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1 **Systematic bioinformatic analysis of nutrigenomic data of flavanols in cell models of**  
2 **cardiometabolic disease**

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**53 Abbreviations**

54	ABCA1	ATP Binding Cassette Subfamily A Member 1
55	ADIPOQ	Adiponectin
56	AGE	Advanced Glycation Endproducts
57	APOA1	Apolipoprotein A1
58	APOB	Apolipoprotein B
59	BAX	BCL2 Associated X, Apoptosis Regulator
60	BCL2	BCL2 Apoptosis Regulator
61	CCL2	C-C Motif Chemokine Ligand 2
62	CEBPA	CCAAT Enhancer Binding Protein Alpha
63	CRP	C-Reactive Protein
64	CXCL8	C-X-C Motif Chemokine Ligand 8
65	EDN1	Endothelin 1
66	EGCG	Epigallocatechin gallate
67	FOXC1	Forkhead Box C1
68	GATA2	GATA Binding Protein 2
69	GDF	Growth Differentiation Factor
70	HIF	Hypoxia Inducible Factor
71	HMOX1	Heme Oxygenase 1
72	IBD	Inflammatory Bowel Disease
73	ICAM1	Intercellular Adhesion Molecule 1

74	IL2	Interleukin 2
75	IL4	Interleukin 4
76	IL6	Interleukin 6
77	IL10	Interleukin 10
78	ITGAM	Integrin Subunit Alpha M
79	ITGB1	Integrin Subunit Beta 1
80	JUN	Jun Proto-Oncogene: AP-1 Transcription Factor Subunit
81	KEGG	Kyoto Encyclopedia of Genes and Genomes
82	LDL	Low Density Lipoprotein
83	LDLR	Low Density Lipoprotein Receptor
84	LPL	Lipoprotein Lipase
85	LPS	Lipopolysaccharide
86	MAPK8	Mitogen-Activated Protein Kinase 8
87	miRNA	MicroRNA
88	MMP9	Matrix Metalloproteinase 9
89	MT-CO3	Mitochondrially Encoded Cytochrome C Oxidase III
90	NAFLD	Non-Alcoholic Fatty Liver Disease
91	NFKB1	Nuclear Factor Kappa B Subunit 1
92	NLRP3	NLR Family Pyrin Domain Containing 3
93	NOS2	Nitric Oxide Synthase 2
94	NOS3	Nitric Oxide Synthase 3

95	PBMC	Peripheral Blood Mononuclear Cell
96	PECAM1	Platelet and Endothelial Cell Adhesion Molecule 1
97	PPARA	Peroxisome Proliferator Activated Receptor Alpha
98	PPARG	Peroxisome Proliferator Activated Receptor Gamma
99	PPARs	Peroxisome Proliferator Activated Receptors
100	PPI	Protein-Protein Interaction
101	PTGS2	Prostaglandin-Endoperoxide Synthase 2
102	RAGE	Receptor for AGE
103	RETN	Resistin
104	ROCK1	Rho Associated Coiled-Coil Containing Protein Kinase 1
105	SELE	Selectin E
106	SERPINE1	Serpin Family E Member 1
107	SP1	Sp1 Transcription Factor
108	SREBF1	Sterol Regulatory Element Binding Transcription Factor 1
109	STAT1	Signal Transducer and Activator of Transcription 1
110	STAT3	Signal Transducer and Activator of Transcription 3
111	TGF-beta	Transforming Growth Factor Beta
112	TLDA	Taqman Low Density Array
113	TLR4	Toll Like Receptor 4
114	TNF	Tumor Necrosis Factor
115	TOLLIP	Toll Interacting Protein

116 VEGF Vascular Endothelial Growth Factor

117 VCAM1 Vascular Cell Adhesion Molecule 1

118 YY1 Yin Yang 1 Transcription Factor

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134 **Abstract:** Flavanols intake positively influences several cardiometabolic risk factors in  
135 humans. However, the specific molecular mechanisms of action of flavanols, in terms of  
136 gene regulation, in the cell types relevant to cardiometabolic disease have never been  
137 systematically addressed. On this basis, we conducted a systematic literature review and a  
138 comprehensive bioinformatic analysis of genes which expression is affected by flavanols in  
139 cells defining the cardiometabolic health: hepatocytes, adipocytes, endothelial, smooth  
140 muscle and immune cells. A systematic literature search was performed using the following  
141 pre-defined criteria: treatment with pure compounds and metabolites (no extracts), at low  
142 concentrations that are close to their plasma concentrations. Differentially expressed genes  
143 were analyzed using bioinformatics tools to identify gene ontologies, networks, cellular  
144 pathways and interactions, as well as transcriptional and post-transcriptional regulators. The  
145 systematic literature search identified 54 differentially expressed genes at mRNA level in  
146 *in vitro* models of cardiometabolic disease exposed to flavanols and their metabolites.  
147 Global bioinformatic analysis revealed that these genes are predominantly involved in  
148 inflammation, leukocyte adhesion and transendothelial migration, and lipid metabolism. We  
149 observed that, although the investigated cells responded differentially to flavanol exposure,  
150 the involvement of anti-inflammatory responses is a common mechanism of flavanol action.  
151 We also identified potential transcriptional regulators of gene expression: transcriptional  
152 factors, such as GATA2, NFKB1, FOXC1 or PPARG, and post-transcriptional regulators:  
153 miRNAs, such as mir-335-5p, let-7b-5p, mir-26b-5p or mir-16-5p. In parallel, we analyzed  
154 the nutrigenomic effects of flavanols in intestinal cells and demonstrated their predominant  
155 involvement in the metabolism of circulating lipoproteins. In conclusion, the results of this  
156 systematic analysis of the nutrigenomic effects of flavanols provides a more comprehensive  
157 picture of their molecular mechanisms of action and will support the future setup of genetic  
158 studies to pave the way for individualized dietary recommendations.

159 **Keywords:** flavanols; cardiometabolic; gene expression; in vitro; bioinformatics; cell  
160 signaling

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## 177 **1. Introduction**

178       Cardiometabolic disease is a cluster of metabolic dysfunctions including insulin  
179 resistance, impaired glucose tolerance, dyslipidemia, hypertension and central adiposity that,  
180 over time, may translate in type 2 diabetes and cardiovascular disease [1]. Unhealthy eating  
181 habits leading to overweight and obesity have been recognized as key determinants in the  
182 development of cardiometabolic disease [2]. Since dietary factors interfere with  
183 cardiometabolic disease progression in connection to individual genetic setting [3], the  
184 understanding of the impact of nutrients and bioactives on the complex networking of human  
185 genes has been long envisaged as a recommended research goal [4]. Even though this  
186 research focus has produced novel results to date, the recent application of bioinformatics  
187 and molecular biology tools to nutritional science has produced a large body of new exciting  
188 evidence on how food and food bioactives may interact with the genome to control health  
189 and wellness [5].

190

191       Among plant food bioactives, the most impressive advancements have been achieved in  
192 the field of polyphenols [6]. Polyphenols are secondary plant metabolites, which are  
193 classified into flavonoids and non-flavonoid compounds. The main subclasses of flavonoids  
194 include flavanols (flavan-3-ols), flavonols, flavones, flavanones, isoflavonoids, and  
195 anthocyanins [7]. Flavanols, the focus of our study, are among the most abundant  
196 polyphenols in the human diet [8] with main dietary sources in green tea, cocoa, apples and  
197 grapes. From a chemical point of view, flavanols represent a complex subclass of flavonoids,  
198 which encompass a variety of monomeric, oligomeric and polymeric compounds. The main  
199 monomeric forms include: (+)-catechin, (-)-epicatechin, (+)-gallocatechin, (-)-  
200 epigallocatechin, (-)-epicatechin-3-*O*-gallate and (-)-epigallocatechin-3-*O*-gallate.  
201 Proanthocyanidins (also known as condensed tannins) are oligomers or polymers of

202 flavanols, whereas polymers composed exclusively of catechin or epicatechin are called  
203 procyanidins. In foods, flavanols exist predominantly as aglycones [9].

204

205 The metabolism of dietary flavanols in the human body includes series of biochemical  
206 transformations that involve both host-microbiome interactions in the large intestine and  
207 microbiome independent routes. Flavanol absorption largely depends on their  
208 physicochemical properties; monomers can be absorbed in the small intestine but most of  
209 ingested flavanols reach intact the large intestine [10,11]. In enterocytes, most of the absorbed  
210 monomers are subjected to initial phase II metabolism, which include conjugation reactions  
211 such as glucuronidation, sulfatation and methylation. Exception are (-)-epicatechin-3-O-  
212 gallate and (-)-epigallocatechin-3-O-gallate [12], where 3-O-galloyl moiety is considered to  
213 interfere the enzymes of phase II metabolism [9], and as such they reach the circulation as  
214 parent compounds. Some of the phase II metabolites are transported back from the  
215 enterocytes to the intestinal lumen, whereas the others are transported to the liver, where their  
216 metabolism by phase II enzymes continues [13]. Since conjugation reactions facilitate the  
217 excretion of flavanol derivatives, the plasma concentrations and half-life of flavanol phase II  
218 metabolites result to be very low: their maximal plasma concentrations are usually found in  
219 the range of nanomolar to low micromolar [14], which are reached approximately two hours  
220 post-ingestion and followed by a rapid elimination [12]. A small number of dimeric  
221 compounds are also absorbed in the small intestine. Most of the ingested flavanols reach the  
222 large intestine where, together with the residual products of intestinal and liver phase II  
223 metabolism, they are catabolized by the microbiome. Small phenolic and aromatic acids, such  
224 as phenyl- $\gamma$ -valerolactones, are generated through the biochemical transformations of  
225 flavanols by gut microbiota. These metabolites can be absorbed and further subjected to  
226 phase II metabolism before their elimination from the human body [9,15]. Therefore, besides  
227 epicatechin-3-O-gallate and (-)-epigallocatechin-3-O-gallate that appear in the systemic

228 circulation as parent compounds, several flavanol glucuronidated, sulfatated and methylated  
229 metabolites, and phenolic acids represent the most common forms traceable in the systemic  
230 circulation and are those that likely mediate the beneficial health effects of their parent  
231 compounds. These metabolites are chemically and, in many instances, functionally distinct  
232 from the parent dietary forms, and such features determine their biological effectiveness [16].  
233 In particular, conjugated forms of flavonoids were shown to have a significantly lower  
234 capacity for donating hydrogens and scavenging free radicals compared to the parent  
235 compounds [17].

236

237 Growing evidence from cohort studies and randomized trials indicate that higher dietary  
238 intake of polyphenols reduces the risk of cardiovascular mortality [18] and positively  
239 influences some of the key cardiometabolic risk factors, such as blood glucose, blood lipids,  
240 blood pressure, endothelial dysfunction and arterial stiffness [19-21]. Despite the large body  
241 of clinical and experimental data [22], evidence regarding the role of polyphenols in  
242 cardiometabolic protection remains not entirely consistent. This inconsistency can be  
243 explained by differences in study designs and polyphenols tested [23,24]. However, recent  
244 findings are also pinpointing role of sex, age, gut microbiome, life-style but also genotype  
245 and more recently epigenetic variations as potential factors contributing to heterogeneity in  
246 the individual response to the consumption of polyphenols [25-27].

247

248 Although cardiovascular benefits of polyphenols have been in the past attributed to their  
249 antioxidant properties (as free radical scavengers) [28], this view was not in agreement with  
250 available knowledge about their bioavailability and *in vivo* metabolism [29]. Complementary  
251 evidence suggests that their protective activities may mainly occur through genomic effects,  
252 by interfering with the expression of genes [29]. Nutrigenomics can be defined as approach  
253 to elucidate the diet-gene interaction by assessing gene or protein expression and gene

254 regulation [30,31]. The capacity of polyphenols to modulate gene expression has been  
255 identified in different cell types and for different families of polyphenols. For example, in  
256 endothelial cells, flavanone metabolites have been shown to affect the expression of a number  
257 of genes related to atherogenesis and especially those involved in cell adhesion, cytoskeleton  
258 organization, inflammation, and chemotaxis [32]. Similarly, the exposure of endothelial cells  
259 to curcumin before applying a pro-inflammatory stress, induced positive changes in the  
260 expression of genes involved in the control of cytoskeleton and endothelial junction  
261 dynamics, and in the pro-inflammatory redox-sensitive transcription factor NF-kappa B [33].  
262 In a complementary fashion, the adoption of untargeted approaches has shown that plasma  
263 epicatechin metabolites affect the expression of more than two hundred of genes, some of  
264 them involved in endothelial permeability and interaction with immune cells, thus  
265 demonstrating a multi-targeted mode of action for flavanols [34]. Together with *in vitro*  
266 investigations, nutrigenomic modifications of polyphenols have also been demonstrated in  
267 several *in vivo* models of cardiometabolic disease. Curcumin [35] and naringin [36]  
268 modulate, in an anti-atherogenic manner, the gene expression profile in the aorta of mice  
269 model of atherosclerosis. Naringin is also able to modulate the expression of genes related to  
270 lipid metabolism, inflammation and insulin signaling in the liver of mice fed a high-fat diet  
271 [37]. Finally, in rats, quercetin was shown to affect the expression of genes involved in fatty  
272 acids metabolism in lung tissue [38]. In humans, several studies have confirmed the capacity  
273 of many of these food bioactives, including flavanols [39] and flavanones [40] to exert  
274 nutrigenomic regulation. However, most nutrigenomic findings with polyphenols are from *in*  
275 *vitro* studies focusing on expression of few target genes (targeted approaches), and using  
276 non-physiologically relevant conditions, that is high concentrations of non-circulating  
277 compounds for long period of time, conditions that do not take into account the  
278 bioavailability and metabolism of polyphenols following their intake. **For these reasons we**  
279 **decided to work only on studies that were performed in physiologically relevant conditions,**

280 that is use of circulating forms and right concentrations, studies that provided findings that  
281 are possible to happen *in vivo*. Furthermore, several studies reported opposite effects  
282 depending on concentrations used, for example significant effect at physiologically relevant  
283 concentrations on prevention of monocyte adhesion to endothelial cells, which is not  
284 observable at higher concentrations [22].

285

286 On this background, experts involved in the COST POSITIVE network  
287 (<https://www6.inrae.fr/cost-positive>) [41] aimed to identify the most significant target genes  
288 and cellular pathways of flavanols underlying their cardiometabolic health properties by  
289 performing systematic bioinformatic analyses of available nutrigenomic data. To this aim,  
290 we conducted a systematic literature search for gene expressions modulated by flavanols in  
291 cellular models of cardiometabolic disease. We included hepatocytes, adipocytes,  
292 endothelial, smooth muscle and immune cells, selecting only studies adopting research  
293 protocols testing monomeric or dimeric compounds or related metabolites at concentrations  
294 in the range of those found in the plasma after flavanol intake. The identified differentially  
295 expressed genes were then subjected to a comprehensive and integrative bioinformatic  
296 analysis among the different cell models to decipher and characterize key target genes and  
297 mechanisms of action of flavanols within a new, more holistic perspective. In parallel, we  
298 also analyzed the nutrigenomic effects of flavanols in intestinal cells exposed to high  
299 concentrations of extracts or oligomeric compounds, as occurring after the ingestion of  
300 flavanols rich sources. The results of these analyses will pave the way for the identification  
301 of genes and pathways underlying the health effects of flavanols. This knowledge will allow  
302 us to identify potential genes which polymorphisms can be investigated in humans with the  
303 aim to better explain some aspects of the inter-individual variability in response to  
304 consumption of flavanols. It will also guide the setup of future nutrigenetic studies aiming to

305 identify flavanol responsive genotypes, whereby flavanol intake will be optimized to reduce  
306 the disease risk.

307

## 308 **2. Methods**

### 309 *2.1. Data sources and search strategy*

310 Literature searches were performed using two main scientific repositories, PubMed  
311 (<https://www.ncbi.nlm.nih.gov/pubmed>) and Web of Science  
312 (<https://www.webofknowledge.com>). Both databases were searched for all relevant studies  
313 published until January 23, 2018. Search terms included, as “plant food bioactives”, catechin  
314 OR epicatechin OR epigallocatechin OR procyanidin OR proanthocyanidin AND, as “cells”,  
315 endothelial OR endothelial cells OR endothelium OR pancreatic OR pancreatic cells OR  
316 adipose OR adipose cells OR adipocyte OR intestinal OR intestinal cells OR intestinal  
317 enteroendocrine cells OR monocytoid OR monocytoid cells OR monocytes OR macrophagic  
318 OR macrophagic cells OR macrophage OR hepatic OR hepatic cells OR liver cell OR  
319 hepatocyte OR smooth muscle cell OR muscle cells OR caco-2 OR PBMC AND, as  
320 “gene/gene expression”, gene expression OR miRNA OR transcript OR nutrigenomic OR  
321 TLDA OR microarray OR genomic OR mRNA.

322

### 323 *2.2. Study selection and data extraction*

324 To be eligible, the studies had to meet the following criteria: (1) published in English;  
325 (2) assess the effects of flavanols in *in vitro* cell models suitable to study cardiometabolic  
326 dysfunction, including endothelium, adipocytes, monocytes/macrophages, pancreatic,  
327 smooth muscle, hepatic and intestinal cells, as primary cells or cell lines; (3) show lack of  
328 toxicity at the tested concentrations; (4) evaluate data on gene expression in terms of mRNA



329 and miRNA modulation, but not proteins; (5) assess cardio-metabolic health outcomes. The  
330 exclusion criteria were the following: (1) treatment of the cells with bioactive compounds at  
331 concentrations higher than 10  $\mu$ M (except for the intestinal cells); (2) studies performed using  
332 extracts (again with the exception of the intestinal cells); (3) redundant publications; (4)  
333 incomplete information; (5) insufficient or insignificant statistical analysis, (6) outcomes  
334 unrelated to the study objectives; (7) lack of appropriate controls; (8) studies in animal  
335 models, in humans and reviews. Also, we aimed to identify papers that showed an effect on  
336 cellular function together with changes in the expression of genes to associate genomic  
337 modifications with potential health impact. The initial lists of titles, as retrieved from  
338 PubMed and Web of Science, were merged by using EndNote X6 reference manager  
339 software, and duplicates were discarded. The resulting list of papers was screened twice, by  
340 two different co-authors, to identify those that fulfilled the predefined criteria. Data were  
341 extracted using a standardized template. The template was pilot-tested on a small subset of  
342 studies to identify and reduce misinterpretations. Extracted data from the eligible studies  
343 included: name of the first author, title, year of publication, accession number, cell type with  
344 detailed description, type of challenge, associated disease, cell function evaluated, bioactive  
345 compounds (if single or mixed; if pure or extract) and their concentrations, number of genes  
346 studied, number of differentially expressed genes, modulation (up/down), official gene  
347 symbols and full names of the differentially expressed genes, and species. Data were  
348 extracted only for those genes that were identified as modulated by flavanols exposure with  
349 a p-value  $<0.05$ . Extracted data were then further crosschecked by two co-authors; in case of  
350 doubts and/or disagreement, a third co-author was consulted.

