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REVIEW

Addressing the inter-individual variation in response to consumption of plant food bioactives: Towards a better understanding of their role in healthy aging and cardiometabolic risk reduction

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Bioactive compounds in plant-based foods have health properties that contribute to the prevention of age-related chronic diseases, particularly cardiometabolic disorders. Conclusive proof and understanding of these benefits in humans is essential in order to provide effective dietary recommendations but, so far, the evidence obtained from human intervention trials is limited and contradictory. This is partly due to differences between individuals in the absorption, distribution, metabolism and excretion of bioactive compounds, as well as to heterogeneity in their biological response regarding cardiometabolic health outcomes. Identifying the main factors underlying inter-individual differences, as well as developing new and innovative methodologies to account for such variability constitute an overarching goal to ultimately optimize the beneficial health effects of plant food bioactives for each and every one of us. In this respect, this position paper from the COST Action FA1403-POSITIVE examines the main factors likely to affect the individual responses to consumption of plant food bioactives and presents perspectives for assessment and consideration of inter-individual variability.

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1 Introduction

Cardiometabolic disease, which comprises cardiovascular diseases, type 2 diabetes mellitus and their associated risk factors including metabolic syndrome and obesity, is the

world's leading cause of death and a major public health concern. Observational studies provide convincing evidence that most cases of cardiometabolic disease could be avoided through dietary and lifestyle changes [1,2]. It is therefore necessary to establish effective preventive strategies targeting physical activity, smoking patterns, and dietary behaviours of

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Colour Online: See the article online to view Fig. 1 in colour.

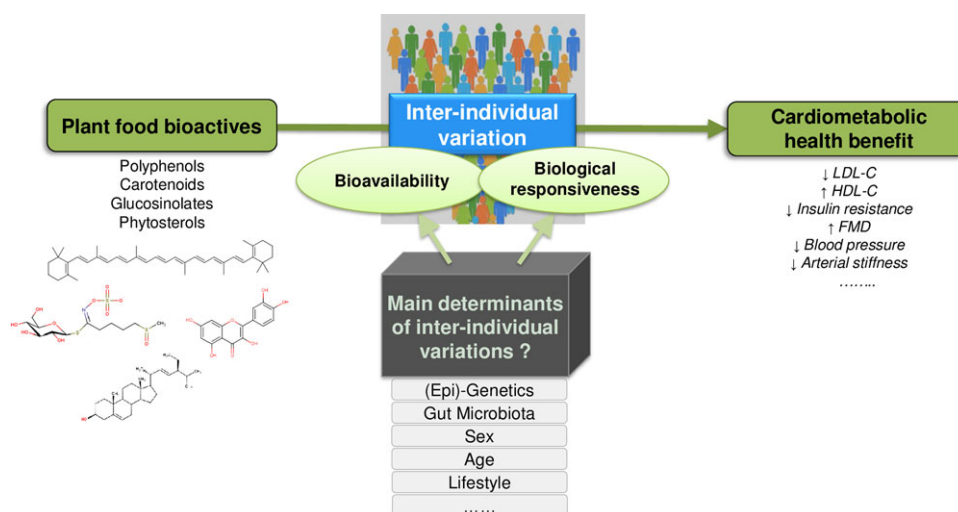


Figure 1. Potential factors responsible for inter-individual variability in bioavailability and biological responsiveness to consumption of plant food bioactives in relation to cardiometabolic health.

populations and to drive the food industry to produce healthier foods.

A high intake of plant-based foods has shown beneficial effects on cardiometabolic health, both in large population studies [3–6] and randomized controlled trials [7–9]. Plant-based foods typically have a low caloric density and provide a range of beneficial nutrients and low-energetic constituents, such as dietary fibres, essential vitamins and minerals, and are exclusive and rich sources of a wide variety of phytochemicals, including polyphenols, carotenoids, plant sterols, and glucosinolates. Although not strictly essential micronutrients, some phytochemicals have been described as “lifespan essential”, as they promote optimal health during ageing [10]. There is increasing evidence from cohort studies and randomized controlled trials that many of these bioactive compounds may help to reduce the risk of chronic diseases, particularly of cardiometabolic diseases [11–13]. Plant food bioactives are widely distributed in the diet but specific groups of compounds can be particularly abundant in some foods for which specific dietary recommendations may be developed: e.g. glucosinolates in cruciferous vegetables, isoflavones in soya, anthocyanins in berries or lycopene in tomatoes. Specific physiological effects with implications for health have been attributed to different bioactive compounds. For example, polyphenol-rich foods and beverages can improve endothelial function, platelet function, insulin sensitivity and decrease blood pressure [14–16]. In 2012, the European Food Safety Authority (EFSA) panel on Dietetic Products, Nutrition and Allergies has approved a health claim related to cocoa flavanols and maintenance of normal endothelium-dependent vasodilation, according to regulation (EC) No 1924/2006 [17]. Despite intense and active research investigating the health effects of plant food bioactives over the last years, the real impact of most compounds and their mechanisms of action have not yet been fully elucidated. Plant food bioactives have been shown to exert antioxidant, anti-inflammatory, anti-atherogenic activities through a large panel of mechanisms,

including modulation of enzyme activities, binding to cell receptors and interfering with signalling pathways and transcription factors ultimately regulating gene expression, altering DNA methylation or affecting the gut microbial population and functionality [18–21].

