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Prebiotic potential of polyphenols, its effect on gut microbiota and anthropometric/clinical markers: a systematic review of randomised controlled trials

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ABSTRACT

Background: Polyphenols have been implicated to have numerous health benefits, and much of these are attributed to the metabolism of phenolic compounds by gut microbiota**.** The aim of this systematic review was to examine the effects of polyphenol consumption in modulating gut microbiota and anthropometric variables/clinical markers in randomised controlled trials (RCTs).

Scope and approach: We systematically searched PubMed, Scopus, Embase, Cochrane library and Web of Science databases from inception to31st July 2019 following the PRIMSA guidelines.RCTs reporting on the effects of polyphenol consumption on gut microbes, and anthropometric variables (body weight, BMI, waist circumference, hip circumference)/clinical markers (CVD markers, and colon cancer markers) were included in this review. The methodological quality of the studies was assessed using the Cochrane Collaboration's risk of bias tool and Jadad scale.

Key findings and conclusion: Seventeen RCTs met the inclusion criteria. Ten studies highlighted significant changes in the microbial profile, while 15 reported significant changes in CVD and colon cancer markers. The univariate correlation data showed a significant correlation between certain genera with clinical markers, specifically TNFα, cholesterol, HDL, CRP, and LPS. In the multivariate analysis, negative correlations were reported between *Lactobacillus* and TAG, CRP, *Bacteroides* with TAG, HDL, DBP, and SBP, and *Bifidobacterium* with cholesterol and CRP. This review supports the notion of polyphenols as prebiotics as significant modulation of intestinal microbes affecting mainly CVD markers were found in most of the RCTs. Further well-structured trials with larger sample size, longer duration, and high-throughput molecular techniques, will provide more conclusive results.

Protocol registration number: PROSPERO; CRD42017077577

Keywords: Systematic review, randomised controlled trials, polyphenols, prebiotic, gut microbiota, CVD markers

Introduction

Polyphenols are phytochemicals (Farhat, Drummond, & Al-Dujaili, 2017; Van Duynhoven et al., 2011) found in fruits, vegetables, and plant-derived products such as tea, coffee, wine, and chocolates (Amiot, Riva, & Vinet, 2016; Farhat et al., 2017; Scalbert, Morand, Manach, & Rémésy, 2002). At present, they garner much attention, due to their health-promoting activities, some of which include protection against cardiovascular diseases (CVD) (Arab, Liu, & Elashoff, 2009; George et al., 2019; Rienks, Barbaresko, & Nothlings, 2017; Wang, Ouyang, Liu, & Zhao, 2014), as well as their anti-carcinogenic (Poschner, Maier-Salamon, Thalhammer, & Jäger, 2019; Singh, Bhui, Singh, & Shukla, 2013; Walle, Wen, & Walle, 2007), anti-inflammatory (Adesso et al., 2016; Joseph, Edirisinghe, & Burton-Freeman, 2014; Van de Velde, Esposito, Grace, Pirovani, & Lila, 2019), and anti-oxidant properties (Alvarez-Suarez et al., 2011; Shen et al., 2016). However, it is estimated that only 5–10% of the total polyphenol intake is absorbed in the small intestine. The remaining 90–95% may accumulate in the large intestinal lumen where they are subjected to the enzymatic activities of the gut microbial community (S. Lin et al., 2019; Ozdal et al., 2016a; Ozdal et al., 2016b; Fulgencio Saura-Calixto, Serrano, & Goñi, 2007).

The colonic microbiota are, therefore, responsible for the extensive breakdown of polyphenols into low-molecular-weight absorbable metabolites that may be responsible for the health effects derived from the consumption of polyphenol-rich food (Cardona, Andrés-Lacueva, Tulipani, Tinahones, & Queipo-Ortuño, 2013; S. Lin et al., 2019; Ozdal et al., 2016a). These findings highlight the prebiotic potential of polyphenols as defined by the consensus document of the International Scientific Association for Probiotics and Prebiotics (ISAPP) (Gibson et al., 2017).

This definition expands the concept of prebiotics to include non-carbohydrate substances such as polyphenols, however, it states the need for a convincing weight of evidence to support such claims.

A growing number of animal and *in vitro* models have described the reciprocal interaction between polyphenols and gut microbiota and its resulting health benefits, some of which include protection against cancer, obesity, insulin resistance, hepatic inflammation, sleep deprivation, and atherosclerosis. Research involving human participants on the same is by far limited, whereas several RCTs published recently, have not as yet been systematically reviewed. In view of the aforementioned health benefits, the existing gap between human and animal/in vitro studies, and to uncover findings of human trials, we have undertaken a systematic review of RCTs to decipher the effects of polyphenol consumption on the modulation of gut microbiota and various anthropometric (body weight, BMI, waist circumference, hip circumference) and clinical markers [CVD – C-reactive protein (CRP), tumor necrosis factor-alpha (TNFα), IL-6, IL-1β,blood pressure, glucose, total cholesterol (TC), triacylglyceride (TAG), high density lipoprotein (HDL), low density lipoprotein (LDL); other markers – bile acid, faecal pH]

Approach

The protocol for this systematic review was registered with PROSPERO (www.crd.york.ac.uk/PROSPERO; CRD42017077577). Reporting of this review was done in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Liberati et al., 2009).

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Data sources and search strategy

A systematic search of the literature was undertaken independently by MM and UDP in the following databases: PubMed, Scopus, Excerpta Medica Database **(**Embase), Cochrane library, and Web of Science to include papers written in all languages. We included studies from database inception to 31st July 2019. A combination of search terms pertaining to 'polyphenols' and 'gut microbiota' were used (Table 1 – search term, Supplementary table 2 -search strategy). The bibliographic records from each database were exported to Endnote X8 (Thomson Reuters, New York, NY) where duplicates were removed. Additionally, a manual search of bibliographies of reviews and included studies were undertaken to identify pertinent studies.

Table 1 Search terms

Key words	
Microbiota	Dietary polyphenols
Gut microbiota	Polyphenols
Colonic microbiota	Flavonoids
Gastrointestinal microbiota	Fruits
Intestinal microbiota	Vegetables
Gut organism	Plant extracts
Microbial consortia	Herbal drugs
Gut bacterium	Medicinal plant
Gut flora	Antioxidants
Gastrointestinal flora	Anthocyanins
Intestinal flora	Chalcones
	Catechin
	Flavanones
	Proanthocyanidins
	Ellagitannins
	Functional food
	Green tea
	Puerh tea
	Cocoa
	Chocolate
	Myo-inositol
	Soy isoflavones
	Blueberries
	Berries

	Grape Quercetin Citrus Cinnamon Red wine Resveratrol Natural s-equol

Selection criteria

The inclusion criteria were i) randomised controlled trials, (ii) studies that reported on the consumption of dietary polyphenols (vegetables/fruits/drinks) or pure compounds of polyphenols or both, and (iii) studies reporting outcomes of modulation of gut microbiota and anthropometric variables (body weight, BMI, waist circumference, hip circumference) and clinical markers [CVD – C-reactive protein (CRP), tumor necrosis factor-alpha (TNFα), IL-6, IL-1β,blood pressure, glucose, total cholesterol (TC), triacylglyceride (TAG), high density lipoprotein (HDL), low density lipoprotein (LDL); other markers – bile acid, faecal pH]. The PICOS criteria are presented in Table 3.

