circunference

		n	Std diff in means	95% CI (lower, upper)		P-value	
Total CHOL	overweight	6	-0.052	-0.300	0.192	0.680	
	mixed	9	-0.199	-0.355	-0.043	0.012	⇦
	Overall	16	-0.183	-0.310	-0.055	0.005	High quality P= 0.111
HDL	overweight	6	0.131	-0.117	0.379	0.300	
	mixed	7	0.270	0.105	0.435	0.001	⇦
	Overall	16	0.214	0.083	0.346	0.001	High quality P= 0.05
LDL	overweight	6	-0.084	-0.332	0.163	0.505	
	mixed	7	-0.257	-0.433	-0.081	0.004	⇦
	Overall	16	-0.191	-0.328	-0.054	0.006	High quality P=0.068
TAG	overweight	6	-0.184	-0.432	0.064	0.147	
	mixed	9	-0.202	-0.358	-0.047	0.011	⇦
	Overall	16	-0.351	-0.596	-0.107	0.005	High quality P=0.076