An increasing number of studies investigating the health effects of plant food bioactives have revealed substantial between-subject variations in response to dietary interventions, suggesting that the consumption of particular foods or bioactive constituents may benefit some individuals more than others. The major determinants responsible for the between-subject variability may include genetic and non-genetic factors which are only beginning to be explored and may differ depending on the compounds (Fig. 1). The aim of this position paper is to highlight the complexity of this issue and the need of increasing knowledge in the field, both to better understand the contribution of phytochemicals in the health effects of plant-based foods and to develop effective strategies and refined dietary recommendations to optimize the beneficial effects of bioactive-containing foods for all population groups.

1.1 Inter-individual variation in metabolism and bioavailability of plant food bioactives

After decades of active research, much about the absorption, distribution, metabolism, and excretion (ADME) patterns of the main plant food bioactives is now known and has been reviewed in humans [22–24]. However, key information on many of the enzymes, carrier proteins or bacterial strains critical for the absorption and metabolism of these bioactive compounds and the factors that may modulate their activities is still lacking. Some plant bioactives can be absorbed in their native form from the stomach or gut, but most of the glycosylated, polymeric, or esterified native plant compounds have to be hydrolyzed before absorption, a step partly

carried out by the gut microbiota [25]. The specific bacterial strains responsible for this initial hydrolysis have not been extensively studied yet. For the intestinal uptake of the bioactive compounds both passive and active mechanisms have been described, as well as efflux transporters that lower the absolute influx of bioactives through the intestinal absorption. Nevertheless, our knowledge of the carriers involved for the various compounds is still very sparse. When absorbed, hydrophilic plant food bioactives typically undergo first pass metabolism in the intestine and in the liver with phase I (oxidation/reduction reactions) and mainly phase II (β -glucuronidation, sulfation, methylation, glutathione conjugation) biotransformations [22]. A substantial enterohepatic recirculation exists for some metabolites. Lipophilic bioactive compounds may be absorbed through the lymphatic system and thereby escape the first pass metabolism by the liver. For a number of plant food bioactives, the gut microbiota in addition to the intestine and liver handles the production of a wide range of metabolites, which can be more or less specific of their precursors [26]. The gut microbiota composition, specific bacteria strains and the functionalities responsible for all these metabolic biotransformations, as well as their occurrence and distribution in humans have not been extensively investigated. Further research in this specific area will be essential for a better understanding of the inter-individual variation.

In human intervention studies that target the bioavailability of diverse plant food bioactives or in large-scale observational studies, researchers consistently observed that the plasma concentrations and urinary excretion levels of plant bioactives or their derived metabolites can markedly differ between individuals upon a similar dietary intake. Several examples of clinical intervention studies that demonstrated inter-individual variability in bioavailability of different families of plant food bioactives are presented in Table 1 [27–41]. However, a systematic analysis of the magnitude of this inter-individual variability has not been performed so far. Between-subject differences could arise due to variances in absorption, metabolism, tissue distribution and turnover, excretion or a combination of these parameters. This has been clearly demonstrated for carotenoids and has led researchers to propose that some subjects could be considered “non-responders” to carotenoids on the basis of their low plasma concentration after intake [42]. Several studies recently demonstrated an association between the carotenoid status and variants of genes encoding proteins involved in the absorption and metabolism of carotenoids. These include *SR-BI* (Scavenger Receptor Class B, Member 1) and *CD36* (*CD36* Molecule, Thrombospondin Receptor) both involved in the uptake of carotenoids by intestinal cells, as well as *BCO1* (Beta-Carotene Oxygenase 1) which catalyzes the oxidative cleavage of carotenoids into vitamin-A [43,44].

In addition, it is most likely that host genetic and epigenetic variations in metabolizing enzymes can be a major determinant of individual phenotypes. Pharmacogenomics