Data extraction

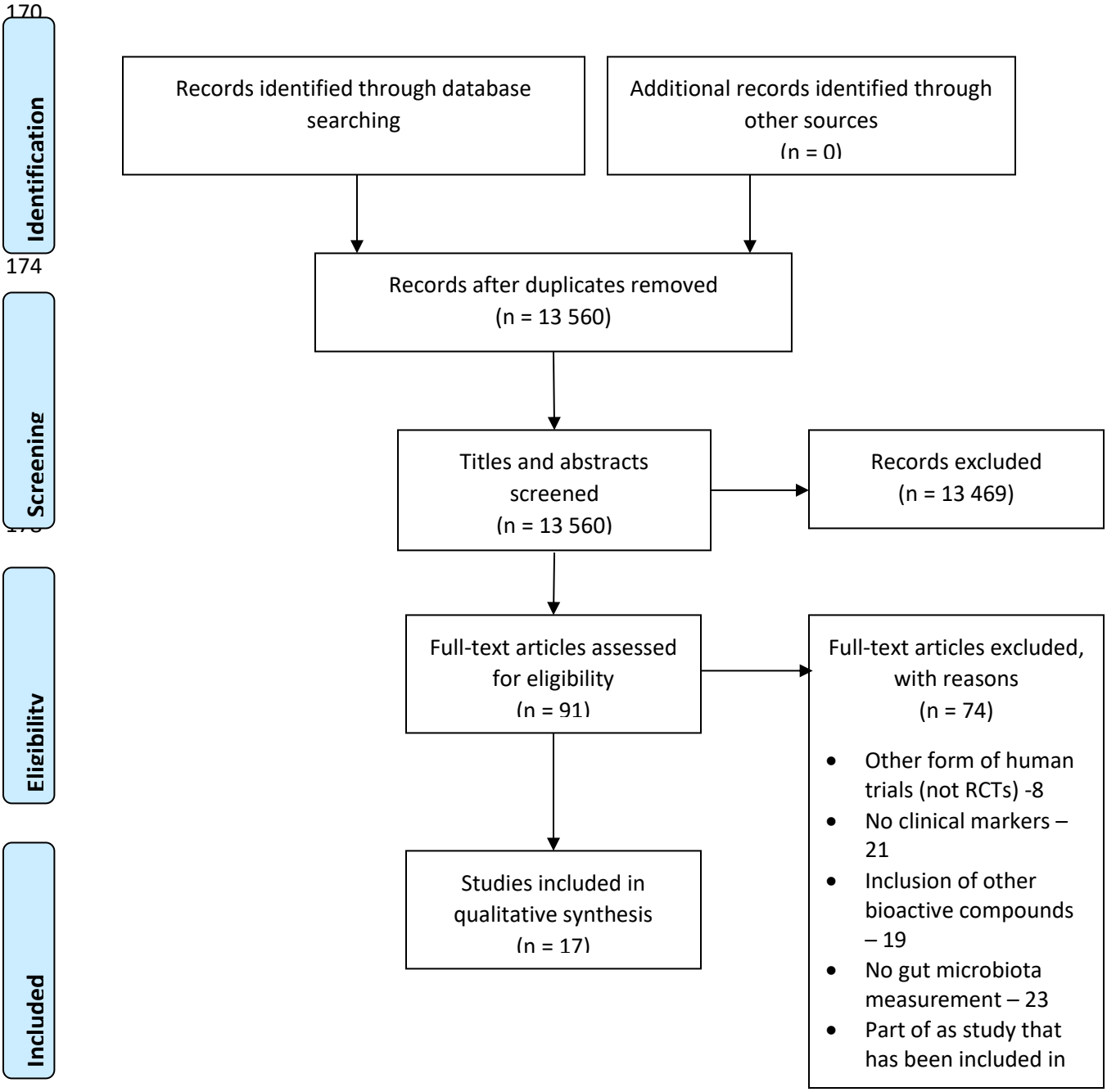
Two reviewers, MM and UDP independently screened titles and abstracts for eligibility based on the selection criteria before evaluating full texts. Any disagreements or discrepancies were discussed with a third reviewer (SAJ) and/or resolved by consensus. Data extraction included study design, subject characteristics, intervention (type of polyphenols), total polyphenols, and results (gut microbes, anthropometric and biochemical variables, univariate and multivariate correlation results between gut microbes and biochemical variables). Where data was unavailable or incomplete, the authors were contacted.

Quality assessment

The internal quality of each study was assessed both qualitatively using the Cochrane Collaboration's tool for assessing the risk of bias (RoB) (Higgins et al., 2011) and quantitatively using the Jadad scale (Jadad, Moore, Carroll, Jenkinson, & Reynolds, 1996). The Cochrane Collaboration's tool consists of six domains: sequence generation, allocation concealment, blinding, incomplete outcome data, and selective outcome reporting. Each study was graded as having low risk, high risk or unclear risk for each domain.The methodological quality of the studies was further evaluated using the Jadad scale which provides a summary score for each RCT, whereby two points were given for randomization, two points for blinding, and one point for the fate of all patients. A total score of ≥ 3 indicates high quality while a score of ≤ 2 indicates low quality. If double blinding is not feasible due to the nature of the intervention, then a score of ≥ 2 indicates high quality (Chung, Kang, Jo, & Lee, 2012). This score was used alongside the Cochrane Collaboration's tool as it allows easier interpretation of the study quality (Olivo et al., 2008). The methodological quality assessment was undertaken independently by MM and UDP. Any disagreements or discrepancies were discussed with a third reviewer (SAJ) and/or resolved by consensus.

Main FindingsA total of 16 304 articles were identified from the database search. Following removal of duplicates and screening of titles and abstracts, 91full texts and their references were screened, and of these, only 17 citations meeting the inclusion criteria were included in this review (Figure 1). The characteristics of included studies are outlined in Table 4.

169 *Figure 1* PRISMA flowchart of study selection



Study characteristics

All 17 studies included in this review were published in the English language. Twelve of these trials were carried out in Europe: Spain, n=4 (Gonzalez-Sarrias et al., 2017; Martín-Peláez et al., 2017; Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012), United Kingdom, n=4 (Geoffrey Istas et al., 2019; Klinder et al., 2017; Lear et al., 2019; Tzounis et al., 2011), Finland, n=1 (Puupponen-Pimiä et al., 2013), Denmark, n=1 (Ravn-Haren et al., 2013), Netherlands, n=1 (Janssens et al., 2016) and Italy, n=1 (Vitaglione et al., 2015)). Three studies were carried out in the USA (Mai et al., 2004; Rodriguez-Morato, Matthan, Liu, de la Torre, & Chen, 2018; Walker et al., 2018), while the remaining studies were conducted in Korea (Song, Wang, Eom, & Kim, 2015), and New Zealand (Molan, Liu, & Plimmer, 2014). The study designs were as follows: five randomised double-blind crossover studies (Gonzalez-Sarrias et al., 2017; Mai et al., 2004; Martín-Peláez et al., 2017; Rodriguez-Morato et al., 2018; Tzounis et al., 2011), three randomised double-blind parallel studies (G. Istas et al., 2019; Song et al., 2015; Walker et al., 2018), one randomised double-blind study (Lear et al., 2019), one randomised single-blind crossover study (Ravn-Haren et al., 2013), one randomised single-blind parallel study (Janssens et al., 2016), four randomised parallel studies (Klinder et al., 2017; Molan et al., 2014; Puupponen-Pimiä et al., 2013; Vitaglione et al., 2015) and two randomised crossover studies (Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012). Six of the studies included overweight/obese participants (Gonzalez-Sarrias et al., 2017; Janssens et al., 2016; Puupponen-Pimiä et al., 2013; Song et al., 2015; Vitaglione et al., 2015; Walker et al., 2018), while seven involved healthy subjects (G. Istas et al., 2019; Lear et al., 2019; Molan et al., 2014; Queipo-Ortuño et al., 2012; Ravn-Haren et al., 2013; Rodriguez-Morato et al., 2018; Tzounis et al., 2011). Klinder et al. (2017) conducted the study on participants with CVD risk, Moreno-Indias et

al. (2015) on those with metabolic syndrome, and Martín-Peláez et al. (2017) on those with high cholesterol. No participant information was given in the study by Mai et al. (2004). The age range of the participants was between 18 - 80 years old.

The polyphenol interventions included: trans-resveratrol (Walker et al., 2018), pomegranate extract (PE) (Gonzalez-Sarrias et al., 2017), green tea (GT) extract (Janssens et al., 2016), aronia berry extract (without fibre and organic acids) and aronia whole fruit powder (G. Istas et al., 2019), blackcurrant extract (FL and CAM 30) (Molan et al., 2014), fruits and vegetables (F&V) (Klinder et al., 2017), olive oil (OO) (Martín-Peláez et al., 2017), red wine (RW) and dealcoholized red wine (DRW) (Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012), black tea (BT) (Mai et al., 2004), berries (strawberry, raspberries, cloudberries) (Puupponen-Pimiä et al., 2013), freeze-dried cranberry powder (Rodriguez-Morato et al., 2018), apple and apple products (Ravn-Haren et al., 2013), Schisandra chinensis (SCF) (Song et al., 2015), cocoa drinks (Tzounis et al., 2011), Montmorency cherry drink (Lear et al., 2019) and whole grain (WG) wheat products (Vitaglione et al., 2015). The duration of the intervention ranged from 5 days - 24 weeks.