351

352 *2.3. Bioinformatic analysis*

353 To identify gene ontologies of the differentially expressed genes extracted from *in vitro*  
354 studies, David database has been used (<https://david.ncifcrf.gov>) [42,43], and the identified  
355 gene ontologies were plotted in treemap plot using Revigo tool (<http://revigo.irb.hr/>) [44].  
356 Gene network analyses were searched using a text-mining algorithm of MetaCore software  
357 from Clarivate Analytics (<https://portal.genego.com>). To identify pathways that are  
358 significantly associated with the genes, we used the web tool GeneTrail2  
359 (<https://genetrail2.bioinf.uni-sb.de/>) [45], version 1.6, as a platform to access **Kyoto**  
360 **Encyclopedia of Genes and Genomes** (KEGG) and BioCarta databases, using the following  
361 settings: over-representation analysis; null hypothesis (for p-value computation) – two-sided;  
362 method to adjust p-values – Benjamini-Yekutieli; significance level – 0.05. Interactions  
363 between functional groups of genes were searched using the online tool Metascape  
364 (<http://metascape.org>), using the option “Multiple Gene List” [46]. The network obtained was  
365 further visualized using Cytoscape platform for molecular interaction networks visualization  
366 (<https://cytoscape.org/>) [47]. Bioinformatic analysis on **protein-protein interaction** (PPI)  
367 between the proteins that are coded by the differentially expressed genes, including their  
368 neighboring proteins, was conducted using the database STRING, version 10.5  
369 (<https://string-db.org/>) [48]. For protein-protein interaction in adipocytes, hepatocytes,  
370 immune, smooth muscle and endothelial cells we applied the following settings: confidence;  
371 text-mining, experiments, databases, co-expression; high confidence – 0.700; no more than  
372 20 interactions in the first shell and no more than 20 interactions in the second shell, without  
373 clustering. STRING settings for the intestinal cells were the following: confidence; text-  
374 mining, experiments, databases, co-expression; high confidence – 0.700; no more than 15  
375 interactions in the first shell and no more than 15 interactions in the second shell. The  
376 resulting protein network was organized in two clusters. For integrated functional analyses  
377 of identified genes and their associated transcription factors and potential miRNAs involved  
378 in their post-transcriptional regulation, we used OmicsNet online tool from MetaboAnalyst

379 (<https://www.omicsnet.ca/faces/home.xhtml>) [49,50]. miRNet 2.0 was used for identification  
380 of potential miRNAs (<https://www.mirnet.ca>). For identification of official names and  
381 symbols of flavanol modulated genes, we used GeneCards (<https://www.genecards.org/>) [51]  
382 and NCBI (<https://www.ncbi.nlm.nih.gov/>) databases.

383

### 384 **3. Results**

#### 385 *3.1. Literature search and characteristics of papers selected for bioinformatic analysis*

386 The initial systematic search in PubMed and Web of Science using the pre-defined words  
387 identified more than 1500 publications. Publications that were out of scope or in duplicate  
388 were removed. The remaining 658 papers were distributed among the co-authors for  
389 screening. The screening based on title and abstract retrieved 79 papers as eligible for data  
390 extraction. Following a detailed analysis of the full text, 41 papers were considered for  
391 bioinformatic analysis (Table 1 and supplemental Table S1), that is *in vitro* studies in which  
392 cells have been exposed to flavanols (from tea, cocoa, apple or grape seed), at concentrations  
393 lower than 10  $\mu$ M (intestinal cells were an exception), and for which expression of genes at  
394 mRNA level had been analyzed. The flow diagram of the literature search and data extraction  
395 is summarized in Figure 1.

396

397 The majority of the studies, 26 out of 41 (63.4%), were conducted on cells of human  
398 origin, and 15 (36.6%) of studies were conducted on rodent cells, 10 from mouse and 5 from  
399 rat. Out of 41 studies, 37 reported results from *in vitro* studies using different cell models  
400 related to cardiometabolic disease: adipocytes, hepatocytes, immune, smooth muscle, and  
401 endothelial cells, and 5 used intestinal cells (in one paper both hepatocytes and intestinal cells  
402 were used [52]). Although pancreatic cells were included in the search criteria, we were not  
403 able to identify any eligible study conducted on this type of cells. As shown in Table 1, within

404 the 37 papers, the majority of experiments were conducted on cells that were challenged with  
405 dysmetabolic or pro-inflammatory stimuli, while the others examined the effects of flavanols  
406 under resting (basal) conditions. Most of these studies were carried out on endothelial cells  
407 (37.8%), followed by immune cells (27%), adipocytes (13.5%), smooth muscle cells (13.5%),  
408 and finally hepatocytes (8.1%). About half of the studies were conducted using primary cells,  
409 while the others used cell lines. Flavanols that were used for treatment of the cells include  
410 monomers, such as catechin, epicatechin, epicatechin gallate, epigallocatechin and  
411 **epigallocatechin gallate** (EGCG), the dimer procyanidin B2, and various flavanol  
412 metabolites. As shown in Table 1, flavanol metabolites were analyzed only in a small number  
413 of studies. Concentrations of flavanols and their metabolites varied from 0.1 to 10  $\mu\text{mol/L}$ ,  
414 in average 5  $\mu\text{mol/L}$ , and the cells were treated for a period from 3 hours to over 24 hours.

415

416 In experiments conducted on intestinal cells, Caco-2 cells were used as an exclusive cell  
417 model. In these experiments, cells were exposed to grape seed extract or oligomeric  
418 compounds, most often at high concentrations (Table 1), which is out of our pre-established  
419 inclusion criteria for the other cell types. However, because these experimental conditions  
420 resemble physiological conditions for the intestinal cells, these papers were included in our  
421 study, but the differentially expressed genes were analyzed separately.

422

### 423 *3.2. Identification of differentially expressed genes in cell models of cardiometabolic* 424 *disease*

425 Most of the retrieved studies adopted a targeted approach, analyzing the expression of a  
426 selection of targeted genes at the mRNA level. Only two studies adopted an untargeted  
427 (holistic) approach, using microarray methods [22,53]. However, for these studies, only RT-  
428 PCR data, used to validate microarray data, have been extracted and used for global  
429 systematic analysis.

430

431 Detailed analysis of human and rodent cell models of cardiometabolic disease  
432 (adipocytes, hepatocytes, immune, smooth muscle, and endothelial cells) exposed to  
433 flavanols (monomers, dimers, or their metabolites) identified 92 differentially expressed  
434 genes at the mRNA level. An overview of data extracted from the papers reporting  
435 experiments on human and rodent cell models of cardiometabolic disease is given in Table  
436 1, while more detailed information can be found in the supplemental Table S1. We observed  
437 that some genes had been studied more frequently than others, which results in their more  
438 frequent identification as differentially expressed. For example, *CCL2* has been identified as  
439 differentially expressed by flavanols in seven different studies, *APOAI* in five experiments,  
440 *TNF* in four different studies, whereas *MMP9*, *IL6*, *LDLR*, *APOB*, *ABCA1*, *PPARG* and *CRP*  
441 were identified as differentially expressed three times each (Figure 2A). After removal of the  
442 duplicates, a total number of genes whose expression was modulated by flavanols was 54,  
443 which were subjected to bioinformatic processing. Of these 54 genes, 42 genes were  
444 identified as having expression modulated by flavanols using human cells, 14 in mouse cell  
445 models, and 3 in cells of rat origin (Figure 2B). The analysis of papers examining the effects  
446 of flavanols in intestinal cells identified 15 differentially expressed genes (Table 1 and  
447 supplemental Table S1), i.e., 14 genes after removal of one duplicate, which were analyzed  
448 through a separate bioinformatic analysis.

449

### 450 3.3. Global gene enrichment and functional annotation analysis of differentially expressed 451 genes

452 In order to identify biological functions of the genes differentially expressed by flavanols  
453 in adipocytes, hepatocytes, immune, smooth muscle and endothelial cells, we first performed  
454 a global gene ontology analysis. As shown in Figure 3, the analysis suggests that these genes

455 are involved in the regulation of different biological processes, including cell signal  
456 transduction, biosynthesis, immune response, cell adhesion, and cell proliferation/death.

457

458       Aiming to deepen the identification of biological processes in which these genes are  
459 involved in, we performed gene network analysis using a text-mining approach. We used the  
460 list of differentially expressed genes identified in different studies to construct gene-gene  
461 networks. The networks were grouped in clusters representing specific biological processes,  
462 which are presented in the pie slice (Figure 4). As shown in Figure 4, flavanol modulated  
463 genes are involved in processes regulating inflammation, immune response, cell adhesion,  
464 apoptosis and cell signaling. Within the inflammation network cluster are pathways that  
465 include IL-2, 4, 6 signaling, chemotaxis, or IL-10 anti-inflammatory response. Within the  
466 signal transduction network cluster are pathways involved in insulin signaling, nitric oxide  
467 signaling or TGF-beta, GDF and activin signaling. The cell adhesion network cluster includes  
468 processes regulating cell junctions, integrin-mediated cell-matrix adhesion, leucocyte  
469 chemotaxis, or platelet-endothelium-leucocyte interactions. Overall, this analysis suggests  
470 that flavanols can modulate the expression of genes identified from different cell models of  
471 cardiometabolic disease that are collectively implicated in the regulation of inflammation,  
472 cell adhesion and metabolic processes.

473

474       To further investigate the functional role of flavanol modulated genes, we aimed to  
475 search for cellular pathways in which these genes are involved using the online platform  
476 GeneTrail2, which allows accesses to KEGG and BioCarta databases. Of 54 genes that were  
477 found differentially expressed at mRNA level in adipocytes, hepatocytes, immune, smooth  
478 muscle and endothelial cells, 53 genes were mapped in GeneTrail2, whereas *MT-CO3* failed  
479 the identification. The enquiring of KEGG database revealed that the differentially expressed  
480 genes are placed within pathways related to both cellular processes and human diseases.

481 Among the top pathways related to cellular processes are those involved in cell signaling and  
482 endothelial cell permeability, including cell adhesion, regulation of cytoskeleton  
483 organization, or focal adhesion (Figure 5). The top five KEGG pathways related to cellular  
484 processes are all involved in cell signaling and include “TNF signaling pathway”, which  
485 encompasses eleven differentially expressed genes (*CCL2, EDN1, ICAMI, IL6, JUN, MMP9,*  
486 *NFKB1, PTGS2, SELE, TNF and VCAMI*), “NF-kappa B signaling pathway” encompassing  
487 eight genes (*BCL2, CXCL8, ICAMI, NFKB1, PTGS2, TLR4, TNF and VCAMI*), “HIF-1  
488 signaling pathway”, also with eight genes (*BCL2, EDN1, HMOX1, IL6, NFKB1, NOS2,*  
489 *SERPINE1 and TLR4*), “Toll-like receptor signaling pathway” with seven genes (*CXCL8,*  
490 *IL6, JUN, NFKB1, TLR4, TNF and TOLLIP*) and “NOD-like receptor signaling pathway”  
491 with six genes (*CCL2, CXCL8, IL6, NFKB1, NLRP3 and TNF*). Among pathways related to  
492 regulation of the endothelial cell permeability, the highest number of encompassed genes  
493 modulated by flavanols have been found in “leukocyte transendothelial migration” (six  
494 genes: *ICAMI, ITGAM, ITGB1, MMP9, ROCK1 and VCAMI*) and “cell adhesion molecules”  
495 (five genes: *ICAMI, ITGAM, ITGB1, SELE and VCAMI*). Among top KEGG pathways  
496 related to human diseases, infectious diseases were predominant, but **non-alcoholic fatty liver**  
497 **disease** (NAFLD), which is a consequence of complex metabolic dysfunctions, was also  
498 present encompassing nine genes (*ADIPOQ, BAX, CEBPA, CXCL8, IL6, JUN, NFKB1,*  
499 *SREBF1 and TNF*).

500

501 Accordingly, the enquiring of BioCarta database returned pathways involved in  
502 inflammation, lipid metabolism and cell signaling (Figure 5). Top BioCarta pathways related  
503 to inflammation include “cells and molecules involved in local acute inflammatory  
504 response”, which encompasses six differentially expressed genes (*CXCL8, ICAMI, IL6,*  
505 *ITGB1, TNF and VCAMI*), “monocyte and its surface molecules”, encompassing four genes  
506 (*ICAMI, ITGAM, ITGB1 and SELE*), “adhesion and diapedesis of granulocytes” (*CXCL8,*

507 *ICAMI, ITGAM and TNF*), and “adhesion and diapedesis of lymphocytes” (*CXCL8, ICAMI,*  
508 *ITGB1 and VCAMI*), also encompassing four genes each. Top BioCarta pathways related to  
509 lipid metabolism are the following: “visceral fat deposits and the metabolic syndrome”,  
510 encompassing five genes (*ADIPOQ, LPL, PPARG, RETN and TNF*), “mechanism of gene  
511 regulation by PPARA”, encompassing six genes (*APOA1, JUN, LPL, NOS2, PTGS2 and*  
512 *TNF*) and “LDL pathway during atherogenesis”, with four genes (*CCL2, IL6, LDLR and*  
513 *LPL*).

514 Together with the identification of cellular pathways in which the genes are involved in, to  
515 facilitate their biological interpretation, we also performed network meta-analysis of  
516 interactions between functional groups of genes using text-mining approach implemented in  
517 the Metascape online tool. This analysis reveals not only the list of functions of the genes but  
518 also functional interaction between them in different cellular processes. This analysis has  
519 been performed using the option “Multiple Gene List”, that is lists of genes identified as  
520 modulated by flavanols in different cell types: adipocytes, smooth muscle cells, immune  
521 cells, endothelial cells and hepatocytes, allowing us to identify which functions are specific  
522 to which cell types. Global analysis has shown that flavanol modulated genes are involved in  
523 processes regulating lipid metabolism, inflammatory response, cellular response to TNF,  
524 AGE-RAGE pathway in diabetes, or regulation of binding. Some of the functions are  
525 common to all cell types studied, such as inflammatory response and cellular response to  
526 TNF. Functions such as steroid metabolic response are more specific to hepatocytes, or HIF-1  
527 signaling to endothelial cells (Figure 6). These analyses showed that exposure of cells to  
528 flavanols could modulate different cellular processes that are interacted at the cellular level.  
529

530 For analysis of functional links between proteins coded by the differentially expressed  
531 genes extracted from the literature and their neighboring proteins, we used the database  
532 STRING. All 54 differentially expressed genes were identified as valid by STRING software.



533 The network obtained consists of 94 nodes (proteins) having 515 edges (interactions) with  
534 PPI enrichment value  $<1.0e-16$  (Figure 7). Notably, some of the proteins have more  
535 interactions with other proteins within the network than others, indicating their key role in  
536 the cellular response to flavanols. For example, TNF, IL6, JUN, TLR, NFKB1, and MAPK8  
537 are on the top of the list with  $\geq 30$  interactions (Table 2).

538

### 539 *3.4. Transcriptional and post-transcriptional regulation of gene expression by flavanols*

540 Our next step of analyses aimed to identify potential transcriptional and post-  
541 transcriptional regulators involved in the observed modulation of gene expression by  
542 flavanols. Expression of genes can be regulated at the transcriptional level by the activity of  
543 transcription factors or post-transcriptionally by non-coding RNAs such as miRNAs. Using  
544 the bioinformatics tool OmicsNet, we first searched for protein-protein interactions followed  
545 by the evaluation of potential transcription factors and then potential miRNAs that could bind  
546 to mRNA of the identified protein-protein network to exert post-transcriptional regulations.  
547 Top 20 transcription factors and miRNAs, with the highest number of interactions in  
548 adipocytes, hepatocytes, immune, smooth muscle and endothelial cells are presented in Table  
549 3. Among the most significant transcription factors identified are GATA2, NFKB1, FOXC1,  
550 or PPARG. The miRNAs identified to interact with flavanol modulated genes identified are  
551 mir-335-5p, let-7b-5p, mir-26b-5p or mir-16-5p. Visualization of the interaction between the  
552 proteins of protein-protein interaction network with miRNAs and transcription factors is  
553 presented in a 3-layer 3D mode in Figure 8. These analyses showed a “dense” interaction  
554 between proteins and the regulatory elements, with each miRNA being able to regulate  
555 several proteins and one protein being potentially regulated by several miRNAs. The same is  
556 observed for transcription factors. This analysis revealed potential regulators of gene  
557 expression whose activity or level might be affected by flavanols, which determines the  
558 observed nutrigenomic modifications.

559

560 *3.5. Nutrigenomic effects of flavanols in intestinal cells*

561 Fifteen differentially expressed genes have been identified in the intestinal cells, i.e., 14  
562 different genes, after removal of one duplicate (Table S1; Table 1). Bioinformatic analysis  
563 demonstrated that these genes are most significantly associated with “PPAR signaling  
564 pathway”, which encompasses seven of 14 differentially expressed genes, and the  
565 “adipocytokine signaling pathway”, encompassing four of 14 genes. Other KEGG pathways  
566 that are significantly related to the differentially expressed genes in the intestinal cells include  
567 “fat digestion and absorption”, “fatty acid degradation”, “fatty acid metabolism”, “bile  
568 secretion” and “peroxisome”, all of them encompassing 3 different genes, as well as “vitamin  
569 digestion and absorption”, encompassing two genes. The enquiring of BioCarta database  
570 revealed only “mechanism of gene regulation by peroxisome proliferators via PPARA”  
571 (Figure 9A). By analyzing the protein-protein interactions using the STRING database, two  
572 protein clusters were identified for the intestinal cells. One of them includes proteins that are  
573 mostly involved in the metabolism of circulating lipoproteins. Proteins that belong to this  
574 cluster are shown in red. The second cluster is connected to the previous one through NOS2  
575 and NOS3 and covers mainly proteins that are involved in calcium signaling. Proteins that  
576 belong to this cluster are shown in green (Figure 9B). Proteins that have the highest number  
577 of interactions within the clusters are lipoprotein lipase, apolipoproteins, and calmodulins  
578 (Table S2).

579

580 Transcriptional and post-transcriptional regulation of flavanol modulated genes in the  
581 intestinal cells was also analyzed using the bioinformatics tool OmicsNet. This analysis  
582 revealed that master regulators of proteins that belong to the protein-protein interaction  
583 network emerging from the differentially expressed genes extracted from the literature

584 include SP1, NFKB1, STAT3, PPARG or STAT1 among the transcription factors, and mir-  
585 335-5p, mir-26b-5p, mir-16-5p, mir-124-3p or mir-92a-3p among the miRNAs. A 3-Layer  
586 3D presentation of this regulatory network is given in Figure 9C.

587

#### 588 **4. Discussion**

589 Facing an unprecedented increase of cardiometabolic, neurodegenerative and other non-  
590 communicable diseases, contemporary science strives to find effective strategies for their  
591 prevention and treatment. In this context, there is a growing body of scientific evidence about  
592 the role of diet in general, as well as of various food constituents, including bioactives, as  
593 important modulators of the cardiometabolic risk. In this review, we have systematically  
594 examined the effects of flavanols in terms of modulation of gene expressions relevant to the  
595 pathogenesis of cardiometabolic disease and identified potential pleiotropic pathways and  
596 cellular and molecular mechanisms underlying their protective actions.

597

598 Living in the era of personalized medicine, we are witnessing an enhanced awareness of  
599 the need for a personalized approach to dietary recommendations. This applies to the general  
600 population in terms of good health preservation, and secondary prevention in patients with  
601 various non-communicable diseases. As recently reviewed, variability in the cardiometabolic  
602 response to consumption of plant food bioactives, including polyphenols, is considered as  
603 one major cause of inconsistency in the results of human intervention studies [26]. This  
604 variability is determined by a number of factors, among which a central role is ascribed to  
605 the genetic variability beside to gut microbiome composition and functionality, sex, age,  
606 lifestyle and various comorbidities (overweight and obesity, diabetes, hypertension,  
607 dyslipidemia, etc.). Aiming to take the pioneering step towards the ultimate goal - identify  
608 genetic variants in the human population underlying the individual metabolic response to the

609 consumption of dietary flavanols - we conducted this systematic literature search to identify  
610 target genes involved in the protective effect of these compounds and which polymorphism  
611 expressions may explain the inter-individual variability in response to flavanols  
612 consumption. This is the first-ever systematic analysis of nutrigenomic data about the effects  
613 of flavanols in cell models relevant for cardiometabolic health. In order to provide  
614 physiologically relevant data, we applied rigorous criteria for inclusion/exclusion of the  
615 studies, resulting in the retrieval of relatively small number of relevant papers and  
616 differentially expressed genes.