studies have demonstrated that for particular drugs, including some phytochemicals of the alkaloid family like codeine, individuals can be categorized into poor, intermediate or extensive and ultra-rapid metabolizers and dosing has to be adapted to achieve equivalent plasma or tissue concentration of the active metabolite [45]. Differences have been attributed to specific genes and enzymes of the xenobiotic metabolism pathways and the genetic variants associated have been compiled in online databases such as the Pharmacogenomics knowledgebase (PharmGKB) [46] and on the FDA Table of Pharmacogenomic Biomarkers in Drug Labeling (<http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/ucm083378.htm>, accessed 26/08/16). Most plant food bioactives are absorbed and metabolized through the same polymorphic carriers and enzymatic systems as some of these drugs, and their pharmacokinetics is thus also likely to depend on genetic background. Caffeine is a plant bioactive for which the importance of genetic polymorphisms has been demonstrated. It is mainly metabolized by *CYP1A2* in the liver. Subjects with the *CYP1A2*1F* variant (carriers of the -163 C allele) associated with a low enzyme inducibility are considered to be slow caffeine metabolizers in comparison with the rapid caffeine metabolizers carrying the wild-type allele [47]. Slow caffeine metabolizers have been shown to be at increased risk of hypertension and myocardial infarction, whereas for rapid caffeine metabolizers it might be more safe to drink coffee [48]. Plant food bioactives are also extensively metabolized by phase II conjugation enzymes. Several variants exist for genes encoding glutathione S-transferases (GSTs), which play an important role in the metabolism of glucosinolates present in cruciferous vegetables. After broccoli consumption, the bioavailability of isothiocyanates was shown to be 20% higher in *GSTM1* null subjects than in *GSTM1* positive subjects [30]. This is of significance as the prevalence of the null allele for *GSTM1* has been estimated to be 40–60% [49]. Variants of UDP-glucuronosyltransferases, sulfotransferases and catechol-O-methyltransferase, may also contribute to variability in plant food bioactives metabolism, clearance and efficacy although it is less documented [50]. The tissue:blood partition and the excretion kinetics of the metabolites produced, for example glucuronides, is likely to be different from that of the native compounds due to differences in compound polarity. The modulation of phase II enzymes may thus affect the duration of exposure to bioactive metabolites and consequently the biological response.

Gut microbiota obviously plays an important role in inter-individual variation in the metabolism of some plant bioactives. A well-known example is the conversion of the soy isoflavones daidzin/daidzein into equol by the gut microbiota. Only 25–35% of the Western population and up to 50–70% of the vegetarian and Asian populations possess the ability to produce equol from these precursors [36], and producers have been reported to experience more beneficial health effects from soy consumption than equol non-producers [51–53]. The equol-producing bacteria identified so far include

Table 1. Clinical intervention studies demonstrating interindividual variability in bioavailability of plant food bioactives

Bioactives/ food rich in bioactives	Study products	Study type	Nb volunteers	Volunteers	Objectives	Results	Reference
Carotenoids	Watermelon juice providing 20 mg lycopene, 2.5 mg b-carotene (Treatment 1) or 40 mg lycopene + 5 mg b-caroten (Treatment 2) or Tomato juice providing 18 mg lycopene + 0.6 mg b-carotene (Treatment 3) Daily intake of each treatment for 3 weeks	Chronic parrallel study	N = 10 for Treatment 1 N = 23 for treatment 2 N = 12 for treatment 3	Healthy non-smokers	Use of cluster analysis to examine individual temporal response of plasma carotenoids and provide basis for classifying subjects as strong or weak responders	Various carotenoids can produce various plasma responses for a given subject. Individual responsiveness was associated with genetic variants of the carotenoid metabolizing enzyme b-caroten 15,15' monooxygenase 1.	[41]
Lutein	Meal 1: providing lutein as a supplement (15 mg) Meal 2: providing the same amount of lutein as tomato puree	Acute cross-over study	N = 39	Healthy non-obese, non-smoker men	Characterization of the interindividual variability in lutein bioavailability by measuring lutein in plasma chylomicrons sampled over 8h postprandially and identification of SNPs involved	The CV of the postprandial lutein response was 75% after lutein supplement and 137% after tomato puree consumption. Most of this variance was explained by 29 SNPs in 15 genes related to both lutein and chylomicron metabolism.	[29]
Lycopene	Test meal containing 100 g tomato puree providing 19.7 mg all-trans Lycopene	Acute study	N = 39	Healthy non-obese, non-smoker men	Characterization of the interindividual variability in lycopene bioavailability by measuring lycopene in plasma chylomicrons sampled over 8h postprandially and identification of SNPs involved	The CV of the postprandial concentrations of lycopene in plasma chylomicrons was of 70%. 72% of this variance was explained by 28 SNPs in 16 genes involved in lycopene and lipid metabolism.	[28]
Cocoa flavanols	5.3 and 10.7 mg total Cocoa Flavanols (CF)/kg BW	Acute parrallel study	N = 20 young (25–35 yrs) N = 20 elderly (65–80 yrs)	Healthy men	Follow-up of concentrations in total structurally-related EC metabolites (SREM) and of individual metabolites in blood and urine over 24 h	The interindividual variations in total SREM were 38 and 39% in AUC (0–6 h) and Cmax, respectively without effect of age. Small differences observed between young and elderly for some specific metabolites.	[34]