Ten studies aimed at investigating the cardio-metabolic effects of polyphenols (Gonzalez-Sarrias et al., 2017; G. Istas et al., 2019; Klinder et al., 2017; Lear et al., 2019; Martín-Peláez et al., 2017; Moreno-Indias et al., 2015; Puupponen-Pimiä et al., 2013; Ravn-Haren et al., 2013; Song et al., 2015; Walker et al., 2018), four studies on the prebiotic effect of polyphenols (Janssens et al., 2016; Queipo-Ortuño et al., 2012; Rodriguez-Morato et al., 2018; Tzounis et al., 2011), while the remaining three studies looked at markers of colon cancer (Molan et al., 2014),

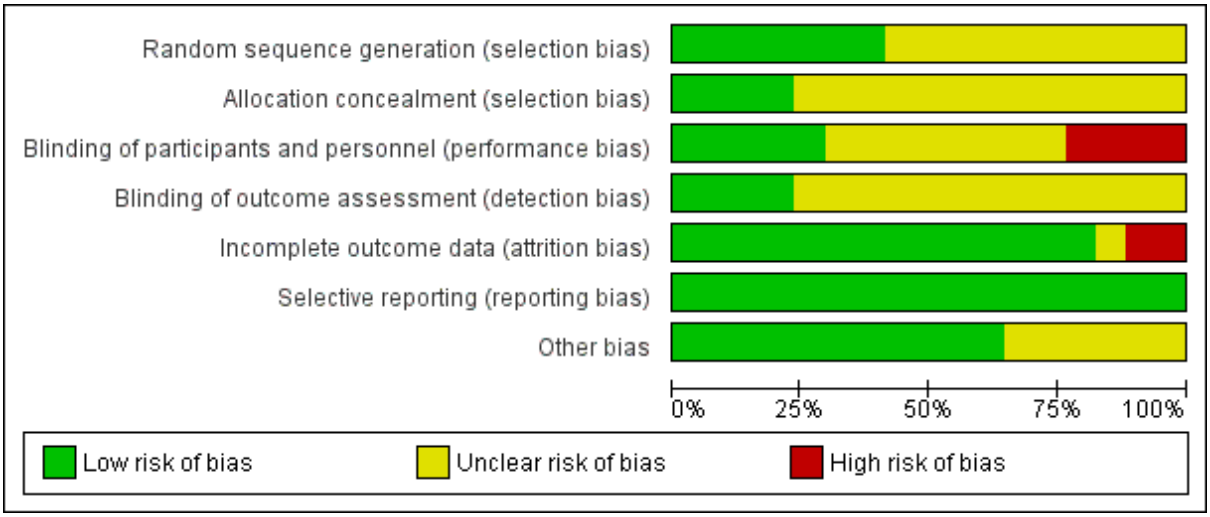
fecal bile acids (Mai et al., 2004), and chronic diseases (assessment of inflammatory and metabolic markers) (Vitaglione et al., 2015).

The primary outcome of fourteen studies was modulation of gut microbiota investigated via various molecular methods. Among these 14 studies, 12 reported changes in anthropometric variables and/or cardio-metabolic markers (Gonzalez-Sarrias et al., 2017; Janssens et al., 2016; Klinder et al., 2017; Martín-Peláez et al., 2017; Moreno-Indias et al., 2015; Puupponen-Pimiä et al., 2013; Queipo-Ortuño et al., 2012; Ravn-Haren et al., 2013; Rodriguez-Morato et al., 2018; Song et al., 2015; Tzounis et al., 2011; Vitaglione et al., 2015) , one on enzyme activities (Molan et al., 2014), and one on faecal bile acid (Mai et al., 2004) as their secondary outcomes. The main objective of the remaining three studies was to investigate the changes in various clinical markers (insulin resistance, glucose, systemic inflammation, endothelial function) following polyphenol intake while, the secondary outcome of interest was gut microbiota modulation (G. Istas et al., 2019; Lear et al., 2019; Walker et al., 2018)

Quality Assessment

Eight studies were graded as high quality and the remaining nine as low quality using the Jadad scale. (Table 4) Assessment of study quality using the Cochrane risk of bias tool showed two RCTs to be of low risk, while 10 RCTs showed low to unclear risk.. Five studies were evaluated to have a high risk for two domains: blinding of participants and personnel (Janssens et al., 2016; Klinder et al., 2017; Ravn-Haren et al., 2013; Vitaglione et al., 2015) and incomplete outcome data (Song et al., 2015; Vitaglione et al., 2015). The risk of bias graph and summary are illustrated in Figure 2 and Supplementary Figure 3 respectively.

Figure 2 Risk of bias graph: Review authors' judgments about each risk of bias item presented as percentages across all included studies



Effects of polyphenols consumption on gut microbiota

Ten studies (Gonzalez-Sarrias et al., 2017; G. Istas et al., 2019; Klinder et al., 2017; Martín-Peláez et al., 2017; Molan et al., 2014; Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012; Rodriguez-Morato et al., 2018; Tzounis et al., 2011; Walker et al., 2018) highlighted significant modulation of various gut microbiota with the consumption of trans-resveratrol (Walker et al., 2018), pomegranate extract,(Gonzalez-Sarrias et al., 2017), aronia berry extract and whole fruit powder (G. Istas et al., 2019), fruits and vegetables (Klinder et al., 2017), phenolic compound enriched olive oil containing thyme (Martín-Peláez et al., 2017), blackcurrant (Molan et al., 2014), freeze-dried cranberry powder (Rodriguez-Morato et al., 2018), red wine and dealcoholized red wine (Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012), and cocoa flavanol drink (Tzounis et al., 2011) (Table 4). Song et al. (2015) found a positive impact of Schisandra chinensis fruit on certain genera. AlthoughVitagoline et al. did not find significant variation between groups, substantial modulation in individual taxa were observed in relation to diet and sex. The remaining five studies involving the consumption of green tea extract (Janssens et al., 2016), black tea (Mai et al., 2004), berries (Puupponen-Pimiä et al., 2013), montmorency berry drink (Lear et al., 2019), and apple and apple products (Ravn-Haren et al., 2013) discovered non-significant modulation of microbes. Consumption of fruit and vegetables (Klinder et al., 2017), blackcurrant extract (CAM 30) (Molan et al., 2014), Schisandra chinensis fruit (Song et al., 2015), red wine(Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012), and cocoa drink (Tzounis et al., 2011) improved the abundance of *Bifidobacterium* , while *Lactobacillus* responded positively to blackcurrant extract (CAM 30) (Molan et al., 2014), red wine (Moreno-Indias et al., 2015), and cocoa drink (Tzounis et al., 2011). There were also other beneficial microbes that were regulated by polyphenols. Gonzales et al. highlighted a significant

increase of *Gordonibacter* in all participants with the intake of pomegranate extract. Interestingly, the author stratified the participants into three different groups based on their ability to produce urolithins (urolithin metobotypes). Urolithins are metabolites of dietary ellagic acid following microbial breakdown of ellagitannins found in pomegranate extract. The three clusters are: UM-A -producers of urolithin A, UM-B -producers of urolithin- B, and UM-O which are non-producers. The UM-A individuals had a significantly higher abundance of *Gordonibacter* compared to UM-B and UM-O. Apart from that, 50% of UM-O participants were identified to have become either UM-A or UM-B following pomegranate extract consumption. It was further highlighted that UM-A (urolithin metabotype) individuals have a significantly higher abundance of *Gordonibacter* compared to UM-B and UM-O. Klinder and group, on the other hand, reported up-regulation in *C. leptum-R. bromii/flavefaciens* and *Bacteroides/Prevotella* in the low flavonoid group following an additional six servings of fruits and vegetables (F&V) which was attributed to both polyphenols and other substances found in F&V such as fibre. Walker et al reported significant improvements in *Gammaproteobacteria, Gemellaceae, Turicibacter, Atopobium* genera following the consumption of trans-resveratrol. Intervention with freeze-dried cranberry powder significantly improved *Lachnospira,* and*Anaerostipes*. Istas et al on other hand reported significant modulation in *Anaerostipes* and *Bacteroides*. Song et al as well observed positive changes in *Akkermansia, Roseburia, Prevotella, and Bacteroides* following the intake of Schisandra chinensis fruit compared to placebo. Interestingly, the study conducted by Moreno and group reduced the differences in bacterial composition between MetS and healthy subjects following 30 days consumption of RW and DRW. Gut microbiota of Mets belonging to enterotype 1 (*Bacteroides*) was significantly improved following RW and DRW intake similar to that of healthy participants which belong to enterotype 2 (*Prevotella*). Queipo

and co (Queipo-Ortuño et al., 2012) also conducted an RCT with RW, DRW, and gin in healthy participants for 20 days. Their study indicated a significant improvement in *Enterococcus,* *Bacteroides*, and *Prevotella*, *Blautia coccoides–Eubacterium rectale* group, *Eggerthella lenta,* and *Bacteroides Uniformis* genera following RW/DRW intake. A significant reduction in intestinal microbes was reported by Molan and group for *Bacteroides* and *Clostridia*, Walker et al., for *Rikenellaceae*, *Ruminococcus, Oscillospira*, *Clostridium*, *Alistipes, Odoribacter*, and *Butyricimonas*, Rodriguez-Morato et al. for *Oribacterium*, Queipo et al. for*Clostridium* genera and the *Clostridium histolyticum* group and Tzounis et al. for the *Clostridium histolyticum* group. The results of each study are presented in Table 4.