617

618 The complex pathogenesis of cardiometabolic disease development, in terms of many  
619 different cell types and cellular processes involved, makes the choice of relevant *in vitro*  
620 models to be assessed rather challenging. Indeed, one single cell model would not be able to  
621 replicate the entire pathogenesis of the disease and/or may not be sufficient to intercept the  
622 therapeutic potential of a given product. Rather, taking into account different cell models,  
623 evaluated together, was needed to cover the wide spectrum of different cellular processes.  
624 Thus, to obtain comprehensive assessment of the genomic effects of flavanols, we extracted  
625 gene expression data from intestinal cells besides to five cell types known for their major  
626 contribution in cardiometabolic dysfunction, such as adipocytes, hepatocytes, endothelial  
627 cells, immune cells and smooth muscle cells. We examined results from cells exposed to  
628 flavanols in the presence or absence of dysmetabolic and/or pro-inflammatory stimuli (such  
629 as lipopolysaccharide (LPS), glucose or cytokines), classically used to better simulate the *in*  
630 *vivo* dysmetabolic conditions, and processed the gene dataset retrieved by integrated  
631 functional analysis tools. The assessment of the flavanol effects in these cell models of  
632 cardiometabolic disease allow circumventing several important confounding factors inherent  
633 to *in vivo* studies, such as age, diet, use of drugs, and chronobiological variations. For this  
634 reason, cell models are useful to unveil all those metabolic alterations induced by a treatment

635 with flavanol that might not be revealed in studies using animal models or human subjects,  
636 due to biological sample complexity. Notwithstanding, these *in vitro* models present some  
637 limitations, particularly the fact that cultured cells fail to reproduce the complex cell-cell and  
638 cell-matrix interactions recognized as a key determinant in the definition of the final cell  
639 homeostasis. In the attempt to interpret the data extracted in a more complex cell networking  
640 and circumvent the use of monotype cell models, data were also subjected to an integrated  
641 bioinformatic analysis among different cell models. Nevertheless, the findings obtained from  
642 these *in vitro* studies need confirmation and validation in animal models and human studies.  
643

644 To understand the biological role of the differentially expressed genes extracted from the  
645 literature, they were subjected to a global bioinformatic analysis. By integrating the relatively  
646 small amount of data scattered across different cell models on the one hand, and applying the  
647 powerful bioinformatics tools driven by a large amount of information on the other, we have  
648 been able to obtain a broader and more complex insight into the molecular effects of flavanols  
649 on the cardiometabolic health. This strategy allowed us to overcome the limitation of the  
650 targeted-approach (i.e., analysis of a selected, limited and predefined target genes) featuring  
651 most of the studies selected. The global analysis using the bioinformatics tools allowed us to  
652 identify, quantify and describe their role in the cellular functions. Furthermore, by integrating  
653 data from different cell types, the derived model could mimic, to some extent, the whole  
654 organism, which is particularly important for the cardiometabolic disease where several  
655 organs and tissues are implicated, connected with complex causal links.

656

657 This systematic review has identified 37 *in vitro* studies with 54 different genes up- or  
658 down-regulated by flavanol exposure in adipocytes, hepatocytes, immune, smooth muscle,  
659 and endothelial cells. Global bioinformatic analysis of differentially expressed genes  
660 extracted from literature has demonstrated that flavanols primarily modulate different

661 pathogenic aspects of cardiometabolic disease particularly processes of inflammation, cell  
662 adhesion and transendothelial migration, or lipid metabolism (Figure 10).

663

664 Low-grade inflammation is a risk factor that induces endothelial dysfunction in medium-  
665 and large-sized arterial blood vessels [54]. Dysfunctional endothelium is characterized by an  
666 increased permeability to atherogenic lipoproteins [54] and circulating immune cells [55].  
667 Under such conditions, endothelial cells increase the expression of leukocyte adhesion  
668 molecules on their surface [55]. In particular, ICAM1 and VCAM1, along with a plethora of  
669 adhesion molecules and ligands, play major roles in the process of adhesion and  
670 transendothelial migration of circulating monocytes, which includes a series of complex  
671 sequential events, such as capture, slow rolling, firm adhesion, adhesion strengthening,  
672 intraluminal crawling and finally, the transendothelial migration [55]. Flavanols have been  
673 shown to decrease the expression of leukocyte adhesion biomarkers in humans [56], as well  
674 as the leukocyte rolling over endothelium in an animal model of inflammation [34]. However,  
675 a more in-depth analysis of molecular mechanisms underlying the protective effects of  
676 flavanols on the arterial endothelium has been made only recently, demonstrating a high level  
677 of modulation of pathways defining cell adhesion and transendothelial migration [34].  
678 Concordantly, we also identified several regulators of cell adhesion, such as the “platelet-  
679 endothelium-leucocyte interaction” and “cell adhesion molecules”, including *ICAMI*,  
680 *ITGAM*, *ITGB1*, *SELE* and *VCAMI* genes as primarily affected by flavanols. The interaction  
681 between immune and endothelial cells requires the attraction of immune cells to endothelium.  
682 This process is regulated by several chemokines, which are involved in “leucocyte  
683 chemotaxis” and “chemokine signaling” pathways. In line with previous results, these  
684 pathways have also been recognized to be affected by flavanols. Upon adhesion to  
685 endothelium, immune cells migrate in sub-endothelial space, predominantly following  
686 paracellular routes [55]. Paracellular transendothelial migration requires the reorganization

687 of endothelial cytoskeleton, which is mediated by several genes, including *ROCK1* [57].  
688 Interestingly, our bioinformatic analyses identified pathways and gene networks regulating  
689 the monocyte transmigration, such as “leukocyte transendothelial migration pathway”,  
690 “regulation of actin cytoskeleton”, “focal adhesion” or “cell junctions”. “Leukocyte  
691 transendothelial migration pathway” exhibited the highest statistical significance among the  
692 pathways defining the endothelial cell function and include the following genes extracted  
693 from *in vitro* studies: *ICAM1*, *ITGAM*, *ITGB1*, *MMP9*, *ROCK1* and *VCAM1*. Concordantly,  
694 bioinformatic analysis of protein-protein interactions of extracted genes that are placed in the  
695 modulated cellular pathways responsible for endothelial cell function, demonstrated that  
696 TNF, MAPK8 and NFKB1 are central to the network of protein-protein interactions, also  
697 revealing the role of inflammation as a common underlying mechanism of cardiometabolic  
698 disease. Taken together, these systematic bioinformatic analyses showed that regulation of  
699 endothelium by flavanols is one of the key molecular mechanisms of these bioactives  
700 underlying their health properties. Genes regulating this function present potential candidates  
701 for further analyses of their importance for the inter-individual variability in response to  
702 consumption of dietary flavanols.

703

704 The enquiring of BioCarta database identified pathways linked to lipid metabolism  
705 including “visceral fat deposits and the metabolic syndrome”, “mechanism of gene regulation  
706 by peroxisome proliferators via PPARA” and “LDL pathway during atherogenesis”. It is well  
707 known that adipose tissue exerts immune-metabolic functions. Besides functioning as an  
708 energy storage tissue (storing energy in the form of lipid) and controlling the lipid  
709 mobilization and distribution in the body, it acts as an active endocrine organ by releasing a  
710 cluster of active molecules, named adipokines with autocrine and paracrine functions and  
711 modulating a range of metabolic pathways [58]. It is now widely recognized that adipose  
712 tissue dysfunction, as in terms of adipose hypertrophy and deregulated release of adipokines,

713 plays a prominent role in the development of obesity and its related disorders such as insulin  
714 resistance or cardiovascular disease [59]. Visceral fat accumulation, linked with levels of  
715 some adipokines, induces chronic inflammation and metabolic disorders, including glucose  
716 intolerance, hyperlipidemia, and arterial hypertension. Together, these conditions contribute  
717 to a diagnosis of metabolic syndrome, directly associated with the onset of cardiovascular  
718 disease [60]. Our data suggest that flavanols significantly interfere with the pathway related  
719 to “visceral fat deposits and the metabolic syndrome” regulating the expression of five  
720 interesting genes within this pathway: *PPARG*, *LPL*, *TNF*, *RETN* and *ADIPOQ*. Several  
721 epidemiological and experimental studies have shown robust hypolipidemic and anti-  
722 obesogenic effects by flavanols [61,62]. Regulation of **peroxisome proliferator-activated**  
723 **receptors** (PPARs) activity and expression by these compounds has been largely suggested  
724 as the primary mechanism of hypolipidemic and anti-obesogenic effects exerted by most  
725 flavanols [63]. PPARs are nuclear hormone receptors that function as transcription factors  
726 [64]. Up to now, three PPARs have been identified, PPARA, D/B, and G with different tissue  
727 distribution and pharmacological ligand activation profile [64]. Among them, PPARG is  
728 abundantly expressed in adipose tissue and muscle cells whereas it mediates the expression  
729 of genes associated with adipogenesis and insulin sensitivity [65], thus making it a molecular  
730 target of choice for the development of therapeutic treatments of both synthetic and natural  
731 origin.

732

733 Bioinformatic analyses of the extracted nutrigenomic data were not focused only to gene  
734 ontology analysis and identification of cellular pathways significantly associated to  
735 differentially expressed genes, but also to the gene network analyses, analysis of interactions  
736 between functional groups of genes and protein-protein interactions. Furthermore, we have  
737 also taken a step forward by analyzing the transcriptional (transcription factors) and post-  
738 transcriptional (miRNAs) regulation of differentially expressed genes. Among the most



739 significant transcription factors identified, we recognized PPARG and GATA2. Previous  
740 studies demonstrated that in addition to its role in hematopoietic stem cell development [66],  
741 GATA2 also has an important role in mediating cardiovascular disease development [67]. It  
742 is abundantly expressed in vascular endothelial cells and regulates endothelial-specific genes,  
743 such as *VCAM1*, *P-selectin* and *PECAM1*, involved in endothelial activation and dysfunction  
744 that can lead to development of atherosclerosis and cardiovascular disease [67]. It has also  
745 been observed that inactivation of GATA2 decreases the expression of cell adhesion  
746 molecules, and that it plays an essential role in endothelial cell activation by acting together  
747 with NF-kappa B, which is a critical factor in the molecular pathogenesis of atherosclerosis  
748 [67]. Our results, suggesting a role for flavanols in modulating *GATA2*, reveal a new potential  
749 regulatory site for flavanol effects. The PPARs modulate several biological processes that  
750 are perturbed in obesity, including inflammation, lipid and glucose metabolism and overall  
751 energy homeostasis. PPARs agonists have some efficacy in reducing cardiovascular risk in  
752 patients with type 2 diabetes who also have pro-atherogenic dyslipidemia [68]. Use of PPARs  
753 agonists, such as aleglitazar, was shown to improve insulin sensitivity, glucose control and  
754 lipid levels in people with type 2 diabetes [69]. Interestingly, two studies have suggested that  
755 polyphenols could act as PPARs agonists and prevent risk factors for obesity-related  
756 metabolic disorders and cardiovascular disease, such as polyphenols from plum [70] or grape  
757 seeds [71]. Together with these 2 transcription factors, our systematic bioinformatic analyses  
758 also identified other ones that present key players in the genomic response to flavanol intake,  
759 like YY1, FOXC1 or NFKB1.

760

761 Along with the identification of transcriptional regulators, we also searched for potential  
762 post-transcriptional regulators, particularly miRNAs. miRNAs are endogenous small non-  
763 coding RNAs that can interact with mRNAs, in this way exerting post-transcriptional  
764 regulation activities [72]. It has been shown that they play an important role in the regulation

765 of lipid metabolism, endothelial function, and consequently, in the development of chronic  
766 diseases such as cardiometabolic disorders [72] or cancer. Our bioinformatic analysis  
767 identified the mir-335-5p as the most significant miRNAs affected by flavanols. It has been  
768 shown that mir-335-5p plays a role in regulating endothelial function [73], insulin secretion  
769 and diabetes development [74], and in suppressing lower extremity deep venous thrombosis  
770 [75]. Concordantly to our results, in mouse models of atherosclerosis catechins, hesperidin,  
771 quercetin, curcumin, or anthocyanins were shown to modulate the expression of this miRNA  
772 [76]. Among the other miRNAs identified by our bioinformatic analysis, there is the mir-16-  
773 5p. mir-16-5p has been interestingly suggested to be associated with insulin sensitivity and  
774 cardiometabolic risk factors in humans [77]. Capacity of polyphenols to regulate the  
775 expression of this miRNA has been described in a few studies, such as with epigallocatechin  
776 gallate and quercetin [78,79]. For let-7b-5p or mir-193b-3p, no role has been reported before  
777 in regulation of cardiometabolic disorders, whereas mir-26b-5p is involved in the regulation  
778 of inflammation in myocardial infarction [80]. Taken together, this systematic analysis of  
779 genomic data of flavanols related to cardiometabolic effects revealed potential transcriptional  
780 and post-transcriptional regulators involved in the genomic modifications of flavanols and  
781 therefore novel mechanisms of action and key players in the observed effects.

782

783       Conducting this systematic bioinformatic analysis of published nutrigenomic data about  
784 the effects of flavanols in cellular models of relevance for cardiometabolic health, such as  
785 adipocytes, hepatocytes, immune, smooth muscle and endothelial cells, we demonstrated that  
786 only in a small number of studies that were identified as eligible for inclusion in our analysis,  
787 the cells were treated with flavanol metabolites (Table 1). Given the growing scientific  
788 evidence that flavanol phase II and gut microbiota metabolites represent the main circulating  
789 forms of the majority of these bioactives and mediate the effects of their parent compounds  
790 at cellular level [9], this finding identifies a major gap in the literature limiting the power of

791 the available *in vitro* studies to demonstrate the true molecular effects of flavanols. This gap  
792 in the literature should be addressed in future.

793

794 Intestinal cells are not only mediators of macro- and micronutrients absorption, but they  
795 also exhibit various functions that may affect the cardiometabolic health. By synthesizing  
796 triglycerides [129] and apolipoproteins [52], intestinal cells actively contribute to the  
797 regulation of plasma lipoprotein pools. Noteworthy, an increased atherogenic risk features  
798 patients with inflammatory bowel disease (IBD) [81]. A recent literature review has indeed  
799 suggested that patients with IBD may be at an increased risk of cardiovascular diseases  
800 [82,83]. Several studies have shown that chronic systemic inflammation in IBD can lead to  
801 endothelial dysfunction and increased platelet activation, conditions preceding the  
802 development of atherosclerotic vascular disease [84] or favoring its clinical manifestations.  
803 High levels of **tumor necrosis factor** (TNF), **C-reactive protein** (CRP) and vascular  
804 endothelial growth factor (VEGF) are characteristic of IBD and may therefore contribute to  
805 endothelial dysfunction and atherogenesis [85]. Furthermore, in both cardiovascular disease  
806 and IBD pro-inflammatory angiogenesis is recognized as a common trait sustaining both  
807 atherosclerotic plaque growth and intestinal inflammation [86-88]. Finally, during IBD  
808 flares, the adhesion of circulating monocytes to the intestinal microvascular endothelial cells,  
809 as well as their infiltration and transformation into macrophages occurs, in tight analogy with  
810 what happens in the early phases of arterial atherosclerosis [89]. Results of our bioinformatic  
811 analysis suggest that flavanols may reduce cardiovascular risk also affecting the intestinal  
812 homeostasis. For example, our data suggest that flavanols affect the expression of genes  
813 involved in PPAR signaling pathway. Beside to adipose tissue and muscle cells, PPARG is  
814 also abundantly expressed in colonic epithelial cells whereas it seems to play important anti-  
815 inflammatory and anti-carcinogenic effects [90]. In experimental animal model of IBD, the  
816 activation of PPARG by synthetic agonist rosiglitazone was shown to reduce the expression

817 of inflammatory genes by interfering with the activation of NF-kappa B transcription factor  
818 [91]. Several experimental evidences suggest that dietary polyphenols possess both  
819 protective and therapeutic effects in the management of IBD [92]. However, further  
820 preclinical and clinical studies are needed in order to understand the efficacy of dietary  
821 polyphenols in IBD patients.

822

823 Although cellular models do not reflect the variability across individuals within  
824 population, in this work, by integrating the mechanistic *in vitro* data, we gain insights on  
825 which genes or proteins are of major importance in mediating the anti-inflammatory and  
826 vasoprotective effects of flavanols. Our integrative bioinformatic meta-analyses of the  
827 existing genomic data from the literature allow us to better identify molecular mechanisms  
828 underlying cardiometabolic health properties of flavanols and identify major molecular  
829 pathways and target genes involved. Nevertheless, from the data here presented, as well as  
830 from the data in the literature, there is no doubt that *TNF* and *IL6* are among the key gene  
831 players in mediating flavanol anti-inflammatory activity, since their polymorphisms have  
832 already been associated with lifestyle dependent cardiometabolic risk factors [93]. Our data  
833 confirm and suggest the need to systematically investigate flavanol effects in relation to *TNF*  
834 and *IL6* polymorphic expressions. Deeper analyses of our data and the data from the literature  
835 may also identify other potential key target genes and polymorphisms that are worth further  
836 studying in the context of inter-individual variability of the effects of flavanols on  
837 cardiometabolic health. In conclusion, integrative biology approaches allow to identify  
838 potential key players of flavanols involved in cardiometabolic disease prevention associated  
839 to gene-protein-miRNA networks, which can be exploited for personalized nutritional  
840 recommendations in cardiometabolic disease prevention.

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842

843 **Figure legends**

844

845 **Figure 1. Data collection flowchart. For search criteria, see Methods section.**

846

847 **Figure 2. A) Number of genes repeated in studies conducted on adipocytes, hepatocytes,**  
848 **immune, smooth muscle and endothelial cells exposed to flavanols. B) Number of**  
849 **differentially expressed genes extracted from the studies on adipocytes, hepatocytes,**  
850 **immune, smooth muscle and endothelial cells exposed to flavanols.**

851

852 **Figure 3. Gene ontology for adipocytes, hepatocytes, immune, smooth muscle and**  
853 **endothelial cells exposed to flavanols.** Each rectangle is a single cluster representative, and  
854 they are joined into ‘superclusters’ of related terms, represented with different colors. Size of  
855 the rectangles reflects the p-value of the GO.

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857 **Figure 4. Gene network pie chart for adipocytes, hepatocytes, immune, smooth muscle**  
858 **and endothelial cells exposed to flavanols.**

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860 **Figure 5. BioCarta and KEGG pathways related to cellular processes in adipocytes,**  
861 **hepatocytes, immune, smooth muscle and endothelial cells exposed to flavanols. \*:**  
862 **KEGG; \*\*: BioCarta.**

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864 **Figure 6. Functional enrichment and interactome meta-analysis based on gene lists for**  
865 **different cell types exposed to flavanols.** Enrichment network visualization of the results

866 from the lists of genes identified for adipocytes, smooth muscle cells, immune cells,  
867 endothelial cells and hepatocytes. Nodes are functional groups represented by pie charts  
868 indicating their associations with each cell type. Cluster labels were added manually. Color  
869 code represents the identities of gene lists (adipocytes: red, endothelial cells: blue,  
870 hepatocytes: green, immune cells: violet) and size of each color is proportional to the  
871 percentage of the genes from different types of cells.

872

873 **Figure 7. Protein-protein interactions in adipocytes, hepatocytes, immune, smooth**  
874 **muscle and endothelial cells exposed to flavanols.** Colored nodes: query proteins and first  
875 shell of interactors; white nodes: second shell of interactors; filled nodes: some 3D structure  
876 is known or predicted; empty nodes: proteins of unknown 3D structure.

877

878 **Figure 8. Regulation of protein-protein interaction network by transcription factors**  
879 **and miRNAs in adipocytes, hepatocytes, immune, smooth muscle and endothelial cells**  
880 **exposed to flavanols.**

881

882 **Figure 9. A) KEGG and BioCarta (marked with \*) pathways for the intestinal cells**  
883 **exposed to flavanols. B) Protein-protein interactions for the intestinal cells exposed to**  
884 **flavanols. Protein network is organized in two clusters: in red – proteins that are mostly**  
885 **involved in the metabolism of circulating lipoproteins; in green – proteins that are**  
886 **mainly involved in calcium signaling. C) Regulation of protein-protein interaction**  
887 **network by transcription factors and miRNAs in the intestinal cells exposed to**  
888 **flavanols.**

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890 **Figure 10. Summary of identified differentially expressed genes modulated by flavanol**  
891 **and related to cardiometabolic health.**

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921

922 **Conflict of interest**

923 The authors do not have conflict of interest

924

925 **Contribution of authors**

926 All authors contributed to conceptualization, methodology, data extraction and validation of  
927 the last version of the manuscript. TR, MM, DM contributed to writing, reviewing and editing  
928 of the manuscript and preparation, creation and/or presentation of the published work.