Table 1. Continued

Bioactives/ food rich in bioactives	Study products	Study type	Nb volunteers	Volunteers	Objectives	Results	Reference
Orange juice - Flavanones	400 mL fresh, homogenized and pasteurized orange juice	Acute cross-over study	N = 18	Healthy subjects (20–50 yrs)	Effect of orange juice processing on flavanone bioavailability as measured in 24 h urine	High excretors (N = 4), medium excretors (N = 7) and low excretors (N = 7) had mean flavanone excretion values of 15, 9, and 3% respectively after fresh juice intake. Type of food processing had an impact in high excretors but not in medium and low excretors	[40]
Hop prenyl- flavonoids - 8 prenylharingenin	3 hop-derived supplements per day for 5 days	Short-term study	N = 50	Healthy post- menopausal women (46–74 yrs)	Examine the extent of interindividual variation in urinary excretion of hop-derived prenylflavonoids	Subjects were classified into poor (60%), moderate (25%) and strong (15%) 8-prenylharingenin (8-PN) producers based on urinary excretion and <i>in vitro</i> microbial bioactivation capacity (conversion of isoxanthohumol to 8-PN).	[27]
Soy isoflavones	Soy milk challenge (240 ml twice daily for 3.5 days)	Short-term study	N = 159 (89 from USA and 70 from Australia)	Healthy adults	Follow-up of urinary excretion of S-equal (24h urine) to identify equal producers and search for association between equal producer status and dietary factors	The observed overall frequency of equal producer is 29.6% without any significant difference between USA and australian participants. Subtle effects of some dietary factors (PUFAs, maltose, VitA, Vit E).	[36]
Soy Isoflavones	Soy protein bar (containing 38 mg Daidzein) per day on 3 consecutive days	Short-term study	N = 91 Korean American (KA) and N = 222 Caucasian American (CA)	Healthy Women and girls	Use a soy challenge test to confirm results from observational studies suggesting that the prevalence of equal producers is higher in Asian than in Western populations	Prevalence of Equal-producer phenotype higher in KA (51%) than in CA (36%), of ODMA-producer phenotype lower in KA (84%) than in CA (92%). No differences in dietary habits between equal and non-equal producers.	[38]
Soy Isoflavones	Soy milk challenge (250 ml twice daily) on 3 consecutive days	Short-term study	N = 41 including 29 vegetarians and 12 non- vegetarians	Healthy adults	Comparison of the frequency of equal producers in the two dietary patterns	The frequency of equal producers was higher in vegetarians (59%) than in nonvegetarians adults (25%).	[37]

Table 1. Continued

Bioactives/ food rich in bioactives	Study products	Study type	Nb volunteers	Volunteers	Objectives	Results	Reference
Lignans- enterolactone	Purified secoisolaricresinol diglucoside (SDG) (1,31 micromol/kg BW)	Acute study	N = 12	Healthy men and women	Pharmacokinetics study of enterolignans (enterodiol (END)), enterolactone (ENL) in volunteers after consumption of their plant precursor	Substantial variation observed among subjects in plasma concentration and urinary excretion of enterolignans: In 5 subjects AUC of ENL was twice that of END; in other 5 subjects AUC of ENL was only 1–2 times that of END; In 2 subjects AUC of END exceeded that of ENL. No differences between men and women.	[32]
Raspberries- Ellagitannins	300 g raspberries	Acute study	N = 10	Healthy subjects (25–48 yrs)	Follow-up of plasma (24h kinetics) and urine (48h kinetics) concentrations of ellagic acid and its microbial metabolites uroolithins	Large interindividual variation in timing, quantity and type of urolithins-O-glucuronides excreted in urine. One subject produced no uroolithins, whereas the other nine excreted quantities ranging from 1.3 to 44 μ mol. Only one subject produced uroolithin B-glucuronide.	[31]
Walnuts and pomegranate extract -Ellagitannins	Two interventions: 1. 30 g walnuts per day for 3 days; 2. pomegranate extract 0.9g per day for 3 days	Short-term study	1. N = 20 2. N = 49	1. Healthy subjects (21–55 yrs) 2. Healthy, overweight subjects (40–65 yrs)	Characterization of the interindividual variability in urolithin production assessed by measuring derivatives in 24 h urine; Analysis of fecal microbiota composition	Three metabolotypes identified: metabotype A subjects (1. 65%; 2. 60%) excreted only uroolithin A metabolites, metabotype B subjects (1. 20%; 2. 30%) in addition to UA also excreted urolithin B and isourolithin A, metabotype 0 subjects (1. 15%; 2. 10%) did not excrete uroolithins. Higher level of <i>Gordoniabacter</i> were found in metabotype A individuals. Metabotype B prevailed in overweight-obese versus normoweight subjects.	[35]

Table 1. Continued

Bioactives/ food rich in bioactives	Study products	Study type	Nb volunteers	Volunteers	Objectives	Results	Reference
Standard broccoli and high- glucosinolate broccoli	3 test meals (150 ml): broccoli soup, super broccoli soup, or water	Acute study	N = 16	Healthy subjects	Investigate the impact of <i>GSTM1</i> genotypes on sulforaphane metabolism and excretion	Significant increase in plasma AUC and urinary excretion of total sulforaphane metabolites observed in <i>GSTM1</i> -null subjects compared with the <i>GSTM1</i> -positive subjects. 62% of <i>GSTM1 null</i> individuals had high ITC excretion whereas only 39% of individuals with the <i>GSTM</i> present genotype were high ITC excretors.	[30]
broccoli- Isothiocyanates	Test meal consisting in 2.5 g broccoli/kg BW with pasta	Acute study	N = 114	Healthy subjects (18–50 yrs)	Investigate the effect of <i>GSTM1</i> genotypes on sulforaphane metabolism and excretion	Differences in gut microbiota composition contribute to observed variation in glucosinolate metabolism in low- (N = 5) vs high-ITC excretors (N = 5).	[33]
broccoli- glucosinolates	Standardized meal containing 200 g cooked broccoli	Acute study	N = 23	Healthy subjects	Examine the association between glucosinolate metabolism and gut bacterial community composition		

BW, body weight; CV, coefficient of variation; SNP, single nucleotide polymorphism; CF, cocoa flavanols; EC, epicatechin; PUFA, poly-unsaturated fatty acids; ODMA, O-desmethylangolensin, AUC, area under the curve; Cmax, peak plasma concentration; *GSTM1*, glutathione S-transferase M1; ITC, isothiocyanate.