Effects of polyphenols on anthropometric variables and clinical markers

As presented in Table 4, amongst the anthropometric variables evaluated (body weight, BMI, waist circumference, hip circumference) only the study by Song et al. (2015) showed a significant drop in waist circumference. As for the clinical markers, 12 RCTs showed significant improvement in various CVD markers (CRP, TNFα, IL-6, IL-1β, leptin, blood pressure, glucose, TC, TAG, HDL, LDL, FMD) following polyphenol consumption: total cholesterol (Gonzalez-Sarrias et al., 2017; Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012; Tzounis et al., 2011), TAG (Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012; Song et al., 2015; Tzounis et al., 2011), CRP (Klinder et al., 2017; Moreno-Indias et al., 2015; Tzounis et al., 2011), BP (Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012), flow-mediated dilation (FMD) (Istas et al., 2019), glucose (Moreno-Indias et al., 2015; Song et al., 2015), oral glucose tolerance (120 min) (Walker et al., 2018), Insulin-AUC (Lear et al., 2019), inflammatory markers (TNFα, Il-6) (Vitaglione et al., 2015), VCAM, e-selectin and nitric oxide (Klinder et al., 2017), and oxLDL

(Martin- Peláez et al.,2017), and leptin (Puuponen- Pimiä et al., 2013). This significant modulation of CVD-related markers was achieved following intervention with trans-resveratrol (Walker etal., 2018), pomegranate extract (Gonzalez-Sarrias et al., 2017), aronia berry extract and whole fruit powder (Istas et al., 2019), montmorency cherry drink (Lear et., 2019), fruits and vegetables (Klinder et al., 2017), red wine and dealcoholized red wine (Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012), Schisandra chinensis fruit (Song et al., 2015), berries (Puuponen- Pimiä et al., 2013). cocoa flavanol drink (Tzounis et al., 2011), phenolic compound/thyme enriched olive oil (Martin- Peláez et al.,2017), and whole grain products (Vitaglione et al., 2015)... Molan et al. (2014) highlighted significant changes in markers of colon cancer (β-glucuronidase and fecal pH, Rodriquez-Morato et al identified significant improvement in secondary bile acids and faecal pH, while Ravn-Haren et al related the lipid-lowering effects of apple and apple-related products to fibre and pectin. Two studies reported nonsignificant findings in any of the clinical markers following intervention with green tea extract (Janssens et al., 2016) and black tea.(Mai et al., 2004) (Table 4).

Univariate and multivariate correlation analysis investigating the relationship between gut microbiota and clinical markers

As shown in Table 5, only seven studies (G. Istas et al., 2019; Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012; Rodriguez-Morato et al., 2018; Song et al., 2015; Tzounis et al., 2011; Vitaglione et al., 2015) presented univariate correlation data investigating the relationship between microbes and various clinical markers. Whereas, multivariate regression analysing the independent relationship between gut microbes and clinical markers was presented in three trials (Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012; Tzounis et al., 2011). (Table 6)

The univariate analysis highlighted a significant correlation between certain intestinal microbes with metabolic parameters as indicated in Table 5. Of these, the genera that showed negative correlations with various parameters were *Bacteroides* with TNFα (Vitaglione et al., 2015), TAG (Queipo-Ortuño et al., 2012), cholesterol (Queipo-Ortuño et al., 2012), HDL (Queipo-Ortuño et al., 2012), DBP (Queipo-Ortuño et al., 2012), SBP (Queipo-Ortuño et al., 2012), fat mass (Song et al., 2015), and aspartate aminotransferase (Song et al., 2015), *Lactobacillus* with TNFα (Vitaglione et al., 2015), TAG (Moreno-Indias et al., 2015), cholesterol (Moreno-Indias et al., 2015), HDL (Queipo-Ortuño et al., 2012) and CRP (Queipo-Ortuño et al., 2012; Tzounis et al., 2011), *Bifidobacterium* with cholesterol (Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012), LPS and CRP (Tzounis et al., 2011), *Phascolarctobacterium* and *Roseburia* with FMD (Istas et al., 2019), *Ruminococcus* with HDL (Song et al., 2015) and glucose (Song et al., 2015), and lastly, *Anaerostipes* with deoxycholic acid (Rodriguez-Morato., 2018) Whereas positive correlations were shown between *Clostridium* with TAG (Moreno-Indias et al., 2015) and CRP

(Moreno-Indias et al., 2015), *E. coli* with TAG (Moreno-Indias et al., 2015) and cholesterol (Moreno-Indias et al., 2015), *Dialister* with FMD (Istas et al., 219), ,*Bifidobacterium* with HDL (Moreno-Indias et al., 2015), and *Fusobacterium* with deoxycholic acid (Rodriguez-Morato., 2018) Multivariate analysis on the other hand, showed negative correlations between , *Lactobacillus* andTAG, CRP, *Bacteroides* with TAG, HDL, DBP, and SBP, and *Bifidobacterium* with cholesterol, CRP, and LPS (Table 6).

Polyphenols, the "new" prebiotics

The preventative role of polyphenols' against chronic diseases, particularly CVD (George et al., 2019; Rienks et al., 2017; Wang et al., 2014), type 2 diabetes (Grosso et al., 2017; Spínola, Llorent-Martínez, & Castilho, 2019) and certain cancers (colorectal and breast cancer) (S.-T. Lin et al., 2016; Poschner et al., 2019; Teixeira et al., 2017) is well documented. Its efficacy has been known to be dependent on its bioavailability, structural complexity, and its composition in the food matrix (Appeldoorn, Vincken, Gruppen, & Hollman, 2009; Cardona et al., 2013; Walle, 2004). Recent findings, however, are linking its bio-efficacy to the gut microbiota-polyphenol interplay (Cardona et al., 2013; Duenas et al., 2015; Kumar Singh et al., 2019; Ozdal et al., 2016b) and with the latest definition of prebiotics, i.e *"a substrate that is selectively utilized by host microorganisms and conferring a health benefit"* (Gibson et al., 2017), polyphenols are beginning to emerge as the "new" prebiotics. This review was carried out with the intention of studying the prebiotic potential of polyphenols in RCTs.

The findings of the RCTs reviewed here indicate it is likely that the consumption of polyphenols could have a positive effect on gut microbiota and markers associated with CVD as 59% of the

reviewed RCTs support our hypothesis. However, this needs to be interpreted with caution as the quality of the RCTs assessed varied.

Overall, out of the 17 studies, 10 indicated a significant modulation of gut microbiota following polyphenol intervention (Table 4), two reported non-significant improvement of few genera, and the remaining five pointed out no significant findings. In particular, six studies reported bifidogenic effects and three RCTs stated positive modulation of *Lactobacillus* similar to conventional prebiotics: the non - digestible oligosaccharides fructans and galactans (Rastall & Gibson, 2015). The health benefits of *Bifidobacteria* and *Lactobacilli* are well established (Arboleya, Watkins, Stanton, & Ross, 2016; Begley, Hill, & Gahan, 2006; Cani et al., 2007; Guo et al., 2019; O'Callaghan & van Sinderen, 2016; Yan et al., 2019). *Lactobacillus* and *Bifidobacterium* were identified to increase in the presence of phenolic compounds such as anthocyanin found in red wine,(Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012) polyphenols from grape pomace and grape seed (Hervert-Hernandez, Pintado, Rotger, & Goni, 2009), and flavanols in cocoa (Tzounis et al., 2011). *Bifidobacterium* was highlighted to play a significant role in the maintenance of intestinal barrier and therefore improve gut integrity. This, in turn, prevents the leakage of pathogenic substances such as LPS from the intestinal lumen to the systemic circulation (Cani et al., 2007). Therefore, *Bifidobacteria* probably offers protection against low-grade inflammation associated with metabolic syndrome, type II diabetes, non-alcoholic fatty liver disease (NAFLD) and CVDs (Minihane et al., 2015). *Lactobacillus* and *Bifidobacteria* collectively have been shown to halt the growth of pathogenic microbes (Gotteland et al., 2008; D. M. Saulnier, S. Kolida, & G. R. Gibson, 2009), relieve lactose intolerance, improve cholesterol level due to their bile salt hydrolysing capabilities (Begley et al.,

2006; Ejtahed et al., 2011; Hervert-Hernández & Goñi, 2011; Huang & Zheng, 2010), and stimulate the production of lactate and acetate (D. Saulnier, S. Kolida, & G. Gibson, 2009).