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933 **References**

- 934 1. Kirk, E.P.; Klein, S. Pathogenesis and pathophysiology of the cardiometabolic  
935 syndrome. *J Clin Hypertens (Greenwich)* **2009**, *11*, 761-765, doi: 10.1111/j.1559-  
936 4572.2009.00054.x.
- 937 2. Mozaffarian, D. Dietary and Policy Priorities for Cardiovascular Disease, Diabetes,  
938 and Obesity: A Comprehensive Review. *Circulation* **2016**, *133*, 187-225,  
939 doi:10.1161/CIRCULATIONAHA.115.018585.
- 940 3. de Toro-Martin, J.; Arsenault, B.J.; Despres, J.P.; Vohl, M.C. Precision Nutrition: A  
941 Review of Personalized Nutritional Approaches for the Prevention and Management  
942 of Metabolic Syndrome. *Nutrients* **2017**, *9*, pii: E913. doi: 10.3390/nu9080913.
- 943 4. Juma, S.; Imrhan, V.; Vijayagopal, P.; Prasad, C. Prescribing Personalized Nutrition  
944 for Cardiovascular Health: Are We Ready? *J Nutrigenet Nutrige* **2014**, *7*, 153-160,  
945 doi:10.1159/000370213.
- 946 5. Mozaffarian, D.; Wu, J.H.Y. Flavonoids, Dairy Foods, and Cardiovascular and  
947 Metabolic Health A Review of Emerging Biologic Pathways. *Circ Res* **2018**, *122*,  
948 369-384, doi:10.1161/Circresaha.117.309008.
- 949 6. Rodriguez-Mateos, A.; Vauzour, D.; Krueger, C.G.; Shanmuganayagam, D.; Reed,  
950 J.; Calani, L.; Mena, P.; Del Rio, D.; Crozier, A. Bioavailability, bioactivity and  
951 impact on health of dietary flavonoids and related compounds: an update. *Arch*  
952 *Toxicol* **2014**, *88*, 1803-1853, doi:10.1007/s00204-014-1330-7.
- 953 7. Neveu, V.; Perez-Jiménez, J.; Vos, F.; Crespy, V.; du Chaffaut, L.; Mennen, L.;  
954 Knox, C.; Eisner, R.; Cruz, J.; Wishart, D., et al. Phenol-Explorer: an online  
955 comprehensive database on polyphenol contents in foods. *Database* **2010**, *2010*,  
956 bap024, doi:10.1093/database/bap024.
- 957 8. Scalbert, A.; Williamson, G. Dietary Intake and Bioavailability of Polyphenols. *J*  
958 *Nutr* **2000**, *130*, 2073S-2085S, doi:10.1093/jn/130.8.2073S.

- 959 9. Del Rio, D.; Rodriguez-Mateos, A.; Spencer, J.P.; Tognolini, M.; Borges, G.;  
960 Crozier, A. Dietary (poly)phenolics in human health: structures, bioavailability, and  
961 evidence of protective effects against chronic diseases. *Antioxid Redox Signal* **2013**,  
962 *18*, 1818-1892, doi:10.1089/ars.2012.4581.
- 963 10. Clifford, M.N.; van der Hooft, J.J.; Crozier, A. Human studies on the absorption,  
964 distribution, metabolism, and excretion of tea polyphenols. *Am J Clin Nutr* **2013**, *98*,  
965 1619S-1630S, doi: 10.3945/ajcn.113.058958.
- 966 11. Cifuentes-Gomez, T.; Rodriguez-Mateos, A.; Gonzalez-Salvador, I.; Alañon, M.E.;  
967 Spencer, J.P. Factors Affecting the Absorption, Metabolism, and Excretion of Cocoa  
968 Flavanols in Humans. *J Agric Food Chem* **2015**, *63*, 7615-7623, doi:  
969 10.1021/acs.jafc.5b00443.
- 970 12. Stalmach, A.; Troufflard, S.; Serafini, M.; Crozier, A. Absorption, metabolism and  
971 excretion of Choladi green tea flavan-3-ols by humans. *Mol Nutr Food Res* **2009**, *53*  
972 *Suppl 1*, S44-53, doi:10.1002/mnfr.200800169.
- 973 13. Oracz, J.; Nebesny, E.; Zyzelewicz, D.; Budryn, G.; Luzak, B. Bioavailability and  
974 metabolism of selected cocoa bioactive compounds: A comprehensive review. *Crit*  
975 *Rev Food Sci Nutr* **2019**, 10.1080/10408398.2019.1619160, 1-39,  
976 doi:10.1080/10408398.2019.1619160.
- 977 14. Gómez-Juaristi, M.; Sarria, B.; Martínez-López, S.; Bravo Clemente, L.; Mateos, R.  
978 Flavanol Bioavailability in Two Cocoa Products with Different Phenolic Content. A  
979 Comparative Study in Humans. *Nutrients* **2019**, *26*, pii: E1441, doi:  
980 10.3390/nu11071441.
- 981 15. Ottaviani, J.I.; Borges, G.; Momma, T.Y.; Spencer, J.P.E.; Keen, C.L.; Crozier, A.;  
982 Schroeter, H. The metabolome of [2-14C](–)-epicatechin in humans: implications  
983 for the assessment of efficacy, safety, and mechanisms of action of polyphenolic  
984 bioactives. *Scientific reports* **2016**, *6*, 29034, doi:10.1038/srep29034.

- 985 16. Kroon, P.A.; Clifford, M.N.; Crozier, A.; Day, A.J.; Donovan, J.L.; Manach, C.;  
986 Williamson, G. How should we assess the effects of exposure to dietary polyphenols  
987 in vitro? *The American journal of clinical nutrition* **2004**, *80*, 15-21,  
988 doi:10.1093/ajcn/80.1.15.
- 989 17. Duenas, M.; Gonzalez-Manzano, S.; Gonzalez-Paramas, A.; Santos-Buelga, C.  
990 Antioxidant evaluation of O-methylated metabolites of catechin, epicatechin and  
991 quercetin. *Journal of pharmaceutical and biomedical analysis* **2010**, *51*, 443-449,  
992 doi:10.1016/j.jpba.2009.04.007.
- 993 18. Rienks, J.; Barbaresko, J.; Nothlings, U. Association of Polyphenol Biomarkers with  
994 Cardiovascular Disease and Mortality Risk: A Systematic Review and Meta-  
995 Analysis of Observational Studies. *Nutrients* **2017**, *9*, doi:10.3390/nu9040415.
- 996 19. Menezes, R.; Rodriguez-Mateos, A.; Kaltsatou, A.; Gonzalez-Sarrias, A.; Greyling,  
997 A.; Giannaki, C.; Andres-Lacueva, C.; Milenkovic, D.; Gibney, E.R.; Dumont, J., et  
998 al. Impact of Flavonols on Cardiometabolic Biomarkers: A Meta-Analysis of  
999 Randomized Controlled Human Trials to Explore the Role of Inter-Individual  
1000 Variability. *Nutrients* **2017**, *9*, doi:10.3390/nu9020117.
- 1001 20. González-Sarriás, A.; Combet, E.; Pinto, P.; Mena, P.; Dall'Asta, M.; Garcia-Aloy,  
1002 M.; Rodríguez-Mateos, A.; Gibney, E.R.; Dumont, J.; Massaro, M., et al. A  
1003 Systematic Review and Meta-Analysis of the Effects of Flavanol-Containing Tea,  
1004 Cocoa and Apple Products on Body Composition and Blood Lipids: Exploring the  
1005 Factors Responsible for Variability in Their Efficacy. *Nutrients* **2017**, *9*, 746, doi:  
1006 10.3390/nu9070746.
- 1007 21. Del Bo', C.; Deon, V.; Campolo, J.; Lanti, C.; Parolini, M.; Porrini, M.; Klimis-  
1008 Zacas, D.; Riso, P. A serving of blueberry (*V. corymbosum*) acutely improves  
1009 peripheral arterial dysfunction in young smokers and non-smokers: two randomized,

- 1010 controlled, crossover pilot studies. *Food & Function* **2017**, *8*, 4108-4117,  
1011 doi:10.1039/C7FO00861A.
- 1012 22. Claude, S.; Bobby, C.; Rodriguez-Mateos, A.; Spencer, J.P.E.; Gerard, N.; Morand,  
1013 C.; Milenkovic, D. Flavanol metabolites reduce monocyte adhesion to endothelial  
1014 cells through modulation of expression of genes via p38-MAPK and p65-Nf-kB  
1015 pathways. *Mol Nutr Food Res* **2014**, *58*, 1016-1027, doi:10.1002/mnfr.201300658.
- 1016 23. Cassidy, A.; Mukamal, K.J.; Liu, L.; Franz, M.; Eliassen, A.H.; Rimm, E.B. High  
1017 anthocyanin intake is associated with a reduced risk of myocardial infarction in  
1018 young and middle-aged women. *Circulation* **2013**, *127*, 188-196, doi:  
1019 10.1161/CIRCULATIONAHA.112.122408.
- 1020 24. Jacques, P.F.; Cassidy, A.; Rogers, G.; Peterson, J.J.; Meigs, J.B.; Dwyer, J.T. Higher  
1021 dietary flavonol intake is associated with lower incidence of type 2 diabetes. *J Nutr*  
1022 **2013**, *143*, 1474-1480, doi: 10.3945/jn.113.177212.
- 1023 25. Gibney, E.R.; Milenkovic, D.; Combet, E.; Ruskovska, T.; Greyling, A.; Gonzalez-  
1024 Sarrias, A.; de Roos, B.; Tomas-Barberan, F.; Morand, C.; Rodriguez-Mateos, A.  
1025 Factors influencing the cardiometabolic response to (poly)phenols and phytosterols:  
1026 a review of the COST Action POSITIVE activities. *European journal of nutrition*  
1027 **2019**, *58*, 37-47, doi:10.1007/s00394-019-02066-6.
- 1028 26. Milenkovic, D.; Morand, C.; Cassidy, A.; Konic-Ristic, A.; Tomas-Barberan, F.;  
1029 Ordovas, J.M.; Kroon, P.; De Caterina, R.; Rodriguez-Mateos, A. Interindividual  
1030 Variability in Biomarkers of Cardiometabolic Health after Consumption of Major  
1031 Plant-Food Bioactive Compounds and the Determinants Involved. *Adv Nutr* **2017**, *8*,  
1032 558-570, doi: 10.3945/an.116.013623.
- 1033 27. Szarc vel Szic, K.; Declerck, K.; Vidakovic, M.; Vanden Berghe, W. From  
1034 inflammaging to healthy aging by dietary lifestyle choices: is epigenetics the key to

- 1035 personalized nutrition? *Clinical epigenetics* **2015**, *7*, 33, doi:10.1186/s13148-015-  
1036 0068-2.
- 1037 28. Naderi, G.A.; Asgary, S.; Sarraf-Zadegan, N.; Shirvany, H. Anti-oxidant effect of  
1038 flavonoids on the susceptibility of LDL oxidation. *Mol Cell Biochem* **2003**, *246*, 193-  
1039 196.
- 1040 29. Ruskovska, T.; Maksimova, V.; Milenkovic, D. Polyphenols in human nutrition:  
1041 from the in vitro antioxidant capacity to the beneficial effects on cardiometabolic  
1042 health and related inter-individual variability - an overview and perspective. *The*  
1043 *British journal of nutrition* **2019**, *123*, 241-254, doi:10.1017/s0007114519002733.
- 1044 30. Sharma, P.; Dwivedi, S. Nutrigenomics and Nutrigenetics: New Insight in Disease  
1045 Prevention and Cure. *Indian journal of clinical biochemistry : IJCB* **2017**, *32*, 371-  
1046 373, doi:10.1007/s12291-017-0699-5.
- 1047 31. Fenech, M. Genome health nutrigenomics and nutrigenetics--diagnosis and  
1048 nutritional treatment of genome damage on an individual basis. *Food and chemical*  
1049 *toxicology : an international journal published for the British Industrial Biological*  
1050 *Research Association* **2008**, *46*, 1365-1370, doi:10.1016/j.fct.2007.06.035.
- 1051 32. Chanet, A.; Milenkovic, D.; Claude, S.; Maier, J.A.; Kamran Khan, M.;  
1052 Rakotomanomana, N.; Shinkaruk, S.; Berard, A.M.; Bennetau-Pelissero, C.; Mazur,  
1053 A., et al. Flavanone metabolites decrease monocyte adhesion to TNF-alpha-activated  
1054 endothelial cells by modulating expression of atherosclerosis-related genes. *The*  
1055 *British journal of nutrition* **2013**, *110*, 587-598, doi:10.1017/s0007114512005454.
- 1056 33. Monfoulet, L.E.; Mercier, S.; Bayle, D.; Tamaian, R.; Barber-Chamoux, N.; Morand,  
1057 C.; Milenkovic, D. Curcumin modulates endothelial permeability and monocyte  
1058 transendothelial migration by affecting endothelial cell dynamics. *Free radical*  
1059 *biology & medicine* **2017**, *112*, 109-120, doi:10.1016/j.freeradbiomed.2017.07.019.

- 1060 34. Milenkovic, D.; Berghe, W.V.; Morand, C.; Claude, S.; van de Sandt, A.; Gorressen,  
1061 S.; Monfoulet, L.E.; Chirumamilla, C.S.; Declerck, K.; Szic, K.S.V., et al. A systems  
1062 biology network analysis of nutri(epi)genomic changes in endothelial cells exposed  
1063 to epicatechin metabolites. *Scientific reports* **2018**, *8*, 15487, doi:10.1038/s41598-  
1064 018-33959-x.
- 1065 35. Coban, D.; Milenkovic, D.; Chanet, A.; Khallou-Laschet, J.; Sabbe, L.; Palagani, A.;  
1066 Vanden Berghe, W.; Mazur, A.; Morand, C. Dietary curcumin inhibits  
1067 atherosclerosis by affecting the expression of genes involved in leukocyte adhesion  
1068 and transendothelial migration. *Mol Nutr Food Res* **2012**, *56*, 1270-1281,  
1069 doi:10.1002/mnfr.201100818.
- 1070 36. Chanet, A.; Milenkovic, D.; Deval, C.; Potier, M.; Constans, J.; Mazur, A.;  
1071 Bennetau-Pelissero, C.; Morand, C.; Berard, A.M. Naringin, the major grapefruit  
1072 flavonoid, specifically affects atherosclerosis development in diet-induced  
1073 hypercholesterolemia in mice. *J Nutr Biochem* **2012**, *23*, 469-477,  
1074 doi:10.1016/j.jnutbio.2011.02.001.
- 1075 37. Chanet, A.; Wizinska, P.; Polakof, S.; Mazur, A.; Bennetau-Pelissero, C.; Morand,  
1076 C.; Berard, A.M.; Milenkovic, D. Naringin at a nutritional dose modulates expression  
1077 of genes related to lipid metabolism and inflammation in liver of mice fed a high-fat  
1078 diet. *Nutrition and Aging* **2012**, *1*, 113-123. doi: 10.3233/NUA-2012-0010.
- 1079 38. de Boer, V.C.; van Schothorst, E.M.; Dihal, A.A.; van der Woude, H.; Arts, I.C.;  
1080 Rietjens, I.M.; Hollman, P.C.; Keijer, J. Chronic quercetin exposure affects fatty acid  
1081 catabolism in rat lung. *Cellular and molecular life sciences : CMLS* **2006**, *63*, 2847-  
1082 2858, doi:10.1007/s00018-006-6316-z.
- 1083 39. Milenkovic, D.; Vanden Berghe, W.; Boby, C.; Leroux, C.; Declerck, K.; Szarc vel  
1084 Szic, K.; Heyninck, K.; Laukens, K.; Bizet, M.; Defrance, M., et al. Dietary flavanols  
1085 modulate the transcription of genes associated with cardiovascular pathology without

- 1086 changes in their DNA methylation state. *PloS one* **2014**, *9*, e95527,  
1087 doi:10.1371/journal.pone.0095527.
- 1088 40. Milenkovic, D.; Deval, C.; Dubray, C.; Mazur, A.; Morand, C. Hesperidin displays  
1089 relevant role in the nutrigenomic effect of orange juice on blood leukocytes in human  
1090 volunteers: a randomized controlled cross-over study. *PloS one* **2011**, *6*, e26669,  
1091 doi:10.1371/journal.pone.0026669.
- 1092 41. Manach, C.; Milenkovic, D.; Van de Wiele, T.; Rodriguez-Mateos, A.; de Roos, B.;  
1093 Garcia-Conesa, M.T.; Landberg, R.; Gibney, E.R.; Heinonen, M.; Tomás-Barberán,  
1094 F.; Morand, C. Addressing the inter-individual variation in response to consumption  
1095 of plant food bioactives: Towards a better understanding of their role in healthy aging  
1096 and cardiometabolic risk reduction. *Mol Nutr Food Res* **2017**, *61*, 1600557, doi:  
1097 10.1002/mnfr.201600557.
- 1098 42. Huang da, W.; Sherman, B.T.; Lempicki, R.A. Systematic and integrative analysis  
1099 of large gene lists using DAVID bioinformatics resources. *Nature protocols* **2009**, *4*,  
1100 44-57, doi:10.1038/nprot.2008.211.
- 1101 43. Huang da, W.; Sherman, B.T.; Lempicki, R.A. Bioinformatics enrichment tools:  
1102 paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids*  
1103 *Res* **2009**, *37*, 1-13, doi:10.1093/nar/gkn923.
- 1104 44. Supek, F.; Bosnjak, M.; Skunca, N.; Smuc, T. REVIGO summarizes and visualizes  
1105 long lists of gene ontology terms. *PloS one* **2011**, *6*, e21800,  
1106 doi:10.1371/journal.pone.0021800.
- 1107 45. Stöckel, D.; Kehl, T.; Trampert, P.; Schneider, L.; Backes, C.; Ludwig, N.; Gerasch,  
1108 A.; Kaufmann, M.; Gessler, M.; Graf, N., et al. Multi-omics enrichment analysis  
1109 using the GeneTrail2 web service. *Bioinformatics* **2016**, *32*, 1502-1508,  
1110 doi:10.1093/bioinformatics/btv770.