Adlercreutzia, *Eggerthella*, *Paraeggerthella*, and *Slackia* species all of them belonging to the family Coriobacteriaceae, a family that has been associated with beneficial metabolic processes for obesity and diabetes [54]. Different combinations of bacteria can perform the successive steps of the conversion of soy isoflavones into equol, but the determinants that govern the daidzein-metabolizing phenotype are not elucidated yet. Identification of these determinants may lead to the development of intervention strategies or food processes to stimulate equol production in equol non-producers. The gut microbiota also play an important role in explaining the variability in the metabolism of other plant food bioactives, in particular lignans [55] and ellagitannins [56, 57]. Recent advances in metagenomics, and increased knowledge of the intestinal microbiota, open up new perspectives to further investigate the implication of the microbiota in the inter-individual variation of the bioavailability of plant food bioactives. The European MetaHit project (FP7, <http://www.metahit.eu/>) proposed that the microbial communities could be classified into three main enterotypes characterised by the predominant bacterial population: *Bacteroides* (enterotype 1), *Prevotella* (enterotype 2) and *Ruminococcus* (enterotype 3) [58]. While it is nowadays clear that the enterotype concept follows a continuum rather than discrete values, it must be emphasized that a functional stratification, rather than a phylogenetic stratification in microbiome profile also needs to be made [59]. Enterotypes as well as functionality differences in microbiota (putatively grouped in “metabotypes”) may be both important for explaining the inter-individual variation in plant bioactives ADME and putative health effects as well as for stratifying subjects [60]. Furthermore, the extent to which dietary interventions can modulate the gut microbiota and consequently affect the metabolism of plant food bioactives will have to be determined.

Besides genetic background and gut microbiota composition, other factors such as sex, age, dietary habits, may affect the bioavailability of plant food bioactives. For example, sex-dependent differences in the glucuronidation of resveratrol, a polyphenol present in grapes and wine, may be explained by sex-specific UDP-glucuronosyltransferase isoenzyme expression profiles regulated by sex hormones [61]. Important pharmacokinetic changes are known to occur with aging, for example the volume of distribution is reduced for polar compounds resulting in higher plasma concentrations [62]. How these changes may affect plant food bioactive ADME needs to be investigated in details. Dietary habits are known to influence microbiota diversity and activity [63]. Furthermore, some dietary phytochemicals such as polyphenols and glucosinolates themselves can induce or inhibit the activity of phase I and phase II enzymes [50].

As illustrated by the examples above, there is a body of evidence that various factors can control the bioavailability and metabolism of plant food bioactives in individuals. These factors must now be studied in a more systematic and integrative way to determine their relative importance for each family of plant food bioactives.

1.2 Inter-individual variation in biological response regarding cardiometabolic health outcomes

Compared with the inter-individual variation in ADME and bioavailability, the inter-individual variation in efficacy of plant food bioactives to modulate physiological outcomes has been less studied. The etiological basis for this variability has rarely been considered and individual data are seldom published. The biological effects of plant food bioactives that might be occurring only in a subgroup of subjects can be overlooked when data are averaged for an entire sample population without an appropriate stratification of the participants. Several clinical intervention studies describing inter-individual variability in the bioactivity of plant food bioactives and the associated determinants are presented in Table 2. For example, in post-menopausal women, consumption of soy isoflavones resulted in decreased systolic and diastolic blood pressure and improvement of the endothelial function, but only in 30% of the volunteers taking part in the study and who were also found to be equol producers [64]. The effect would not have been observed when just considering average values. Similarly, a recent clinical trial which prospectively recruited male equol producers and non-equol producers with moderate cardiovascular disease risk, provided evidence of the importance of the equol phenotype, and thereby of the gut microbial activity in mediating the positive effect of dietary isoflavones on arterial stiffness [53]. This would suggest a clear link between bioavailability and efficacy of plant bioactives but this is not necessarily the case. Indeed, a recent controlled clinical trial showed that consumption of flavan-3-ol-enriched dark chocolate resulted in decreased platelet aggregation in males but not females volunteers, however this effect could not be correlated with plasma or urine concentrations of flavan-3-ols metabolites [65]. This observation suggests that inter-individual variation in biological responses is not solely due to variation in the bioavailability of plant food bioactives but can depend on other factors, such as sex.