Earlier, the term prebiotic was most often related to the positive modulation of *Bifidobacteria* and *Lactobacilli* (Gibson et al., 2017). With advancement in molecular techniques, other beneficial microbial groups are now being recognised for their prebiotic effect. Through this systematic review, we were able to showcase the modulation of other gut microbes some of which were correlated with certain health markers. *Ruminococcus bromii* plays a significant role in the breakdown of resistant starch in human gut (Ze, Duncan, Louis, & Flint, 2012), while a reduction in *Bacteroides/Prevotella* has been associated with obesity (Hjorth et al., 2019; Kong et al., 2014; Ley, 2010), in particular an inverse relationship was seen between these genera and fat mass (Fava et al., 2013). *Faecalibacterium prausnitzii*,*Roseburia,* and *Anaerostipes* are butyrate producers (Burger-van Paassen et al., 2009; Hao, Wang, Guo, & Liu, 2019) - a component that induces mucin production which in turn improves gut integrity (Peng, Li, Green, Holzman, & Lin, 2009). *Eggerthella lenta,* and *Bacteroides uniformis* can breakdown resveratrol into dihydroresveratrol (Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012). The resulting dihydroresveratrol possess anti-proliferative effects against human prostate cancer cells (Queipo-Ortuño et al., 2012). *Proteobacteria* has been observed to metabolise uric acid (Self, 2002), while *Akkermansia* was reported to improve diet- induced weight gain and fat mass (Everard et al., 2013). The antimicrobial effect of polyphenols was mainly observed against *Clostridium* (Molan et al., 2014; Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012; Tzounis et al., 2011). This observation was related to the ability of flavan-3-ol and procyanidins to bind to the

bacterial membranes of this genus (Kemperman, Bolca, Roger, & Vaughan, 2010; Sirk, Brown, Friedman, & Sum, 2009).

As for the studies that reported non-significance in GM modulation, few observations are worth highlighting in this review. Janssens et al. reported non-significance in GM modulation following 12 weeks of intervention with green tea extract. The authors related the findings to normo-weight participants who may have a stable gut microbial profile unlike that of obese patients or those with chronic illnesses. We also noted that this study only analysed three phyla, therefore may have missed genus level GM modification. Ravn-Haren and co observed insignificant changes with apple and apple products and proposed a longer duration with a larger population. Similar to Janssen and co's study, this RCT confined their GM assessment to only a few genera (*Bifidobacteria*, *Clostridium* and *Bacteroides*). As for the RCT by Mai et al., fecal samples were collected from six participants while 13 volunteers were assessed for bile acid (the total number of participants was not reported). Thus, there is a possibility of inadequate sampling which may have resulted in a negative observation. Puupponen-Pimia et al only utilised DGGE which is a semi-quantitative method to analyse GM composition. DGGE is a useful first-line test that is prone to incomplete separation of different bacterial DNA (Tabit, 2016). Lastly, Lear and co also reported non-significant changes in GM modulation following the consumption of montmorency cherry drink. The author pointed out inappropriate storage methods of collected faecal samples, the number of study participants as well as the study duration to be the underlying cause of the non-significant results obtained.

Besides the GM-modulatory property, the ISAPP consensus statement also iterated the importance of a substance to improve host health in order to be classified as a prebiotic. Through

this review it is evident that polyphenols subscribe to such properties, as 10 studies (Gonzalez-Sarrias et al., 2017; G. Istas et al., 2019; Klinder et al., 2017; Martín-Peláez et al., 2017; Molan et al., 2014; Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012; Rodriguez-Morato et al., 2018;; Tzounis et al., 2011;; Walker et al., 2018) reported improvements in both intestinal microbiota and various clinical markers particularly CVD-related parameters The prebiotic capacity of polyphenols was further proven with the provision of a correlation analysis in some of the studies. The genera *Bacteroides*, *Lactobacillus*, and *Bifidobacterium* were correlated to inflammatory and lipid profile parameters. With the existing correlation analysis, most notable changes were seen following red wine and high flavonal cocoa interventions.

Effect of gut metabotypes on clinical markers

Two RCTs which investigated the effects of red wine observed differences in the modulation of clinical markers: Moreno et al. reported significant improvement in glucose, total cholesterol, and HDL, which were not observed by Queipo et al. This may be related to the unique gut metabotypes of participants. Gut metabotype refers to differences in metabolizing phenotype due to an individual's distinctive gut microbiome. This results in the release of gut microbiota specific metabolites (Espin, Gonzalez-Sarrias, & Tomas-Barberan, 2017). In their RCT with pomegranate extract, Gonzales et al. investigated the gut metabotype concept and reported improved CVD markers based on different urolithins metabotypes (UMs) as previously reported daidzein-metabolizing phenotypes (Frankenfeld, 2017; Reverri, Slupsky, Mishchuk, & Steinberg, 2017). Pomegranate extract administration improved blood lipid levels only in UM-B

participants who were at risk of developing CVDs. Therefore, this group postulated that with ellagitannin-containing food, higher effects may only be seen in individuals with specific microbial communities, i.e. urolithins-mediated activity only in UM-B subjects (Gonzalez-Sarrias et al., 2017). Such observations were also noted with red wine (Vazquez-Fresno et al., 2016), whereby participants were clustered based on 4-hydroxyphenylacetate production. Therefore, stratification of individuals based on their ability to produce certain metabolites following polyphenol consumption probably will provide a more promising result and be able to diminish the inter-individual variations reported with the same phenolic interventions.

We would also like to highlight the need to have more studies on cancer-related markers as there was only one which investigated both intestinal microbes and markers of colon cancer. Thus, we are unable to conclude the effect of polyphenols on gut microbes and parameters related to cancer.

Challenges and Trends Though polyphenol intervention improved intestinal microbes and CVD related markers, there were, however, few challenges in summarizing the findings of this review. Firstly, the heterogeneity present due to differences in the study population: some studies involved healthy subjects while others involved overweight/obese individuals, those presenting with CVD risk, hypercholesteraemia, and metabolic syndrome. The age range was wide in most studies, andindeed it has been previously postulated that age may affect the gut microbial composition of individuals (Kristensen et al., 2016; Seganfredo et al., 2017). The exclusion criteria of participants were not clear in two studies (Janssens et al., 2016; Moreno-Indias et al., 2015) and not mentioned in one (Mai et al., 2004). As such, the presence of other comorbidities

may affect the gut microbial profile in these RCTs. All of these factors may influence the baseline gut microbial composition (Duda-Chodak, Tarko, Satora, & Sroka, 2015; Zhu, Sunagawa, Mende, & Bork, 2015) leading to differences in bioavailability and bio-efficacy of polyphenols. The study duration varied tremendously and some RCTs were only conducted from 5 days-4 weeks (Gonzalez-Sarrias et al., 2017; Lear et al., 2019; Martín-Peláez et al., 2017; Molan et al., 2014; Moreno-Indias et al., 2015; Queipo-Ortuño et al., 2012; Rodriguez-Morato et al., 2018; Tzounis et al., 2011). Moreover, heterogeneity in the polyphenols used in these studies may lead to variation in the modulation of intestinal microbes and therefore the health benefits /clinical markers. It's also noteworthy that only one polyphenol discussed in this review was a pure compound, while the remaining 16 were extracts of food/food ingredients/formulations containing polyphenols. Thus, the presence of dietary fibre in the interventions may be a contributory factor in modulating gut microbiota or rather may be a prebiotic itself (Edwards et al., 2017; F. Saura-Calixto, 2011).

As for the method utilised in investigating GM, most RCT used a targeted approach to evaluate the changes in gut microbiota, thus there is a possibility of missing out on the untargeted groups. The different molecular techniques used to analyse gut microbes (FISH, FISH-FC, qPCR, DGGE, IS-profiling, 16s rRNA sequencing) also presented a huge variation in the taxonomic level reported. Though eight studies investigated CVD-related markers, the markers analysed varied between studies which posed a vast challenge for us to make a definite conclusion on the effect of polyphenols. Due to the above-mentioned limitations, a meta-analysis was not plausible.

Strength

To the best of our knowledge, this is the first systematic review that looked at the effectiveness of polyphenol interventions in improvements in both gut microbiota and various clinical markers. This review was undertaken by two reviewers who independently searched, screened, and assessed the internal quality of RCTs using two different tools, thereby, reducing the risk of error and bias. We also included RCTs of all languages during the initial search process. The findings of this review involve RCTs which should be considered more reliable as they are assumed as "gold standards" in measuring the efficacy of an intervention.

Conclusion

In summary, this review supports the notion of polyphenols as prebiotics capable of modulating intestinal microbes which in turn affect markers mainly associated with CVD. Besides *Bifidobacterium* and *Lactobacillus*, polyphenols positively modulated other gut microbes that were shown to improve human health. Nevertheless, due to the varying internal quality of the studies included in this review further well-structured trials with larger samples, longer duration, and high-throughput molecular techniques are warranted to obtain conclusive results.