- 1111 46. Zhou, Y.; Zhou, B.; Pache, L.; Chang, M.; Khodabakhshi, A.H.; Tanaseichuk, O.;  
1112 Benner, C.; Chanda, S.K. Metascape provides a biologist-oriented resource for the  
1113 analysis of systems-level datasets. *Nature Communications* **2019**, *10*, 1523,  
1114 doi:10.1038/s41467-019-09234-6.
- 1115 47. Shannon, P.; Markiel, A.; Ozier, O.; Baliga, N.S.; Wang, J.T.; Ramage, D.; Amin,  
1116 N.; Schwikowski, B.; Ideker, T. Cytoscape: a software environment for integrated  
1117 models of biomolecular interaction networks. *Genome Res* **2003**, *13*, 2498-2504,  
1118 doi:10.1101/gr.1239303.
- 1119 48. Szklarczyk, D.; Morris, J.H.; Cook, H.; Kuhn, M.; Wyder, S.; Simonovic, M.; Santos,  
1120 A.; Doncheva, N.T.; Roth, A.; Bork, P., et al. The STRING database in 2017: quality-  
1121 controlled protein-protein association networks, made broadly accessible. *Nucleic  
1122 Acids Res* **2017**, *45*, D362-D368, doi:10.1093/nar/gkw937.
- 1123 49. Zhou, G.; Xia, J. OmicsNet: a web-based tool for creation and visual analysis of  
1124 biological networks in 3D space. *Nucleic Acids Res* **2018**, *46*, W514-w522,  
1125 doi:10.1093/nar/gky510.
- 1126 50. Zhou, G.; Xia, J. Using OmicsNet for Network Integration and 3D Visualization.  
1127 *Current Protocols in Bioinformatics* **2019**, *65*, e69, doi:10.1002/cpbi.69.
- 1128 51. Stelzer, G.; Rosen, N.; Plaschkes, I.; Zimmerman, S.; Twik, M.; Fishilevich, S.;  
1129 Stein, T.I.; Nudel, R.; Lieder, I.; Mazor, Y., et al. The GeneCards Suite: From Gene  
1130 Data Mining to Disease Genome Sequence Analyses. *Current Protocols in  
1131 Bioinformatics* **2016**, *54*, 1.30.31-31.30.33, doi:10.1002/cpbi.5.
- 1132 52. Yasuda, A.; Natsume, M.; Osakabe, N.; Kawahata, K.; Koga, J. Cacao polyphenols  
1133 influence the regulation of apolipoprotein in HepG2 and Caco2 cells. *J Agric Food  
1134 Chem* **2011**, *59*, 1470-1476, doi:10.1021/jf103820b.
- 1135 53. Hong Byun, E.; Fujimura, Y.; Yamada, K.; Tachibana, H. TLR4 Signaling Inhibitory  
1136 Pathway Induced by Green Tea Polyphenol Epigallocatechin-3-Gallate through 67-



- 1137 kDa Laminin Receptor. *The Journal of Immunology* **2010**, *185*, 33-45,  
1138 doi:10.4049/jimmunol.0903742.
- 1139 54. Park, K.H.; Park, W.J. Endothelial Dysfunction: Clinical Implications in  
1140 Cardiovascular Disease and Therapeutic Approaches. *Journal of Korean medical*  
1141 *science* **2015**, *30*, 1213-1225, doi:10.3346/jkms.2015.30.9.1213.
- 1142 55. Gerhardt, T.; Ley, K. Monocyte trafficking across the vessel wall. *Cardiovascular*  
1143 *research* **2015**, *107*, 321-330, doi:10.1093/cvr/cvv147.
- 1144 56. Esser, D.; Mars, M.; Oosterink, E.; Stalmach, A.; Muller, M.; Afman, L.A. Dark  
1145 chocolate consumption improves leukocyte adhesion factors and vascular function  
1146 in overweight men. *FASEB journal : official publication of the Federation of*  
1147 *American Societies for Experimental Biology* **2014**, *28*, 1464-1473,  
1148 doi:10.1096/fj.13-239384.
- 1149 57. Zhou, Q.; Gensch, C.; Liao, J.K. Rho-associated coiled-coil-forming kinases  
1150 (ROCKs): potential targets for the treatment of atherosclerosis and vascular disease.  
1151 *Trends Pharmacol Sci* **2011**, *32*, 167-173, doi:10.1016/j.tips.2010.12.006.
- 1152 58. Luo, L.; Liu, M. Adipose tissue in control of metabolism. *J Endocrinol* **2016**, *231*,  
1153 R77-R99, doi:10.1530/JOE-16-0211.
- 1154 59. Ha, E.E.; Bauer, R.C. Emerging Roles for Adipose Tissue in Cardiovascular Disease.  
1155 *Arterioscler Thromb Vasc Biol* **2018**, *38*, e137-e144, doi:  
1156 10.1161/ATVBAHA.118.311421.
- 1157 60. Alberti, K.G.; Eckel, R.H.; Grundy, S.M.; Zimmet, P.Z.; Cleeman, J.I.; Donato,  
1158 K.A.; Fruchart, J.C.; James, W.P.; Loria, C.M.; Smith, S.C., Jr., et al. Harmonizing  
1159 the metabolic syndrome: a joint interim statement of the International Diabetes  
1160 Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and  
1161 Blood Institute; American Heart Association; World Heart Federation; International

- 1162 Atherosclerosis Society; and International Association for the Study of Obesity.  
1163 *Circulation* **2009**, *120*, 1640-1645, doi:10.1161/CIRCULATIONAHA.109.192644.
- 1164 61. Hursel, R.; Westerterp-Plantenga, M.S. Catechin- and caffeine-rich teas for control  
1165 of body weight in humans. *The American journal of clinical nutrition* **2013**, *98*,  
1166 1682S-1693S, doi:10.3945/ajcn.113.058396.
- 1167 62. Freisling, H.; Pisa, P.T.; Ferrari, P.; Byrnes, G.; Moskal, A.; Dahm, C.C.; Vergnaud,  
1168 A.-C.; Boutron-Ruault, M.-C.; Fagherazzi, G.; Cadeau, C., et al. Main nutrient  
1169 patterns are associated with prospective weight change in adults from 10 European  
1170 countries. *European journal of nutrition* **2016**, *55*, 2093-2104, doi:10.1007/s00394-  
1171 015-1023-x.
- 1172 63. Lee, S.J.; Jia, Y. The effect of bioactive compounds in tea on lipid metabolism and  
1173 obesity through regulation of peroxisome proliferator-activated receptors. *Curr Opin*  
1174 *Lipidol* **2015**, *26*, 3-9, doi:10.1097/MOL.000000000000145.
- 1175 64. Grygiel-Górniak, B. Peroxisome proliferator-activated receptors and their ligands:  
1176 nutritional and clinical implications - a review. *Nutrition Journal* **2014**, *13*, 17,  
1177 doi:10.1186/1475-2891-13-17.
- 1178 65. Gregoire, F.M. Adipocyte differentiation: from fibroblast to endocrine cell. *Exp Biol*  
1179 *Med (Maywood)* **2001**, *226*, 997-1002, doi:10.1177/153537020122601106.
- 1180 66. Fujiwara, T. GATA Transcription Factors: Basic Principles and Related Human  
1181 Disorders. *Tohoku J Exp Med* **2017**, *242*, 83-91, doi:10.1620/tjem.242.83.
- 1182 67. Qiu, C.; Wang, Y.; Zhao, H.; Qin, L.; Shi, Y.; Zhu, X.; Song, L.; Zhou, X.; Chen, J.;  
1183 Zhou, H., et al. The critical role of SENP1-mediated GATA2 deSUMOylation in  
1184 promoting endothelial activation in graft arteriosclerosis. *Nat Commun* **2017**, *8*,  
1185 15426, doi:10.1038/ncomms15426.

- 1186 68. Gross, B.; Pawlak, M.; Lefebvre, P.; Staels, B. PPARs in obesity-induced T2DM,  
1187 dyslipidaemia and NAFLD. *Nat Rev Endocrinol* **2017**, *13*, 36-49,  
1188 doi:10.1038/nrendo.2016.135.
- 1189 69. Stirban, A.O.; Andjelkovic, M.; Heise, T.; Nosek, L.; Fischer, A.; Gastaldelli, A.;  
1190 Herz, M. Aleglitazar, a dual peroxisome proliferator-activated receptor- $\alpha/\gamma$  agonist,  
1191 improves insulin sensitivity, glucose control and lipid levels in people with type 2  
1192 diabetes: findings from a randomized, double-blind trial. *Diabetes, Obesity and*  
1193 *Metabolism* **2016**, *18*, 711-715, doi:10.1111/dom.12620.
- 1194 70. Noratto, G.; Martino, H.S.; Simbo, S.; Byrne, D.; Mertens-Talcott, S.U.  
1195 Consumption of polyphenol-rich peach and plum juice prevents risk factors for  
1196 obesity-related metabolic disorders and cardiovascular disease in Zucker rats. *J Nutr*  
1197 *Biochem* **2015**, *26*, 633-641, doi:10.1016/j.jnutbio.2014.12.014.
- 1198 71. Pascual-Serrano, A.; Blade, C.; Suarez, M.; Arola-Arnal, A. Grape Seed  
1199 Proanthocyanidins Improve White Adipose Tissue Expansion during Diet-Induced  
1200 Obesity Development in Rats. *Int J Mol Sci* **2018**, *19*, doi:10.3390/ijms19092632.
- 1201 72. Najafi-Shoushtari, S.H. MicroRNAs in cardiometabolic disease. *Curr Atheroscler*  
1202 *Rep* **2011**, *13*, 202-207, doi:10.1007/s11883-011-0179-y.
- 1203 73. Yue, J.; Wan, F.; Zhang, Q.; Wen, P.; Cheng, L.; Li, P.; Guo, W. Effect of  
1204 glucocorticoids on miRNA expression spectrum of rat femoral head microcirculation  
1205 endothelial cells. *Gene* **2018**, *651*, 126-133, doi:10.1016/j.gene.2018.01.057.
- 1206 74. Vienberg, S.; Geiger, J.; Madsen, S.; Dalgaard, L.T. MicroRNAs in metabolism. *Acta*  
1207 *Physiol (Oxf)* **2017**, *219*, 346-361, doi:10.1111/apha.12681.
- 1208 75. Bao, C.X.; Zhang, D.X.; Wang, N.N.; Zhu, X.K.; Zhao, Q.; Sun, X.L. MicroRNA-  
1209 335-5p suppresses lower extremity deep venous thrombosis by targeted inhibition of  
1210 PAI-1 via the TLR4 signaling pathway. *J Cell Biochem* **2018**, *119*, 4692-4710,  
1211 doi:10.1002/jcb.26647.

- 1212 76. Milenkovic, D.; Deval, C.; Gouranton, E.; Landrier, J.F.; Scalbert, A.; Morand, C.;  
1213 Mazur, A. Modulation of miRNA expression by dietary polyphenols in apoE  
1214 deficient mice: a new mechanism of the action of polyphenols. *PLoS One* **2012**, *7*,  
1215 e29837, doi: 10.1371/journal.pone.0029837.
- 1216 77. Ma, E.; Fu, Y.; Garvey, W.T. Relationship of Circulating miRNAs with Insulin  
1217 Sensitivity and Associated Metabolic Risk Factors in Humans. *Metab Syndr Relat*  
1218 *Disord* **2018**, *16*, 82-89. doi: 10.1089/met.2017.0101.
- 1219 78. Tsang, W.P.; Kwok, T.T. Epigallocatechin gallate up-regulation of miR-16 and  
1220 induction of apoptosis in human cancer cells. *J Nutr Biochem* **2010**, *21*, 140-146,  
1221 doi:10.1016/j.jnutbio.2008.12.003.
- 1222 79. Sonoki, H.; Sato, T.; Endo, S.; Matsunaga, T.; Yamaguchi, M.; Yamazaki, Y.;  
1223 Sugatani, J.; Ikari, A. Quercetin Decreases Claudin-2 Expression Mediated by Up-  
1224 Regulation of microRNA miR-16 in Lung Adenocarcinoma A549 Cells. *Nutrients*  
1225 **2015**, *7*, 4578-4592, doi:10.3390/nu7064578.
- 1226 80. Ge, Z.W.; Zhu, X.L.; Wang, B.C.; Hu, J.L.; Sun, J.J.; Wang, S.; Chen, X.J.; Meng,  
1227 S.P.; Liu, L.; Cheng, Z.Y. MicroRNA-26b relieves inflammatory response and  
1228 myocardial remodeling of mice with myocardial infarction by suppression of MAPK  
1229 pathway through binding to PTGS2. *Int J Cardiol* **2019**, *280*, 152-159, doi:  
1230 10.1016/j.ijcard.2018.12.077.
- 1231 81. Aarestrup, J.; Jess, T.; Kobylecki, C.J.; Nordestgaard, B.G.; Allin, K.H.  
1232 Cardiovascular Risk Profile Among Patients With Inflammatory Bowel Disease: A  
1233 Population-based Study of More Than 100 000 Individuals. *Journal of Crohn's and*  
1234 *Colitis* **2018**, *13*, 319-323, doi:10.1093/ecco-jcc/jjy164.
- 1235 82. Singh, S.; Singh, H.; Loftus, E.V., Jr.; Pardi, D.S. Risk of cerebrovascular accidents  
1236 and ischemic heart disease in patients with inflammatory bowel disease: a systematic

- 1237 review and meta-analysis. *Clin Gastroenterol Hepatol* **2014**, *12*, 382-393,  
1238 doi:10.1016/j.cgh.2013.08.023.
- 1239 83. Theocharidou, E.; Gossios, T.D.; Giouleme, O.; Athyros, V.G.; Karagiannis, A.  
1240 Carotid intima-media thickness in patients with inflammatory bowel disease: a  
1241 systematic review. *Angiology* **2014**, *65*, 284-293, doi:10.1177/0003319713477471.
- 1242 84. Gimbrone, M.A. Jr.; García-Cardena, G. Endothelial Cell Dysfunction and the  
1243 Pathobiology of Atherosclerosis. *Circ Res* **2016**, *118*, 620-636, doi:  
1244 10.1161/CIRCRESAHA.115.306301.
- 1245 85. Scaldaferri, F.; Lancellotti, S.; Pizzoferrato, M.; De Cristofaro, R. Haemostatic  
1246 system in inflammatory bowel diseases: new players in gut inflammation. *World J*  
1247 *Gastroenterol* **2011**, *17*, 594-608, doi:10.3748/wjg.v17.i5.594.
- 1248 86. Moreno, P.R.; Purushothaman, M.; Purushothaman, K.R. Plaque neovascularization:  
1249 defense mechanisms, betrayal, or a war in progress. *Ann N Y Acad Sci* **2012**, *1254*,  
1250 7-17, doi:10.1111/j.1749-6632.2012.06497.x.
- 1251 87. Danese, S.; Sans, M.; de la Motte, C.; Graziani, C.; West, G.; Phillips, M.H.; Pola,  
1252 R.; Rutella, S.; Willis, J.; Gasbarrini, A., et al. Angiogenesis as a novel component  
1253 of inflammatory bowel disease pathogenesis. *Gastroenterology* **2006**, *130*, 2060-  
1254 2073, doi:10.1053/j.gastro.2006.03.054.
- 1255 88. Chidlow, J.H., Jr.; Shukla, D.; Grisham, M.B.; Kevil, C.G. Pathogenic angiogenesis  
1256 in IBD and experimental colitis: new ideas and therapeutic avenues. *Am J Physiol*  
1257 *Gastrointest Liver Physiol* **2007**, *293*, G5-G18, doi:10.1152/ajpgi.00107.2007.
- 1258 89. Bain, C.C.; Mowat, A.M. Macrophages in intestinal homeostasis and inflammation.  
1259 *Immunol Rev* **2014**, *260*, 102-117, doi:10.1111/imr.12192.
- 1260 90. Speca, S.; Dubuquoy, L.; Desreumaux, P. Peroxisome proliferator-activated receptor  
1261 gamma in the colon: inflammation and innate antimicrobial immunity. *J Clin*  
1262 *Gastroenterol* **2014**, *48 Suppl 1*, S23-27, doi:10.1097/MCG.0000000000000253.

- 1263 91. Sanchez-Hidalgo, M.; Martin, A.R.; Villegas, I.; de la Lastra, C.A. Rosiglitazone, a  
1264 PPARgamma ligand, modulates signal transduction pathways during the  
1265 development of acute TNBS-induced colitis in rats. *Eur J Pharmacol* **2007**, *562*, 247-  
1266 258, doi:10.1016/j.ejphar.2007.01.047.
- 1267 92. Farzaei, M.H.; Rahimi, R.; Abdollahi, M. The role of dietary polyphenols in the  
1268 management of inflammatory bowel disease. *Current pharmaceutical biotechnology*  
1269 **2015**, *16*, 196-210, doi:10.2174/1389201016666150118131704.
- 1270 93. Curti, M.L.R.; Pires, M.M.; Barros, C.R.; Siqueira-Catania, A.; Rogero, M.M.;  
1271 Ferreira, S.R.G. Associations of the TNF-alpha -308 G/A, IL6 -174 G/C and AdipoQ  
1272 45 T/G polymorphisms with inflammatory and metabolic responses to lifestyle  
1273 intervention in Brazilians at high cardiometabolic risk. *Diabetology & Metabolic*  
1274 *Syndrome* **2012**, *4*, 49, doi:10.1186/1758-5996-4-49.
- 1275 94. Chan, C.Y.; Wei, L.; Castro-Munozledo, F.; Koo, W.L. (-)-Epigallocatechin-3-  
1276 gallate blocks 3T3-L1 adipose conversion by inhibition of cell proliferation and  
1277 suppression of adipose phenotype expression. *Life Sci* **2011**, *89*, 779-785,  
1278 doi:10.1016/j.lfs.2011.09.006.
- 1279 95. Vazquez-Prieto, M.A.; Bettaieb, A.; Haj, F.G.; Fraga, C.G.; Oteiza, P.I. (-)-  
1280 Epicatechin prevents TNFalpha-induced activation of signaling cascades involved in  
1281 inflammation and insulin sensitivity in 3T3-L1 adipocytes. *Arch Biochem Biophys*  
1282 **2012**, *527*, 113-118, doi:10.1016/j.abb.2012.02.019.
- 1283 96. Yan, J.; Zhao, Y.; Suo, S.; Liu, Y.; Zhao, B. Green tea catechins ameliorate adipose  
1284 insulin resistance by improving oxidative stress. *Free radical biology & medicine*  
1285 **2012**, *52*, 1648-1657, doi:10.1016/j.freeradbiomed.2012.01.033.
- 1286 97. Shin, D.W.; Kim, S.N.; Lee, S.M.; Lee, W.; Song, M.J.; Park, S.M.; Lee, T.R.; Baik,  
1287 J.H.; Kim, H.K.; Hong, J.H., et al. (-)-Catechin promotes adipocyte differentiation in

- 1288 human bone marrow mesenchymal stem cells through PPAR gamma transactivation.  
1289 *Biochem Pharmacol* **2009**, *77*, 125-133, doi:10.1016/j.bcp.2008.09.033.
- 1290 98. Chani, B.; Puri, V.; Chander Sobti, R.; Puri, S. Epigallocatechin Gallate Inhibits  
1291 Mouse Mesenchymal Stem Cell Differentiation to Adipogenic Lineage. *J Stem Cells*  
1292 *Regen Med* **2016**, *12*, 16-24.
- 1293 99. Hong, M.H.; Kim, M.H.; Chang, H.J.; Kim, N.H.; Shin, B.A.; Ahn, B.W.; Jung, Y.D.  
1294 (-)-Epigallocatechin-3-gallate inhibits monocyte chemotactic protein-1 expression in  
1295 endothelial cells via blocking NF-kappaB signaling. *Life Sci* **2007**, *80*, 1957-1965,  
1296 doi:10.1016/j.lfs.2007.02.024.
- 1297 100. Pasten, C.; Olave, N.C.; Zhou, L.; Tabengwa, E.M.; Wolkowicz, P.E.; Grenett, H.E.  
1298 Polyphenols downregulate PAI-1 gene expression in cultured human coronary artery  
1299 endothelial cells: molecular contributor to cardiovascular protection. *Thromb Res*  
1300 **2007**, *121*, 59-65, doi:10.1016/j.thromres.2007.02.001.
- 1301 101. Perez-de-Arce, K.; Foncea, R.; Leighton, F. Reactive oxygen species mediates  
1302 homocysteine-induced mitochondrial biogenesis in human endothelial cells:  
1303 modulation by antioxidants. *Biochem Biophys Res Commun* **2005**, *338*, 1103-1109,  
1304 doi:10.1016/j.bbrc.2005.10.053.
- 1305 102. Pullikotil, P.; Chen, H.; Muniyappa, R.; Greenberg, C.C.; Yang, S.; Reiter, C.E.; Lee,  
1306 J.W.; Chung, J.H.; Quon, M.J. Epigallocatechin gallate induces expression of heme  
1307 oxygenase-1 in endothelial cells via p38 MAPK and Nrf-2 that suppresses  
1308 proinflammatory actions of TNF-alpha. *J Nutr Biochem* **2012**, *23*, 1134-1145,  
1309 doi:10.1016/j.jnutbio.2011.06.007.
- 1310 103. Reiter, C.E.; Kim, J.A.; Quon, M.J. Green tea polyphenol epigallocatechin gallate  
1311 reduces endothelin-1 expression and secretion in vascular endothelial cells: roles for  
1312 AMP-activated protein kinase, Akt, and FOXO1. *Endocrinology* **2010**, *151*, 103-  
1313 114, doi:10.1210/en.2009-0997.