Age is the strongest independent cardiovascular risk factor for CVD and is also associated with increased vascular stiffness, endothelial dysfunction and systolic hypertension [66]. So far, few studies have investigated the impact of age on the cardiometabolic effects of food bioactives (Table 2). Consumption of a flavanol-rich drink reversed age-related increase in systolic blood pressure together with aortic augmentation index (a measure of vascular stiffness) in healthy elderly but not in young men [67]. In this study, the plasma levels of flavanol metabolites were not significantly different between young and elderly individuals, suggesting that differences in bioavailability could not explain the differences observed in biological response [34]. In another intervention study, the effect of the consumption of a flavanol-rich cocoa drink on blood pressure was also higher in the elderly than in young volunteers [68]. An age-dependent effect of quercetin on blood pressure was also reported [69].

Inter-individual variability in the response to the consumption of plant food bioactives has also been described

Table 2. Clinical intervention studies with plant food bioactives demonstrating factors of interindividual variability in their bioactivity related to cardiometabolic outcomes

Bioactives/food rich in bioactives	Quantity (per day)	Duration	Number of volunteers	Volunteers	Biomarkers presenting interindividual variability	Factors of inter-individual variability	Reference
Isoflavones	99 mg	12 months	202	Postmenopausal women	Systolic/diastolic blood pressure and endothelial function	Equol producers	[64]
Isoflavones	80 mg	Acute	28	Men at moderate cardiovascular risk	Arterial stiffness	Equol producers	[53]
Cocoa Flavanols	907 mg	Acute	42	Healthy subjects	Platelet function	Gender	[65]
Cocoa Flavanols	450 mg	14 days	42	Young and elderly healthy men	Systolic blood pressure and arterial stiffness	Age	[67]
Cocoa Flavanols	821 mg	4–6 days	34	Healthy young and elderly subjects	Blood pressure and endothelial function	Age	[68]
Quercetin	150 mg	6 weeks	93	Overweight or obese young and elderly subjects	Systolic blood pressure	Age and diseases state (hypertension)	[69]
Plant sterols	2–3.2 g	4–8 weeks	67	Men and women with normal or increased blood cholesterol levels	Total cholesterol	CYP7A1 polymorphism	[72]

for other phytochemicals (Table 2). For example, despite the abundance of data of clinical trials demonstrating the cholesterol-lowering action of plant sterol supplements, substantial variability in efficacy exists in responsiveness among individuals [70]. Genetic polymorphisms of genes associated with cholesterol trafficking processes may be responsible for this inter-individual variability. For example, a significant inter-individual variation in the LDL-cholesterol lowering response to plant sterol consumption has been associated with a polymorphism in the *ABCG8* gene. The subjects carrying the A allele of the gene were reported to get a greater benefit from plant sterol intake [71]. The bioactivity of plant sterols was also associated with polymorphism in cholesterol 7 alpha-hydroxylase (*CYP7A1*) gene with -204A>C variant presenting greater CYP7A1 activity [72].

Environmental factors, including dietary habits, can also affect our genetic imprint through a range of epigenetic mechanisms that can impact gene transcription and the subsequent cellular responses. These epigenetic mechanisms involving DNA methylation, histone modifications, and non-coding RNAs (microRNAs, Long noncoding RNAs) regulation emerge as important players in cardiovascular diseases [73–75]. Recent review papers reported that dietary polyphenols induced epigenetic changes that may promote cardiovascular health benefit [19, 76]. For example, consumption of a cocoa extract for two weeks significantly reduced the global DNA methylation levels of peripheral leukocytes in subjects with cardiovascular risk factors [77]. Another study demonstrated that one-year supplementation with a grape extract containing resveratrol modulated the expression of microRNAs involved in the regulation of the inflammatory response in circulating immune cells of hypertensive male patients with type 2 diabetes [78]. However, the role of the individual epigenome in the variation of the responses to plant food bioactives is still unexplored.

Recently, modest progress has been made toward a better understanding of the role of genetic polymorphisms in the variability of the response to diet. For example, the benefit of the Mediterranean diet (MedDiet) on cardiovascular-related outcomes was shown to depend on genetic variants of the Transcription factor 7-like 2 (*TCF7L2*) gene [79]. This gene encodes a protein acting as a transcription factor, and the *TCF7L2*-rs7903146 polymorphism is known as a strong genetic determinant of type-2 diabetes risk and fasting glucose concentrations [80]. The prevalence of the 7903146T allele is associated with higher type-2 diabetes risk. In their study, Corella et al. showed that an intervention with the MedDiet was more beneficial toward cardiovascular risk factors and stroke incidence for individuals with the 7903146T allele [79]. However, it has to be noted that most nutrigenetics studies thus far are association studies and results have not been confirmed in studies.

The development of high-throughput “omics” technologies (epigenomics, metagenomics, transcriptomics, proteomics, metabolomics) and bioinformatic tools has enabled researchers to go deeper in the analysis of the complex

mechanisms that are involved in the way the organism responds to plant food bioactives and ultimately impact human health and well-being. For example, the consumption of hesperidin from orange juice or of a resveratrol-containing grape extract have been shown to change leukocyte gene expression toward anti-atherogenic and anti-inflammatory profiles in healthy men [81] and in patients with stable coronary artery disease [82], respectively. The inter-individual variation in the overall expression profile of genes involved in the regulation of cardiometabolic health in response to plant food bioactives has not been evaluated in humans. However preliminary evidence of a large between-subject variability in the expression levels of genes and microRNAs related to inflammation and cardiovascular diseases in humans has been reported [78]. Nutrigenomics, particularly transcriptomic studies, will help to reveal genes and non-coding RNA expression profiles that may be used as new biomarkers of responsiveness to plant food bioactives. Furthermore, investigations focused on the identification of the molecular mechanisms of action in response to plant food bioactives are still necessary to identify genes and (or) non-coding RNAs underlying the health effects of these compounds. These genes may become candidates for future nutrigenetic studies aiming to identify gene variants involved in inter-individual variability in responsiveness to plant bioactive intake.