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892 *Table 3* **PICOS criteria used to perform the systematic review**

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Parameter	**Description**
Population	Human subjects with no restriction on age, gender and ethnicity.
Intervention,	Dietary polyphenols or pure compounds of polyphenols or both
Comparator	Placebo or any other form of therapy
Outcome	1. Gut microbiota modulation/ any type of anthropometric variables (body weight, BMI, waist circumference, hip circumference) or clinical markers [CVD – C-reactive protein (CRP), tumor necrosis factor alpha (TNFα), IL-6, IL-1β,blood pressure, glucose, total cholesterol (TC), triacylglyceride (TAG), high density lipoprotein (HDL), low density lipoprotein (LDL); other markers – bile acid, faecal pH] 2. Any type of anthropometric variables (body weight, BMI, waist circumference, hip circumference) or clinical markers [CVD – C-reactive protein (CRP), tumor necrosis factor alpha (TNFα), IL-6, IL-1β,blood pressure, glucose, total cholesterol (TC), triacylglyceride (TAG), high density lipoprotein (HDL), low density lipoprotein (LDL); other markers – bile acid, faecal pH] / Gut microbiota modulation
Study design	Randomized controlled trials

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901 *Table 4* **Characteristics of reviewed studies**

Lead author, year, country	Design	Jadad Scale	Participants	Intervention	Total polyphenol	Duration	Method/Modulation of gut microbes	Modulation of clinical markers (Significant/highlights)
Pure compound								
Walker et al. (2018) United States of America	Pilot RCT, DB, parallel, placebo Two groups: trans-resveratrol (RES), control (CT)	3	Obese men with MetS (BMI: 30–40 kg/cm^2) Age 30-70 N=31 (DO =3, N = 28, RES= 14, CT = 14)	trans-resveratrol (RES) subjects –Mega-RES capsules, 2x CT- 500mg placebo capsules, 2x	500mg RES/capsule	30 days	**16s rRNA sequencing (V4 region):** Significant improvement - Alpha diversity (compared to CT) No significance - beta diversity (compared to CT) Significant improvement - Alpha and beta diversity (compared to baseline) Relative abundance (taxonomy) (compared to baseline): Sigficant ↑ in *Gammaproteobacteria*, *Gemellaceae*, *Turicibacter*, *Atopobium*. Significant ↓ in *Rikenellaceae*, *Ruminococcus*, *Oscillospira*, *Clostridium*, *Alistipes*, *Odoribacter*, *Butyricimonas*	No significant in MetS characteristics (FBG, HDL, TAG, SBP, DBP, waist circumference) significant ↓in OGTT (120 min) compared to CT Gene expression (subcutaneous white adipose tissue): No significance – gene associated with glucose metabolism compared to baseline and CT

Extracts								
Gonzalez-Sarrias et al. (2017) Spain	RCT, DB, CO, placebo	5	Overweight-obese males and females (BMI > 27 kg/m^2) with no diagnosed chronic diseases Age > 40 N=50 (DO =1, N=49)	450mg pomegranate extract in gelatin capsule placebo – maltodextrin in hard gelatin capsule	106.4 mg/capsule	Six months; Intervention- 3 weeks, WO-3 weeks	**Real-time qPCR:** Significant ↑ *Gordonibacter, Bacteroides, E. coli*	4 capsules/day- significant ↓ oxLDL in both arms but only, with more pronounced effects in UM-B individuals 1 capsule/day – significant ↓ TC, small LDL subfraction, Apo B, and oxLDLc in UM-B individuals 4 capsules/day – significant ↓TC, small LDL subfraction, Apo B, and oxLDL, LDL and non-HDL
Janssens et al. (2016) Netherlands	RCT, SB, parallel, placebo Two groups - green tea (GT), placebo (PL)	0	Healthy, Normal weight (BMI 18–25 kg/ m^2)/ overweight/obese (BMI >25 kg/m^2) Caucasians Age :18–50 N=65 subjects (4 DO, N = 58, GT=30, CT=28)	GT extract 9 capsules/day	230-270 mg/capsule	12 weeks	**IS-profiling:** *No significant findings	* No significant findings
Molan et al. (2014) New Zealand	RCT, parallel Two groups: First leaf (FL) and Cassis Anthomix 30 (CAM30)	0	Healthy Age: 20–60 N=30 (FL=15, CAM 30 = 15)	FL (blackcurrant extract powder, lactoferrin, lutein in gelatin capsule) CAM30 (blackcurrant extract powder in gelatin capsule)	No data	Baseline-2weeks, intervention - 2weeks, WO-2weeks	**FISH analysis** After intervention with CAM 30:	After intervention with CAM 30: Significant ↓ in β-glucuronidase Significant ↑ in β-glucosidase

				Group one – FL (1500 mg/day; 375 mg 4 capsules), Group 2 - CAM30 (672 mg/day; 168 mg, 4 capsules) - both products contain same amount of blackcurrant powder (672mg)			Significant ↑ in *Lactobacillus spp*. and *Bifidobacterium spp* Significant ↓ - *Clostridium spp*. and *Bacteroides spp*.	Significant ↓ in stool pH
Drinks								
Song et al. (2015) Korea	RCT, DB, placebo Two groups: Schisandra chinensis (SCF) and placebo (CT)	3	Obese (BMI ≥25 kg/m^2) N=40 (DO=12, N=28, SCF =13, CT=15)	SCF (100ml) x 2 pouches /day	11.9 mg/ 100 mL	12 weeks	**DGGE & qPCR:** ↑ *Akkermansia, Roseburia, Bacteroides, Prevotella, Bifidobacterium* (CT) ↓*Ruminococcus* (CT)	Significant ↓ in waist circumference Significant ↓in fat mass ↓ FBG, TAG, AST, ALT (SCF)
Mai et al. (2004) United States of America	RCT, DB, CO	3	Information not given	Black tea (BT) (amount/dose - not given)	No data	Run-in -2 weeks, treatment - not given, WO - 4 weeks	**FISH** * No significant findings	*No significant difference
Moreno-Indias et al. (2015) Spain	RCT, CO Two groups: Mets and control (CT)	0	Healthy and participants with Mets Men Age: 45-50	Red wine (RW) -272 mL/d Dealcoholized red wine (DRW) -272 mL/d	RW – 797.86 mg/272 mL	Initial WO - 2weeks Intervention – 30 days WO – 15 days	**Real-time quantitative PCR:** Baseline: MetS vs. CT	MetS (RW &DRW) compared to baseline: Significant ↓ in SBP, DBP, glucose, TAG, TC, CRP and LPS

	N= 20	DRW – 733.02 mg/272 mL	Significant ↑ - *Proteobacteria, Firmicutes* No significance after RW & DRW After intervention, in MetS compare to baseline (RW, DRW): Within Firmicutes: Significant ↓ in *Clostridium, Clostridium histolyticum* Significant ↑in *Blautia coccoides–Eubacterium rectale* group, *Faecalibacterium prausnitzii, Roseburia, Lactobacillus* Within *Bacteroidetes*: Significant ↑in *Prevotella* Significant ↓in *Bacteroides* Within *Actinobacteria*: Significant ↑ in *Bifidobacterium, Eggerthella lenta* Within *Proteobacteria*: Significant ↓in *Escherichia coli, Enterobacter cloacae* CT compared to baseline (RW, DRW):	Significant ↑in HDL

							Within *Firmicutes*: Significant ↑ in *Faecalibacterium prausnitzii, Roseburia* Significant ↓in *Bacteroides uniformis* Within *Actinobacteria*: Significant ↑ in *Bifidobacterium, Eggerthella lenta*	
Queipo-Ortuño et al. (2012) Spain	RCT, CO	0	Healthy adult men Age 45–50 N=10	Red wine (RW) -272 mL/d Dealcoholized red wine (DRW) -272 mL/d Gin – 100mL/d	RW – 797.86 mg/272 mL DRW – 733.02 mg/272 mL Gin – not detected	WO – 15days (baseline), intervention - 3 consecutive periods of 20 days	**DGGE& q-PCR** After intervention (compared to baseline): Significant ↑ in *Proteobacteria, Fusobacteria, Firmicutes, Bacteroidetes* (RW) Significant ↑ in *Fusobacteria* (DRW) Within *Firmicutes*: Significant ↑ in *Enterococcus, Blautia coccoides–Eubacterium rectale* (RW, DRW) Significant ↑ in *Clostridium, Clostridium histolyticum* (gin) Within *Bacteroidetes*: Significant ↑ in *Bacteroides, B.*	After intervention (compared to baseline): Significant↓ in DBP (RW) Significant ↓ in uric acid, TC (RW) Significant ↓ in SBP (RW, DRW) Significant ↓in glutamate-oxaloacetate transaminase (RW, DRW) Significant ↓ in c-glutamyl transpeptidase (RW, DRW) Significant ↓ in TAG (RW. DRW) Significant ↓ in HDL, CRP (RW, DRW)