- 1314 104. Rodriguez, S.K.; Guo, W.; Liu, L.; Band, M.A.; Paulson, E.K.; Meydani, M. Green  
1315 tea catechin, epigallocatechin-3-gallate, inhibits vascular endothelial growth factor  
1316 angiogenic signaling by disrupting the formation of a receptor complex. *Int J Cancer*  
1317 **2006**, *118*, 1635-1644, doi:10.1002/ijc.21545.
- 1318 105. Wang, Z.M.; Gao, W.; Wang, H.; Zhao, D.; Nie, Z.L.; Shi, J.Q.; Zhao, S.; Lu, X.;  
1319 Wang, L.S.; Yang, Z.J. Green tea polyphenol epigallocatechin-3-gallate inhibits  
1320 TNF- $\alpha$ -induced production of monocyte chemoattractant protein-1 in human  
1321 umbilical vein endothelial cells. *Cell Physiol Biochem* **2014**, *33*, 1349-1358,  
1322 doi:10.1159/000358702.
- 1323 106. Yamagata, K.; Tanaka, N.; Suzuki, K. Epigallocatechin 3-gallate inhibits 7-  
1324 ketocholesterol-induced monocyte-endothelial cell adhesion. *Microvasc Res* **2013**,  
1325 *88*, 25-31, doi:10.1016/j.mvr.2013.03.006.
- 1326 107. Yamagata, K.; Xie, Y.; Suzuki, S.; Tagami, M. Epigallocatechin-3-gallate inhibits  
1327 VCAM-1 expression and apoptosis induction associated with LC3 expressions in  
1328 TNF $\alpha$ -stimulated human endothelial cells. *Phytomedicine : international journal*  
1329 *of phytotherapy and phytopharmacology* **2015**, *22*, 431-437,  
1330 doi:10.1016/j.phymed.2015.01.011.
- 1331 108. Yang, H.; Xiao, L.; Yuan, Y.; Luo, X.; Jiang, M.; Ni, J.; Wang, N. Procyanidin B2  
1332 inhibits NLRP3 inflammasome activation in human vascular endothelial cells.  
1333 *Biochem Pharmacol* **2014**, *92*, 599-606, doi:10.1016/j.bcp.2014.10.001.
- 1334 109. Yang, J.; Han, Y.; Chen, C.; Sun, H.; He, D.; Guo, J.; Jiang, B.; Zhou, L.; Zeng, C.  
1335 EGCG attenuates high glucose-induced endothelial cell inflammation by suppression  
1336 of PKC and NF-kappaB signaling in human umbilical vein endothelial cells. *Life Sci*  
1337 **2013**, *92*, 589-597, doi:10.1016/j.lfs.2013.01.025.
- 1338 110. Liu, Y.; Zhao, B.; Mao, G.; Fang, X.; Liu, Y.; Huang, Y.; Wang, N. Epigallocatechin-  
1339 3-O-gallate, a green tea polyphenol, induces expression of pim-1 kinase via



- 1340 PPARgamma in human vascular endothelial cells. *Cardiovascular toxicology* **2013**,  
1341 *13*, 391-395, doi:10.1007/s12012-013-9220-4.
- 1342 111. Schnorr, O.; Brossette, T.; Momma, T.Y.; Kleinbongard, P.; Keen, C.L.; Schroeter,  
1343 H.; Sies, H. Cocoa flavanols lower vascular arginase activity in human endothelial  
1344 cells in vitro and in erythrocytes in vivo. *Arch Biochem Biophys* **2008**, *476*, 211-215,  
1345 doi:10.1016/j.abb.2008.02.040.
- 1346 112. Oleaga, C.; Ciudad, C.J.; Izquierdo-Pulido, M.; Noe, V. Cocoa flavanol metabolites  
1347 activate HNF-3beta, Sp1, and NFY-mediated transcription of apolipoprotein AI in  
1348 human cells. *Mol Nutr Food Res* **2013**, *57*, 986-995, doi:10.1002/mnfr.201200507.
- 1349 113. Zhao, J.; Liu, J.; Pang, X.; Zhang, X.; Wang, S.; Wu, D. Epigallocatechin-3-gallate  
1350 inhibits angiotensin II-induced C-reactive protein generation through interfering with  
1351 the AT1-ROS-ERK1/2 signaling pathway in hepatocytes. *Naunyn-Schmiedeberg's*  
1352 *archives of pharmacology* **2016**, *389*, 1225-1234, doi:10.1007/s00210-016-1279-6.
- 1353 114. Chokor, R.; Lamy, S.; Annabi, B. Transcriptional targeting of sphingosine-1-  
1354 phosphate receptor S1P2 by epigallocatechin-3-gallate prevents sphingosine-1-  
1355 phosphate-mediated signaling in macrophage-differentiated HL-60  
1356 promyelomonocytic leukemia cells. *OncoTargets and therapy* **2014**, *7*, 667-677,  
1357 doi:10.2147/ott.s62717.
- 1358 115. Vezina, A.; Chokor, R.; Annabi, B. EGCG targeting efficacy of NF-kappaB  
1359 downstream gene products is dictated by the monocytic/macrophagic differentiation  
1360 status of promyelocytic leukemia cells. *Cancer immunology, immunotherapy : CII*  
1361 **2012**, *61*, 2321-2331, doi:10.1007/s00262-012-1301-x.
- 1362 116. Yen, G.C.; Duh, P.D.; Huang, D.W.; Hsu, C.L.; Fu, T.Y. Protective effect of pine  
1363 (*Pinus morrisonicola* Hay.) needle on LDL oxidation and its anti-inflammatory  
1364 action by modulation of iNOS and COX-2 expression in LPS-stimulated RAW 264.7  
1365 macrophages. *Food and chemical toxicology : an international journal published for*

- 1366 *the British Industrial Biological Research Association* **2008**, *46*, 175-185,  
1367 doi:10.1016/j.fct.2007.07.012.
- 1368 117. Marinovic, M.P.; Morandi, A.C.; Otton, R. Green tea catechins alone or in  
1369 combination alter functional parameters of human neutrophils via suppressing the  
1370 activation of TLR-4/NFkappaB p65 signal pathway. *Toxicology in vitro : an*  
1371 *international journal published in association with BIBRA* **2015**, *29*, 1766-1778,  
1372 doi:10.1016/j.tiv.2015.07.014.
- 1373 118. Liu, S.H.; Lu, T.H.; Su, C.C.; Lay, I.S.; Lin, H.Y.; Fang, K.M.; Ho, T.J.; Chen, K.L.;  
1374 Su, Y.C.; Chiang, W.C., et al. Lotus leaf (*Nelumbo nucifera*) and its active  
1375 constituents prevent inflammatory responses in macrophages via JNK/NF-kappaB  
1376 signaling pathway. *The American journal of Chinese medicine* **2014**, *42*, 869-889,  
1377 doi:10.1142/s0192415x14500554.
- 1378 119. Wang, Q.M.; Wang, H.; Li, Y.F.; Xie, Z.Y.; Ma, Y.; Yan, J.J.; Gao, Y.F.; Wang,  
1379 Z.M.; Wang, L.S. Inhibition of EMMPRIN and MMP-9 Expression by  
1380 Epigallocatechin-3-Gallate through 67-kDa Laminin Receptor in PMA-Induced  
1381 Macrophages. *Cellular physiology and biochemistry : international journal of*  
1382 *experimental cellular physiology, biochemistry, and pharmacology* **2016**, *39*, 2308-  
1383 2319, doi:10.1159/000447923.
- 1384 120. Kumazoe, M.; Yamashita, M.; Nakamura, Y.; Takamatsu, K.; Bae, J.; Yamashita, S.;  
1385 Yamada, S.; Onda, H.; Nojiri, T.; Kangawa, K., et al. Green Tea Polyphenol EGCG  
1386 Upregulates Tollip Expression by Suppressing Elf-1 Expression. *J Immunol* **2017**,  
1387 *199*, 3261-3269, doi:10.4049/jimmunol.1601822.
- 1388 121. Kumazoe, M.; Nakamura, Y.; Yamashita, M.; Suzuki, T.; Takamatsu, K.; Huang, Y.;  
1389 Bae, J.; Yamashita, S.; Murata, M.; Yamada, S., et al. Green Tea Polyphenol  
1390 Epigallocatechin-3-gallate Suppresses Toll-like Receptor 4 Expression via Up-

- 1391 regulation of E3 Ubiquitin-protein Ligase RNF216. *The Journal of biological*  
1392 *chemistry* **2017**, *292*, 4077-4088, doi:10.1074/jbc.M116.755959.
- 1393 122. Li, Y.F.; Wang, H.; Fan, Y.; Shi, H.J.; Wang, Q.M.; Chen, B.R.; Khurwolah, M.R.;  
1394 Long, Q.Q.; Wang, S.B.; Wang, Z.M., et al. Epigallocatechin-3-Gallate Inhibits  
1395 Matrix Metalloproteinase-9 and Monocyte Chemotactic Protein-1 Expression  
1396 Through the  $\alpha_6\beta_4$  Laminin Receptor and the TLR4/MAPK/NF- $\kappa$ B  
1397 Signalling Pathway in Lipopolysaccharide-Induced Macrophages. *Cellular*  
1398 *physiology and biochemistry : international journal of experimental cellular*  
1399 *physiology, biochemistry, and pharmacology* **2017**, *43*, 926-936,  
1400 doi:10.1159/000481643.
- 1401 123. Cheng, X.W.; Kuzuya, M.; Sasaki, T.; Kanda, S.; Tamaya-Mori, N.; Koike, T.;  
1402 Maeda, K.; Nishitani, E.; Iguchi, A. Green tea catechins inhibit neointimal  
1403 hyperplasia in a rat carotid arterial injury model by TIMP-2 overexpression.  
1404 *Cardiovascular research* **2004**, *62*, 594-602, doi:10.1016/j.cardiores.2004.01.023.
- 1405 124. Hwang, K.C.; Lee, K.H.; Jang, Y.; Yun, Y.P.; Chung, K.H. Epigallocatechin-3-  
1406 gallate inhibits basic fibroblast growth factor-induced intracellular signaling  
1407 transduction pathway in rat aortic smooth muscle cells. *Journal of cardiovascular*  
1408 *pharmacology* **2002**, *39*, 271-277, doi:10.1097/00005344-200202000-00014.
- 1409 125. Peng, N.; Liu, J.T.; Guo, F.; Li, R. Epigallocatechin-3-gallate inhibits interleukin-6-  
1410 and angiotensin II-induced production of C-reactive protein in vascular smooth  
1411 muscle cells. *Life Sci* **2010**, *86*, 410-415, doi:10.1016/j.lfs.2010.01.010.
- 1412 126. Wang, C.J.; Liu, J.T.; Guo, F. (-)-epigallocatechin gallate inhibits endothelin-1-  
1413 induced C-reactive protein production in vascular smooth muscle cells. *Basic Clin*  
1414 *Pharmacol Toxicol* **2010**, *107*, 669-675, doi:10.1111/j.1742-7843.2010.00557.x.

- 1415 127. Lu, L.H.; Lee, S.S.; Huang, H.C. Epigallocatechin suppression of proliferation of  
1416 vascular smooth muscle cells: correlation with c-jun and JNK. *British journal of*  
1417 *pharmacology* **1998**, *124*, 1227-1237, doi:10.1038/sj.bjp.0701912.
- 1418 128. Erlejman, A.G.; Jagers, G.; Fraga, C.G.; Oteiza, P.I. TNFalpha-induced NF-kappaB  
1419 activation and cell oxidant production are modulated by hexameric procyanidins in  
1420 Caco-2 cells. *Arch Biochem Biophys* **2008**, *476*, 186-195,  
1421 doi:10.1016/j.abb.2008.01.024.
- 1422 129. Quesada, H.; Pajuelo, D.; Fernandez-Iglesias, A.; Diaz, S.; Ardevol, A.; Blay, M.;  
1423 Salvado, M.J.; Arola, L.; Blade, C. Proanthocyanidins modulate triglyceride  
1424 secretion by repressing the expression of long chain acyl-CoA synthetases in Caco2  
1425 intestinal cells. *Food Chemistry* **2011**, *129*, 1490-1494,  
1426 doi:10.1016/j.foodchem.2011.05.125.
- 1427 130. Gonzalez-Abuin, N.; Martinez-Micaelo, N.; Blay, M.; Pujadas, G.; Garcia-Vallve,  
1428 S.; Pinent, M.; Ardevol, A. Grape seed-derived procyanidins decrease dipeptidyl-  
1429 peptidase 4 activity and expression. *J Agric Food Chem* **2012**, *60*, 9055-9061,  
1430 doi:10.1021/jf3010349.
- 1431 131. Heidker, R.M.; Caiozzi, G.C.; Ricketts, M.L. Dietary procyanidins selectively  
1432 modulate intestinal farnesoid X receptor-regulated gene expression to alter  
1433 enterohepatic bile acid recirculation: elucidation of a novel mechanism to reduce  
1434 triglyceridemia. *Mol Nutr Food Res* **2016**, *60*, 727-736,  
1435 doi:10.1002/mnfr.201500795.
- 1436
- 1437

1438  
1439**Table 1.** Overview of data extraction for cell models exposed to different flavanols or flavanol metabolites at physiological concentrations.

Flavanol tested	Concentration	Challenge	Differentially expressed genes; p<0.05	Reference
<i>Adipocytes</i>				
EGCG	10 $\mu$ M	adipogenic cocktail	<i>CEBPA, PPARG</i>	[94]
Epicatechin	0.5 – 10 $\mu$ M	TNF	<i>IL6, CCL2, RETN, TNF</i>	[95]
EGCG	1 – 5 $\mu$ M	dexamethasone	<i>ADIPOQ, RETN</i>	[96]
Catechin	10 $\mu$ M	adipogenic cocktail	<i>ADIPOQ, FABP4, LPL, PPARG</i>	[97]
EGCG	1 $\mu$ M	adipogenic cocktail	<i>CFD</i>	[98]
<i>Endothelial cells</i>				
EGCG	10 $\mu$ M	phorbol-12-myristate-13-acetate	<i>CCL2</i>	[99]
Catechin	0.1 – 10 $\mu$ M	no challenge	<i>SERPINE1</i>	[100]
Catechin	10 $\mu$ M	homocysteine	<i>NRF1, TFAM, MT-CO3</i>	[101]
EGCG	2.5 – 10 $\mu$ M	no challenge	<i>EDN1, HMOX1</i>	[102]
EGCG	10 $\mu$ M	no challenge	<i>EDN1</i>	[103]
EGCG	0.5 – 10 $\mu$ M	vascular endothelial growth factor	<i>CXCL8</i>	[104]
EGCG	10 $\mu$ M	TNF	<i>CCL2</i>	[105]
EGCG	10 $\mu$ M	no challenge	<i>ICAM1, CCL2</i>	[106]
EGCG	10 $\mu$ M	TNF	<i>ICAM1, VCAM1, CCL2, BCL2, BAX, CASP9</i>	[107]
Procyanidin B2	1 – 2 $\mu$ M	LPS and ATP	<i>NLRP3</i>	[108]
EGCG	10 $\mu$ M	glucose	<i>VCAM1</i>	[109]
EGCG	10 $\mu$ M	no challenge	<i>PIM1</i>	[110]
Epicatechin, Flavanol metabolites	1 – 10 $\mu$ M 0.4 – 7.8 $\mu$ M	no challenge	<i>ARG2</i>	[111]

Flavanol metabolites	1 μM	TNF	<i>CALD1, TJP1, ARHGEF7, CASK, NFKB1, SELE, CCL2, ITGB1, ROCK1</i>	[22]
<b><i>Hepatocytes</i></b>				
Epicatechin, Catechin, Procyanidin B2	0.1 – 10 μM	no challenge	<i>APOA1, APOB, LDLR, ABCA1, SREBF1, SCARB1, SCAP</i>	[52]
Epicatechin, Flavanol metabolites	10 μM	no challenge	<i>APOA1, FOXA2</i>	[112]
EGCG	1 – 10 μM	angiotensin II	<i>AGTR1, PPARG</i>	[113]
<b><i>Immune cells</i></b>				
EGCG	3 – 10 μM	phorbol-12-myristate-13-acetate	<i>S1PR2</i>	[114]
EGCG	3 μM	phorbol-12-myristate-13-acetate	<i>MMP9, PTGS2</i>	[115]
Epicatechin	2 μg/mL	LPS	<i>NOS2, PTGS2</i>	[116]
Epicatechin gallate	3 μM	no challenge	<i>ITGAM</i>	[117]
Catechin	10 μM	LPS	<i>IL6, TNF</i>	[118]
EGCG	10 μM	phorbol-12-myristate-13-acetate	<i>MMP9, BSG</i>	[119]
EGCG	2.5 μM	no challenge	<i>TOLLIP</i>	[120]
EGCG, (-)-epigallocatechin-3-O-(3-O-methyl)-gallate	5 μM 1 μM	no challenge; palmitic acid	<i>RNF216, TNF</i>	[121]
EGCG	1 μM	LPS	<i>MMP9, CCL2</i>	[122]
EGCG	1 μM	LPS; no challenge	<i>TNF, IL6, TLR4, TOLLIP</i>	[53]
<b><i>Smooth muscle cells</i></b>				
EGCG	0.1 – 10 μM	no challenge	<i>TIMP2</i>	[123]

EGCG	10 $\mu$ M	basic fibroblast growth factor	<i>JUN</i>	[124]
EGCG	3 – 10 $\mu$ M	IL-6;	<i>CRP</i>	[125]
EGCG	1 – 10 $\mu$ M	angiotensin II	<i>CRP</i>	[126]
EGCG	3 – 10 $\mu$ M	endothelin 1	<i>CRP</i>	[126]
Epigallocatechin	10 $\mu$ M	serum	<i>JUN</i>	[127]
<b><i>Intestinal cells</i></b>				
Hexameric procyanidins	20 $\mu$ M	TNF	<i>NOS2</i>	[128]
Grape seed extract	100 mg/L 25 – 100 mg/L	fasted state medium; postprandial state medium	<i>ACSL5, ACSL3, FABP2, PPARA, CPT1A</i>	[129]
Cinnamtannin A2	1 – 10 $\mu$ M	no challenge	<i>APOA1, APOB</i>	[52]
Grape seed extract	100 mg/L	no challenge	<i>DPP4</i>	[130]
Grape seed extract	20 – 100 mg/L	chenodeoxycholic acid	<i>SLC10A2, FABP6, FGF19, SLC51A, SLC51B</i>	[131]

1441  
1442**Table 2.** Proteins with the highest number of interactions within the network ( $\geq 15$ ).

<b>Symbol</b>	<b>Name</b>	<b>Number of interactions</b>
TNF	Tumor necrosis factor	40
IL6	Interleukin-6	39
JUN	Transcription factor AP-1	37
TLR4	Toll-like receptor 4	30
NFKB1	Nuclear factor NF-kappa-B p105 subunit	30
MAPK8	Mitogen-activated protein kinase 8	30
IL8	Interleukin-8	26
CCL2	C-C motif chemokine 2	24
MMP9	Matrix metalloproteinase-9	23
PPARG	Peroxisome proliferator-activated receptor gamma	22
BCL2	Apoptosis regulator Bcl-2	22
MMP2	72 kDa type IV collagenase	21
CYCS	Cytochrome c	21
FOS	Proto-oncogene c-Fos	21
ICAM1	Intercellular adhesion molecule 1	20
CRP	C-reactive protein	19
PTGS2	Prostaglandin G/H synthase 2	19
ADIPOQ	Adiponectin	19
CASP3	Caspase-3	18
NOS3	Nitric oxide synthase, endothelial	17
BCL2L1	Bcl-2-like protein 1	17
MYD88	Myeloid differentiation primary response protein MyD88	16
XIAP	E3 ubiquitin-protein ligase XIAP	16
VCAM1	Vascular cell adhesion protein 1	16
BAX	Apoptosis regulator BAX	15
EDN1	Endothelin-1	15
ITGAM	Integrin alpha-M	15

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1449 Table 3: Top 20 transcription factors and miRNAs that regulate the protein-protein  
 1450 interaction network in adipocytes, hepatocytes, immune, smooth muscle and endothelial cells  
 1451 exposed to flavanols.