In summary, a range of studies have indicated that inter-individual variation in biological responsiveness to plant food bioactives exists and that it may be influenced by several factors including bioavailability and metabolism, gut microbiome composition, (epi)-genetic profiles, age, or sex.

1.3 How to tackle the complexity of the inter-individual variability through a multi-dimensional approach?

Research in the field of inter-individual variation in response to consumption of plant bioactives and the determinants involved is presently highly fragmented and many research gaps remain. Addressing this question requires the participation of experts from a range of disciplines. The first initiative to this end is the creation of a dedicated Action called “FA1403-POSITIVE” (<https://www6.inra.fr/cost-positive>) within the framework of the European Cooperation in Science and Technology (COST). This open network gathers nutritionists, clinicians, geneticists, epidemiologists, microbiologists, experts in nutrigenomics, bioinformaticians, molecular biologists and biochemists from more than 70 research institutions in 31 countries. Their common goal is to analyse in a systematic way the available knowledge to evaluate the extent of inter-individual variation for some of the main families of plant food bioactives and to identify the key determinants of the inter-individual variation regarding both bioavailability and bioactivity related to cardiometabolic health outcomes. A major expected outcome of this COST Action will be to define

strategies and methods to stratify populations into subgroups according to their ability to respond to plant food bioactives.

From the findings of the POSITIVE network and of other complementary initiatives, the relative contribution and possible interactions of the main determinants of inter-individual variability identified should be further validated through dedicated randomized controlled trials and large-scale prospective studies for the different families of plant food bioactives. The power of these studies will have to be calculated to allow different 'responsive' subgroups analyses and/or wise targeted recruitments must be performed based on the factors likely to affect the individual response. In upcoming studies assessing the health effects of plant foods and their bioactives, publication of individual data should be strongly recommended and as far as possible the volunteers should be phenotyped for the determinants that have already been identified as important.

Because of the inter-individual variation in ADME, the internal exposure to plant food bioactive compounds and (or) derived metabolites is not directly correlated to the dietary intake and will have to be characterized in depth in future studies. To date in the vast majority of epidemiological studies, health outcomes were related to food or nutrient intake data, estimated from dietary questionnaires and tables of food composition without taking into account the bioavailability. In intervention studies, the background diet and the supplementation are usually standardized but this does not guarantee equal internal exposures among volunteers due to the inter-individual variability in absorption and metabolizing capacity. Known metabolites can be quantified in biofluids such as plasma and urine or in tissue biopsies using targeted methods of analysis based on UV or mass spectrometry detection. However, the scope of targeted methods of analysis is usually restricted to a limited number of known metabolites of a particular family of compounds. Non-targeted metabolomics with high-resolution mass spectrometry (Q-TOF and Orbitrap instruments) has emerged as a holistic and sensitive approach to analyse in human biofluids the food metabolome which encompasses all the metabolites derived from food intake and digestion, including the plant food bioactive metabolites [83,84]. Comprehensive profiles of metabolites present in urine, plasma or other biospecimens, including still unknown metabolites of plant food bioactives, can be obtained and compared in nutritional intervention studies for subjects consuming the same diet but stratified according to factors such as age, sex, gut microbiota composition, and genotype. The extent and the nature of the differences existing between the metabolomic profiles of the study groups, for example, between males and females after the same plant food intake is likely to provide valuable new insights for a better understanding of the inter-individual variation.

Some technological and methodological improvements will have to be implemented to facilitate the use of metabolomics for plant food bioactive research. First initiatives have been launched in the framework of the COST Action POSITIVE with the development of a standardized

combination of methods ensuring a wide coverage of plant food metabolites, the sharing of standards of rare metabolites, and the compilation of metabolism and analytical data in the online database PhytoHub dedicated to dietary phytochemicals (www.phytohub.eu). The objective is to make possible the use of comprehensive annotated profiles of plant food bioactive metabolites in intervention trials and large-scale observational studies such as wide-association studies.

Furthermore, despite the knowledge accumulated on the metabolism of plant bioactives in humans, and while most plant biosynthetic pathways of these compounds are accessible on-line at KEGG (<http://www.genome.jp/kegg/pathway.html>) or MetaCyc (<http://metacyc.org/>) databases, their metabolic pathways in humans or mammals have not yet been published in online databases. Up to now, caffeine is the only plant food bioactive for which the metabolism in humans is described in the KEGG database (<http://www.genome.jp/kegg/pathway/map/map00232.html>). As modern biology increasingly relies on bioinformatics, there is an urgent need to fill this gap. We need to compile existing knowledge in online resources, with data on proteins, genes, polymorphisms, gut bacteria involved as well as to design new research projects aiming to find out the missing information. In this way, the updated and complete human metabolic pathways of the plant bioactive compounds will be made available for systems biology approaches to study the inter-individual variability in an integrative way.