							uniformis, Prevotella (RW) Significant ↓ in *Prevotella* (gin) Within *Actinobacteria*: Significant ↑ in *Bifidobacterium, Eggerthella lenta* (RW) Significant ↓ in *Bacteroidetes, Firmicutes* (DRW compared to RW)	
Tzounis et al. (2011) United Kingdom	RCT, DB, CO	5	Healthy volunteers BMI: 20.2- 25.4 Age 18–50 N=20 (2 DO, N= 18)	Cocoa drinks -low cocoa flavanol (LCF) drink or high–cocoa flavanol (HCF) drink	LCF – 29mg flavanol HCF – 495 mg flavanol	Intervention- 4weeks WO- 4 weeks	**FISH:** Significant ↑ in *Lactobacillus, Enterococcus spp, Bifidobacterium spp* (HCF) Significant ↑ in *E. rectale–C. coccoides* (HCF, LCF) Significant ↓ in *C. histolyticum* group (HCF) Significant ↑ in *C. histolyticum* group (LCF)	Significant ↓ in TC ((LCF, HCF) Significant ↓ in TAG (HCF) (compared to baseline and LCF) Significant ↓ in CRP (HCF)
Lear et al. (2019) United Kingdom	RCT, DB, placebo	1	Healthy men and women Age 40-60 N=30 (DO =2, N=28, MC = 13, CT = 15)	Montmorency cherry (MC) drink, 30mL 2x/day	AC - 296mg/ day PP- 1040mg/ day	Intervention – 4weeks	**16s rRNA sequencing (V3-V4 regions):** No significance - richness and diversity	Significant ↓ in insulin AUC (compared to baseline) No significance – blood glucose, OGTT, serum insulin, HOMA-IR, RER, plasma CRP, IL-6

Dietary polyphenols								
Klinder et al. (2017) Macready et al. (2014) (both are part of FLAVURS trial) United Kingdom	RCT, parallel Three groups: high-flavonoid (HF), low-flavonoid (LF), control (CT)	2	Subject with elevated CVD risk Age 30–70 N = 122 (HF=39, LF=42, CT=41)	Daily intake of fruits and vegetables (F&V) - gradient manner with an additional 2 portions per day every 6 weeks resulting in 6 extra portions per day during weeks 12–18. High-flavonoid (HF) fruit & vegetables (≥15 mg/100 g) Low-flavonoid (LF) fruit & vegetables (<5 mg/100 g)	2 portions – LF: 7mg/day HF: 98 mg/day 4 portions: LF: 12mg/day HF: 243 mg/day 6 portions: LF: 13 mg/day HF: 394 mg/day	18 weeks	**FISH:** Significant ↑ *in C. leptum-R. bromii/flavefaciens* (week 18, LF) Significant ↑ in *Bacteroides/Prevotella* (week 6,12,18, LF) Significant ↑ in *Bacteroides/Prevotella* (week 18, HF) Significant ↑ in *Bifidobacterium* (week 18, LF)	*No significant findings – anthropometric variable Significant ↓ in CRP -HF men +4portion of F&V compared to baseline, LF and HF with +2 and +4 compared to CT Significant ↓ in VCAM with +4 compared to CT Significant ↓in E-selectin –HF men with +4, LF men +2 and LF women +4 Significant ↑ in nitric oxide in HF with +4 compared to LF and CT
Puupponen-Pimiä et al. (2013) Finland	RCT	0	Overweight male and female N= 37 (DO=5, N = 17)	300 g fresh berries comprising of 100 g of strawberry puree, 100 g of frozen raspberries, and 100 g of frozen cloudberries/day	~ 863.8mg/ 300 g	Baseline- 4-weeks intervention - 8weeks, Recovery -4 weeks	**DGGE:** *No significant differences in similarity values or diversity of predominant bacterial populations	After intervention: Slight significant in leptin Borderline positive effects on SBP, LDL, 8-isoprostane, TRAP, and resistin
Others								
Martín-Peláez et al. (2017) Spain	RCT, DB, CO (subsample from VOHF (Virgin Olive Oil and HDL (high density	3	Hypercholesterolemic participants (total cholesterol >200 mg/dL) Age 35 - 80	Daily dose of 25 mL of three raw virgin olive oil (OO)	VOO– 2.88 mg/25 mL	Intrevention-3 weeks, WO - 2weeks	**FISH-FC** Significant ↑ - most *Bifidobacterium spp, Parascardovia*	Significant ↓in ox-LDL post-FVOOT intervention

	lipoprotein) Functionality) study		N = 10	Virgin OO naturally containing 80 mg phenolic compound (PC)/kg (VOO) PC-enriched virgin OO containing 500 mg PC/kg from OO (FVOO) PC-enriched virgin OO containing a mixture of 500 mg PC/kg from OO and thyme, 1:1 (FVOOT)	FVOO-12.61 mg/25 mL FVOOT-12.1 mg/25 mL		*denticolens* (FVOOT compared to VOO)	
Ravn-Haren et al. (2013) Denmark	RCT, SB, 5 x 4 weeks CO Five periods consisted of a control period where only the restricted diet was allowed and four periods where the restricted diet was supplemented with one of four apple products.	0	Healthy men and women Age 18–69 N=34, (DO=11, N=23)	whole apples (WA) (550 g/day), apple pomace (AP) (22 g/day), clear (CL J) and cloudy apple (CJ) juices (500 ml/day),	WA – 239 mg/day AP – 75 mg/day CJ -145 mg/day CLJ- 108 mg/day	Restricted diet- 4 weeks-, restricted diet + one of four apple products - 4 weeks	**DGGE & q-PCR:** DGGE findings could be verified by qPCR, which showed no effect of any diet for any of the primers tested	After intervention: Significant ↓ in TC and LDL (WP, AP, CJ) Significant ↑ in TC and LDL (CL J compared to WA & AP) Significant ↓ in LCA (AP, CJ compared to CT) Significant ↑ in FRAP (WA, CJ compared to CT) Significant ↓ in FRAP (AP compared to WA, CJ, CLJ) Significant ↓ in ORAC (WA compared to CJ, CLJ) Significant ↑ in erythrocyte GPX1 (AP compared to CT, CLJ, CJ) Significant ↓ GPX1 (CLJ, CJ compared to WA)
Vitaglione et al. (2015) Italy	RCT, parallel, placebo Two groups: whole grain (WG) and control (CT)	2	Overweight/obese (BMI: 25–35) Age > 18 N=80 (DO =12, N = 68, WG=36, CT=32)	WG subjects- 70 g/d (3 biscuits/d) of WG product CT - 1 package (33 g) of crackers and 3 slices of toasted bread (w27 g).	138.7 mg/70 g of WG	8 weeks	**16S rRNA gene sequencing:** * No significant difference in response to intervention.	Significant ↓ in TNF-a (8weeks compared to CT and baseline) Significant ↑ in IL-10 (4weeks compared to CT, baseline) ↓ IL-6 (8 weeks compared to CT)

Istas et al. (2019) United Kingdom	RCT, DB, three arms, parallel whole fruit (WF), extract (EX), control (CT),	5	Healthy male Age 18-45 N=66 (DO =2, N=64)	Aronia berry extract - 500mg/capsule Aronia whole fruit – 500mg/capsule	EX – total polyphenol – 116mg/capsule WF – 12mg/capsule	Intervention – 12weeks	**16s rRNA sequencing (V3-V4 regions)** No significance - alpha and beta diversity Relative abundance (random forest): Significantly ↑ in *Anaerostipes* in EX (compared to baseline and CT) Significantly ↑ in *Bacteroides* in WF (compared to baseline)	Significant ↑ -in FMD (WF, EX compared to CT at week 12) Significantly ↑ -in FMD (EX compared to CT at 2 h, day 1) Significant ↑ in FMD (EX compared to CT at 2h, week 12) No significance – blood pressure, arterial stiffness, TC, HDL, LDL, TG, FBG, body weight, body fat, BMR
J. Rodríguez-Morató et al. (2018) United States of America	RCT, DB, CO	1	Healthy men and women Age 25-54 N=11, (DO =11, N=23)	30 g/day of freeze-dried whole cranberry powder	LG-1.665g PA-706.2mg HC-45.6mg FL-138.9mg AC-83.7mg	Intervention – 5days, WO – 2weeks	**16S rRNA pyrosequencing:** No significance - alpha and beta diversity (compared to CT) Relative abundance (phylum) Significant ↑ in *Bacteroidetes* Significant ↓ in *Firmicutes* Relative abundance (LDA > 2) compared to CT: Significantly ↑ in genera *Lachnospira*. *Anaerostipes* Significant ↓ in genus *Oribacterium*	No significance- IFN-γ, IL-1β, IL-6, TNF-α Significant ↓ in lithocholic, deoxycholic (secondary faecal bile acid) (compared to CT) No significance- TMA and TMAO (plasma, urine, faeces) Significant improvement - faecal pH (compared to baseline) No significance -faecal water content (compared to CT , baseline)