Symbol	Name	Number of hits
<b><i>Transcription factor</i></b>		
FOXC1	Forkhead box protein C1	362
GATA2	Endothelial transcription factor GATA-2	266
YY1	Transcriptional repressor protein YY1	186
E2F1	Transcription factor E2F1	160
FOXL1	Forkhead box protein L1	149
USF2	Upstream stimulatory factor 2	141
RELA	Transcription factor p65	138
PPARG	Peroxisome proliferator-activated receptor gamma	137
NFKB1	Nuclear factor NF-kappa-B p105 subunit	136
CREB1	Cyclic AMP-responsive element-binding protein 1	134
TFAP2A	Transcription factor AP-2-alpha	131
TP53	Cellular tumor antigen p53	127
NFIC	Nuclear factor 1 C-type	123
POU2F2	POU domain, class 2, transcription factor 2	115
SRF	Serum response factor	115
HINFP	Histone H4 transcription factor	114
JUN	Transcription factor AP-1	113
SREBF1	Sterol regulatory element-binding protein 1	106
STAT3	Signal transducer and activator of transcription 3	106
MEF2A	Myocyte-specific enhancer factor 2A	92
<b><i>micro RNA</i></b>		
mir-335-5p	microRNA-335-5p	105
mir-16-5p	microRNA-16-5p	83
mir-124-3p	microRNA-124-3p	80
mir-26b-5p	microRNA-26b-5p	79
mir-17-5p	microRNA-17-5p	77
let-7b-5p	let-7b-5p	74
mir-155-5p	microRNA-155-5p	70
mir-92a-3p	microRNA-92a-3p	70
mir-93-5p	microRNA-93-5p	66
mir-20a-5p	microRNA-20a-5p	64
mir-106b-5p	microRNA-106b-5p	61
mir-1-3p	microRNA-1-3p	53
let-7c-5p	let-7c-5p	52

mir-193b-3p	microRNA-193b-3p	51
mir-20b-5p	microRNA-20b-5p	51
mir-34a-5p	microRNA-34a-5p	51
mir-615-3p	microRNA-615-3p	50
mir-218-5p	microRNA-218-5p	49
mir-519d-3p	microRNA-519d-3p	49
mir-21-5p	microRNA-21-5p	48

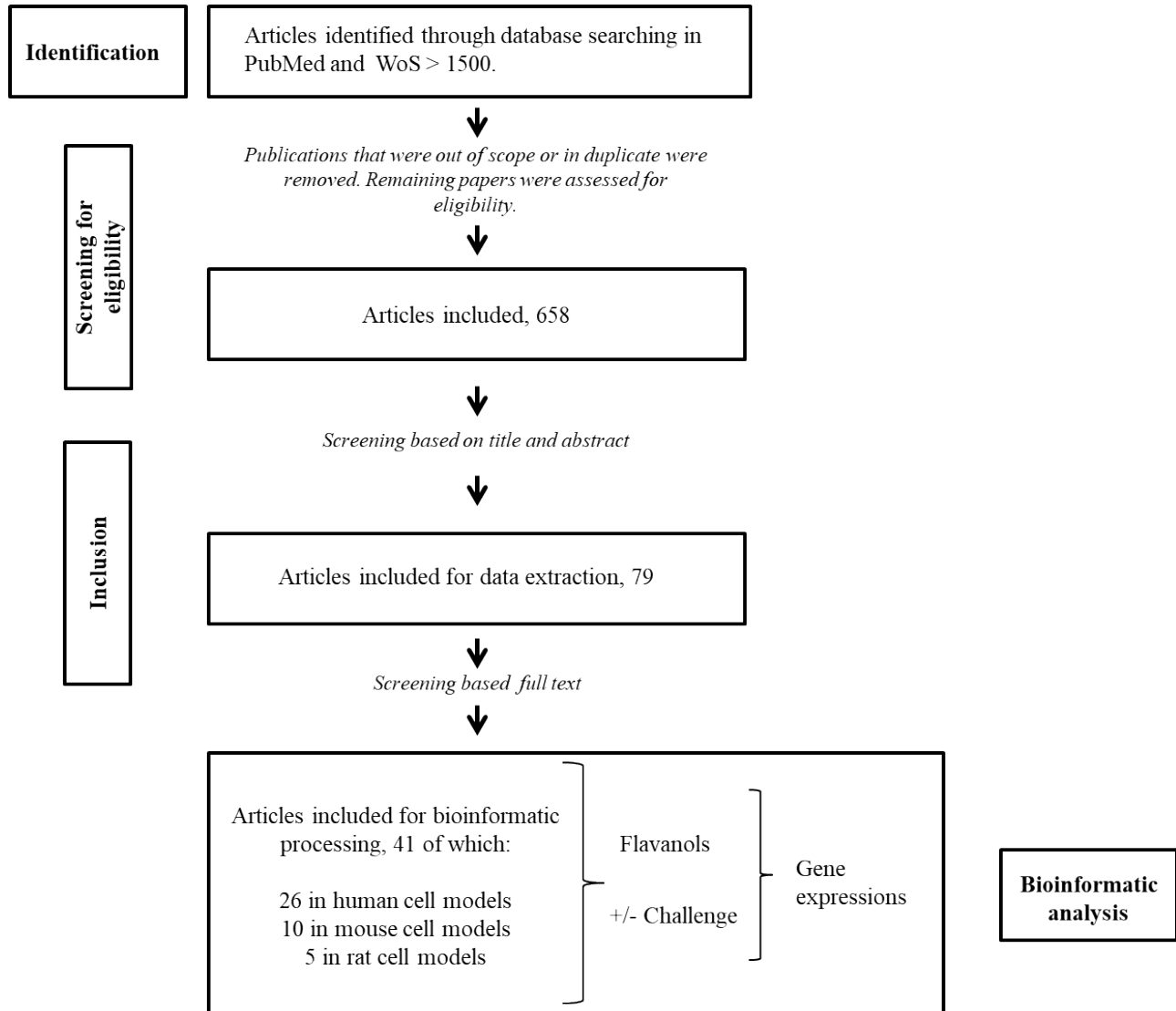
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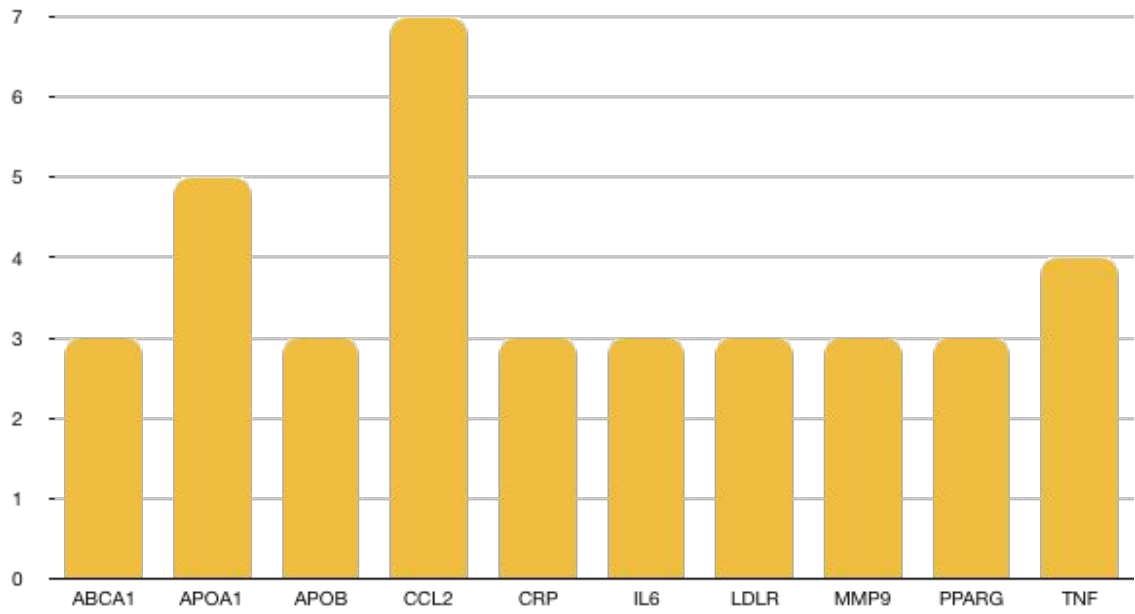
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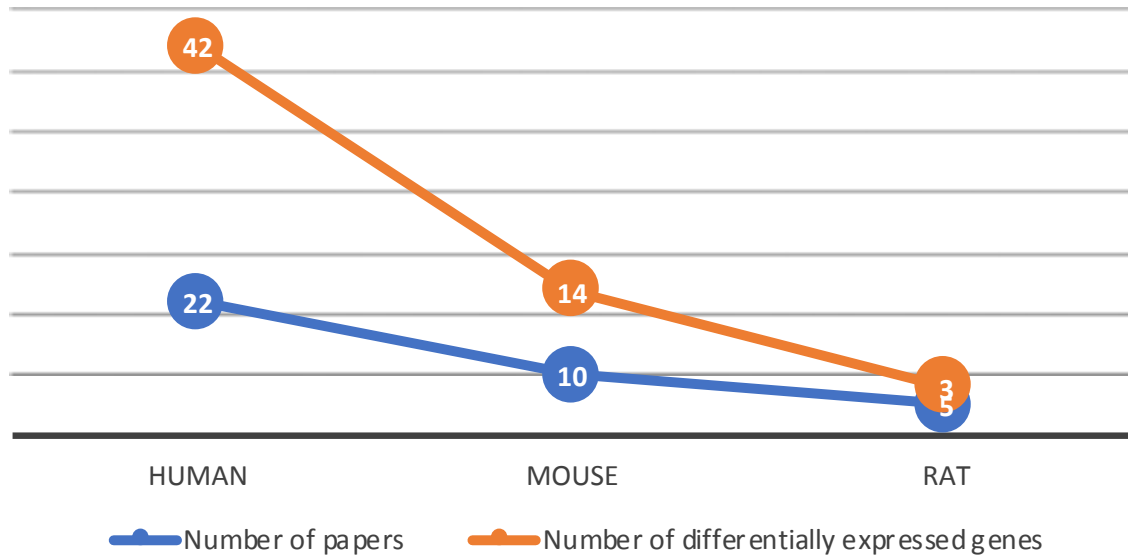
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**Figure 1. Data collection flowchart.** For search criteria, see Methods section.



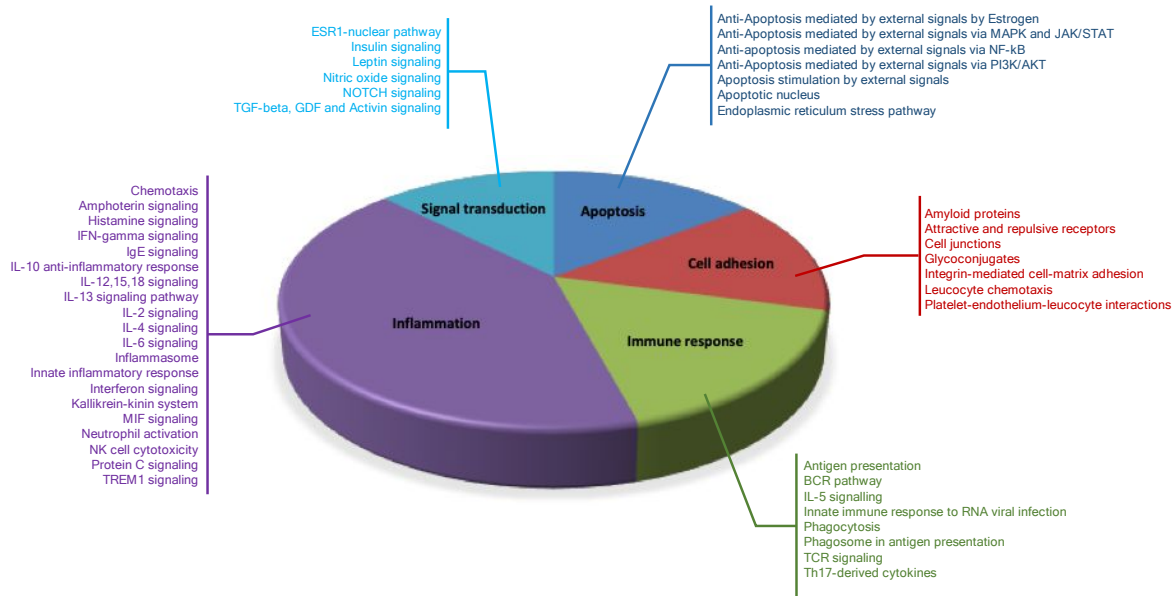
**Figure 2A.** Number of genes repeated in studies conducted on adipocytes, hepatocytes, immune, smooth muscle and endothelial cells exposed to flavanols.



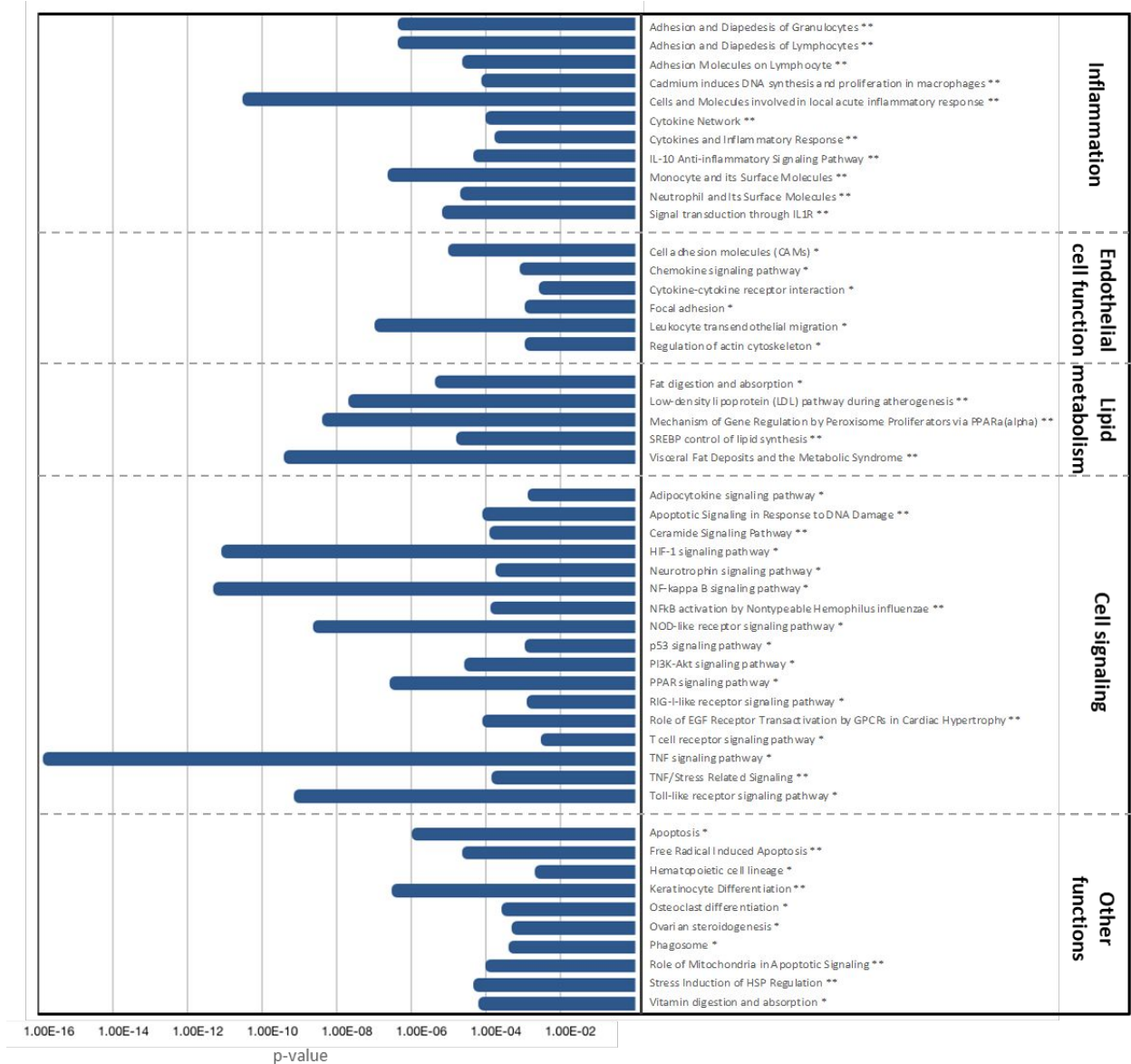
**Figure 2B.** Number of differentially expressed genes extracted from the studies on adipocytes, hepatocytes, immune, smooth muscle and endothelial cells exposed to flavanols.



**Figure 3.** Gene ontology for adipocytes, hepatocytes, immune, smooth muscle and endothelial cells exposed to flavanols. Each rectangle is a single cluster representative, and they are joined into ‘superclusters’ of related terms, represented with different colors. Size of the rectangles reflects the p-value of the GO.



**Figure 4.** Gene network pie chart for adipocytes, hepatocytes, immune, smooth muscle and endothelial cells exposed to flavanols.

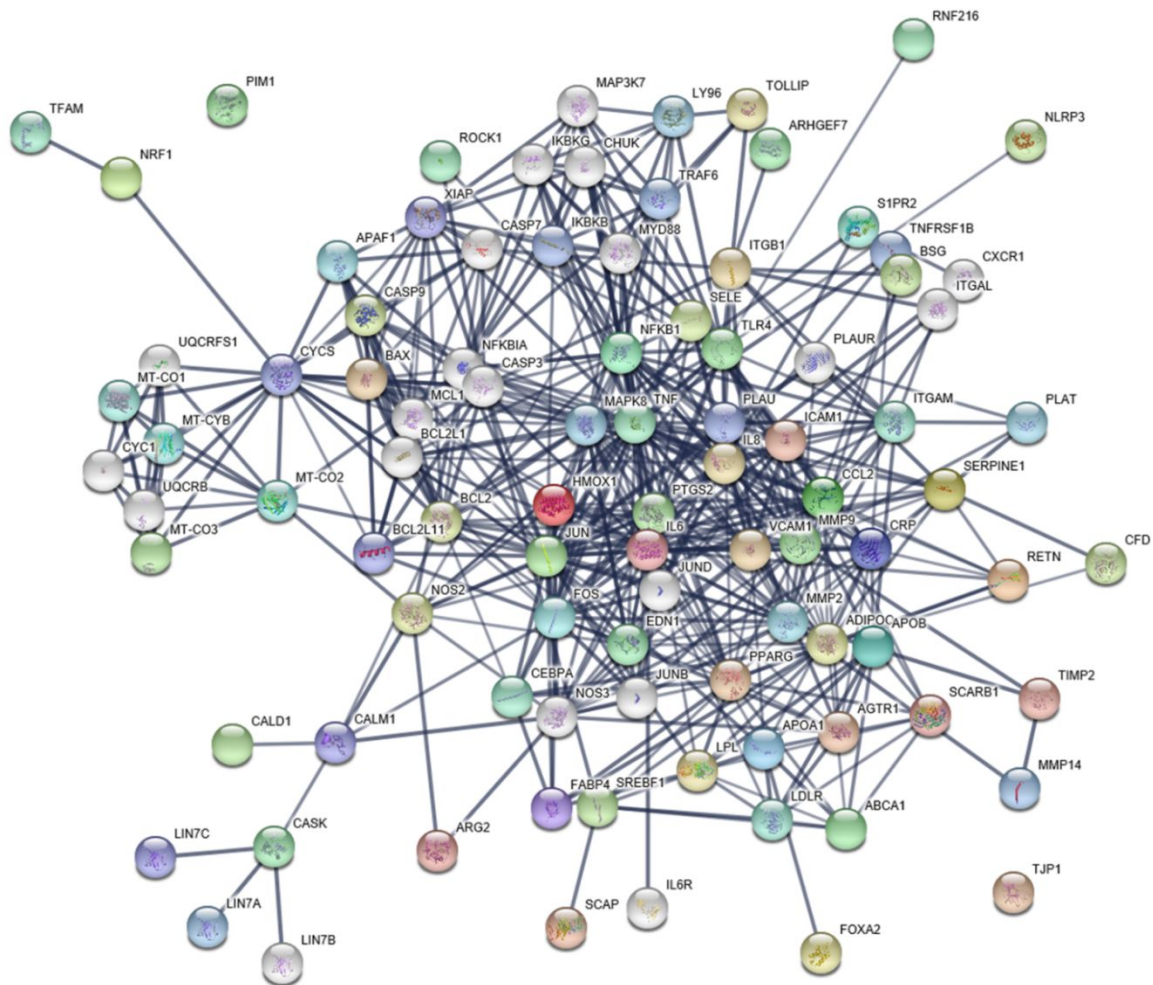


**Figure 5.** BioCarta and KEGG pathways related to cellular processes in adipocytes, hepatocytes, immune, smooth muscle and endothelial cells exposed to flavanols. \*: KEGG; \*\*: BioCarta.