Well-designed intervention and prospective studies, integrating omics approaches for a better phenotyping of individuals will show the relative contribution of various factors to control the bioavailability of different families of plant food bioactives and the individual biological responsiveness to their consumption. A major challenge will be to develop methods and tools to phenotype and stratify individuals based on their ability to respond to plant food bioactives intake. An in-depth characterisation of the subjects, including genotyping, gut microbiota analysis, food metabolome profiling has become achievable, although still quite expensive, making feasible the search for correlations between information-rich datasets and health outcomes in intervention and prospective studies. New bioactives as well as new molecular targets in population subgroups may be identified. However, for the majority of studies investigating the health effects of plant foods and for clinical applications, the subject stratification methods should be more cost-effective and easy to handle. Several approaches can be foreseen, including modeling and challenge tests. Models may be built from the acquired knowledge to predict the internal exposure of individuals based on their declared food intake and their status for the main factors controlling inter-individual variation in ADME (e.g. genotyping and gut microbiota). These models will be improved as new data and knowledge are generated and could be validated through measurement of actual exposure in controlled intervention studies. Together with models aiming to predict the internal exposure, more complex ones should also be developed to predict biological

responsiveness to plant food bioactives intake and thus estimate the personal health benefits that an individual can gain from different bioactives compounds. This estimation would be made by taking into account the bioavailability of bioactives together with other factors identified as involved in the inter-individual variability in responsiveness, such as age, sex, specific genetic polymorphisms or physiological status. Before obtaining enough knowledge to develop such models, another option for stratification of the population into responders and non-responders may be considered. This could consist of designing challenge tests in which subjects would consume a standardized supplement containing various bioactives. The plasma concentrations and/or urinary excretion of key metabolites would be monitored according to a precise sampling schedule over a defined timeframe, as it has already been proposed for equal-production capacity [85]. In addition, standardized postprandial nutritionally challenging conditions could be defined to induce disturbances impacting cardiometabolic outcomes to assess the individual capacity to respond to plant food bioactives intake. This would be done by testing the ability of subjects receiving specific bioactives to attenuate/counterbalance the induced disturbances by measuring the impact on well-established and emerging biomarkers of cardiometabolic risk. This challenge test approach will be helpful both to strengthen the scientific knowledge of the determinants of inter-individual variability and to stratify responders/non-responders.

2 Conclusion and perspective

Within the context of improving the health of populations and promoting sustainable economic growth of the food sector, research policies emphasize the importance of developing high quality and healthy foods and refining dietary recommendations to decrease the burden of diet-related chronic diseases. The challenge is to stimulate both the consumers to select foods that fit into a healthy diet and the agro-food sector to develop healthier foods. Dietary patterns rich in plant foods have been largely shown to be healthier than those rich in animal products [86]. Consequently, high consumption of fruits, vegetables and whole-grains is widely encouraged in many countries through national public health policies. However, thus far the recommendations are applied to the general population in a “one-size-fits-all approach” without considering the possible high between-subject variations in response to plant food bioactives. As a consequence, this approach does not necessarily ensure adequate exposure to protective compounds for all individuals.

Improving our knowledge of the factors (such as age, sex, (epi)genotype and gut microbiota) that influence whether plant food bioactives are more or less effective in individuals, together with the development of methods to identify responsiveness profiles will be invaluable to progress in the development of effective and innovative solutions leading to health improvements. There is a high potential for the

development of new functional foods or optimized traditional foods with higher effectiveness for general or targeted populations. For example, adapting processing methods may improve the bioavailability of key bioactive compounds for individuals identified as poor absorbers/metabolizers. This research will also provide the scientific evidence to help the public health bodies to elaborate a new generation of nutritional recommendations for improving the prevention of cardiometabolic diseases in the long run. In particular, it may lead to refined recommendations toward foods particularly rich in specific bioactives and to public health advice targeting specific populations. As recently demonstrated in the European FP7-Food4me project, personalized dietary advice is more efficient to positively affect dietary behaviours (see white paper at: www.food4me.org/). In a global context of an increasing prevalence of low intake of fruits and vegetables, with less than 25% of the population consuming at least five portions per day (400 g) in most countries, more personalized messages regarding this food group could be of high benefit [87]. Acquired knowledge might also be integrated for individual counseling along personal profiles of determinants affecting the bioavailability and responsiveness to plant food bioactives.

Addressing the inter-individual variation in response to plant food bioactives perfectly fits one of the main European research priorities identified for foods and diets toward 2050, which is to contribute to the understanding of human variation in response to nutrition factors and to better translate this knowledge into innovative individualised food and nutrition solutions to improve citizens' health [88]. This position paper elaborated in the framework of the COST POSITIVE Action proposes some priorities for tackling the challenging research question of inter-individual variation, with the ultimate goal of ensuring that the cardiometabolic health-promoting effects associated with bioactives present in plant foods are applicable for everyone.

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