AC, Anthocyanins; AST, Aspartate Transaminase; ALT, Alanine Aminotransferase; ApoB, Apoprotein B; AUC, Area Under Curve; BMI, Body Mass Index; BMR, Basal Metabolic Rate; CRP, C-Reactive Protein; CVD, Cardiovascular Disease; CO, Crossover; DB, Double Blind; DBP, Diastolic Blood Pressure; DO, Drop-Out; DGGE, Denaturing Gradient Gel Electrophoresis; FBG, Fasting Blood Glucose; FISH, Fluorescent In situ Hybridization ; FISH-FC, Fluorescent In situ Hybridization Flow Cytometry; FL, Flavonols; FMD, Flow Mediated Dilation; FRAP, Ferric Reducing Ability of Plasma; Gpx1, Glutathione Peroxidase; HC, Hydroxycinnmate; HDL, High Density Lipoprotein; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; IL6, Interleukin 6; IL10, Interleukin 10;IL-1β, Interleukin 1 Beta; IFNγ, Interferon Gamma; IS-profiling, Interspacer profiling; LCA, Lithocholic Acid; LDA, linear discriminant analysis; LDL, Low density Lipoprotein; LG, Lignin; LPS, Lipopolysaccharide; MetS, Metabolic Syndrome; OGTT, Oral Glucose Tolerance Test, ORAC, Oxygen Radical Absorbance Capacity; oxLDL, Oxidized Low Density Lipoprotein; PA,

Proanthocyanidins; q-PCR, Quantitative Polymerase Chain reaction; RCT, Randomised Controlled Trial; RER, Respiratory Exchange Ratio; SB, Single Blind; SBP, Systolic Blood Pressure; TC, Total Cholesterol; TG, Triacylglyceride; TMA, Trimethylamine, TMAO, Trimethylamine N-oxide; TNFα, Tumor Necrosis Factor Alpha; TRAP, Total Radical-Trapping Antioxidant Parameter; UM-B, Urolithin Metabotype B; VCAM, Vascular Cell Adhesion Protein; WO, Wash-Out; ↑, Increase; ↓, Decrease

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	Bacteroides	***Lactobacillus***	***Actinobacterias***	***Clostridium***	***Escherichia coli***	***Bacteroidetes***	***Bacteroides uniformis***	***Bifidobacterium***	***Bifidobacterium Eggerthella lenta***	***Enterobacter cloacae***	***Faecalibacterium prausnitzii***	***Roseburia***	***Enterococcus***	***Ruminococcus***	***Phascolarctobacterium***	***Dialister***	***Fusobacterium***	***Anaerostipes***
TNF-a	r = -0.637, P = 0.002(Vitaglione et al., 2015)	(r = -0.572, P = 0.021, n = 31(Vitaglione et al., 2015)																
TAG	(r = -0.364, P = 0.048(Queipo-Ortuño et al., 2012)	r = −0.915, P =0.030(Moreno-Indias et al., 2015)	r = −0.989 , P =0.001(Moreno-Indias et al., 2015)	r= 0.882 , P=0.048(Moreno-Indias et al., 2015)	r = 0.972,P= 0.006 (Moreno-Indias et al., 2015)	r = −0.916, P = 0.029 (Moreno-Indias et al., 2015)													
TC	r = -0.363, P = 0.049(Queipo-Ortuño et al., 2012)	r = −0.992, P =0.007(Queipo-Ortuño et al., 2012)			r = 0.942, P = 0.005 (Moreno-Indias et al., 2015)		r = −0.956 , P = 0.011(Moreno-Indias et al., 2015)	(r = −0.908, P =0.033) (Moreno-Indias et al., 2015) (r = -0.401, P = 0.028) (Queipo-Ortuño et al., 2012)										
HDL	r = -0.469, P = 0.009(Queipo-Ortuño et al., 2012)	r = -0.447, P = 0.013(Queipo-Ortuño et al., 2012)						r = 0.917, P = 0.028 (Moreno-Indias et al., 2015)	r = 0.901, P = 0.037 (Moreno-Indias et al., 2015)					R = neg, P < .05)(Song et al., 2015)				

LPS			r = 0.915, P = 0.029 (Moreno-Indias et al., 2015)	−0.906 , P = 0.034 (Moreno-Indias et al., 2015)	r = 0.971, P =0.029 (Moreno-Indias et al., 2015)			
CRP	(r = -0.492, P , 0.01)(Tzounis et al., 2011) r = -0.405, P = 0.027) (Queipo-Ortuño et al., 2012)	r = 0.882, P =0.048 (Moreno-Indias et al., 2015)		(r =- 0.438, P , 0.05)(Tzounis et al., 2011) r =- 0.430, P = 0.018) (Queipo-Ortuño et al., 2012)				
FBG					r = −0.997 , P = 0.001 (Moreno-Indias et al., 2015)	r = −0.937, P =0.030 (Moreno-Indias et al., 2015)	r = neg, (P < .05) (Song et al., 2015)	
DBP	r = -0.406,P = 0.026 (Queipo-Ortuño et al., 2012)							
SBP	r = -0.362, P = 0.049 (Queipo-Ortuño et al., 2012)							r = -0.362, P = 0.049(Queipo-Ortuño et al., 2012)

FM	r = neg, P < .01 (Song et al., 2015)	r = neg, P < .05 (Song et al., 2015)				
ALT		R = neg, P < .05 (Song et al., 2015)				
AST	r = neg, P < .05 (Song et al., 2015)	r = neg, P < .01 (Song et al., 2015)				
FMD			r= −0.45 (Istas et al., 2019)	r= −0.45 (Istas et al., 2019)	r=0.42 (Istas et al., 2019)	
Deoxycholic acid					r=0.74 (Rodriguez-Morato et al., 2018)	r=−0.50 (Rodriguez-Morato et al., 2018)

ALT, Alanine Transaminase; AST, Aspartate Transaminase; CRP, C - Reactive Protein; DBP, Diastolic Blood Pressure; FBG, Fasting Blood Glucose; FM, Fat Mass; FMD, Flow Mediated Dilation; HDL, High Density Lipoprotein; LPS-Lipopolysaccharide; SBP, Systolic Blood Pressure, TAG, Triacyglyceride; TC, Total Cholesterol; TNFα, Tumor Necrosis Factor Alpha

Table 6 **Multivariate regression analysis of gut microbiota and clinical markers**

	Actinobacteria	***Lactobacillus***	***Clostridium histolyticum***	***Clostridium***	***Escherichia coli***	***Bacteroides***	***Bifidobacterium***	***Faecalibacterium prausnitzii***	***Enterobacter cloacae***
TAG	P = 0.001, R^2 = -0.99 (Moreno-Indias et al., 2015)	P < 0.001, β = 0.224, R^2 =- 0.99 (Moreno-Indias et al., 2015)	P = 0.029, β = −0.194, R^2 = 0.99 (Moreno-Indias et al., 2015)		P = 0.029, β = −0.194, R^2 = 0.99 (Moreno-Indias et al., 2015)	P= 0.048, R^2 = -0.364 (Queipo-Ortuño et al., 2012)			
TC							P = 0.001, β = 1.004, R^2 = -0.99 (Moreno-Indias et al., 2015); P = 0.012, R^2 = -0.583 (Queipo-Ortuño et al., 2012)		
HDL						P = 0.001, R^2 = -0.732 (Queipo-Ortuño et al., 2012)			
CRP		P = 0.05, R^2 = -0.33(Tzounis et al., 2011)		P = 0.040, β = −0.762 R^2 = 0.97 (Moreno-Indias et al., 2015)			P = 0.018, R^2 =-0.430 (Queipo-Ortuño et al., 2012)		
Glucose								P = 0.001, β = 1.10, R^2 = -0.99 (Moreno-Indias et al., 2015)	
LPS							P = 0.015, β = 0.342, R^2 = -0.750 (Moreno-Indias et al., 2015)		P = 0.032, β = −0.564, R^2 = 0.98 (Moreno-Indias et al., 2015)
DBP						P = 0.48, R^2 = -0.364 (Queipo-Ortuño et al., 2012)			
SBP						P = 0.03, R^2 = - 0.369 (Queipo-Ortuño et al., 2012)			

CRP, C-Reactive Protein; DBP, Diastolic Blood Pressure; HDL, High Density Lipoprotein; LPS, Lipopolysaccharide binding protein; SBP, Systolic Blood Pressure, TAG,Triacyglyceride; TC, Total Cholester

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