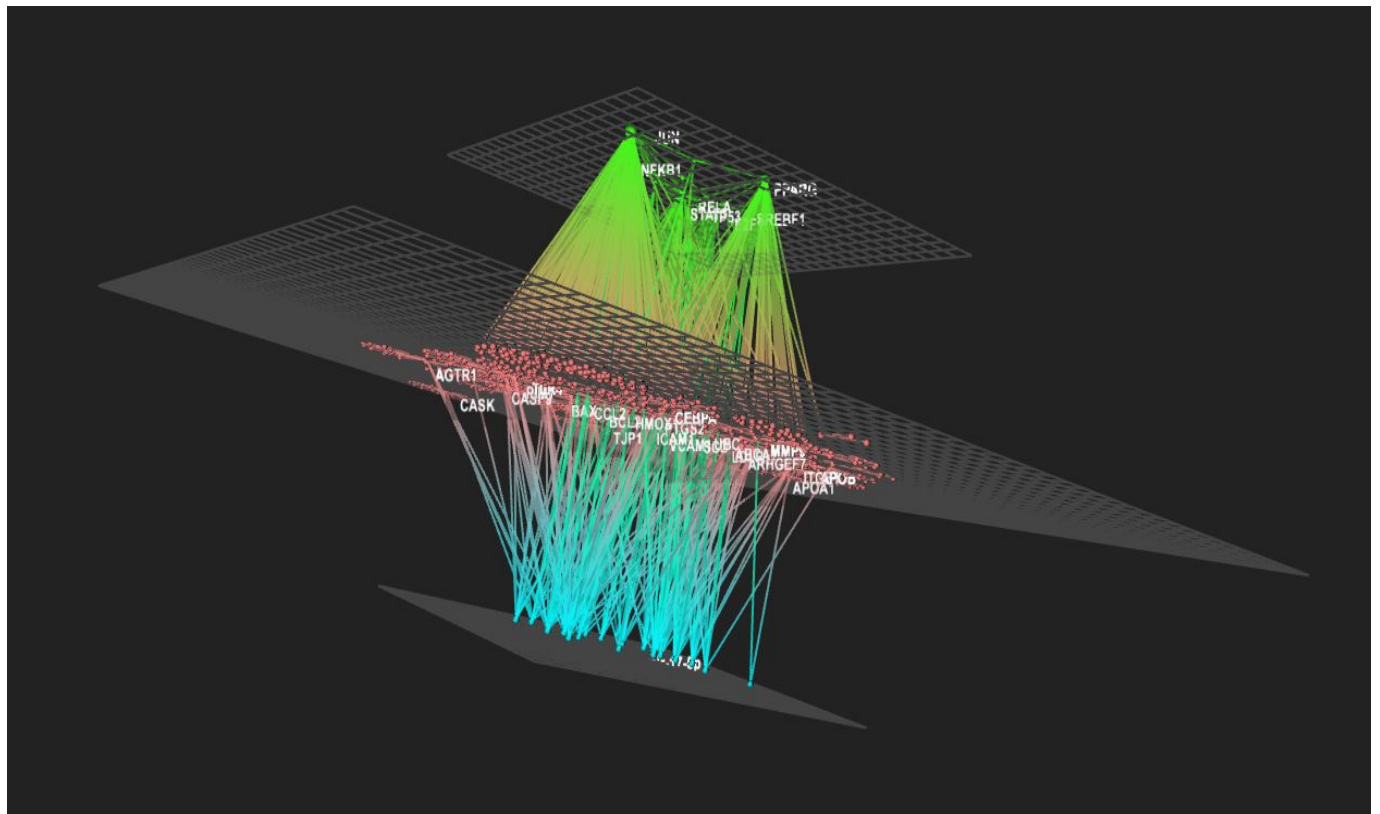




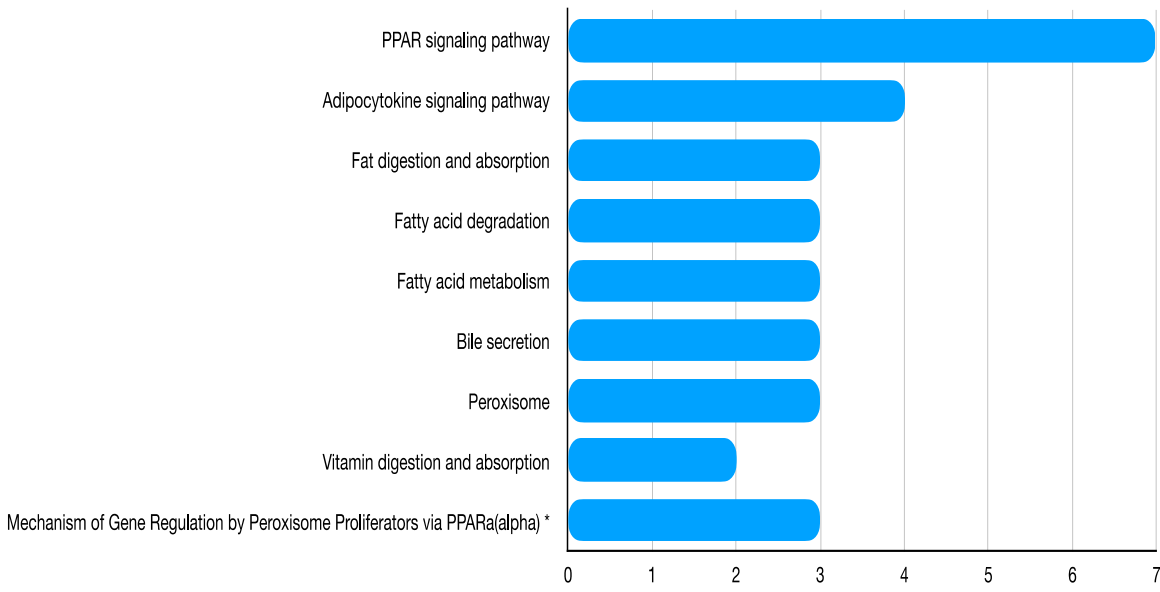
**Figure 6.** Functional enrichment and interactome meta-analysis based on gene lists for different cell types exposed to flavanols. Enrichment network visualization of the results from the lists of genes identified for adipocytes, smooth muscle cells, immune cells, endothelial cells and hepatocytes. Nodes are functional groups represented by pie charts indicating their associations with each cell type. Cluster labels were added manually. Color code represents the identities of gene lists (adipocytes: red, endothelial cells: blue, hepatocytes: green, immune cells: violet) and size of each color is proportional to the percentage of the genes from different types of cells.



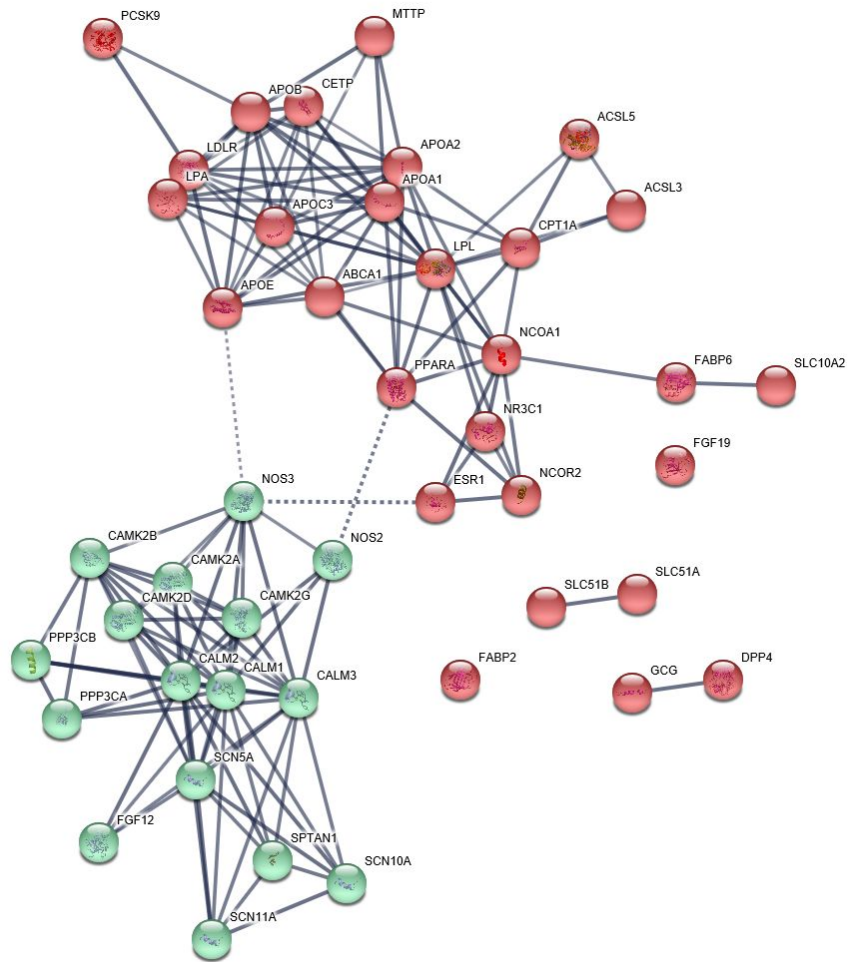
**Figure 7.** Protein-protein interactions in adipocytes, hepatocytes, immune, smooth muscle and endothelial cells exposed to flavanols. Colored nodes: query proteins and first shell of interactors; white nodes: second shell of interactors; filled nodes: some 3D structure is known or predicted; empty nodes: proteins of unknown 3D structure.



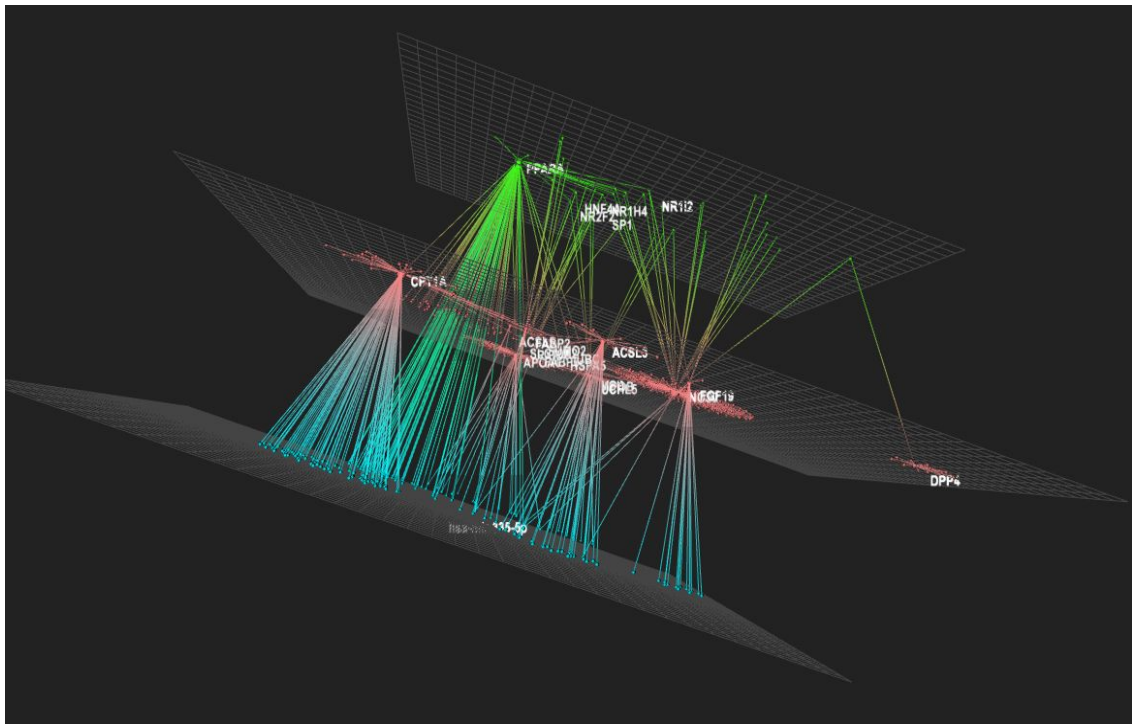
**Figure 8.** Regulation of protein-protein interaction network by transcription factors and miRNAs in adipocytes, hepatocytes, immune, smooth muscle and endothelial cells exposed to flavanols.



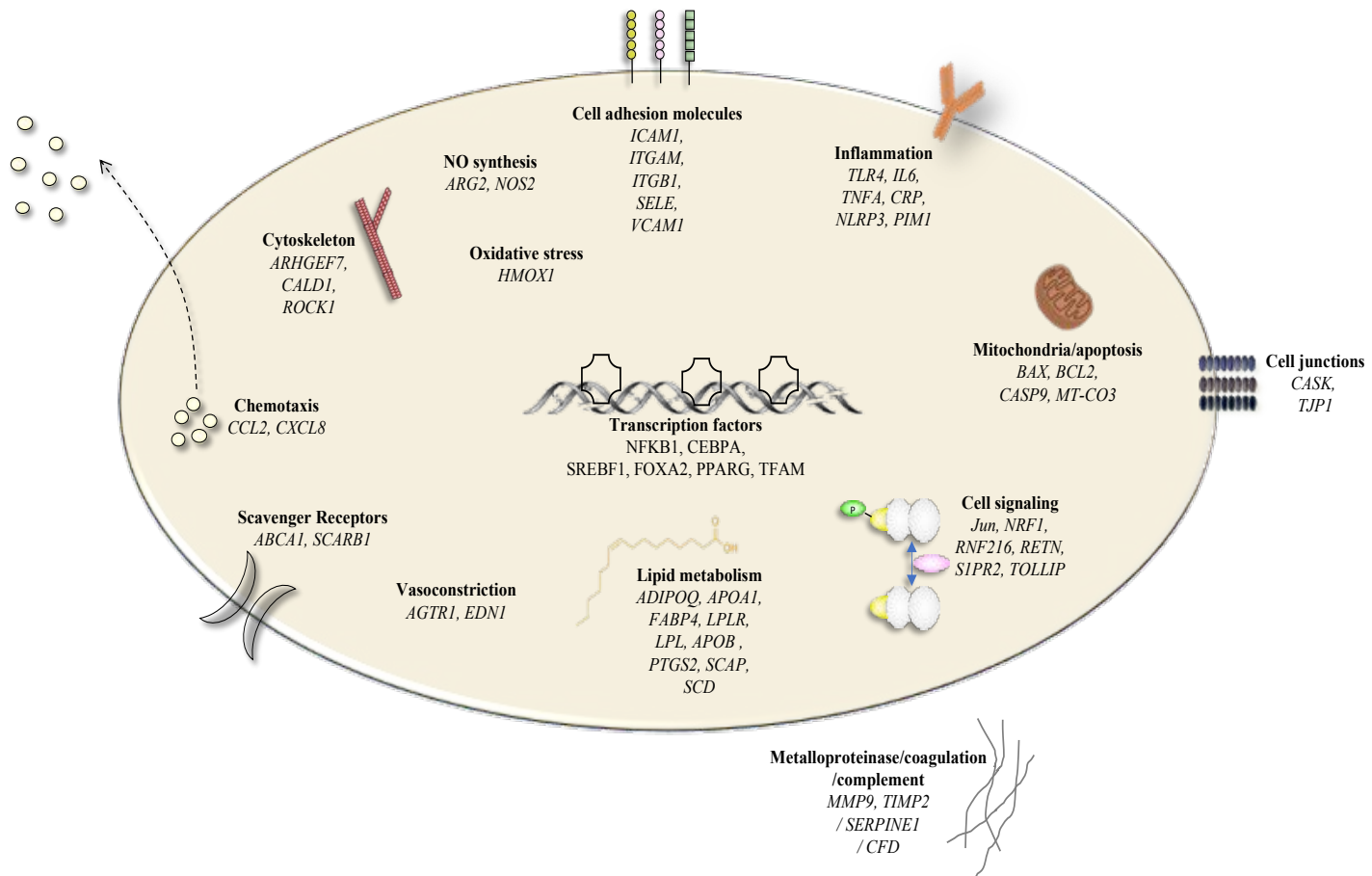
**Figure 9A.** KEGG and BioCarta (marked with \*) pathways for the intestinal cells exposed to flavanols.



**Figure 9B.** Protein-protein interactions for the intestinal cells exposed to flavanols. Protein network is organized in two clusters: in red – proteins that are mostly involved in the metabolism of circulating lipoproteins; in green – proteins that are mainly involved in calcium signaling.



**Figure 9C.** Regulation of protein-protein interaction network by transcription factors and miRNAs in the intestinal cells exposed to flavanols.



**Figure 10.** Summary of identified differentially expressed genes modulated by flavanol and related to cardiometabolic health.



**Table S2: Proteins with the highest number of interactions within the network ( $\geq 7$ ), for the intestinal cells.**

Gene symbol	Name	Number of interactions
<i>LPL</i>	Lipoprotein lipase	16
<i>APOA1</i>	Apolipoprotein A-I	13
<i>APOA2</i>	Apolipoprotein A-II	12
<i>APOB</i>	Apolipoprotein B-100	11
<i>APOE</i>	Apolipoprotein E	10
<i>APOC3</i>	Apolipoprotein C-III	10
<i>NCOA1</i>	Nuclear receptor coactivator 1	10
<i>ABCA1</i>	ATP-binding cassette sub-family A member 1	9
<i>CETP</i>	Cholesteryl ester transfer protein	9
<i>LDLR</i>	Low-density lipoprotein receptor	9
<i>PPARA</i>	Peroxisome proliferator-activated receptor alpha	9
<i>LPA</i>	Apolipoprotein(a)	8
<i>CPT1A</i>	Carnitine O-palmitoyltransferase 1, liver isoform	7
<i>CALM3</i>	Calmodulin-3	15
<i>CALM1</i>	Calmodulin-1	15
<i>CALM2</i>	Calmodulin-2	15
<i>SCN5A</i>	Sodium channel protein type 5 subunit alpha	11
<i>CAMK2B</i>	Calcium/calmodulin-dependent protein kinase type II subunit beta	10
<i>NOS3</i>	Nitric oxide synthase, endothelial	10
<i>CAMK2G</i>	Calcium/calmodulin-dependent protein kinase type II subunit gamma	8



<i>CAMK2A</i>	Calcium/calmodulin-dependent protein kinase type II subunit alpha	8
<i>CAMK2D</i>	Calcium/calmodulin-dependent protein kinase type II subunit delta	8